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Bioactive compounds of ethanol extract from agarwood leaves (*Aquilaria malaccensis*) and antimicrobial activity against bacteria and fungi growing on the skin

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Abstract. *Batubara R, Wirjosentono B, Siregar AH, Harahap U, Tamrin. 2021. Bioactive compounds of ethanol extract from agarwood leaves (*Aquilaria malaccensis*) and antimicrobial activity against bacteria and fungi growing on the skin. Biodiversitas 22: 2884-2890.* Agarwood plants (*Aquilaria malaccensis* Lamk.) provide numerous benefits and have been cultivated by the people. The leaves containing various compounds, such as flavonoids, glycosides, triterpenoids and tannins with antibacterial and antifungal activities, were reported to be potential for flesh wound healing. Therefore, research on these activities against microorganisms that cause infection in wounds was carried out in vitro. This research aims to determine the antibacterial and antifungal activities of agarwood leaves ethanol extract against three bacteria species (*Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes*) and two fungi species (*Candida albicans* and *Trichophyton* sp.) that commonly cause skin infection. The results of this research show that the concentration of ethanol extract of agarwood leaves affected the bacterial and fungal growth inhibition zone's diameter. The best inhibitory potential occurred at 5% concentration in activity against bacteria and fungi. The extract's active compounds contributing to the antimicrobial activity were the flavonoids and tannins. Furthermore, GC-MS identification shows that the ethanol extract from agarwood leaves contained Disulfide, dioctyl; 6,7-Dimethylquinoxaline; (E)-1,3-Di-m-tolylallyl ethyl Carbonate; 5-(2-Methoxyphenyl)-1,2-dihydropyrazol-3-one; 9-[(tert-Butyldimethylsilyl)oxy]benzo[3,4]cyclod ec-3-ene-1,5-diyne; Neophytadiene, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-; Hexadecanoic acid, methyl ester and a-(3-chloro-4-hydroxyphenyl)-aphenoxy-a-phenyl acetonitrile.

Keywords: Agarwood leaves, bacteria, fungi, inhibition zone's diameter, skin

INTRODUCTION

Several studies have reported the benefits of *Aquilaria* leaves for humans. The leaves contain various chemical constituents including 2- (2-phenylethyl) chromones, phenolic acids, steroids, fatty acids, benzophenones, xanthonoids, flavonoids, terpenoids, and alkanes. They are assumed to be associated with beneficial pharmacological properties, including analgesic, anti-rheumatic, anti-inflammatory, anticancer, antitumor, antioxidant, antibacterial, antifungal, antidiabetic, antihistamine, lipid-lowering, laxative, acetylcholinesterase (pain) inhibition, and hepatoprotective (Adam et al. 2017). It indicates the potentials of agarwood leaves as a source of medicine.

Previous studies have explained the antimicrobial properties of agarwood leaves. The Minimum Inhibitory Concentration (MIC) of leaves extract in inhibiting the growth of *Staphylococcus aureus* ATCC 25923 was 2.5 mg/mL with an inhibitory diameter of 7.00 ± 0.00 mm (Liana 2017). Agarwood leaves extract with a concentration of 30% showed the best antibacterial activity with an inhibition zone area of 9.65 mm against *Pseudomonas aeruginosa* and 13.50 mm against *S. aureus* (Jansen 2017). The ethanol extract from agarwood leaves with the concentration of 300 mg/mL, 400 mg/mL, and 500 mg/mL inhibited *S. aureus* with the average of inhibition zone diameter of 12.50 mm, 13.51 mm, and 15.80 mm, respectively, and also inhibited *Proteus mirabilis* with the average yield of inhibition zone diameter of 10.17 mm, 11.62 mm, and 13.41 mm, respectively (Sari et al. 2017). The most excellent performance of the extract in inhibiting *S. aureus* bacteria was at the concentration of 450 mg/mL with an average inhibition zone diameter of 21.17 ± 3.32 mm (Wahid and Ittiqo 2019).

