

**THE ANTIBACTERIAL ACTIVITY OF FRACTIONS OF ETHYL ACETATE *GARCINIA LATISSIMA* MIQ. STEM BARK EXTRACTS AGAINST *BACILLUS SUBTILIS* AND *PSEUDOMONAS AERUGINOSA***NENENG SITI SILFI AMBARWATI<sup>1,2</sup>, AMARILA MALIK<sup>3\*</sup>, ELFIRA AMALIA DEBORAH<sup>3</sup>, CAKRA HAGAI ARPATI SM<sup>3</sup>, MA'ULFI HANIF<sup>3</sup>, BERNA ELYA<sup>2</sup>, MUHAMMAD HANAFI<sup>4</sup>

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**ABSTRACT**

**Objective:** This research focuses on the antibacterial activities of fractions of ethyl acetate *Garcinia latissima* Miq. extracts against *Bacillus subtilis* and *Pseudomonas aeruginosa*.

**Methods:** For each bacteria, the values of the inhibitory zone diameter were determined using paper discs method, while the minimum inhibitory concentration (MIC) was determined by microdilution and antibacterial assay was determined by bioautography. Fractionation was carried out using gradient elution with silica gel column as stationary phase successively increasing the separation according to the polarity (using combination of eluents; n-hexane, ethyl acetate, and methanol).

**Results:** To determine the diameter of the inhibitory zone for each fraction, the fractions were diluted with dimethyl sulfoxide until the concentration of each fraction was 20,000 ppm. The results showed that fraction B had no inhibitory zone against *B. subtilis*. The inhibitory zone diameters of fractions A, C, D, E, F, and G against *B. subtilis* were 7.600±0.000, 6.767±0.202, 7.950±0.477, 7.883±0.901, and 9.233±0.231 mm, respectively. Only Fraction G had an inhibition zone diameter of 7.200±0.173 mm against *P. aeruginosa*. The active fractions were tested by contact bioautography using silica gel (60 GF254) plate and hexane:chloroform (2:3) as mobile phase for Fractions A, C, D, E, and F and chloroform:ethyl acetate (4:1) as mobile phase for Fraction G. The thin layer chromatography plates were observed under the ultraviolet light at wavelengths of 254 and 366 nm, while the others plate (using in contact bioautography) placing on agar medium that had been inoculated with the bacteria, so the compounds could be diffuse to the medium. The active compound will appeared inhibitory zones in agar medium. The determination of the MIC values using microdilution with methylthiazol tetrazolium salt showed that fractions of the ethyl acetate extracts of *G. latissima* Miq. stem bark inhibited the growth of *B. subtilis* more actively than *P. aeruginosa*. The highest activity of the fractions against *B. subtilis* was shown by fraction G, which had a MIC value of 78.125 ppm, followed by fractions F (156.25 ppm), E (312.5 ppm), D (625 ppm), C (1250 ppm), B (2500 ppm), and A (5000 ppm). The highest activity of the fractions against *P. aeruginosa* was Fraction G (5000 ppm), while the MIC values of other fractions were >5000 ppm.

**Conclusion:** Further investigations should be conducted to obtain the new antibacterial compounds in fractions of ethyl acetate extract of *G. latissima* Miq. stem bark that effective to inhibit the growth of *B. subtilis* and *P. aeruginosa*.

**Keywords:** Antibacterial activities, *Garcinia latissima* Miq., Ethyl acetate fractions, Stem bark, *Bacillus subtilis*, *Pseudomonas aeruginosa*.

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**INTRODUCTION**

Medicinal plants contain thousands of active substances that can be used for therapeutic purposes. These substances are a precursor for the synthesis of drugs that are useful and safe for human health [1]. With recent population growth, concerns about health problems have led to the need for the development of natural antimicrobials to control microbial disease. Antimicrobial activity found in plant extracts is associated with some secondary metabolites contained there in *Garcinia latissima* Miq. is a plant belonging to the *Clusiaceae* tribe that can be found on Seram Island in the Maluku Islands and Papua Island [2]. This plant, which has been cultivated in the Bogor Botanical Gardens of West Java, *G. latissima* Miq. and which is better known as *Dolo magota* (Maluku), is commonly used to treat wounds [2]. Ethyl acetate extracts of *G. latissima* Miq. stem bark have been found to contain flavonoids and alkaloids [3]. Specifically, the stem bark from *G. latissima* Miq. which grows in the Central Province of Papua New Guinea, has been found to contain latisxanton-A, latisxanton-B, latisxanton-C, and latisxanton-D compounds belonging to the pyrano xanthone group [4].

