

## Antibacterial Potential of Agarwood Leaf Extract (*Aguilaria Microcarpa* Baill) Against *Salmonella Typhi*

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### ABSTRACT

*Aguilaria microcarpa* Baill, commonly known as the Agarwood plant, is commonly used as a medicinal plant that is thought to be able to treat certain diseases because it contains chemical compounds in the form of alkaloids, flavonoids, glycosides, and tannins which can act as antioxidants and antibacterials and can be used as raw materials for the medicinal industry natural herbs. Testing in this research nature is semi-quantitative and is laboratory research in testing *Aguilaria microcarpa* Baill leaf extract based on characterization tests, chemical content tests, and antibacterial potential tests on growth activity of *Salmonella typhi* with inhibition zone parameters produced by the agar diffusion method. The results showed that Agarwood leaf extract (*Aguilaria microcarpa* Baill) with concentrations of 3%, 6%, 9%, and 12% w/v had the potential as an antibacterial against growth activity *Salmonella thypi*. The higher the concentration of the extract tested, the greater the inhibitory activity against bacterial growth. The most effective concentration is at a concentration of 12% w/v with a Sig value.  $0.000 < 0.05$  (*Oneway Anova*  $\alpha = 0.05$ ) in strong category inhibition (10 - 20 mm)

## INTRODUCTION

Indonesia is a country rich in biodiversity. Of the total 28,000 species of medicinal plants in Indonesia, 1,845 have been identified as having medicinal properties. The use of plants as medicine to cure disease has been widely studied. Biodiversity and medicinal plants from various types of scientific research in terms of developing active compounds in them make it possible to provide sources of antimicrobial agents originating from plants or parts of plants, ultimately these results can be used as raw materials for medicines (Indrawati, Isnaeni, & Baharuddin, 2022) .

The use of medicinal plants is also an effort to "back to nature". Apart from that, medicinal plants have no side effects if used correctly and are effective for diseases that are difficult to cure with chemical drugs and are easy to process. Medicinal plants in general are one of the uses of the biodiversity found around us, whether they are still wild plants or plants that are in the development stage. Since ancient times, the process of developing plants (plants) has been used as conventional medicine. This needs to be remembered because the cost of treatment cannot be reached by all levels of society, so medicinal plants have become one of the alternatives used by the community in treatment (Indrawati, Baharuddin, et al., 2022; Mamahani, 2016) .

Indonesia is noted for its plant diversity, even though its management is not yet complete and complete, Indonesia is a mega-biodiversity country with a wealth of medicinal plants and significant development potential. Agarwood is a plant that has benefits in traditional medicine. The general public believes that Gaharu leaves can lower blood pressure and act as an antioxidant (Wahid & Ittiqo, 2019) .

*Aquilaria microcarpa* Baill species, which is commonly known to the public as the Agarwood plant, is also commonly used as a medicinal plant that is thought to be able to treat certain diseases such as constipation, bloating, kidney disease, diarrhea, antibacterial, colds, shortness of breath, and symptoms of hypertension. According to (Halim et al., 2014) stated that Gaharu as a raw material for the natural herbal medicine industry can be used to treat stress, asthma, rheumatism, gastric inflammation, kidney disease, malaria, antibiotics, tuberculosis, liver, cancer, and tumors that are still in the clinical trial process.

Currently, research is developing on agarwood leaves because they are thought to contain higher levels of secondary metabolite compounds due to the increased metabolic processes of agarwood trees infected with fungi. Through metabolic processes, these compounds are distributed to other parts of the tree, especially the leaves (Nasution et al., 2015). According to agarwood farmers, consuming agarwood leaves from this type can provide benefits in improving digestion. Traditionally, agarwood leaves are used in the form of brewed tea which acts as an antioxidant because it is thought to contain chemical compounds from the flavonoid group, namely flavones, flavonols, and isoflavones. Agarwood leaves contain secondary metabolite compounds obtained from the results of phytochemical screening tests in the form of flavonoids, glycosides, tannins, and triterpenoids (Nugraha et al., 2015). This is different from research by Silaban in 2013 (Sari et al., 2017) which stated that *Aquilaria malaccensis* Lamk.

leaf extract contains flavonoids, alkaloids, saponins, terpenoids, and steroids, while research (Misrahanum et al., 2022) states that Gaharu leaves contain chemical compounds in the form of alkaloids, flavonoids, and tannins which have antibacterial properties.

