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Research Article

Antibacterial and Antioxidant Activities of Fractions from *Garcinia latissima* miq. Stem Bark Methanol Extract

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ABSTRACT

Objective: This study aimed to get complete information about the antioxidant and antibacterial against *B. subtilis* and *Staphylococcus aureus* activities of the fractions from *G. latissima* Miq. stem bark methanol extract. Methods: Fractionation was performed by column chromatography. The antibacterial activities of the fractions from *Garcinia latissima* Miq. stem bark were assayed by inhibition zone technique, bioautography, and minimum inhibition concentration. The antioxidant activity were evaluated using DPPH (2,2-diphenyl-1picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) methods. Results: Stem bark methanol extract had higher antioxidant activity (% inhibition = 95.68%) than ethyl acetate extract and n-hexane stem bark extract of *G. latissima* Miq. The stem bark methanol extract obtained ten fractions. The greatest of inhibition zone diameter against *B. subtilis* (ATCC (the American Type Culture Collection) 6633) and *S. aureus* (ATCC 25923) was fraction G (7.83 ± 0.46 mm and 7.43 ± 0.15 mm). The highest antioxidant activity fraction by DPPH method and FRAP method was fraction G. Phytochemical screening showed that fraction G contained flavonoid and tannin. Conclusion: The results obtained reveal that the fraction G of stem bark of *G. latissima* Miq. methanol extract possessed antibacterial activity against *B. subtilis* and *S. aureus* and antioxidant activity.

Keywords: *Garcinia latissima* Miq. stem bark. methanol fractions. *Bacillus subtilis*. *Staphylococcus aureus.* DPPH & FRAP.

INTRODUCTION

Previous research had been conducted on *G. latissima* Miq. stem bark methanol extract that the extract (20,000 ppm) was active against *B. subtilis* bacterium with inhibition zone diameter was 10.70±0.638 mm, and the inhibition zone diameter against *S. aureus* was 10.38±0.653 mm[1]. The MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of the extract against *B. subtilis* were 625 and 5,000 ppm[1]. The MIC and MBC values of the extract against *S. aureus* were 2,500 and 5,000 ppm[1]. This research had been continued with fractionation and tested the fractions against *B. subtilis* and *S. aureus* bacteria.

Free radicals act as toxic compounds because they can be harmful to the body. Free radicals formed in the body can cause damage to nucleic acids, proteins, and lipids in cell membranes and plasma lipoproteins[2]. Free radicals can be produced from normal cell metabolism as well as from outside the body. If the excess free radicals cannot be destroyed gradually, then the accumulation of free radicals in the body produces a phenomenon called oxidative stress. This process plays a role in the development of chronic and degenerative diseases. The human body has several mechanisms to overcome oxidative stress by producing antioxidants, which are produced naturally in the body, or externally administered through food or supplements[3]. Research on natural antioxidants in food has become popular these days, and the choice and availability of natural antioxidants are still limited[4]. Therefore, an antioxidant test was performed on the extract and fractions of *G. latissima* Miq. stem bark.

This study aimed to get completed information about the antibacterial against *B. subtilis* and *Staphylococccus aureus* and antioxidant activities of the fractions from *G. latissima* Miq. stem bark methanol extract.

MATERIALS AND METHODS

Antioxidant Activity Test for Extract

One mL of 100 µg/mL extract solution was added 1.0 mL 100 µg/mL DPPH solution and 2.0 mL methanol p.a. then

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Table 1: The inhibition zone diameter of fractions against *B. subtilis* and *S. aureus.*

Fractions	Inhibition Zone Diameter (mm) against bacteria	
	B. subtilis	*S. aureus*
A	0	7.10 ± 0.00
B	0	6.77 ± 0.21
C	7.07 ± 0.74	7.35 ± 0.14
D	0	0
E	0	0
F	0	0
G	7.83 ± 0.46	7.43 ± 0.15
H	6.97 ± 0.59	0
I	6.60 ± 0.95	0
J	0	0
Positive Control (antibiotic)	29.18 ± 0.98	24.86 ± 0.02

Table 2: The minimum inhibition concentration of fractions against *B. subtilis* and *S. aureus.*

Fractions	Minimum inhibition concentration (ppm) against bacteria	
	B. subtilis	*S. aureus*
A	5.000	5.000
B	>5.000	>5.000
C	2.500	2.500
D	2.500	5.000
E	5.000	5.000
F	5.000	5.000
G	2.500	1.250
H	2.500	>5.000
I	5.000	5.000
J	5.000	>5.000

Table 3: Percentage of inhibition from fractions to DPPH.

