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ANTIBACTERIAL ACTIVITY OF EXTRACT AND FRACTION OF AGARWOOD LEAVES (*Aquilaria malaccensis* Lam) AGAINST *Staphylococcus aureus* AND BIOAUTOGRAPHY TEST

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ABSTRACT

Agarwood plant (*Aquilaria malaccensis* Lam) is one of the forest plants that has medicinal and cosmetic benefits. When skin is broken, it will be easily infected by bacteria, one of which is *Staphylococcus aureus*. This study aimed to evaluate the antibacterial activity of the extract and fraction of Agarwood leaves against *Staphylococcus aureus*. The extract and fraction of Agarwood leaves were assayed for antibacterial activity using microdilution and bioautography methods. The extraction was performed by using maceration method with ethanol 96% as the solvent. The fractionation was carried out using liquid-liquid extraction method with *n*-hexane, ethyl acetate, and methanol-water as the solvents. The results showed that MIC value obtained for the extract,*n*-hexane fraction, ethyl acetate fraction,and methanol-water fraction was 32 µg/mL, 16 µg/mL, 32 µg/mL, and 32 µg/mL, respectively. In addition, MBC value for the extracts, *n*-hexane fraction, and ethyl acetate fraction was 512 µg/mL, 512 µg/mL, and was 256 µg/mL. The results indicate that the ethyl acetate fraction has stronger antibacterial activity than the extract, the *n*-hexane fraction and the methanol-water fraction. The bioautography test found the compounds that are assumed to be active as antibacterial, namely phenolic compounds.

Keywords: Agarwood Leaves, Antibacterial, *Staphylococcus aureus*, Microdilution Method, MIC Value, MBC Value, Bioautography Test

INTRODUCTION

Diseases Agarwood plants (*Aquilaria malaccensis* Lam) have many benefits including raw materials for medicines, cosmetics, perfumes, therefore they become commercial commodities with high economic value. Based on phytochemical screening, Agarwood leaves (*Aquilaria malaccensis* Lam) contains secondary metabolites, namely phenolics, flavonoids and terpenoids **[1]**. *Staphylococcus aureus* is a gram-positive bacteria that are spherical in shape, most of these strains are positive coagulase, when clustered in an irregular arrangement the sides are somewhat flat due to pressure, a diameter between 0.8-1.0 microns. This bacterium is immobile and non-sporical. These gram-positive bacteria are arranged like grapes, non-motile, aerobic, facultative anaerobic, produce coagulase, can be found on the nasal membrane, skin, hair follicles. In addition, *Staphylococcus aureus* can also cause food poisoning, mild to severe skin infections **[2]**. This study aimed to determine the antibacterial activity of the extract and fraction of Agarwood leaves against *Staphylococcus aureus* using microdilution method and to determine the content of active compounds as antibacterial using bioautography test.

MATERIALS AND METHODS

Materials

The tools used in this study were *beaker glass*, *azeotrop* distillation set, boiling stone, rotary evaporator, analytical scales, spatel, volume pipettes, drop pipettes, vaporizer cups, separating funnels, reagent sprayers, chromatographic vessels, UV wavelengths of 254 nm and 366 nm , micropipette, spiritus, incubator, stirring rod, UV-Vis spectrophotometer, cuvette, silicate crucible, filter paper, furnace, autoclave, capillary tube, oven, Moisture Balance, reagent tube, and watch glass.

Simplicia of Agarwood leaves, Aquadest, toluene, chloroform, ethanol 96%, $FeCl_3$, gelatin, dragendorff reagent, Mg powder, HCl solution, Mayer's reagents (HgCl, Kl), Lieberman-Bouchard reagents (anhydrous acetic acid, concentrated sulfuric acid), *n*-hexane, ethyl acetate, methanol, TLC plate silica gel 60 GF_{254} as the stationary phase, pure culture of *Staphylococcus aureus*, physiological NaCl, MHB (Mueller Hinton Broth) media, MHA (Mueller Hinton Agar) media, 5% AlCl in methanol, amyl alcohol, NaOH 1 N, ammonia, and ether.