One way to treat infections is by using antibacterials. Antibacterials are compounds that can inhibit or kill the proliferation of bacteria. Inhibition of these microorganisms prevents the spread of infection and kills bacteria in infected hosts (Wilapangga & Syaputra, 2018). Various research results on Agarwood leaves as antibacterial (antimicrobial) include, Agarwood leaf extract as antibacterial against *S. aureus* (Wahid & Ittiqo, 2019), as antibacterial (anti-acne) against *P. acnes* through the activity of Agarwood leaf fractions (Suhardiman et al., 2020), antibacterial activity against *S. aureus* and *P. mirabilis* (Sari et al., 2017), antibacterial activity against *E. coli* and *S. aureus* bacteria (Misrahanum et al., 2022) and many others.

Based on various literature and research results, it is necessary to carry out further research regarding the antibacterial potential of Agarwood (*Aquilaria microcarpa* Baill) leaf extract against *Salmonella thypi* to know and determine the potential of Agarwood (*Aquilaria microcarpa* Baill) leaf extract as an antibacterial against the growth activity of *Salmonella thypi*.

## LITERATURE REVIEW

Indonesia is one of the producers of agarwood plants apart from several countries such as India, Singapore, Malaysia, Japan, East Central, and the United States. In world trade, the agarwood plant is known as *agarwood*, *aloe wood*, or *eaglewood*, because it has a fragrant aroma, so it is a luxury commodity for industrial purposes, perfumes, cosmetics, incense, medicinal raw materials, and tea. Agarwood is an aromatic substance colored light brown, dark brown, and blackish brown to black that forms on the stems of gaharu-producing wood (*Aquilaria malaccensis* Lamk), as a self-defense response against pathogen attacks (Nasution et al., 2015). Agarwood plants themselves can grow well at an altitude of 300 to 750 meters above sea level have a temperature of 20°C to 30°C with humidity ranging from 77% to 85% and have a light intensity of 56% to 75% in fertile soil, nests, and good drainage. The agarwood planting process also has many versions depending on the results of seed multiplication, seed multiplication, planting, maintenance and transmission (gaharu-forming infections), harvest period, and production results (Halim et al., 2014).

The use of agarwood leaves will be very important considering that the harvest period for agarwood after being infected with fungus (appearing sick) is 3-4 years. During the harvest cycle, which is quite long, agarwood leaves can be used as medicine. The lack of public knowledge about the benefits of gaharu leaves means that the use of gaharu parts such as leaves is not yet popular among the public, especially gaharu farmers themselves. Empirically, aloes are known to have benefits such as anti-inflammatory, antidepressant, nerve stimulant, anti-diarrhea and anti-malarial. Apart from that, the wastewater produced from the agarwood oil refining process is used to smooth facial skin and for facial care (Misrahanum et al., 2022; Nasution et al., 2015).

Agarwood leaves (*Aquilaria microcarpa* Bail) are a natural ingredient that can be used as an antibacterial. Based on the results of research conducted by Liana in 2013, Sriwijaya University, Palembang, it was stated that Agarwood leaf extract has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria. In the world of health, gaharu also has many benefits for the body, such as fighting bacterial infections. In a study of the benefits of gaharu tree leaf extract being able to fight bacterial infections, quoted from Atmajaya University, Yogyakarta, it was revealed that based on research on gaharu leaf extract with methanol, distilled water, and chloroform solvent, it has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. According to Adelina, N. in 2008, secondary metabolite compounds from gaharu leaves revealed that gaharu leaves contain alkaloids, flavonoids, triterpenoids, steroids, and saponins. Compounds from gaharu leaves that act as antibacterials against *Propionibacterium acnes* bacteria are flavonoid compounds (Halim et al., 2014; Suhardiman et al., 2020).

Agarwood plants also have benefits in healing chronic diseases such as diabetes, gout, kidney disease, chronic fatigue, treating headaches, treating stomach problems, treating blood circulation problems, hypertension, cancer, and skin disorders. The results of a quote from the Ministry of Environment and Forestry by Mohamed in 2019 in the Agarwood House Journal, revealed that agarwood plants are also usually used as a main ingredient in medicines, both herbal and chemical medicines. Apart from that, aloes are also used as herbal teas and cosmetics because each part of the aloe plant has its benefits which are beneficial for health. Apart from that, quoted from Marlina (2018) at the University of Lampung, in health sciences, in the pharmacology of gaharu, it contains ingredients that can treat various diseases such as depression, anxiety, stomach or intestinal diseases, skin diseases, cancer, tumors, diabetes, and others. In pharmacology it is said that there are 12 chemical components in aloes, there are also those that reveal as many as 17 components, there are even 31 chemical compound components, such as *noroxo-agarofuran*, *agarospirol*, *dihydroxy*, *dihydrogarufuran*, *p-methoxy-benzylacetone*, *aquilochim*, and so on (Halim et al., 2014).