Fraction	% inhibition
A	8.47
B	3.47
C	92.52
D	92.77
E	31.29
F	27.27
G	93.39
H	93.02
I	72.50
J	12.89

was vortexed for 20 seconds and incubated at 37°C for 30 minutes in dark place. Absorbance was measured using a UV-Vis (Ultraviolet-visible) spectrophotometer at the optimum wavelength. The test was done triplicately[15].

Fractionation of The Most Active from Garcinia latissima Miq. Stem Bark Extract

The fractionation was performed by using column chromatography. The stationary phase used was silica gel, and the mobile phase began with n-hexane: ethyl acetate (1: 1), which was then increased by its polarity. The falling fractions are accommodated using 100 mL glass bottles.

Inhibition Zone Test for Fractions

One mL of bacterial suspension with 106 microbes/mL was fed into a tube containing 4 mL of antibiotic media that has been liquefied by heating at 45-60°C. After being shaken until homogenized using vortex poured into a petri dish that already contains nutrient media to 20 mL that had been poured before and had been solidified. Then flattened by wagging petri dishes slowly, this top layer was a layer of seed. Then allowed to freeze.

A sterile 6 mm diameter paper disc was prepared and placed on a petri dish. Each paper disc was dropped with 20 µL partial solution in DMSO (Dimethyl sulfoxide) (20,000 ppm). Each fraction was dropped on three paper (triplicate). The paper discs that had been depleted with a fractional solution and were already dried, were placed on agar medium and seed layers. One petri dish was mounted 6 paper discs over the surface so that the distance between the discs between 20-35 mm. The center of the petri dish was placed positive control or negative control. For positive control used standard antibiotic discs. Negative controls used 20 µL DMSO bottled discs. Then incubation of petri dish at 37oC for 24 hours. After that, it was observed by measuring the inhibition zone diameter using the calipers[16].

Antioxidant Activity Test Fractions with DPPH method[15,17]

One mL of 100 µg/mL fraction solution in methanol was added 1.0 mL DPPH 100 µg / mL and 2.0 mL methanol, then was vortexed for 20 s. Then was incubated 37oC for 30 minutes in dark place. The absorbance was measured by a UV-Vis spectrophotometer at the optimum wavelength to obtain a percentage of inhibition. The test was done triplicately. The percentage of inhibition from DPPH radical was obtained according to the following formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100\%$$

The fraction with the highest percentage of inhibition was the most active fraction.

IC50 (the concentration at which the curve passes through the 50% inhibition level) Test and Calculation of the Most Active Fractions

The most active fraction solution (100 µg/mL) was diluted to obtain a fraction series concentration of 2.5; 5; 10; 15; 20 and 25 µg / mL. The respective concentration fractions were added 1.0 mL DPPH 100 µg / mL and 2.0 mL methanol, then was vortexed for 20 s. Then incubated 37oC for 30 minutes in the dark place. The absorbance was measured by a UV-Vis spectrophotometer at the optimum wavelength to obtain a percentage of inhibition.

After obtaining the inhibition percentage of each concentration (x), then used the equation $y = a + bx$ with y is the percentage of inhibition (%).

Inhibition Concentration 50% (IC50) was the concentration of test substance required to capture 50% DPPH radical for 30 minutes (operating time) or the time interval required by the test material to ultimately reduce

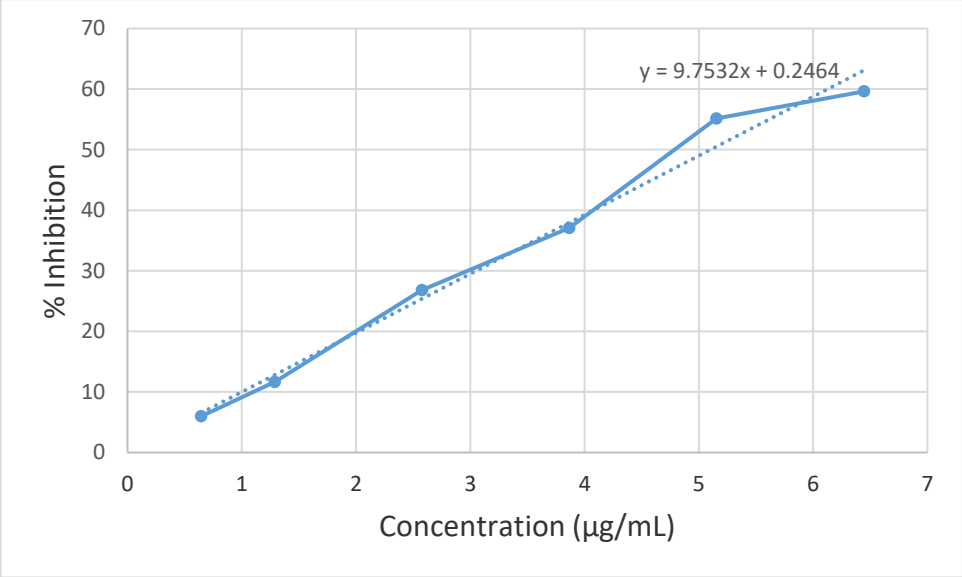

Figure 1: The relationship between concentration and percentage inhibition of fraction G (r = 0.992).