METHODS

In this study, there are several methods carried out such as preparation of materials, characterization of simplicia, phytochemical screening, extraction, fractionation, monitoring of extracts and fractions as well as antibacterial activity

tests. Material preparation includes material collection, determination of materials and processing of materials into simplicia. Simplicia characterization includes determination of drying shrinkage, water content, total ash content, acid insoluble ash, water soluble extracts and ethanol soluble extracts **[3]**. Phytochemical screening includes examination of secondary metabolites compound found in simplicia which include examination of flavonoids, saponins, quinones, tannins, phenolics, alkaloids and steroids or triterpenoids **[4]**. The extraction was carried out using maceration method with 96% ethanol solvent, then the extract was thickened using a rotary evaporator so that a thick extract was obtained. Fractionation was carried out on thick extract using the Liquid-Liquid Extraction method so that three fractions obtained were nonpolar, semipolar and polar. The extract and the three fractions were tested for antibacterial activity. Antibacterial activity tests were performed using microdilution and bioautography methods **[5]**.

RESULTS AND DISCUSSION

1. Simplicia Characterization

The results of drying shrinkage and water content obtained meet the requirements so that the simplicia used had good quality because it could affect the yield of the extract. In water soluble extract content and ethanol soluble extract content tests showed that the simplicia of Agarwood leaves were more soluble in ethanol solvent so that when extracting using ethanol solvent more compounds were found. Flavonoid compounds and phenolics are more easily solved in ethanol solvents **(Table 1)**.

2. Phytochemical Screening

The results of phytochemical screening showed that Agarwood leaves contain flavonoids, saponins, quinones, phenolics, and steroids. In the antibacterial activity test, the compounds that play important roles are flavonoids and phenolics so that the compounds contained in Agarwood leaves can perform activity against bacteria which has mechanism of action by inhibiting bacterial protein denaturation **(Table 2)**.

3. Antibacterial Activity Test

In general, various concentrations of the tested compounds are mixed with media containing bacteria, then incubated. The lowest concentration of the test substance which is not detect the growth of microorganisms is considered as MIC value **[5]**. In **Table 3**, the antibacterial activity of the extract (mother liquor at 1024 µg/mL)of 32 µg/mL has the MIC value of antibiotics was 4 µg/mL.

In **Table 4**, the antibacterial activity test of the fraction obtained the MIC value as follow: the *n*-hexane fraction was 16

µg/mL, the ethyl acetate fraction was 32 µg/mL and the methanol-water fraction was 32 µg/mL with the MIC value of the antibiotic was 4 µg/mL. After obtaining the MIC value, the MBC value was determined by using agar diffusion method. The formation of clear zone in the media occurs because the test agent diffuses into agar and inhibits the germination and growth of microorganisms, then the results are incubated and the zone of growth inhibition can be measured **[5]**. Based on the observations, the MBC value of the extract was 512 µg/mL, the *n*-hexane fraction was 512 µg/mL, the ethyl acetate fraction was 256 µg/mL, whereas the methanol-water fraction did not show MBC, which means the concentration used for MBC test can only inhibit bacterial growth.

4. Bioautography Test

After obtaining the MIC and MBC values, the test was then continued by using bioautography method. Bioautography method is a specific method for detecting spots on thin layer chromatography(TLC) plate and paper chromatography that has antibacterial, antifungal and antibiotic activities **[6]**. The extracts and fractions were spotted on TLC plates then eluted with mobile phase using ethyl acetate: methanol: water (8: 1: 1, v/v), then contacted to a media containing bacteria. The contact bioautography method was chosen because it is easier, simpler and most often used. Contact bioautography obtained the process of moving active compounds into the agar medium which can produce inhibition zones and the ability to distinguish between active compounds with the same Rf value **[7]**. After the plate was contacted with the media, the plate was removed and then incubated. In **Figure 1**, there are spots that have clear zones on agar media with Rf 0.62. The same spot with the rf value of 0.62 is the spot on the plate reacted with $FeCl_3$, so that the compounds that are antibacterial are phenolics or polyphenol compounds. Polyphenol compounds are plant compounds that contain aromatic rings with one or more hydroxyl groups and with the addition of $FeCl_3$, will give a black color. The antibacterial activity of polyphenols is due to the presence of hydroxyl groups which can inhibit bacterial growth by the mechanism of bacterial protein denaturation. Polyphenols consist of simple phenol groups and phenolic acids, quinine, flavonin, flavonoids and flavonols, tannins and coumarin **[8]**.