The development of natural ingredients for wound medicine derived from plants requires further research. The utilization of these raw materials is due to its renewable and relatively small negative impact. Thus, it is necessary to experiment with producing wound medicine ingredients from agarwood leaves extract. During the healing process, the wound is sometimes followed by infection from both fungal and bacterial groups. Therefore, this research's initial step is assessing the antimicrobial properties of agarwood leaves against bacteria and fungi that grow on the skin and commonly cause flesh wound infection. The three bacteria species are *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes*, and the two fungi species are *Candida albicans* and *Trichophyton* sp.

Infectious disease becomes one of the health problems in society that is difficult to overcome completely (Qomar 2018). Infectious diseases, which are easily transmitted, can be caused by bacteria, fungi, viruses, and parasites (Diyantika et al. 2017). One of the bacteria *S. epidermidis* that can be generally responsible for swelling in skin infections is abscesses or acne (Radji 2011). Another bacteria is *S. aureus* which plays an essential role in infection of the hair follicles and sweat glands, acne, abscesses, impetigo, and wounds infection. This bacterium has low invasion ability but is involved in many skin infections (Miller et al. 2012). It is commonly found around the human environment as the cause of most skin infections due to its ability to adapt and its resistance to antimicrobial properties. Although, it is also found on mucous membranes and can cause sore throat and infections of the central nervous system and lungs (Diyantika et al. 2017). Some infection cases caused by *S. aureus* are acne, impetigo, and wound infection (Mardiah 2018).

Propionibacterium acnes is an anaerobic gram-positive bacterium that causes inflammation on the skin (Brzuszkiewicz et al. 2011). This bacterium is the primary organism that plays a role in the formation of acne (Aida et al. 2016). *C. albicans* is the most often isolated species in humans (Listiyawati et al. 2017). It usually causes candidiasis, an acute and subacute fungal disease affecting the skin of the mouth, vagina, nails, skin, bronchi, or lungs (Farizal 2017).

Three main genera of dermatophyte fungi responsible for the fungal disease of the skin are *Epidhermophyton*, *Microsporum*, and *Trichophyton* (Tambayong 2000). *Trichophyton* is fungi that often affect hair, skin, and nails (Sintowati et al. 2008).

This research aims to determine the bioactive compounds of ethanol extract from agarwood leaves *(Aquilaria malaccensis)*. In addition, this research aims to determine the antibacterial and antifungal activities of agarwood leaves ethanol extract against bacteria and fungi species that commonly cause skin infection.

MATERIALS AND METHODS

Time and place of research

This research was conducted from August 2019 to March 2020. The sampling was located at Pekan Bahorok Village, Bahorok Subdistrict, Langkat District, North Sumatra Province, Indonesia. Phytochemical analyses were carried out at the Phytochemical Laboratory, Faculty of Pharmacy, University of Sumatera Utara (USU), Medan, Indonesia. Antimicrobial activity analyses were carried out at the Microbiology Laboratory, Faculty of Pharmacy, USU. The characterization and extraction were carried out at the Forest Products Technology Laboratory, Faculty of Forestry, USU. Whereas. the GC-MS analysis was conducted at Customs and Excise (Bea Cukai) Laboratory of North Sumatra, Medan.

Agarwood leaves sampling

The sample used in this study was *Aquilaria malaccensis* species., cultivated by the local people in the Bahorok Subdistrict, Langkat District, North Sumatra, Indonesia. This species is identified and deposited in Medanense Herbarium.

Raw material preparation

At this step, the agarwood leaves were cleaned by rinsing with running water, spread on parchment paper until the water was absorbed. Afterward, the samples were dried using a drying cabinet with a temperature of 40˚C–50˚C until dry and brittle. Dried leaves were then blended using a blender into powder and stored in the polyethylene plastic and in a place protected from the sun before being extracted and analyzed.

Ethanol extraction of the agarwood leaves powder

The extract was obtained using the maceration method with 96% ethanol solvent. As much as 200 g of simplicia powder was put into a glass container, poured with 1500 mL of the ethanol, covered, kept protected from light aside, and occasionally stirred. After five days, the mixture was filtered. Then the powder was soaked again with a 96% ethanol solution of 1500 mL, left in a place protected from light for 2 more days. After 2 days, the mixture was then filtered again. The first and second filter (macerate) results were then combined. The residue was then soaked with 96% ethanol sufficiently to obtain 2000 mL of the mixture, transferred into a closed vessel, and left in a cool place protected from light for two days, then filtered. All collected filtrates were concentrated using a rotary evaporator at 40°C until a thick liquid was obtained and then dried using a freeze dryer to get a dry extract (Batubara et al. 2020).

