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Antimicrobial and Antioxidant Potential of Some Agarwood Plant Extracts (Aquilaria Malaccensis Lamk.) Against Escherichia coli ATCC 25922, Staphylococcus Aureus ATCC 29213 and Candida Albicans (C.P Robin) Berkhout 1923

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***Abstract* – Research on the Antimicrobial and Antioxidant Potential of some Agarwood Plant Extracts (*Aquilaria malaccensis* Lamk) against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Candida albicans* (C.P Robin) Berkhout 1923 has been carried out at the Microbiology Laboratory of Andalas University in April - July 2023. This study aims to determine the antimicrobial and antioxidant activity of some Agarwood Plant Extracts. The method used in this research is the experimental method of nested pattern. The results showed that each extract gave a significantly different effect on *S. aureus* with the largest inhibition zone in the leaf extract of (9.79 mm) and *E. coli* of (7.37 mm), but did not give a significantly different effect on *C. albicans*, because no inhibition zone was found. The Minimum Inhibitory Concentration (MIC) of the leaf extract against *S. aureus* was 6.25% with a Minimum Bactericidal Concentration (MBC) of 50% and the Minimum Inhibitory Concentration (MIC) value of *E. coli* was 3.125% with a Minimum Bactericidal Concentration (MBC) of 12.5%. The percentage of inhibition of agarwood leaf extract against the growth of *E. coli* and *S. aureus* was (30.18%) and (43.84%) of the positive control chloramphenicol (0.1 mg/ml). While the percentage of inhibition of agarwood leaf extract against the growth of *C. albicans* was (28.70%) from the positive control of fluconazole (0.1 mg/ml). The highest antioxidant activity was found in the leaf extract with an IC50 value of 50.47 µg/ml. The highest polyphenol content was found in the leaf extract at 34.14 mgGAE/ml.**

Keywords — Antimicrobial, Antioxidant, Agarwood, Leaf, Polyphenols.

I. INTRODUCTION

Infectious diseases are an increasing health problem due to factors such as unfavorable environments, densely populated slum areas, and high population density [1]. Traditional management involves antimicrobials such as antibiotics for bacterial infections and antifungals for fungal infections. However, the widespread use of antimicrobials has led to an increase in antimicrobial resistance [2]. This poses a significant threat to public health, prompting interest in exploring traditional medicine and medicinal plants as potential solutions. A variety of bioactive compounds found in medicinal plants have antimicrobial properties, thereby offering a potential source of new antimicrobial agents against resistant strains [3].

Agarwood, also known as *Aquilaria malaccensis* Lamk, is a plant in the Thymelaeaceae family, with a long history of use in culture and medicine, particularly in Asia. It is known by various names, including agarwood, agarwood, or agarwood, and is used in perfumery, cosmetics, medicines, balms, soaps, and body lotions. In Indonesia it is known as gaharu, worthy tree, short

tree, lingua wood, menameng, and terentak. Agarwood leaves are used in religious rituals and ceremonies as incense, and their aromatic properties make them a sought-after ingredient in spiritual and cultural practices [4]. The phytochemical composition of agarwood leaves, which includes alkaloids, flavonoids, triterpenoids, steroids, tannins and saponins, shows that they are rich in bioactive compounds, including alkaloids and terpenoids [5]. Research shows that agarwood leaf extract has potential therapeutic effects, including antimalarial, antidiabetic, antiulcer, anti-inflammatory, and antibacterial activities. A decoction of aloe vera leaves is traditionally used to treat various degenerative diseases, including heart disease, cancer, diabetes, high blood pressure, cholesterol and gout [6].

Agarwood leaves are rich in secondary metabolites such as flavonoids, tannins, triterpenoids and glycosides, which are proven to be a rich source of antioxidants. Agarwood leaves have been evaluated for their antioxidant potential using methods such as DPPH free radical scavenging activity[7]. Agarwood leaf hot water extract has shown strong antioxidant activity, mainly due to the flavonoid content which acts as a hydrogen donor against free radicals. Ethanol extract of *Aquilaria malaccensis* Lamk. has the highest antioxidant activity and the richest flavonoid compounds [8]. Herbal medicines and plant extracts have been used to treat infectious diseases, including viral and bacterial infections. However, research on gaharu leaves and bark is still limited, there has been no research on antimicrobial tests against Escherichia coli, Staphylococcus aureus, and Candida albicans.

II. MATERIAL AND METHODS

2.1 Tools and Materials

The tools used are petri dishes, test tubes, erlenmeyer, ose needle, spirit lamp, test tube rack, measuring cup, sprayer, funnel, mortar, dropper pipette, hot plate, vortex, eppendorf tube, autoclave, incubator, microwave, tissue, digital scales, refrigerator, micropipette, stirring rod, gauze, cotton, disc paper, filter paper, label paper, box, rubber band, paper punch, digital camera, laminar air flow cabinet, vernier term, spectrophotometer and stationery.

The materials used are samples of leaves and bark of agarwood (*Aquilaria malaccensis Lamk*), pure culture of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Candida albicans*, medium Nutrient Agar (NA), Potato Dextrose Agar (PDA), Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Sabouraud Dextrose Agar (SDA), *Sabouraud Dextrose Broth* (SDB), distilled water, 70% alcohol, DPPH solution, *Folin-Ciocalteu* solution, Sodium Carbonate, Methanol p.a and Gallic Acid.

2.2 Research Methods

The method used is a nested pattern experimental method with 2 factors and 3 replicates, where factor A is the treatment of *A. malaccensis* extract and factor B is the microbes to be tested.