*Salmonella typhi* and *paratyphi* are the bacilli that cause typhoid they are members of the genus *Salmonella* and are gram-negative bacteria, are mobile, do not have capsules, do not form spores, but have fimbria, are aerobic and facultative anaerobic. The size is between (2-4) x 0.6µm. The optimum temperature for growth is 37°C with a pH between 6-8. This bacillus can live for several weeks in the wild, such as in water, ice, trash, and dust. Meanwhile, the only reservoir is humans, namely someone who is sick or a carrier. This bacillus can be killed by heating to a temperature of 60°C for 15-20 minutes, pasteurization, boiling, and chlorination. The incubation period for typhoid is 10-14 days and in children this incubation period is more variable, ranging from 5-40 days, with the course of the disease sometimes being irregular. In addition, *Salmonella typhi* is one of the biggest causes of diarrhea after *E. coli*. Diarrhea caused by *Salmonella typhi* is usually invasive diarrhea characterized by

prolonged fever, abdominal pain, and other systemic manifestations such as delirium or headache (Imara, 2020; Putra et al., 2019).

## METHODOLOGY

### Types of Research

This type of research is semi-quantitative research, which in this case is laboratory research to test the antibacterial potential of Agarwood leaf extract (*Aguilaria microcarpa* Baill) with concentrations of 3%, 6%, 9%, and 12% (w/v) on the growth activities of *Salmonella typhi* using the agar diffusion method.

### Research Tools and Materials

The tools used are an autoclave (GEA YX 24 LDJ), a stirring rod (IWAKI Pyrex), Petri dishes (IWAKI Pyrex), porcelain dishes, glass funnels (IWAKI Pyrex), Erlenmeyer (IWAKI Pyrex), measuring cup (IWAKI Pyrex), beaker (IWAKI Pyrex), scissors set, incubator (Mammert T.75), caliper (Mitotuyo 0-150), measuring flask (IWAKI Pyrex), Laminar Air Flow (Esco), spirit lamp, micropipette (*Dragon Med*), round tube, oven (Memmert UN 55 53 L), water bath (Faithful Model DK-2000-IIIL 6 Holes), tweezers (GOOI TS-11), a set of maceration tools, a set of rotary evaporator tools (*Ika*®), a horn spoon, a test tube (IWAKI Pyrex), an analytical balance (AND GR-300), an analog balance (PGB).

The materials used are aqua bides (WaterOne), aluminum foil (Total Wrap), pure culture *Salmonella typhi*, Agarwood leaves (*Aguilaria microcarpa* Baill), Ethanol 96% absolute (Merck), Ether (Merck), sterile cotton, filter paper, Nutrient Agar (Merck), Mueller Hilton Agar (Merck), Natrium Chloride (Merck), Na. CMC 1%, Paper Disk (Oxoid), Sterile Cotton Swab (onemed).

### Research Procedure

- **Taking and Collecting Test Samples**

The test samples were leaves of the Agarwood plant (*Aguilaria microcarpa* Baill) obtained from Marauke Regency, Papua Province. Agarwood leaves are taken in the morning between 9 - 10 local time, namely 5 leaf stalks from the top to the bottom that are still green and picked directly and then collected in one container.

- **Sample Processing**

Processing of test samples refers to research (Latif et al., 2019), the leaves that have been collected are then wet sorted washed with running water, chopped, and then dried. The dried leaves are sorted dry and powdered to obtain dry simplicia powder.

- **Sample Extraction and Evaporation**

± 250 g of dry Simplicia powder test samples, then put into a container and then macerated with 96 % ethanol solvent up to 1000 mL until the samples were all submerged (ratio 1: 4 w/v), then covered and stored for 5 days (5 x 24 hours) protected from light, stirring occasionally, then filtered. After the first extraction process is complete, the dregs are put back into the maceration tool and carried out as before until the liquid is colorless and a perfect extraction process is obtained. The extraction results obtained were then evaporated using a rotary evaporator until a thick ethanol extract was obtained. The thick ethanol extract is free from ethanol by adding 5 drops of aquabides to the thick extract and then heating it again over a water bath until it evaporates until we get

Agarwood leaf extract which is ready to be used (Indrawati, Isnaeni, Baharuddin, et al., 2022; Misrahanum et al., 2022).

### **1. Determination of Test Sample Characterization**

Characterization testing of Agarwood leaf extract (*Aguilaria microcarpa* Baill) was in the form of yield, shape, color, odor, taste, water content, and ash content.

### **2. Examination of the Chemical Content of Test Samples**

A chemical content examination was carried out to determine the secondary metabolites contained in Agarwood (*Aguilaria microcarpa* Baill) leaf extract. Secondary metabolites that will be tested qualitatively include flavonoids, terpenoids, triterpenoids, alkaloids, tannins, saponins, glycosides, steroids (Indrawati, Isnaeni, Baharuddin, et al., 2022; Nugraha et al., 2015; Sari et al., 2017).