Phytochemical screening and tannin content determination

The chemicals used for testing are pro-analysis quality chemicals, produced by E-Merck, Germany. Phytochemical tests include examining flavonoids, alkaloids, saponins, tannins and steroids/triterpenoids, referring to Harborne (1987) and MoHRI (2000). Testing of tannin levels was carried out using the Lowenthal-Procter method (Sudarmadji et al. 1984): (i) A total of 5 g of finely ground material was added to 400 mL of distilled water and then boiled for 30 minutes. (ii) It was then cooled, filtered, and put into a 500 mL measuring flask and added with distilled water until the specified mark (phytate 1). (iii) 10 mL of phytate I was taken and added with 25 mL of indigo carmine solution and 750 mL of distilled water. Then it was titrated with 0.1 N $KMnO_4$ solution until it turned to a golden yellow color. (iv) 100 mL of phytate I was taken and added with 50 mL of gelatin solution, 100 mL of acid salt solution, and 10 g of kaolin powder, respectively. Furthermore, it was shaken vigorously for a few minutes and filtered (filtrate II). (v) 25 mL of phytate II was taken and mixed with 25 mL of indigo carmine solution and 750 mL of distilled water. Then, it was titrated with 0.1 N $KMnO_4$ solution. (vi) Standardization of KMnO4 solution is Na-oxalate.

Calculation:

$$\text{Tannin Level} = \frac{(50\ A - 50\ B) \times 0.1 \times 0.00416}{\text{Sample Weight}} \times 100\%$$

Where:

A : Tannin titration volume (mL)
B : Volume of blank titration (mL)
N : Normality standard KMnO4 (N)
10 : Dilution factor, 1 mL of KMnO4 0.1
N : equivalent with 0.00416 g of tannin

Antimicrobial activity (against bacteria and fungi commonly growing on the skin)

Preparation and evaluation of antibacterial activity using the disc diffusion method (Kirby-Bauer Test)

Preparation steps include bacterial rejuvenation, making bacterial suspensions, making paper discs, preparing negative controls, preparing positive controls, and making series of concentrations. This research is a series of research for applications, so that it takes into account the safety aspect. The initial concentration refers to Surjanto et al. (2019), wherein this research the initial concentration was 1.30%. However, for ease of production and calculation, the initial concentration was reduced to 1.25%. The subsequent concentrations were multiples of 2.5%, 5%, 10% and 20%. Antibacterial activity evaluation used the disc method (Peng and Zhao 2009). The disc method was carried out using 6 mm diameter paper discs and agar media that had been sterilized as bacterial growth media.

The evaluation used three species of bacteria, namely: *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, and *Propionibacterium acnes* ATCC 6919. Bacteria were inoculated in Petri dishes which had been filled with agar media. Afterward, the disc paper was dipped in the ethanol extract from agarwood leaves and then placed on agar media. After being incubated for 48 hours, the inhibition zone (area that was not loaded by bacteria) diameter was measured using calipers. The test was carried out with three replications. The positive control was prepared using amoxicillin, while the negative control was prepared using ketoconazole.

Fungal incubation and assessment of agarwood leaves extract

The assessment of antifungal activity refers to Al-Enazi (2018), where the fungi were grown on Müeller-Hinton and Nutrient agar media on Petri dishes. Furthermore, the disc was added to the ethanol extract of agarwood leaves according to the treatment's concentration. Incubation was carried out at 35°C for 72-120 hours. The extract's effectiveness an antifungal was measured by observing the clear zone formed around the diffusion discs and Whatman paper. *Candida albicans* ATCC 10231 and *Trichophyton* sp. ATCC 18748 were used in this study.

The GC-MS analysis

Identification of compounds from agarwood leaves ethanol extract was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). The instrument is GC-MS 7890B.

RESULTS AND DISCUSSION

Phytochemical screening and tannin content

Phytochemical screening results of agarwood leaves ethanol extract are presented in Table 1, which are presented in detail test results based on the reference literature.