The ethyl acetate extract of *G. latissima* Miq. stem bark (2%) in dimethyl sulfoxide (DMSO) has demonstrated antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* [3]. Therefore, it is necessary to investigate the activity of the ethyl acetate extract fractions of *G. latissima* Miq. further. Liquid-solid column chromatography, which requires column packaging with multiple adsorbents such as silica gel, can be used for the stationary phase. The sample will then be eluted using a solvent, and the force of gravity pushes the solvent downward. Adsorption will cease when a balance is reached between the mobile phase and the stationary phase [5]. Motion phase solvents with different polarities, ranging from n-hexane to methanol, are used in low-pressure column chromatography [6]. Thus, fractions will be obtained that increase polarity.

**METHODS**

The ethyl acetate extracts of *G. latissima* Miq. stem bark that were used in this study existed from previous research conducted at the Phytochemical Laboratory of the Faculty of Pharmacy in the University

of Indonesia [3]. For fractionation, 25 g of the extract and silica gel 60 (S) were used in the silent phase. Successive solvents were used (n-hexane, ethyl acetate, and methanol) with the polarity increasing according to gradient [7]. The fractions collected and tested using thin layer chromatography (TLC) for the separation profile. For each fraction that has similar separation chromatogram profile mixed into one fraction.

The bacteria used in this experiment were *B. subtilis* (ATCC 6633) and *P. aeruginosa* (ATCC 27853). These bacteria were taken from a collection at the Microbiology Laboratory of the Faculty of Pharmacy (University of Indonesia). The first antibacterial test was conducted to determine the diameter of the inhibitory zone. Warm antibiotic media that were inoculated by the bacteria test were poured over the agar medium for *B. subtilis* and the cetrinide agar medium for compacted *P. aeruginosa*. This test used fractions with a 20,000 ppm concentration of DMSO. Each fraction was dropped on a paper disc. Once each fraction dried, it was placed on top of the prepared media. Then, the fractions were incubated at 37°C for 24 hrs for *B. subtilis* and at 28°C for 48 hrs for *P. aeruginosa* [8].

Fractions that had inhibitory zones were further tested using contact bioautography. The 20,000-ppm fraction solution (10 µL) was eluted on a TLC plate using a suitable eluent; then, it was dried slowly and visualized under ultraviolet light at wavelengths of 254 nm and 366 nm, and the Rf values were measured [9]. After each fraction was dried, it was attached to the agar layer and left for approximately 1 hr to allow for diffusion of the compound content. Then, the TLC plate was removed, and the Petri dish was incubated at 37°C for 24 hrs for both *Staphylococcus aureus* and *B. subtilis* and at 28°C for 48 hrs for *P. aeruginosa* [10].

Fractions minimum inhibitory concentration (MIC) test was conducted using a microdilution method. Fractions from a DMSO solution of 20,000 ppm were diluted using bacterial medium or broth to obtain the following concentrations: 10,000 ppm, 5000 ppm, 2500 ppm, 1250 ppm, 625 ppm, 312.5 ppm, and 156.25 ppm. Each 50-µL fraction solution was added to 50 µL of inoculum, which contained bacteria at a concentration of 106 colony-forming unit per mL. Tests for each well of the microplate obtained fraction concentrations of 5000, 2500, 1250, 625, 312.5, 156.25, and 78.125 ppm. Each concentration was placed in 3 wells because the test was done in triplicate. The positive control for *B. subtilis* was erythromycin, while the positive control for *P. aeruginosa* and *S. aureus* was gentamycin.

The microplate was then incubated at 37°C for 24 hrs. For *P. aeruginosa*, the incubation was carried out at a temperature of 28°C for 48 hrs. After incubation, 0.6 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to 10-µL sterile aquifers [11] and then incubated for 20 minutes [12]. The observations were conducted by visually checking the presence or absence of discoloration as the presence of bacterial growth is characterized by a change of color from light yellow to purple or pink. The lowest concentration of non-discolored wells is the MIC [13].