2.3 Medium Preparation

Prepare an Erlenmeyer flask, then weigh 5 grams of NA, 10 grams of PDA, 9.5 grams of MHA, 16.5 grams of SDA, 5.5 grams of MHB, and 7.5 grams of SDB into the Erlenmeyer flask and dilute with distilled water to reach a total volume of 250 ml. After that, the mixture was heated on a hot plate until it reached boiling point, and then sterilized using an autoclave at 121°C and 1 atm pressure for 15 minutes.

2.4 Preparation of Test Microbial Suspension

The test microbes that have been inoculated on the inclined media are then taken with a sterile ose needle and then suspended in a tube containing 2 mL of 0.9% NaCl until the turbidity is the same as the standard turbidity of 0.5 Mc Farland solution.

2.5 Antimicrobial Activity Test With Diffusion Methods

The diffusion method used refers to [9]. MHA medium was poured into Petri dishes aseptically and allowed to solidify. Then 1 ml of bacterial suspension was taken and inoculated on the surface of the medium and leveled with a sterile cotton stick. Then dipped a paper disc with a diameter of 6 mm aseptically into the sample, and dried briefly and then placed the paper disc on the surface of the medium. The disc paper was placed on the surface of the medium with sterile tweezers. After that, it was incubated for 24 hours and the diameter of the clear zone was measured. The positive controls used were chloramphenicol (0.1

mg/mL) and fluconazole (0.1 mg/mL) and the negative control was the solvent used in the extraction, distilled water. Treatment of samples with the highest inhibition zone area was continued to determine the KHM and KBM values in the dilution method.

2.6 Antimicrobial Activity Test With Dilution Methods

The dilution method for the test microbes used refers to [10]. Sterile test tubes were provided, each labeled 1-10 and tubes A, B, for control. Tube 1-10 SDB /MHB medium was inserted as much as 2 ml aseptically and then 2 ml of agarwood leaf extract was added to tube 1 and then homogenized and transferred from tube 1 to tube 2 by 2 ml. Then homogenized, then from tube 2 to tube 3 and so on until tube 10. In tube 10, 2 ml was taken and discarded so that the volume in each tube was 2 ml. As a result, the concentration of agarwood extract was obtained as 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.8%, 0.4%, 0.2% and 0.1% respectively. In tubes 1-10, 1 ml of microbial suspension was added.

Tubes A and B as controls, where tube A was filled with 2 ml of agarwood leaf extract, then added 2 ml of SDB /MHB medium. Tube B was filled with 2 ml of SDB/MHB medium plus 1 ml of test microbial suspension. Furthermore, all tubes were incubated at 37°C for 1 x 24 hours. After 24 hours, the turbid tubes 1-10 were marked and the dilution value was recorded. Positive tubes (clear) were taken 0.1 ml of test microbial suspension and cultured on SDA / MHA medium, incubated at 37º C for 24 hours. After that, it was observed whether there was growth of the test microbes if there was a record of the number of colonies formed and the MIC/ MBC number was determined.

2.7 Determination of Antioxidant Activity (IC50) Using The DPPH Methods

Antioxidant activity test was conducted using DPPH (1,1-Diphenyl-2-Picryl-Hydrazine) method. The DPPH method refers to [11]. Fresh and dried extracts of agarwood leaves and bark were reacted with DPPH solution. The antioxidant activity test was conducted using a spectrophotometer at a wavelength of 517 nm.

2.8 Gallic Acid Standard Curve

Preparation of gallic acid standard curve refers to [12]. Weigh 0.025 g of gallic acid and put into a 100 ml beaker glass. Added 0.2 ml of ethanol p.a, the volume was 100 ml with distilled water (obtained 250 ppm gallic acid solution). Then homogenized with shaking. This solution is called gallic acid mother solution. Standard solutions of gallic acid with various concentrations of 0; 50; 100; 150; 200 ppm were made by taking 0; 2; 4; 6; 8 ml of gallic acid mother solution and put into a 10 ml volumetric flask and made sufficient volume to 10 ml with distilled water. 1 ml of standard gallic acid solution was pipetted, put into a 10 ml flask then added 1 ml of Folin-Ciocalteu reagent and homogenized. After 5 minutes, 1 ml of sodium carbonate (Na2CO3) solution was added and the volume was increased to 10 ml with distilled water. The solution was shaken and incubated for 90 minutes and measured the absorbance value with a wavelength of 725 nm.

2.9 Determination of Total Phenolic Content (TPC) by the Follin-Ciocalteau Method

Phenol compounds in fresh, boiled and brewed extracts of agarwood leaves and bark were carried out by the Folin-Ciocalteu Assay method. A 1 ml sample of fresh, boiled and brewed extracts of agarwood leaves and bark was taken, then mixed with 1 ml of Folin-Ciocalteu reagent. After 5 minutes, 1 ml of 13% sodium carbonate was added to the mixture and sufficed with distilled water until the volume was 10 ml. The tubes were kept in the dark for 90 minutes and the absorbance value was measured using a spectrophotometer at 725 nm.

2.10 Data Analys

Data obtained from the diffusion method were analyzed using a nested pattern. If there were significant differences between treatments, further data analysis was conducted using the DMRT test at the 5% significance level. On the other hand, descriptive analysis was conducted to determine the antioxidant activity and total polyphenol content of all extracts used.

III. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

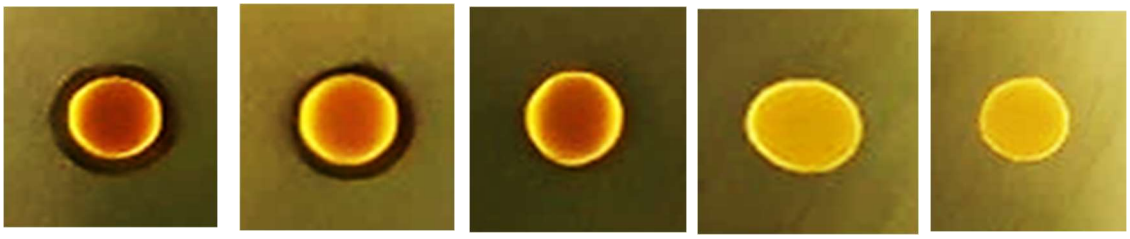
Based on the results of the antimicrobial activity test of *Aquilaria malaccensis* leaf and stem bark extracts using the disc paper diffusion method incubated for 24 hours on Sabouraud Dextrose Agar (SDA) and Mueller Hinton Agar (MHA) medium, the following results were obtained:

Table 1. Average diameter of inhibition zone of leaf and bark extracts of agarwood plants

Number	Extract	Microbial inhibition zone diameter (mm)		
		E. coli	***S. aureus***	***C. albicans***
1.	Leaf Extract	7.37[a]	9.79[a]	6.00
2.	Boiled Dried Leaves	6.65[b]	8.88[b]	6.00
3.	Brewing Dried Leaves	6.00[b]	8.05[c]	6.00
4.	Boiled Dry Bark	6.00[b]	6.14[d]	6.00
5.	Brewing Dry Bark	6.00[b]	6.09[d]	6.00

Notes: Numbers followed by small letters that are not the same in the same column are significantly different at 5% DMRT.

Based on ANOVA statistical analysis and continued with the DMRT test, the effect of several leaf and stem bark extracts of *A. malaccensis* on *S. aureus* and *E. coli* is significantly different, but does not give a significantly different effect on *C. albicans* which has no inhibition zone. The largest inhibition zone on *S. aureus* and *E. coli* is found in leaf extracts with an inhibition zone of 9.79 mm on *S. aureus* and 7.37 mm on *E. coli*. The results of antimicrobial tests of several leaf and stem bark extracts of *A. malaccensis* with the disc diffusion method showed that boiled and brewed extracts of leaves and stem bark of *A. malaccensis* plants can inhibit the growth of *S. aureus*. While the growth of *E. coli* can only be inhibited by leaf extract, boiled leaves and brewed leaves. This can be seen from the formation of inhibition zones caused by the antimicrobial activity produced from *A. malaccensis* leaf and stem bark extracts (Table 1). The antimicrobial activity resulting from this extract forms an inhibition zone, which is a clear area around the disc or paper that is impregnated with substances that inhibit or kill bacteria, thereby preventing bacterial growth in certain areas. The concentration of the extracted substance increases the average diameter of the inhibition zone [13].



(a) (b) (c) (d) (e)

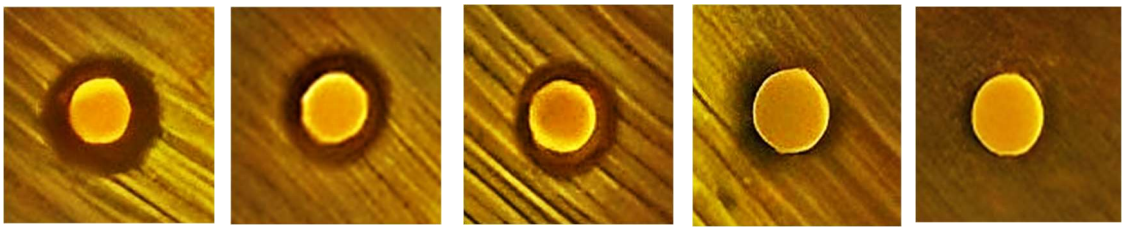
Figure 1. Antimicrobial activity of some *A.malaccensis* leaf and stem bark extracts against *E. coli* growth. Description: (a) Leaf extract, (b) Boil dried leaves, (c) Brew dried leaves, (d) Boil dried stem bark, (e) Brew dried stem bark.

Based on Figure 2, it can be seen that the inhibition zone against *E. coli* was obtained from the treatment of leaf extract with a diameter of (7.37) mm, boiled extract of dried leaves with a diameter of (6.65) mm and brewed extract of dried leaves with a diameter of (6.12) mm. In the treatment *of E. coli*, the largest inhibition zone was produced by the leaf extract. *A. malaccensis* plants contain secondary metabolite compounds like terpenoids, phenolic acids, benzophenones, xanthonoids, sesquiterpenes, chromones, fatty acids, phytosterols, and lignans. These compounds contribute to the plant's antimicrobial and anti-inflammatory activities, including 4-Phenyl-2-butanone, α-bulnesene, α-guaiene, agarospirol, ledene oxide-(II), elemol, and γ-eudesmol [14].

The largest zone of inhibition in E. coli treatment using *Aquilaria malaccensis* leaf extract is caused by the composition of the E. coli cell wall which is composed of lipopolysaccharide (LPS), protein and phospholipids. The outermost layer, LPS,

contains nonpolar organic compounds that are insoluble in polar solvents but soluble in nonpolar solvents such as ether or chloroform [15]. Polar solvents may not effectively dissolve or transport antimicrobial compounds to the target site. Factors that influence the emergence of inhibition zones include the diffusion of antimicrobial agents into the media, interactions with microbes, the number of microbes tested, growth speed, and level of sensitivity [16].