Table 1. Phytochemical screening results of the agarwood leaves ethanol extract

Compound groups	Reagents	Positively results (reference)	The result of the extract
Flavonoids	Mg powder + HCl concentrated + amyl alcohol	Colored solution (red, yellow, orange) on the amyl alcohol layer (MoHRI 2000)	(+) Red color on the amyl alcohol layer
Alkaloids	HCl + Dragendorff/ Meyer reagents + Bouchardat reagents	Red/white precipitate (MoHRI 2000)	(-) Neither white nor yellow precipitate was presented on the Meyer; neither brown nor brownish-orange on the Dragendrorff and neither brown nor dark brown on the Bouchardat reagents
Saponins	Shaked + HCl 2N	Stable foam (MoHRI 2000)	(-) The formed foam was unstable and diminished after the addition of HCl 2 N
Tannins	+ $FeCl_3$ 1%	Blue or dark green color was formed (MoHRI 2000)	(+) The solution became dark blue after the addition of the $FeCl_3$ 1%
Steroids/ triterpenoids	+ Lieberman-Burchard reagents	The blue or greenish-blue color indicated the presence of steroids, while red, pink, or purple color indicated the presence of triterpenoids (Harborne 1987).	(+) The solution became purplish-red after the addition of concentrated sulfuric acid solution

Notes: +: positively contain the compounds, -: negatively contain the compounds

Table 2. Inhibitory zone diameters (mm) of the ethanol extract from agarwood leaves against the tested bacteria *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Propionibacterium acnes*

Concentrations and controls (%)	Tested bacteria		
	S. epidermidis	***S. aureus***	***P. acnes***
20.00	10.43 ± 0.76	9.23 ± 0.97	11.47 ± 0.59
10.00	9.90 ± 0.43	8.33 ± 1.50	10.73 ± 0.98
5.00	8.23 ± 0.11	19.90 ± 2.08	7.93 ± 0.32
2.50	7.80 ± 0.10	16.40 ± 3.11	7.26 ± 0.38
1.25	7.40 ± 0.20	12.7 ± 3.42	6.90 ± 0.17
Positive control (Amoxicillin)	12.73 ± 2.45	12.73 ± 2.45	12.73 ± 2.45
Negative control (Ketoconazole)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

The agarwood leaves extract positively contained flavonoids, tannin, and steroids/triterpenoids compounds. Meanwhile, the leaves extract did not contain saponins because it produced an unstable foam with a thickness of 1 cm which was diminished after the addition of HCl 2 N. The leaves extract did not contain alkaloids either. None of the three reagents tested showed positive results.

Phytochemical screening provided important information about the chemical compounds of agarwood leaves. Screening techniques assisted phytopharmacological steps in the initial selection of the plant analysis to prove the presence of certain chemical compounds and, furthermore, be associated with the biological activity (Farnsworth 1996).

Ethanol extract from agarwood leaves positively contained tannin compounds. The quantitative analyses show that the extract contained 4.64 ± 0.49% of tannin. This tannin content was not much different from the results of the research on agarwood tannin levels of type *W. tenuiramis* of 4.95% and *A. malaccensis* of 5.62% (Batubara et al. 2018).

Evaluation of antimicrobial activity

Antibacterial activity

The maximum inhibitory zones of the agarwood leave ethanol extract against three bacteria species are shown in Table 2. The highest result of *S. epidermis* and *P. acnes* was on 20% concentration whereas *S. aureus* was on 5% concentration.

Statistical analysis shows that extract concentrations affected the inhibitory zone against tested fungi. There was no significant difference between 5.00% concentration and control. The concentrations of 1.25%, 2.50%, 10.00% and 20.00% were insignificant difference either. According to Clinical Laboratory Standard Institute (CLSI) (2013), inhibit zone classified as resistant (inhibitory zone diameters ≤14 mm), intermediate (inhibitory zone diameters 15-18 mm) and susceptible (inhibitory zone diameters ≥19 mm). Based on inhibitory these, the inhibitory zone classifications of *S. aureus* were classified as susceptible (at 5.00% concentration) and intermediate (at 2.5% concentration). In the same way, the rest concentration treatment for all bacteria were classified as resistant.