## RESULTS AND DISCUSSION

Fractionation in column chromatography used 100% n-hexane solvent, which was then added to the ethyl acetate by reducing the n-hexane so that the polarity increased by a gradient to 100% ethyl acetate. Subsequently, methanol was added, and the ethyl acetate was reduced until the solution reached 100% methanol. The chromatographic results of this column were stored in a 100 mL bottle. Each fraction in the bottle was tested with TLC, and the fraction which has similar chromatogram pattern was combined. From the fractionation results, Fraction G had the highest percentage (60.8268%). Fraction G was also the most polarized, followed by Fractions C (9.9968%), D (8.8129%), A (7.5140%), F (7.1154%), E (3.9652%), and B (1.7659%) (Table 1).

The test to determine the diameter of the inhibitory zone was done triplicate with a fraction concentration at 20,000 ppm. Based on the

results of this test, which used the diffusion method on paper discs, the fractions that could indicate the diameter of the inhibitory zone toward *B. subtilis* were G, F, D, E, A, and C, from the bigger diameter of the fraction to smaller diameter consecutively (Table 2). In contrast, the only fraction that could provide the diameter of the inhibitory zone toward *P. aeruginosa* was fraction G.

With the results of the above test to determine whether there was a compound contained in the fraction that could indicate the diameter of the inhibitory zone, a bioautographic test was performed using a 20,000-ppm concentration of test solution (10 µL) (Figs. 1 and 2).

The next antibacterial test was the MIC test, which uses an MTT indicator—to detect microbial growth. This dehydrogenase reagent indicator (tetrazolium salt) works based on the reduction reaction of yellow tetrazolium salt to purple formazan crystals by the action of dehydrogenase enzymes on the mitochondria of the cell (Fig. 3) [14].

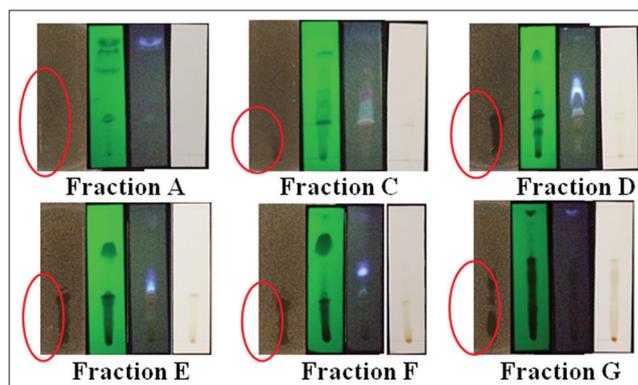


Fig. 1: Bioautography results of active fraction of ethyl acetate extracts of *Garcinia latissima* Miq. stem bark against *B. subtilis* using n-hexane:chloroform (2:3) as eluent for Fractions A, B, C, D, E, and F. For the Fraction G, the chloroform:ethyl acetate (4:1) was used as eluent

Table 1: Fractionation of *G. latissima* Miq. ethyl acetate extracts

Fraction	Bottle number	Fraction weight (g)	Fraction (%)
A	1-4	0.7578	7.5140
B	5-9	0.1784	1.7689
C	10-12	1.0082	9.9968
D	13-15	0.8888	8.8129
E	22-33	0.3999	3.9652
F	34-60	0.7176	7.1154
G	61-223	6.1345	60.8268

Total fraction weight 10.0852. *G. latissima*: *Garcinia latissima*

Table 2: The inhibitory zone diameters results of the ethyl acetate fractions of *G. latissima* against *B. subtilis* and *P. aeruginosa*

Fraction	Diameter of the inhibitory zone (mm) against bacteria	
	<i>B. subtilis</i>	<i>P. aeruginosa</i>
A	7.600±0.000	0
B	0	0
C	6.767±0.202	0
D	7.950±0.477	0
E	7.883±0.901	0
F	9.233±0.231	0
G	9.300±0.173	7.200±0.173
Standard antibiotics	23.58±0.988	17.92±0.549

*G. latissima*: *Garcinia latissima*, *B. subtilis*: *Bacillus subtilis*,  
*P. aeruginosa*: *Pseudomonas aeruginosa*

The succinic dehydrogenase produced by the mitochondria of living cells and contained within these microbes can actively convert the tetrazolium (MTT) salt into purple formazan [10].