Table 1: Results of the simplicia characterization

Simplicia Characterization Test	Results % (w/w)
Drying Shrinkage	9.48
Water Content	5*
Total Ash content	7
Acid Insoluble Ash Content	1,5
Water Soluble extract Content	5
Ethanol Soluble extract Content	6

*measurement in % (v/w)

Table 2: Results of Phytochemical Screening

Phytochemical screening test	Results
Flavonoids	+
Saponin	+
Kuinon	+
Tanin	-
Phenolate	+
Alkaloids	-
Steroid/Triterpenoid	+

Table 3: Test of antibacterial activity of the extract

	1	2	4	8	16	32	64	128	256	512
Extract	+	+	+	+	-	-	-	-	-	-
	+	+	+	+	+	-	-	-	-	-
	+	+	+	+	-	-	-	-	-	-
	+	+	+	+	+	-	-	-	-	-
	+	+	+	+	+	-	-	-	-	-
	+	+	+	+	+	-	-	-	-	-
Antibiotics	+	+	-	-	-	-	-	-	-	-
	+	+	-	-	-	-	-	-	-	-

(+): There is bacterial growth; (-) : There is no bacterial growth

Table 4: Test of antibacterial activity of the fraction

	1	2	4	8	16	32	64	128	256	512
n-hexanefraction	+	+	+	+	-	-	-	-	-	-
	+	+	+	+	-	-	-	-	-	-
Ethyl acetate fraction	+	+	+	+	-	-	-	-	-	-
	+	+	+	+	+	-	-	-	-	-
Methanol-water fraction	+	+	+	+	+	-	-	-	-	-
	+	+	+	+	+	-	-	-	-	-
Antibiotics	+	+	-	-	-	-	-	-	-	-
	+	+	-	-	-	-	-	-	-	-

(+): There is bacterial growth; (-): There is no bacterial growth

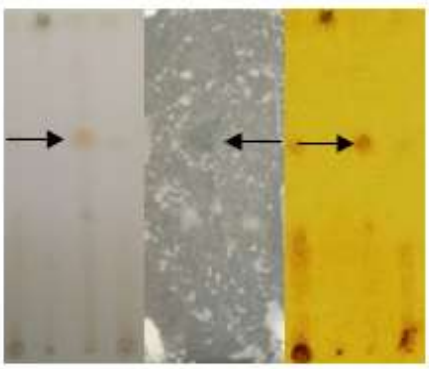

Figure 1: The results of Bioautography of extracts and fractions of Agarwood leaves against *Staphylococcus aureus*

CONCLUSION

The extract and fraction of Agarwood leaves have antibacterial activity against *Staphylococcus aureus* and the results of bioautography tests show that compounds that act as antibacterial are phenolic compounds.

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REFERENCES

[1] Kumphune, S., Prompunt, E., Phaebuaw, K., Sriudwong, P., Pankla R., & Thongyoo, P. Antiinflammatory Effects of The Ethylacetate Extract of *Aquilaria crassna* Inhibits LPS-Induced Tumor Necrosis Factor-Alpha Production by Attenuating P38 MAPK Activation. International Journal Green Pharm, 2011, 5(4), 3-8.

[2] Pelczar, Michael J dan E. C. S. Chan. Fundamentals of Microbiology, University of Indonesia Publisher, 1, 2006, 100-175; 508-543.

[3] Indonesian herbal pharmacopoeia, edition I, 2008

[4] Farnsworth, N. Biological and Phytochemical Screening of Plants. Journal of Pharmaceutical Sciences, 1996, 55(3), 256-264.

[5] Choma, Irena M danGrzelak, Edyta M. Bioautography detection in thin – layer chromatography, Journal of Chromatography A, 2011, 1218, 2684–2691.

[6] Novitasari, *et al*, Chromatographic Profile of Antioxidant and Antibacterial Active Compounds of the Etho Acetate Fraction of Libo Leaves (*Ficus variegata* Blume.), Journal of Science and Health, 2015, 3, 131- 137

[7] Khaerati, *et al*, Antibacterial Effects of Celery Herb (*Apium graveolens* Linn.) Ethanol Extract Against Escherichia coli and *Staphylococcus aureus* and Analysis of TLC Bioautography, Biocelebes, 2011, 5, 19.

[8] Ulfa, *et al*. Antibacterial Activity and TLC Bioautography Ethanol Extract of Naga Scales (*Drymoglossum piloselloides*) Against *Streptococcus* mutans, Journal of Dentistry, 2013, 10, 42.