The prior experiments by Khasanah et al. (2014) showed that the higher a concentration, the higher the active components in it, so the inhibitory zones differed for each concentration. However, the increasing concentration sometimes stops effecting antibacterial activity at a particular level. It can be seen that 5.005 was the optimum concentration for *S. aureus*. Antimicrobial substances are capable of inhibiting or killing living microorganisms (Gobel et al. 2008).

Agarwood leaves are beneficial to health. They can be utilized as sources of antimicrobial substances, since the phytochemical screening result shows that they contained flavonoids, tannins, and steroids/triterpenoids. This is in line with Pelczar and Chan (2008) who stated that antimicrobial substances with bacterial growth and metabolism. can control microorganisms, prevent spoilage, and damage by microorganisms.

Phytochemical screening results show that the extract contained flavonoids and tannins. Flavonoids are active as antibacterials by forming complex compounds with extracellular and dissolved proteins that damaging bacterial cell membranes by the release of intracellular compounds. Flavonoids play a role in inhibiting DNA-RNA synthesis by bonding intercalation or hydrogen with the buildup of nucleic acid. Besides, they also hamper the energy metabolism in a similar way to inhibition of the respiratory system because it requires sufficient energy for the active absorption of various metabolites and macromolecules' biosynthesis. Phenol compound is capable of breaking the peptidoglycan bonds when they go through the cell wall. Peptidoglycan is an essential layer for the survival of bacteria in a hypotonic environment. The breakage of layer results in the stiffening of the bacterial cell and subsequently the death of the bacteria (Dewi 2010). The previous study by Kahraman et al. (2019) found that the three flavonoids obtained from ethyl acetate extract of *Ferula caspica* had the highest antimicrobial and antioxidant activity.

Tannins also contribute to antibacterial activity due to their chemical property which is similar to mild acids because of the phenolic OH-groups (Ismarani 2012). Tannic acid is the simplest form of hydrolyzable tannin. Although tannin acid can function as a natural antimicrobial agent, it is not active against a broad spectrum of fungi and bacteria. Higazy et al. (2010) revealed that the burlap fabric treated to form a metal tannic acid-complex showed an increase in antimicrobial activity compared to samples treated with tannic acid only or metal ions only at the same concentration.

The positive control used amoxicillin, a penicillin-derived antibiotic that has a broad spectrum of action, with a mechanism of inhibiting bacterial cell wall synthesis. The use of positive control aimed to see a picture of tested bacteria death seen from the inhibition zone. The negative control treatment was ketoconazole with an average inhibition zone diameter of 0.00 mm (no clear area around the disc).

Antifungal activity

The maximum inhibitory zone of the ethanol extract from agarwood leaves against two fungi species can be seen in Table 3. It shows that the higher concentration of extracted substances, the higher the average inhibition zone diameter. The inhibitory zone diameters against *C. albicans* were higher than against *Trichophyton* sp.

The highest concentration shows the best result in inhibiting the growth of *C. albanicans* and *Trichophyton* sp. The concentration treatments have statistically affected the fungi inhibitory zone. There was no significant difference between the 5.00% and 20.00% statistic test result concentration treatment and effect on inhibitory zone against fungi. Statistical analysis shows that treatments used 5.00% and 20.00% concentrations and between 2.50% and 10.00% concentrations. Based on CLSI classification, the inhibitory zone of *C. albicans* was classified intermediate (at 20.00% concentrations) and the rest concentration was classified as resistant. The inhibitory zone of *Trichopyiton* sp. for all concentrations was classified as resistant.

Indeed, there are about 200 Candida fungi species, but only a few species to be a concern because some are dangerous to humans, e.g., *C. albicans*. The other dangerous species are *C. glabrata, C. parapsilosition, C. tropicalis*, and *C. krusei*. *C. albicans* can attack or infect human blood called candidiasis, even causing death. (Molero et al. 1998).

Based on the results of antimicrobial activity evaluation, ethanol extract from agarwood leaves (*A. malaccensis* Lamk.) showed potential antimicrobial activity against both the test bacteria and the test fungi. For more details, inhibition of zone diameters can be seen in Figure 1. Based on the test, 5% concentration showed the highest antimicrobial activity of *S. aureus*. On the other side, there was no significant difference between control (Amoxicillin), 20%, and 5% concentration for the tested fungi. Therefore, it can be assumed that the 5% concentration already had antimicrobial activity against bacteria and fungi.