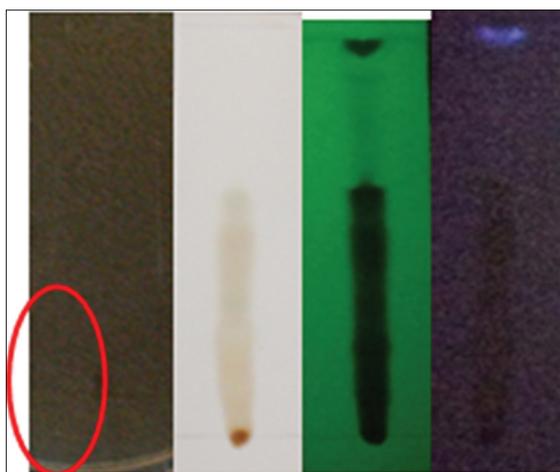
From the MIC test results (Table 3), the MIC value obtained from Fraction G was 78.13 ppm, so Fraction G has strong antibacterial activity against *B. subtilis*. Fractions E and F have MIC values of 312.5 and 156.25 ppm, respectively, and medium antibacterial activity toward *B. subtilis*. Fraction D has weak antibacterial activity toward *B. subtilis* (625 ppm). This is consistent with reference to the MIC values of <100 ppm, 100-500 ppm, 500-1000 ppm, and >1000 ppm indicate strong, moderate, weak, and inactive/absent antimicrobial activity, respectively [15]. The antimicrobial activity of these test fractions was compared with standard antibiotics: Erythromycin for *B. subtilis* and gentamicin for *P. aeruginosa*.

The seven fractions tested showed different inhibitory effects. The results showed that the most susceptible bacteria to Fraction G were

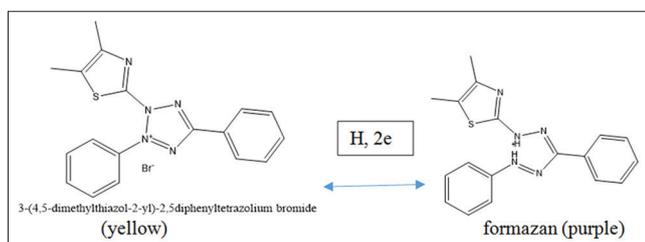
**Table 3: Results of the MIC of the ethyl acetate fraction of *G. latissima* Miq. stem bark (ppm)**

Fractions	<i>B. subtilis</i>	<i>P. aeruginosa</i>
A	5000	>5000
B	2500	>5000
C	1250	>5000
D	625	>5000
E	312.5	>5000
F	156.25	>5000
G	78.13	5000
Standard antibiotics	25	0.39

MIC: Minimum inhibitory concentration, *G. latissima*: *Garcinia latissima*, *B. subtilis*: *Bacillus subtilis*, *P. aeruginosa*: *Pseudomonas aeruginosa*



**Fig. 2: Bioautography results of the active fraction of ethyl acetate extract of *Garcinia latissima* Miq. stem bark against *Pseudomonas aeruginosa* using chloroform:ethyl acetate (4:1) as eluent**



**Fig. 3: The reaction of tetrazolium salt reduction by enzyme succinic dehydrogenase**

*B. subtilis*. Gram-positive bacteria (such as *B. subtilis*) are more susceptible to active fractions than Gram-negative bacteria (such as *P. aeruginosa*). This has been demonstrated by the susceptibility of microbes to various fractions reported in literature that Gram-positive bacteria more susceptibility than Gram-negative bacteria [15]. One explanation is that the cell walls of Gram-positive bacteria are less complex than Gram-negative bacteria and do not have selectivity for large molecules [16]. The walls of these cells are composed of a thick layer of mucopeptides (peptidoglycan) (40-50% of the bacterial body weight), while the cell walls of Gram-negative bacteria contain layers of fat and peptidoglycan (20% and 5-20% of the body weight, respectively) [17].