The smallest zone of inhibition was produced by the boiled extract of dried leaves. The zone of inhibition observed in *Aquilaria malaccensis* extracts is influenced by the extraction method and the metabolite compounds present in the extract. The extraction process is a crucial step in isolating phytochemicals from plant material. The extraction process of bioactive compounds in *Aquilaria malaccensis* is influenced by factors such as the extraction method, sample size, solvent, and presence of interfering substances [17]. The concentration of extracted substances also affects the inhibition zone diameter. Heat generated during extraction can damage cell walls and plasma membranes, preventing the extraction of antimicrobial compounds. The nonpolar nature of lipids in gram-negative bacteria can also affect antimicrobial compounds' ability to penetrate cell walls [18].



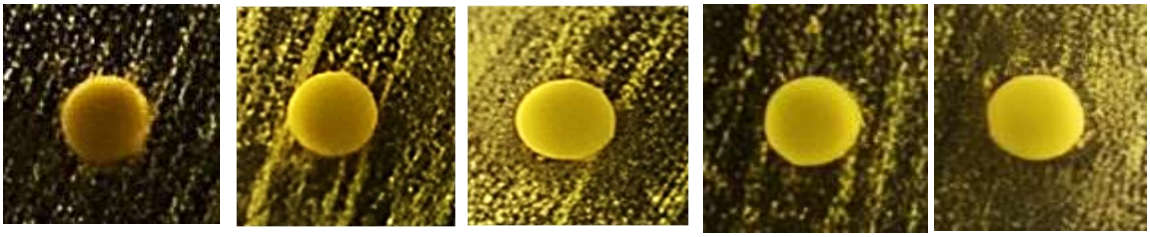
(a) (b) (c) (d) (e)

Figure 2. Antimicrobial activity of some *A.malaccensis* leaf and stem bark extracts against *S.aureus* growth. Description: (a) Leaf extract, (b) Boil dried leaves, (c) Brew dried leaves, (d) Boil dried stem bark, (e) Brew dried stem bark.

Based on Figure 2, it can be seen that the inhibition zone against *S. aureus* was obtained from the leaf extract treatment of (9.79) mm followed by boiled extract of dried leaves at (8.88) mm, brewed extract of dried leaves at (8.05 mm), boiled extract of dried stem bark at (6.14 mm) and brewed extract of dried stem bark at (6.09 mm). *Aquilaria malaccensis* extract contains secondary metabolites like alkaloids, flavonoids, triterpenoids, steroids, tannins, and saponins, which have antibacterial properties. These compounds, including saponins, disrupt bacterial cell membranes and lead to cell death. The extraction process requires polar solvents to isolate these bioactive compounds. The plant's potential for therapeutic applications is further highlighted by its diverse secondary metabolites. The concentration of extracted substances influences the inhibition zone diameter [19].

The largest zone of inhibition in the *S. aureus* treatment was produced by *Aquilaria malaccensis* leaf extract. Fresh extracts produced from plant water without the addition of solvents have a higher concentration of active compounds than dry extracts. This is because the drying or extraction process can reduce the concentration of active compounds. Compounds in fresh extracts, especially those that are soluble in water, have high bioavailability, meaning they are more easily absorbed by microorganisms or target cells, thereby increasing their effectiveness [20]. Polar flavonoid compounds play an important role in *Aquilaria malaccensis* leaf extract as antimicrobials. The mechanism of action of flavonoids as antibacterial agents includes forming complex compounds with extracellular and soluble proteins, damaging bacterial cell membranes, and releasing intracellular compounds [21].

The smallest zone of inhibition in the *S. aureus* treatment was produced by the brewed dry stem bark extract of *Aquilaria malaccensis*. The inhibitory zone diameter in a plant extract is influenced by factors like active compounds, extract concentration, solvent selection, and extraction method. Tannins, polar compounds, are soluble in polar solvents like water, making hot water and brewing duration crucial for effective tannin extraction [22]. *Aquilaria malaccensis* extracts can penetrate Staphylococcus aureus' cell wall due to their water-soluble nature. Teichoic acid, an essential component in the cell wall, facilitates the interaction of positive ions and polar compounds, causing damage to the bacterial cell structure [23].



(a) (b) (c) (d) (e)

Figure 3. Antimicrobial activity of some *A.malaccensis* leaf and stem bark extracts against *C.albicans* growth. Description: (a) Leaf extract, (b) Boil dried leaves, (c) Brew dried leaves, (d) Boil dried stem bark, (e) Brew dried stem bark.

Based on Figure 3, The study found that the absence of an inhibition zone in *C. albicans* leaf extract, boiled dry leaves, brewed dry leaves, boiled stem bark, and brewed dry bark of aloes was due to suboptimal extraction of antifungal compounds, such as alkaloids, flavonoids, tannins, and saponins. This suboptimal extraction may be due to factors such as low extraction efficiency, degradation of antimicrobial compounds, and low active compound content [24].The absence of an inhibition zone in five extracts is due to the difficulty of polar compounds, such as those found in agarwood plants, in penetrating the nonpolar cell walls of Candida albicans. The complex and thick structure of Candida albicans cell walls, including polysaccharides, provides additional protection against certain antimicrobial compounds or chemicals [25]. The dynamic and layered structure of yeast cell walls can also inhibit extract penetration. Test methods and genetic variability between strains can also affect results. The complex and dynamic structure of yeast cell walls, combined with the difficulty of polar compounds penetrating nonpolar cell walls, contribute to the absence of an inhibition zone in the extract [26].