### **3. Preparation of Test Sample Suspension**

The test sample suspension of Agarwood leaf extract (*Aguilaria microcarpa* Baill), was made by referring to research (Indrawati, Isnaeni, Baharuddin, et al., 2022), namely, test samples made with concentrations of 3%, 6%, 9%, and 12% (b/v) by weighing 3, 6, 9, and 12 g of each test sample and then suspending them in Na. CMC 1% (w/v) to 100 mL, then the four concentrations are homogenized and then put into a suitable container and tightly closed.

### **4. Preparation of Test Microbial Suspension**

The test microbial suspension was made by referring to research (Suherman et al., 2018), namely, the test bacteria (*Salmonella thypi*) aged 24 hours were taken using a round tube and then suspended in a sterile 0.9% NaCl physiological solution with a comparison using a standard solution of Mc. Farland 0.5 (the composition of the Mc. Farland 0.5 standard solution is  $BaCl_2$  0.048 M 0.5 mL and  $H_2SO_4$  0.18 M 9.5 mL).

### **Testing of Antibacterial Potential of Test Samples**

The antibacterial potential of agarwood leaf extract (*Aguilaria microcarpa* Baill) against *Salmonella thypi* was obtained by pouring 20 mL of medium Mueller Hinton Agar (MHA) into each sterile petri dish aseptically, then adding 1 ml of suspended *Salmonella thypi* and homogenized and then allowed to solidify. After it solidifies, take and place the paper disk previously soaked in a suspension solution of Agarwood leaf extract with concentrations of 3, 6, 9, and 12% and negative control Na.CMC 1% w/v. Set the distance of the paper disk from the edge of the petri dish to  $\pm 20$  mm then incubate at a temperature of 37°C for 1 x 24 hours and 2 x 24 hours. The resistance area formed in the petri dish was measured with a slide rule. Treatments in effectiveness testing were carried out 3 times, and the average and standard deviation were calculated (Baharuddin & Isnaeni, 2020; Indrawati, Isnaeni, Baharuddin, et al., 2022).

## 5. Data Processing and Analysis

The calculation of yield results is based on calculations (Indrawati, Baharuddin, et al., 2022) :

$$\text{Yield: } \frac{\text{Weight of test sample (Agarwood leaf extract)}}{\text{Weight of Agarwood leaf simplicia}} \times 100\%$$

Observations and measurements of the diameter of the test microbial growth inhibition zone for each concentration were carried out after an incubation period of 1 x 24 hours and 2 x 24 hours at a temperature of 37°C using a slide rule. Next, the data from measurements of the diameter of bacterial growth barriers were analyzed using the analysis of variance method (*one-way* ANOVA) and correlation tests using the IBM SPSS Statistics version 26 program.

## RESULTS

### Yield Value

Table 1. Yield Value from the Extraction of Agarwood Leaves (*Aguilaria Microcarpa* Baill)

Extraction	Weight A. Baill's <i>microcarpa</i> (g)	Extract Weight (g)	Yield Value (%)
Maceration	250	29.87	11.94
	250	30.20	12.08
Average yield value			<b>12.01</b>

### Characterization Testing

Table 2. Results of Characterization Testing of Agarwood Leaf Extract (*Aguilaria Microcarpa* Baill)

Specification	Description (Maceration Method)
Yield(%)	12.01
Form	Concentrated Extract
Color	Blackish brown
Smell	Typical, not pungent
Flavor	Bitter (Sepat)
Water content (%)	1.38
Ash Content (%)	0.14

**Chemical Content Examination**Table 3. Results of Chemical Content Examination (Phytochemical Screening) of Test Samples of Agarwood (*Aguilaria Microcarpa* Baill) Leaves