## CONCLUSION

From the fractionation of ethyl acetate extract of *G. latissima* Miq. stem bark, Fraction G has the largest percentage of total weight fraction (60.8268%). Fraction G also has the greatest diameter of inhibitory zone compare to the other fractions against *B. subtilis* and *P. aeruginosa* using paper disc method. From the results of bioautography test, the active fractions showed the inhibitory zone on the agar medium. The MIC test results showed that Fraction G has the strongest antibacterial activity against *B. subtilis* and *P. aeruginosa*. Therefore, further research is needed to isolate and obtain the new active compound from the active fraction of ethyl acetate extract of *G. latissima* Miq. stem bark.

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## REFERENCES

- Li JJ. Top Drugs-History, Pharmacology, and Synthesis. New York: Oxford University Press; 2014.
- Eisai P. Medicinal Herb Index in Indonesia. 2<sup>nd</sup> ed. Jakarta: Eisai Indonesia; 1995.
- Ambarwati NS, Elya B, Malik A, Hanafi M. Evaluation of antimicrobial activities of *Garcinia latissima* Miq. Stem bark extract. J Young Pharm 2017;9(1):118-21.
- Ito C, Miyamoto Y, Nakayama M, Kawai Y, Rao KS, Furukawa H. A novel depsidone and some new xanthenes from *Garcinia* species. Chem Pharm Bull 1997;45(9):133-40.
- Ahuja S. Separation science and technology. Chromatography and Separation Science. California: Elsevier Science; 2003.
- Reid RG, Sarker SD. Isolation of natural products by low-pressure column chromatography. In: Sarker SD, Nahar L, editors. Natural Product Isolation Methods and Protocols. New York: Humana Press; 2012.
- Martins S, Amorim EL, Sobrinho TJ, Saraiva AM, Pisciotano MN, Aguilar CN, et al. Antibacterial activity of crude methanolic extract and fractions obtained from *Larrea tridentata* leaves. Ind Crops Prod 2013;41:306-11.
- Kochuthressia KP, Britto SJ, Jaseentha MO, Raphael R. *In vitro* antimicrobial evaluation of *Kaempferia galanga* L. Rhizome extract. Am J Biotechnol Mol Sci 2012;2(1):1-5.
- Sarker SD, Latif Z, Gray AI. Natural Products Isolation. 2<sup>nd</sup> ed. New Jersey: Humana Press; 2006.
- Dewanjee S, Gangopadhyay M, Bhattacharya N, Khanra R, Dua TK. Bioautography and its scope in the field of natural product chemistry. J Pharm Anal 2015;5(2):75-84.
- Bilola Messi B, Ho R, Meli Lannang A, Cressend D, Perron K, Nkengfack AE, et al. Isolation and biological activity of compounds from *Garcinia preussii*. Pharm Biol 2014;52(6):706-11.
- Grare M, Fontanay S, Cornil C, Finance C, Duval RE. Tetrazolium salts for MIC determination in microplates: Why? Which salt to select? How? J Microbiol Methods 2008;75(1):156-9.
- Zubair M, Rizwan K, Rasool N, Ahmed V. Antimicrobial potential of various extract and fractions of leaves of *Solanum nigrum*. Int J Phytomed 2011;3(1):63-7.
- Suwantong O, Pankongadisak P, Deachathai S, Supaphol P. The potential of electrospun poly(L-lactic acid) fiber mats containing a crude *Garcinia dulcis* extract for use as wound dressings. Chiang Mai J Sci 2013;40(3):517-33.

15. Fernandes MR, Dias AL, Carvalho RR, Souza CR, Oliveira WP. Antioxidant and antimicrobial activities of *Psidium guajava* L. Spray dried extracts. Ind Crops Prod 2014;60:39-44.
16. Ohadoma SC, Natuanya IV, Amazu LU, Okolo CE. Antimicrobial activity of the leaf extract and fractions of *Lupinus arboreus*. J Med Plant Res 2014;8(8):386-91.
17. Ibrahim A, Ahmad I, Narsa AC, Sastyarina Y. Effect of antimicrobial preparation of skin ointment based on ethyl acetate sungkai leaf extract (*Peronema canencens* Jack.) Against bacterial infectious pathogen burn. J Trop Pharm Chem 2013;2(3):125-31.