The GC-MS analysis

The phytochemical composition of the ethanol extract from agarwood leaves that had antimicrobial potency was identified using Gas Chromatograph-Mass Spectrometry (GC-MS). The fragmentation pattern of each compound obtained from the spectrogram is shown in Figure 2. The structure of each compound was identified based on the pattern of fragmentation and basic peaks. Table 4 shows the nine compounds identified.

The result shows that the compound of ethanol extracts from agarwood leaves contained phenolic group, on the compound of (E)-1,3-Di-m-tolylallyl ethyl Carbonate, 5-(2-Methoxyphenyl)-1,2-dihydropyrazol-3-one, 9-[(tert-Butyl-dimethylsilyl)oxy]benzo[3,4]cyclod ec-3-ene-1,5-diyne and a-(3-chloro-4-hydroxyphenyl)-aphenoxy-a-phenylacetonitrile. According to Gagola (2004), phenolic compounds have antimicrobial activity. Tannins and flavonoids are compounds that contain phenolic groups. The results of the phytochemical test show that the ethanol extract from agarwood leaves contained flavonoids, tannins, and triterpenoids (Surjanto et al. 2019). Tannins and flavonoids are compounds that contain phenolic groups. Tannin and saponin are antiseptic in surface wounds and work as bacteriostatic; while steroids are anti-inflammatory.

Table 3. Inhibitory zone diameters (mm) of the ethanol extract from agarwood leaves against the tested fungi *C. albicans* and *Trichophyton* sp.

Concentrations and controls (%)	Tested fungi	
	C. albicans	***Trichophyton* sp.**
20.00	16.20 ± 1.41	6.53 ± 0.40
10.00	12.16 ± 1.85	6.30 ± 0.40
5.00	13.90 ± 0.87	7.36 ± 0.99
2.50	11.50 ± 0.91	6.86 ± 0.11
1.25	8.30 ± 1.72	6.46 ± 0.15
Positive control (Amoxicillin)	6.70 ± 0.00	6.70 ± 0.00
Negative control (Ketonazole)	0.00 ± 0.00	0.00 ± 0.00

Table 4. Chemical compounds identified of the ethanol extract from agarwood leaves by GC-MS

Retention time	Compound name	Molecular formula	Biological activity
3.235	Disulfide, dioctyl	$C_{16}H_{34}S_2$	Antibacterial (Joondan et al. 2020)
5.858	6,7-Dimethylquinoxaline	$C_{10}H_9FN_2$	Antimicrobial (El-Gaby et al. 2002)
6.652	(E)-1,3-Di-m-tolylallyl ethyl carbonate	$C_{20}H_{22}O_3$	Antioxsidant (Paris et al. 2009)
7.687	5-(2-Methoxyphenyl)-1,2-dihydropyrazol-3-one	$C_{10}H_{10}N_2O_2$	Antimicrobial (Obasi et al. 2016)
8.019	9-[(tert-Butyldimethylsilyl)oxy]benzo[3,4]cyclod ec-3-ene-1,5-diyne	$C_{20}H_{26}Osi$	Antimicrobial (Dunn 2011)
10.106	Neophytadiene	$C_{20}H_{38}$	A substance that kills or slows the growth of microorganisms, including bacteria, viruses, fungi, and protozoans (Palic et al. 2002; Raman et al. 2012; Ceyhan-Güvensen and Keskin 2016; Swamy et al. 2017)
10.402	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	Diarrhea, anemia, anti-inflammatory, hepatitis, and anticancer activity (Babu et al. 2014)
10.697	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Antibacterial and antifungal (Chandrasekaran et al. 2011)
13.376	a-(3-chloro-4-hydroxyphenyl)-aphenoxy-a-phenylacetonitrile	$C_{20}H_{14}ClNO_2$	Thomas et al. 2013; Yusufov et al. 2020