Gynecology Test	Reactor	Test result	References	Note.
Alkaloids	- Mayer	(↓) yellowish-white color is formed	(↓) lumpy white or yellow	Detected (+)
	- Dragendorff	formed (↓) light brown (reddish)	(↓) brown or brownish orange	Detected (+)
Phenol/Phenolic	5 drops FeCl <sub>3</sub> 5% (shaken vigorously)	a blackish-blue color is formed	a blackish-blue color is formed	Detected (+)
Tannin	FeCl <sub>3</sub> 1%	a blackish-blue color is formed	blue or green black	Detected (+)
Flavonoids	0.1 g Mg powder + and 1 mL HCl P + 2 mL amyl alcohol (shaken)	a dark yellow color is formed	red or yellow or orange on the amyl alcohol layer	Detected (+)
	Acetone P + Ace Powder Borate + Oxalate Powder	light yellow fluorescent solution	Intensively yellow fluorescent (under 366 nm UV light)	Detected (+)
Glycosides	2 ml water + 5 drops of Molish fixative then + 2 ml H <sub>2</sub> SO <sub>4</sub> P through the wall Tube	no purple ring is formed	a purple ring forms at the second boundary liquid, indicating the presence of sugar (glycone) or glycoside bonds	Not detected (-)
Terpenoids	Liebermann-Burchard	no color change occurs	a red, dark red, or purple color is formed	Not detected (-)
Saponin	H <sub>2</sub> O (hot) then shake vigorously	2.5 cm high foam is formed	foam is formed stable for no less than 10 minutes as high as 1 to 10 cm and + 1 drop of HCl 2N foam not lost	Detected (+)
Steroids	Liebermann-Burchard	a greenish-blue color appears	a blue or blue-green color is formed	Detected (+)

**Antibacterial Potential Testing**

Table 4. Antibacterial Potential of Agarwood Leaf Extract (*Aguilaria Microcarpa* Baill) Against *Salmonella Typhi* Incubation Period 1 X 24 Hours

Test Bacteria	Obstacle zone diameter (mm)				
	<i>Aguilaria microcarpa</i> Baill leaf extract 1 x 24 hours				
	Control (-)	3 % b/v	6 % b/v	9 % b/v	12 % b/v
<i>Salmonella typhi</i>	6.0	7.5	8.5	9.5	11.0
	6.0	7.5	9.0	9.7	11.5
	6.0	7.3	8.0	9.7	11.8
<b>Amount</b>	<b>18.0</b>	<b>22.3</b>	<b>25.5</b>	<b>28.9</b>	<b>34.3</b>
<b>Average</b>	<b>6.0</b>	<b>7.43</b>	<b>8.50</b>	<b>9.63</b>	<b>11.43</b>
<b>SD</b>	<b>±0.000</b>	<b>±0.115</b>	<b>±0.500</b>	<b>±0.115</b>	<b>±0.404</b>

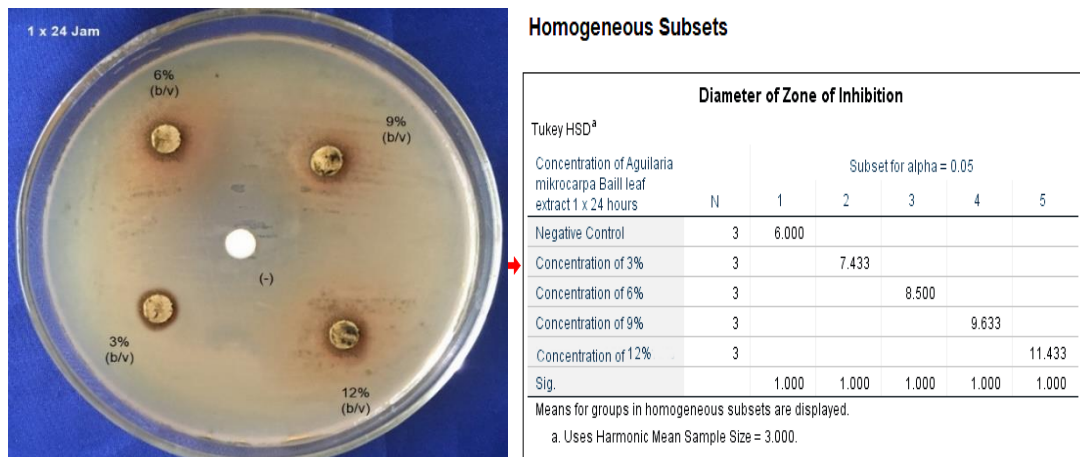


Figure 1. Antibacterial Potential of Agarwood Leaf Extract (*Aguilaria microcarpa* Baill) Against *Salmonella typhi* Incubation Period 1 X 24 Hours

Table 5. Antibacterial Potential of Agarwood Leaf Extract (*Aguilaria Microcarpa* Baill) Against *Salmonella Typhi* Incubation Period 2 X 24 Hours

Test Bacteria	Obstacle zone diameter (mm)				
	<i>Aguilaria microcarpa</i> Baill leaf extract 2 x 24 hours				
	Control (-)	3 % b/v	6 % b/v	9 % b/v	12 % b/v
<i>Salmonella typhi</i>	6.0	7.0	8.0	8.5	10.8
	6.0	7.1	8.0	9.0	10.8
	6.0	6.8	7.6	9.0	11.0
<b>Amount</b>	<b>18.0</b>	<b>20.9</b>	<b>23.6</b>	<b>26.5</b>	<b>32.6</b>
<b>Average</b>	<b>6.0</b>	<b>6.97</b>	<b>7.87</b>	<b>8.83</b>	<b>10.87</b>
<b>SD</b>	<b>±0.000</b>	<b>±0.152</b>	<b>±0.230</b>	<b>±0.288</b>	<b>±0.115</b>