Table 2. Percentage of inhibitory power of agarwood leaf and bark extracts on the growth of *E. coli*, *S. aureus* and *C. albicans*.

	Microbial inhibition zone diameter (mm)		
Treatment	***E. coli***	***S. aureus***	***C. albicans***
Positive control (mm)	24.42	22.33	20.29
Negative control (mm)	0,00	0,00	0,00
Percentage of leaf extract control (%)	30.18	43.84	28.70
Percentage of control boiled dry leaves (%)	27.23	39.76	28.70
Percentage of dry leaf brew control (%)	25.06	36.05	28.70
Percentage of control boiled dry bark (%)	24.57	27.49	28.70
Percentage of dry stem bark brew control (%)	24.57	27.07	28.70

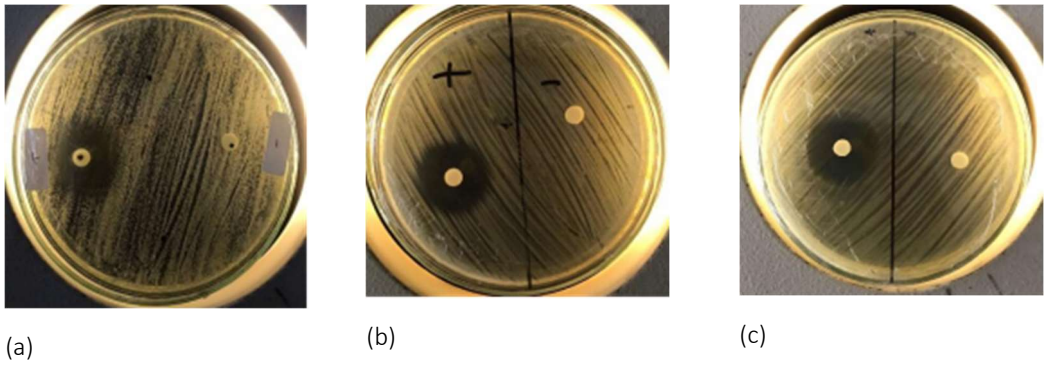


Figure 4 . Microbial Inhibition Zone Test Area of Control Treatment

Description: (a) *C. albicans* control , (b) *S. aureus* control, (c) *E. coli* control

Based on Figure 4, research that has been carried out for positive control treatment against *S. aureus* and *E. coli* using the antibiotic chloramphenicol 0.1 mg/ml and control treatment against *C. albicans* using fluconazole 0.1 mg/ml, while for negative control using distilled water. The diameter of the inhibition zone for the positive control of chloramphenicol on *S. aureus* was 22.33 mm. When compared with the control, the strength of the leaf extract was 43.84%, the dried leaf boiled extract was 39.76%, the dry leaf brewed extract was 36.05%, boiled extract of dry stem bark was 27.49 and brewed extract of dry stem bark was 27.07%. The diameter of the inhibition zone for the positive control of chloramphenicol on *E. coli* was 24.42 mm. When compared with the control, the strength of the leaf extract was 30.18%, the boiled dry leaf extract was 27.23%, the dry leaf brewed extract was 26.06%, boiled extract of dry stem bark was 24.57% and brewed extract of dry stem bark was 24.57%. Chloramphenicol is a broad-spectrum antibiotic that inhibits bacterial growth by interfering with bacterial protein synthesis [27]. The negative control used in the experiment was distilled water to prove that the solvent had no effect on the growth of the test microbes [28].

The diameter of the inhibition zone for the positive control fluconazole on *C. albicans* was 20.29 mm. When compared with the control, it was found that the strength of the leaf extract was 28.70%, boiled dry leaves were 28.70%, brewed dried leaves were 28.70%, boiled the skin dry stems amounted to 28.70%, and brewed dry stem bark amounted to 28.70%. Fluconazole is an antifungal medication that inhibits the synthesis of ergosterol, a key component of fungal cell membranes, by targeting the enzyme 14α-demethylase. It is primarily used to treat fungal infections, including those caused by *Candida albicans*, and is available in different pharmaceutical forms for oral administration [29].

3.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Based on research that has been carried out regarding the MIC and MBC values using the dilution method on fresh agarwood leaf extract, the following results were obtained:

Table 3. MIC and MBC values of Agarwood Leaf Extract against *S. aureus* and *E. coli* Test Microbes

Number	Treatment	Test Microbes	MIC value (%)	MBC value (%)
1.	Fresh Leaves	*Staphylococcus aureus*	6.25 %	50%
		Escherichia coli	3.125 %	12.5%

Based on Table 3, the results showed that fresh leaf extract was able to inhibit *S. aureus* with a Minimum Inhibitory Concentration (MIC) value of 6.25% and was able to kill *S. aureus* with a minimum Bactericidal Concentration (MBC) value of 50%. Meanwhile, fresh leaf extract was able to inhibit *E. coli* with a Minimum Inhibitory Concentration (MIC) value of 3.125% and was able to kill *E. coli* with a Minimum Bactericidal Concentration (MBC) value of 12.5%.