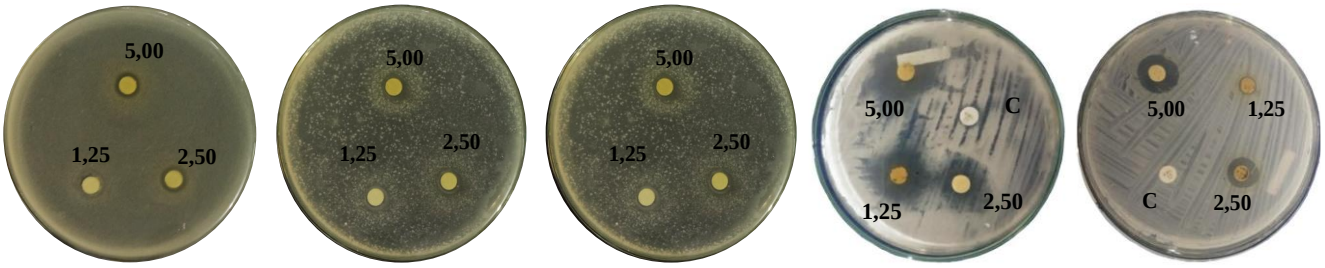

Figure 1. Inhibitory zone of the ethanol extract from agarwood leaves on tested fungi and bacteria (C: Control). A. *Propionibacterium acnes*, B. *Staphylococcus epidermidis*, C. *Trichophyton* sp., D. *Staphylococcus aureus*, E. *Candida albicans*

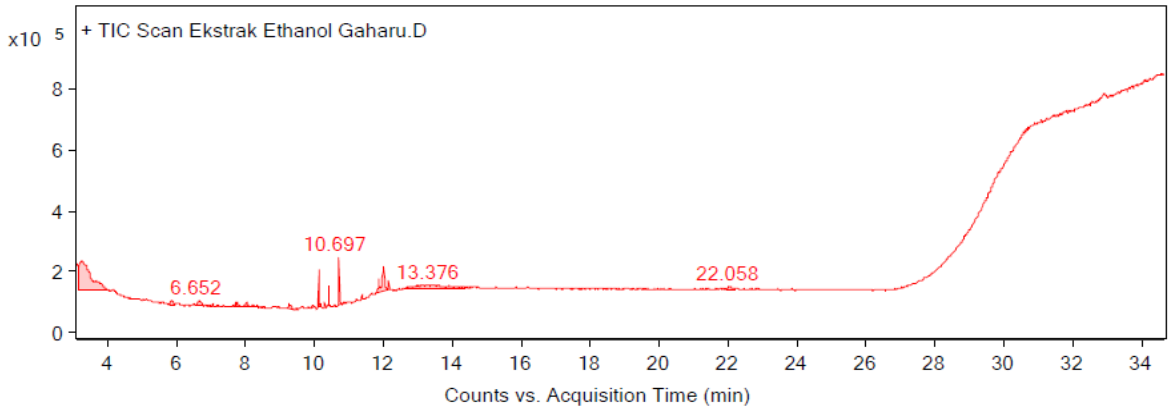

Figure 2. Chromatography of the ethanol extract from agarwood leaves

The biological activity of the chemical compounds contained in the ethanol extract from agarwood leaves can be seen in Table 4, indicating that all compounds identified in the ethanol extract of agarwood leaves have a biological activity based on literature searches. The literature searches show that apart from having antimicrobial properties, the compounds contained in *Aquilaria malaccensis* Lamk leaves have antioxidant and anti-inflammatory properties as well.

The compounds found in the ethanol extract of agarwood (*A. malaccensis*) leaves are potential compounds in the pharmaceutical field such as disulfide, neophytadiene, and hexadecanoic acid, methyl ester. Most of the identified compounds on agarwood leaves had biological activity. References show that besides having antimicrobial properties, the compounds found in the ethanol extract of agarwood leaves are potential in the pharmaceutical field, such as disulfide, neophytadiene, hexadecanoic acid, and methyl ester. it is recommended to study further on the ethanol extract from agarwood leaves as the base material for skin disease medicines.

In conclusion, the ethanol extract from agarwood leaves (*A. malaccensis*) had antibacterial and antifungal activities. The chemical compounds contained in the extract were flavonoids, tannins, and triterpenoids. Identification using GC-MS shows that the ethanol extracts from agarwood leaves had nine biologically active compounds.

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