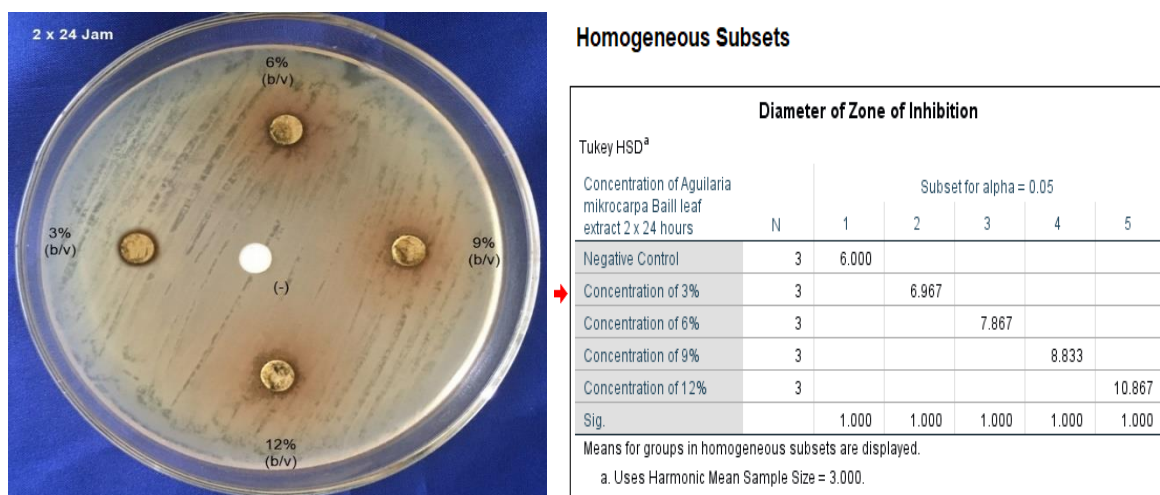


Figure 2. Antibacterial Potential of Agarwood Leaf Extract (*Aguilaria Microcarpa Baill*) Against *Salmonella Typhi* Incubation Period 2 X 24 Hours

## DISCUSSION

Antibacterial potential is the ability of a certain substance to inhibit the growth of bacteria, even in certain concentrations it can usually kill pathogenic bacteria. This can be seen in the compound components of a substance or material that has antibacterial components so that it can be categorized as bacteriostatic in nature which suppresses the growth of bacteria or as bactericidal in nature which can kill bacteria. Bacteriostatic or bactericidal can be determined after carrying out a series of various test results (research) on the substance or material using response parameters for inhibiting bacterial growth with various inhibition categories.

The material or sample for which testing was developed to obtain antibacterial potential results is Agarwood leaves with the scientific name *Aguilaria mikrocarpa* Baill. In this research, the antibacterial potential of Agarwood leaves was tested on the growth activity of *Salmonella thypi*. The main supporting basis for carrying out this test is because the Agarwood plant has been widely used by some people as a traditional medicine in treating various diseases and the parts of the Agarwood plant that are used as a line of treatment are the roots, leaves, skin, and fruit which are often processed to be used as ingredients for potion mixtures traditional medicine. Parts of this material are often used for the treatment or prevention of various diseases, including malaria, high blood pressure (hypertension), rheumatism, diabetes, asthma, kidney problems, and many others. Another supporting factor that forms the basis is that there have been previous studies on the plant which has the name *Aguilaria mikrocarpa* Baill in the field of pharmacognosy testing, phytochemistry, and even pharmacological activity testing such as antioxidant, antidiabetic, anti-inflammatory, antibacterial, sedative, antitrypanosomal, antiallergic, and many more others.

Testing the antibacterial potential of Agarwood leaves (*Aquilaria microcarpa* Baill) against *Salmonella thypi* involves various processes starting from processing the test material, extraction process, determining characteristics, identifying chemical components, and testing antibacterial potential. The process of extracting *Simplicia* Agarwood leaves involves a maceration extraction process, where this process can extract compound components that are thermolabile because the maceration method is carried out without using heating. On the other hand, the maceration method (cold extraction) allows many compounds to be extracted although some compounds have limited solubility in solvents at room temperature.