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism under controlled in vitro conditions. It is typically expressed in micrograms per milliliter (µg/mL) or milligrams per liter (mg/L). MIC values are crucial indicators of a strain's susceptibility or resistance to a drug. MIC is a vital parameter for assessing antimicrobial agent effectiveness and guiding appropriate therapy. The determination of MIC values is performed in both diagnostic and drug discovery laboratories. The MIC is determined by preparing a dilution series of the chemical, adding agar or broth, then inoculating with bacteria or fungi, and incubating at a suitable temperature [30]. The Minimum Bactericidal Concentration (MBC) is the lowest antimicrobial agent concentration needed to kill microorganisms without regrowth after incubation. It's determined by identifying the lowest compound concentration where the growth medium stays clear with no bacterial regrowth post-incubation. MBC values provide significant insights into the bactericidal activity of an antimicrobial agent [31].

3.3 The Correlation of Total Polyphenols with Antimicrobial Activity

Based on research that has been carried out regarding the total polyphenol test of *A. malaccensis* leaf and bark extracts, the following results were obtained:

Table 4. Total Polyphenols from *A. malaccensis* leaf and stem bark extracts

Number	Treatment	Polifenol (mgGAE/ml)
1.	Leaf Extract	34.14
2.	Boil Dried Leaves	20.79
3.	Brew Dried Leaves	20.32
4.	Boil Dried Bark	19.73
5.	Brew Dried Bark	19.16

Based on Table 4, the results show that the polyphenol value in leaf extract is 34.14 mgGAE/ml, boiled dry leaves is 20.79 mgGAE/ml, brewed dry leaves is 20.32 mgGAE/ml, boiled dry bark extract is 19.73 mgGAE /ml and brewed dry stem bark extract of 19.16 mgGAE/ml. Gaharu leaves contain secondary metabolites like flavonoids, alkaloids, triterpenoids, steroids, tannins, glycosides, and saponins, which are linked to their antioxidant and antimicrobial properties. The Folin-Ciocalteu method is used to assess the antioxidant potential of gaharu leaves [32]. High polyphenol content in gaharu leaves is associated with strong antimicrobial activity, making it a promising candidate for antimicrobial agents development [33]. The chemical composition of agarwood leaves can vary based on factors like habitat and growth, influencing the presence and concentration of secondary metabolites. The presence of bioactive compounds in agarwood leaves has increased interest in pharmaceutical and food industries [34].

The high polyphenol content in agarwood leaves has been associated with their strong antimicrobial activity, making them a promising candidate for the development of antimicrobial agents. The chemical composition of agarwood leaves can vary based on factors such as the plant's habitat, growth, and development, which can influence the presence and concentration of secondary metabolites. The presence of these bioactive compounds in agarwood leaves has led to increased interest in their potential applications in the pharmaceutical and food industries. The results also indicate that the enhancement of bioactive phytochemicals in agarwood leaves can be achieved under suitable conditions [35].

3.4 Antioxidant Activity Using the DPPH Method

Based on research that has been carried out regarding the antioxidant activity of *A. malaccensis* leaf and stem bark extract using the DPPH free radical reduction method (1,1-Diphenyl-2-Picryl Hydrazyl), the following results were obtained:

Table 5. Antioxidant Activity (IC50 Value) of *A. malaccensis* Leaf and Bark Extracts

Number	Treatment	Antioxidant Activity (µg/ml)
1.	Leaf Extract	50.47
2.	Boil Dried Leaves	62.13
3.	Brew Dried Leaves	76.64
4.	Boil Dried Bark	77.61
5.	Brew Dried Bark	79.07

Research found that A. malaccensis leaves have strong antioxidant activity with an IC50 value of 50.47 µg/ml, with the DPPH method showing that it is able to overcome DPPH free radicals. Meanwhile, dried stem bark showed moderate antioxidant activity, with IC50 values of 77.61 and 79.07 µg/ml respectively. The methanol extract of *A. malaccensis* leaves has very strong antioxidant activity, while agarwood bark has very weak antioxidant activity. Vitamin C is often used as a positive comparison in antioxidant tests [36]. Environmental factors such as altitude, temperature, lighting, humidity, water, air, soil, and species differences can also influence antioxidant activity and stability. High oxygen pressure, extensive contact with oxygen, heating, or irradiation can reduce antioxidant activity. Disrupted antioxidant stability also affects activity in dealing with free radicals [37].

IV. CONCLUSION

Based on the research conducted, it can be concluded that the *A. malaccensis* plant extracts have significant antimicrobial activity against *S. aureus* and *E. coli*, with the highest inhibition zone shown by the leaf extract against *S. aureus* (9.79 mm) and *E .coli* (7.37 mm). However, the leaf and stem bark extracts do not inhibit the growth of C. albicans. The leaf extract has a Minimum Inhibitory Concentration (MIC) of 6.25% against *S. aureus* with a Minimum Bactericidal Concentration (MBC) of 50%, and a MIC of 3.125% against *E. coli* with a MBC of 12.5%. The leaf extract of agarwood had a percentage inhibition of 30.18% and 43.84% against the growth of *E. coli* and *S. aureus*, respectively, compared to the positive control chloramphenicol (0.1mg/ml). The leaf extract of agarwood also has a percentage inhibition of 28.70% against the growth of *C. albicans* compared to the positive control fluconazole (0.1mg/ml). The highest antioxidant activity is found in the leaf extract with an IC50 value of 50.47 µg/mL, and the highest polyphenol content is found in the leaf extract at 34.14 mgGAE/mL.

V. SUGGESTION

In the next study, further research was carried out using extraction methods other forms and additional variations in the concentration of the extract used and the type different bacteria to prove that extracts of leaves and bark of agarwood can inhibit the growth of certain types of bacteria.