An extraction process involves certain stages such as mixing the extraction material with a solvent, separating the extract solution and raffinate, and isolating the extract from the extract solution. Extraction is usually often used in the process of separating a bioactive content from a plant or plant if you want to know the yield that will be produced, which consists of hot extraction, namely by reflux, soxhletation, and cold extraction viz with the percolation method and maceration method. According to (Indrawati et al., 2023) the maceration extraction process is a simple extraction method by soaking the material in a suitable solvent to obtain active compounds without any heating process.

The results obtained from the extraction process of Agarwood leaves by maceration are in the form of a yield of 12.01% in the form of a concentrated extract with a slightly blackish brown color with a distinctive and not pungent odor, having a bitter (astringent) taste with a water content of 1.38% and ash content of 0.14%. The results of the yield values and characterization tests can be seen in Table 1 and Table 2. A yield of an extract is said to be good and good if the value is more than 10% because the yield value is related to the amount of bioactive content contained. Therefore, the higher the yield produced, the higher the content of interested substances in a *simplicia* or raw material. The same thing was stated by Harbone (1987) in research (Hasnaeni et al., 2019) , that the high level of active compounds present in a sample is influenced by the high yield value produced. The yield value is related to the active compounds contained in a raw material.

Examination of the chemical content (phytochemical screening) of Agarwood (*Aquilaria microcarpa* Baill) leaves showed positive results (detected) according to the literature regarding the content of alkaloids, phenols (phenolics), tannins, flavonoids, saponins, and steroids through qualitative testing using color reagents while glycoside compounds and terpenoids were not found (not detected) (Table 3). The results obtained are not different from the results of the research (Suhardiman et al., 2020) , where the results of phytochemical screening of agarwood leaves showed positive results for the secondary metabolite compound groups tested, namely alkaloids, flavonoids, saponins, tannins, and steroids (triterpenoids). Apart from that, (Sari et al., 2017) also, their research obtained results through tube tests (chemical component identification) from the ethanol extract of *Aquilaria microcarpa* Bail leaves. in the form of phenolic compounds, flavonoids, steroids, tannins, and saponins with positive results.

In general, flavonoids, tannins, saponins, alkaloids, and triterpenoids (steroids) are antimicrobial (antibacterial) agents, where flavonoids have a mechanism of action by forming complex compounds with extracellular proteins that disrupt the integrity of cell membranes, while tannins have a mechanism of action by inhibiting the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot form, because they can inactivate microbial cell adhesins, inactivate enzymes, and disrupt protein transport in the inner layers of cells. This is different from saponin, which acts as an antibacterial by damaging the permeability of cell walls so that it can cause cell death. Alkaloids (pyridine alkaloids) also act as antibacterial agents, where the mechanism of action is thought to be by interfering with the components that make up the peptidoglycan in bacterial cells so that the cell wall layer does not form completely and can cause cell death. Triterpenoid compounds react with porins in the outer membrane of the bacterial cell wall, forming strong polymer bonds thereby reducing the permeability of the bacterial cell wall.

Antibacterial potential of Agarwood leaf extract (*Aguilaria microcarpa* Baill) against the growth activity of *Salmonella thypi* using extract suspension solutions with concentrations of 3%, 6%, 9%, and 12% as well as negative control Na.CMC 1% w/v. The treatment for testing antibacterial potential was carried out with incubation for 1 x 24 hours and 2 x 24 hours. For testing Agarwood leaf extract on the growth activity of *Salmonella thypi* with an incubation period of 1 x 24 hours at a temperature of 37°C, the results obtained were an obstacle zone with an average diameter for a concentration of 3% w/v of 7.43±0.115 mm, a concentration of 6% w/v was 8.50±0.500 mm, 9% w/v concentration was 9.63±0.115 mm, 12% w/v concentration was 11.43±0.404 mm while for the negative control Na.CMC 1% is 6.00±0.00 mm. Meanwhile, for testing Agarwood leaf extract (*Aguilaria microcarpa* Baill) on the growth activity of *Salmonella thypi* with an incubation period of 2 x 24 hours at a temperature of 37°C, the results obtained were an obstacle zone with an average diameter for a concentration of 3% w/v of 6.97±0.152. mm, 6 % w/v concentration was 7.87±0.230 mm, 9% w/v concentration was 8.83 ±0.288 mm, 12% w/v concentration was 10.87 ± 0.115 mm while for the negative control Na. CMC 1% is 6.00 ± 0.00 mm. The test results can be seen in Table 4 and Table 5.

The results of the process of testing the antibacterial potential of Agarwood leaves on the growth of *Salmonella thypi* with an incubation period of 24 hours or 48 hours showed that there was an increase in the inhibitory power that occurred at each concentration of the test sample, thus the Agarwood leaf extract extracted by maceration was effective in inhibiting the growth of *Salmonella thypi*. The results of calculations and data analysis using the analysis of variance test (oneway Anova) obtained SPSS output results with each incubation period obtaining a Sig value equal to 0.000 < 0.05 so that the average variation in concentration and inhibition zone produced is significantly different, which means there is a significant difference between treatments. According to (Indrawati et al., 2023), the results of their research suggest that an antimicrobial activity test means that the higher the concentration of the extract of the test

sample being tested, the greater the diameter of the inhibition zone (inhibition) that results in the growth of a microorganism.

Tests obtained from the antibacterial potential of Agarwood leaves against the growth of *Salmonella typhi* with an incubation period of 2 x 24 hours experienced a decrease in terms of inhibitory power resulting from the results of the inhibition zone obtained with an incubation period of 1 x 24 hours, this is due to the possibility that the Agarwood leaf extract is only bacteriostatic to the growth activity of *Salmonella typhi* because it is influenced by condition factors so that the microorganism is difficult to kill but can only be inhibited through short exposure to an antimicrobial. This was also confirmed by Dewi (2010) in research by Septiani (2017) that the diameter of an obstacle zone does not always increase in proportion to an increase in antibacterial concentration, this possibility occurs due to differences in the speed of diffusion of antibacterial compounds on agar media and the type and concentration of antibacterial compounds used. Different types also provide different inhibition zone diameters for certain periods (Septiani et al., 2017).

Bacteriostatic compounds often inhibit protein synthesis or bind to ribosomes. This is demonstrated by the addition of antimicrobials to microbial cultures that are in the logarithmic phase. After the addition of antimicrobial agents in the logarithmic phase, it was found that the total cell number and the number of viable cells remained constant. Pelczar and Chan (2008) added that the stationary phase is characterized by the exhaustion of the nutrients needed by bacteria for their growth. This is characterized by the production of compounds or toxins that cause some bacterial cells to die, while others grow and divide so that the number of living cells remains constant. In this case, antibacterial is produced in the stationary phase (Septiani et al., 2017). In addition, the longer the incubation period, the greater the opportunity for mutants that are resistant to emerge or that are at least sensitive to antimicrobial drugs to reproduce as the inhibitory ability of antimicrobial drugs decreases (Carroll et al., 2017; Martsiningsih et al., 2023).

In this study, a negative control was also used as a comparison, whereas in the negative control Na. CMC 1% was used, because of Na. CMC 1% apart from acting as a suspending agent to increase suspension stability is also a cellulose derivative compound with a carboxymethyl group (-CH<sub>2</sub> -COOH) which is bound in several hydroxyl groups of glucopyranose monomers which can form the main chain of cellulose so that it can function as a thickening agent for a preparation which will form a colloidal disperse system and can increase viscosity. When Na. CMC is dispersed in water then the grains Na. CMC which is hydrophilic will absorb water and swelling will occur.

Testing the antibacterial potency of Agarwood leaf extract (*Aguilaria microcarpa* Baill) with various extract concentrations ranging from concentrations of 3% w/v, 6% w/v, 9% w/v, and 12% w/v has a different resistance to activity. growth of *Salmonella typhi* with an incubation period of 24 hours and 48 hours, but in terms of classification, *Aguilaria microcarpa* Baill extract as an antibacterial is in the moderate inhibition category at concentrations of 3%, 6%, 9% w/v, and the strong inhibition category at a concentration of 12% w/v. This refers to the

results of the research (Suherman et al., 2018), stating that the category of inhibition is based on the diameter of the inhibitory zone, namely for the inhibitory response in the weak category with an inhibitory zone diameter  $\leq 5$  mm, for the medium category between 5 - 10 mm, the strong category is between 10 - 20 mm and for the inhibitory response in the very strong category  $\geq 20$  mm.

## CONCLUSIONS AND RECOMMENDATIONS

Based on the results of research, data processing, and analysis, it can be concluded that Agarwood leaf extract (*Aguilaria microcarpa* Baill) with concentrations of 3%, 6%, 9%, and 12% w/v has the potential as an antibacterial against the growth activity of *Salmonella thypi*. The higher the concentration of the extract tested, the greater the inhibitory activity against bacterial growth. The most effective concentration is at a concentration of 12% w/v with a Sig value.  $0.000 < 0.05$  (*Oneway Anova*  $\alpha = 0.05$ ) in the strong category of inhibition (10 - 20 mm).

## FURTHER STUDY

The results of research that has been carried out regarding the antibacterial potential of Agarwood leaf extract (*Aguilaria microcarpa* Baill) as well as a review of various literature, further research is recommended in various in vivo tests on living creatures (test animals).

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