REFERENCES

[1] Hsu, V. P., Lukacs, S. L., Handzel, T., Hayslett, J., Harper, S., Hales, T., Semenova, V. A., Romero-Steiner, S., Elie, C., Quinn, C. P., Khabbaz, R., Khan, A. S., Martin, G., Eisold, J., Schuchat, A., & Hajjeh, R. A. (2002, October). The Public Health Response and Epidemiologic Investigation Related to the Opening of aBacillus anthracis–Containing Envelope, Capitol Hill, Washington, D.C. *Emerging Infectious Diseases*, *8*(10), 1039–1043. https://doi.org/10.3201/eid0810.020332

[2] Kongkham, B., Prabakaran, D., & Puttaswamy, H. (2020). Opportunities and challenges in managing antibiotic resistance in bacteria using plant secondary metabolites. *Fitoterapia*, *147*, 104762. https://doi.org/10.1016/j.fitote.2020.104762

[3] Ahmed, M. M., Emad Khadum, H., & Saied Jassam, H. M. (2023). Medicinal Herbs as Novel Therapies against Antibiotic-Resistant Bacteria. *Research Journal of Pharmacy and Technology*, 62–66. https://doi.org/10.52711/0974-360x.2023.00011

[4] Liu, Y. Y., Wei, J. H., Gao, Z. H., Zhang, Z., & Lyu, J. C. (2017). A Review of Quality Assessment and Grading for Agarwood. *Chinese Herbal Medicines*, *9*(1), 22–30. https://doi.org/10.1016/s1674-6384(17)60072-8

[5] Ridwanti Batubara, SURJANTO, T. ISMANELLY HANUM, ARBI HANDIKA, & ODING AFFANDI. (2020). The screening of phytochemical and antioxidant activity of agarwood leaves (*Aquilaria malaccensis*) from two sites in North Sumatra, Indonesia. *Biodiversitas Journal of Biological Diversity*, *21*(4). https://doi.org/10.13057/biodiv/d210440

[6] Jouki, M., & Khazaei, N. Antibacterial activity of essential oil of north west Algerian Eucalyptus camaldulensis against *Escherichia coli* and *Staphylococcus aureus*. (2014). *Journal of Coastal Life Medicine*. https://doi.org/10.12980/jclm.2.201414b57

[7] LU, J. J. (2009). Antioxidant Activity and Structure-activity Relationship of the Flavones from the Leaves of Aquilaria sinensis *Chinese Journal of Natural Medicines*, *6*(6), 456–460. https://doi.org/10.3724/sp.j.1009.2008.00456

[8] Alwi, H., Ali, S. A., Hamid, K. H. K., Shamsudin, M. Z., Radzi, N. C., Md Zaki, N. A., & Muhd, M. N. (2017). Drying effects of vacuum far-infrared on *Aquilaria malaccensis* leaves. *Journal of Physics: Conference Series*, *885*, 012017. https://doi.org/10.1088/1742-6596/885/1/012017

[9] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

[10] Morello, J.A., P.A. Granato & H.E. Mizer. *Laboratory Manual & Workbook in Microbiology.* 7 th Edition. The McGraw-Hill Companie. 2003. New York.

[11] Molyneux, P. The Use of Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakarin Journal of Science & Technology*. 2004. 26, 211-219.

[12] Jeong, S. M., Kim, S. Y., Kim, D. R., Jo, S. C., Nam, K. C., Ahn, D. U., & Lee, S. C. (2004, April 30). Effect of Heat Treatment on the Antioxidant Activity of Extracts from Citrus Peels. *Journal of Agricultural and Food Chemistry*, *52*(11), 3389–3393. https://doi.org/10.1021/jf049899k

[13] Gurusamy, K. S., Koti, R., Toon, C. D., Wilson, P., & Davidson, B. R. (2013). Antibiotic therapy for the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in surgical wounds. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.cd009726.pub2

[14] Tian, J., Lai, D., & Zhou, L. (2017). Secondary Metabolites from Acremonium Fungi: Diverse Structures and Bioactivities. *Mini-Reviews in Medicinal Chemistry*, *17*(7), 603–632. https://doi.org/10.2174/1389557516666160914194134

[15] Verma, A. (2012). Phytochemical Examination and in Vitro Free Radicl Scavinging Activity of Rhizome of Acorus Calamus Linn. Family Araceae. *International Journal of Scientific Research*, *3*(1), 54–55. https://doi.org/10.15373/22778179/jan2014/18

[16] Meredith, T. C., Aggarwal, P., Mamat, U., Lindner, B., & Woodard, R. W. (2006). Redefining the Requisite Lipopolysaccharide Structure in *Escherichia coli*. *ACS Chemical Biology*, *1*(1), 33–42. https://doi.org/10.1021/cb0500015

[17] Zainurin, N., Hashim, Y. Y., Mohamed Azmin, N., & Al-Khatib, M. (2020). Understanding the effects of different parameters of Soxhlet extraction on bioactive compounds from *Aquilaria malaccensis* leaf through GCMS-based profiling. *Food Research*, *4*(S1), 63–73. https://doi.org/10.26656/fr.2017.4(s1).s09.

[18] Loo, A., Jain, K., & Darah, I. (2007). Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, Rhizophora apiculata. *Food Chemistry*, *104*(1), 300–307. https://doi.org/10.1016/j.foodchem.2006.11.048

[19] Weng, A., Thakur, Melzig, & Fuchs. (2011). Chemistry and pharmacology of saponins: special focus on cytotoxic properties. Botanics: Targets and Therapy, 19. https://doi.org/10.2147/btat.s17261

[20] .Ajayi, O., Awala, S., Olalekan, O., & Alabi, O. (2017). Evaluation of Antimicrobial Potency and Phytochemical Screening of Persea americana Leaf Extracts against Selected Bacterial and Fungal Isolates of Clinical Importance. *Microbiology Research Journal International*, *20*(1), 1–11. https://doi.org/10.9734/mrji/2017/24508

[21] Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M. L., De Tommasi, N., Izzo, V., & Dal Piaz, F. (2021). Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids. *Pharmaceutics*, *13*(5), 660. https://doi.org/10.3390/pharmaceutics13050660

[22] MARKOM, M., HASAN, M., DAUD, W., SINGH, H., & JAHIM, J. (2007). Extraction of hydrolysable tannins from Phyllanthus niruri Linn.: Effects of solvents and extraction methods. *Separation and Purification Technology*, *52*(3), 487–496. https://doi.org/10.1016/j.seppur.2006.06.003

[23] van Dalen, R., Peschel, A., & van Sorge, N. M. (2020). Wall Teichoic Acid in *Staphylococcus aureus* Host Interaction. *Trends in Microbiology*, *28*(10), 869. https://doi.org/10.1016/j.tim.2020.07.001

[24] Koyu, H., Kazan, A., Ozturk, T. K., Yesil-Celiktas, O., & Haznedaroglu, M. Z. (2017). Optimizing subcritical water extraction of Morus nigra L. fruits for maximization of tyrosinase inhibitory activity. *The Journal of Supercritical Fluids*, *127*, 15–22. https://doi.org/10.1016/j.supflu.2017.03.007

[25] Ajayi, O., Awala, S., Olalekan, O., & Alabi, O. (2017). Evaluation of Antimicrobial Potency and Phytochemical Screening of Persea americana Leaf Extracts against Selected Bacterial and Fungal Isolates of Clinical Importance. *Microbiology Research Journal International*, *20*(1), 1–11. https://doi.org/10.9734/mrji/2017/24508

[26] Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M. L., De Tommasi, N., Izzo, V., & Dal Piaz, F. (2021). Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids. *Pharmaceutics*, *13*(5), 660. https://doi.org/10.3390/pharmaceutics13050660

[27] Cannon, M., Harford, S., & Davies, J. (1990). A comparative study on the inhibitory actions of chloramphenicol, thiamphenicol and some fluorinated derivatives. *Journal of Antimicrobial Chemotherapy*, *26*(3), 307–317. https://doi.org/10.1093/jac/26.3.307

[28] Chwastowski, J., Wójcik, K., Kołoczek, H., Oszczęda, Z., Khachatryan, K., & Tomasik, P. (2023). Effect of water treatment with low-temperature and low-pressure glow plasma of low frequency on the growth of selected microorganisms. *International Journal of Food Properties*, *26*(1), 502–510. https://doi.org/10.1080/10942912.2023.2169708

[29] Zervos, M., & Meunier, F. (1993). Fluconazole (Diflucan®): a review. *International Journal of Antimicrobial Agents*, *3*(3), 147–170. https://doi.org/10.1016/0924-8579(93)90009-t

[30] Chikezie, I. O. (2017). Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method. *African Journal of Microbiology Research*, *11*(23), 977–980. https://doi.org/10.5897/ajmr2017.8545

[31] Billings, N., & Ribbeck, K. (2014). Minimal Bactericidal Concentration for Biofilms (MBC-B). *BIO-PROTOCOL*, *4*(9). https://doi.org/10.21769/bioprotoc.1115

[32] Leyte-Lugo, M., Britton, E. R., Foil, D. H., Brown, A. R., Todd, D. A., Rivera-Chávez, J., Oberlies, N. H., & Cech, N. B. (2017). Secondary metabolites from the leaves of the medicinal plant goldenseal (Hydrastis canadensis). *Phytochemistry Letters*, *20*, 54–60. https://doi.org/10.1016/j.phytol.2017.03.012

[33] Budi Santoso, Neni Anggraini, Kiki Yuliati, & Din Pangawikan. (2022). Phenol compound content and antibacterial activity of gaharu leaf extract products (*Aquilaria malaccensis*). *Bioscience Journal*, *38*, e37009. https://doi.org/10.14393/bj-v38n0a2022-54813

[34] Kim Ngan, T. T., Thu Thuy, D. T., Tuyen, T. T., Inh, C. T., Bich, H. T., Long, P. Q., Chien, N. Q., Kieu Linh, H. T., Yen Trung, L. N., Tung, N. Q., Nguyen, D. C., Bach, L. G., & Toan, T. Q. (2019). Chemical Components of Agarwood (Aquilaria crassna)Essential Oils Grown in Various Regions of Asia. *Asian Journal of Chemistry*, *32*(1), 36–40. https://doi.org/10.14233/ajchem.2020.22177

[35] de Araújo, F. F., de Paulo Farias, D., Neri-Numa, I. A., & Pastore, G. M. (2021, February). Polyphenols and their applications: An approach in food chemistry and innovation potential. *Food Chemistry*, *338*, 127535. https://doi.org/10.1016/j.foodchem.2020.127535

[36] Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Advances*, *5*(35), 27986–28006. https://doi.org/10.1039/c4ra13315c

[37] Shahidi, F., & Zhong, Y. (2015, October). Measurement of antioxidant activity. *Journal of Functional Foods*, *18*, 757–781. https://doi.org/10.1016/j.jff.2015.01.047