Table 4: Table of Antioxidant Activity Data of Fractions and Equivalence with Ammonium Ferro Sulfate Using FRAP Method.

Fraction	FeEAC fraction (µmol / g)
A	55.35
B	60.24
C	674.87
D	191.25
E	183.25
F	125.54
G	1,189.65
H	507.83
I	182.20
J	141.80

the DPPH radical. After 30 minutes there would be a constant absorbance. The value of IC50 was obtained from the value of x after substituting y = 50.

Antioxidant Activity Test with FRAP method

FRAP test was performed using a microplate reader with normalized results[10]. Normalization of the results was done so that the results were by the data obtained when using the UV-Vis spectrophotometer with pathlength 1 cm. Normalization of FRAP microplate data for 1 cm pathlength was done by a separate FRAP test, using a UV-Vis spectrophotometer. Ammonium ferrous sulfate (AFS) was used as the standard for calibration and absorption measured at 593 nm using a UV-Vis spectrophotometer with a 1 cm pathlength.

A solution of each fraction of 20 µL (1000 µg / mL) was added 280 µL of FRAP solution.

The FRAP solution was prepared by mixing 2.5 mL of FeCl3.6H2O solution with 2.5 ml of 10 mM TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) solution and 25 mL of a 300 mM buffer pH (potential of hydrogen) solution of 3.6 ppm, and CO2 (carbon dioxide)-free aquadest was added to 100.0 mL.

Then it was incubated at 37°C for 30 minutes in darkness and measured its absorbance at 593 nm. The test was done triplicately.

Calculate the following Equivalent Antioxidant activity Ferric ions[12]:

$$\underset{(\mu mol/gram)}{FeEAC} = \frac{\Delta A}{GRAD(AFS)} \times \frac{Av}{SpV} \times D \times \frac{1}{C_{sam}} \times 105$$

Where, FeEAC was Ferric ion equivalent, ΔA was the Net absorption of the sample that has been reduced by a correction factor, GRAD (AFS) was calibration gradient curve, Spv was the volume of sample, Av was total test volume, D was dilution, and Csample was sample concentration.

Phytochemical Screening of The Most Active Fraction as Antioxidant[18]

The test was performed for the identification of flavonoids ($AlCl_3$), saponins (hot water and HCl), tannins (lead acetate)[19].

RESULTS AND DISCUSSION

Antioxidant Activity Test for Extract

The antioxidant activity test was performed by two methods, the first performed with DPPH damping or inhibition method and the second using FRAP method. The DPPH method was used because it was easier, simpler, cheaper, accessible, its use was extensive and accurate for measuring free radical fishing activity[15].

DPPH radical is an organic compound that contains an unpaired electron and has a strong absorption maximum at a wavelength of about 515 nm-519 nm. Radical DPPH (2,2-diphenyl-1-picrylhydrazyl) were colored purple in the presence of unpaired electrons was reduced and the presence of antioxidants or reducing compounds (hydrogen donor), electrons were paired so that a 1,1-diphenyl-2-picrylhidrazyl, which were non-radical and yellow. The color change from purple to yellow was measured by a spectrophotometer[5]. DPPH and antioxidant radical reactions occurred where antioxidants (AH) were

molecular donors and A was a free radical product. Optimum wavelength of DPPH is obtained at 516 nm wavelength.

While the FRAP method is a cheap method, using a simple reagent, the results are highly reproducible, as well as the procedures are easy and fast. The FRAP method is incorporated into the colorimetric method based on the antioxidant ability to reduce Fe^{3+} ions to Fe^{2+} [6].

Acquired % inhibition of methanol extract at concentration of 100 µg/mL was 95.68%, higher than % inhibition of stem bark ethyl acetate extract (93.77%) and *G. latissima* Miq. stem bark n-hexane extract (25.36%), indicating that the methanol extract had the highest antioxidant activity compared to the other two extracts.

Fractionation of G. latissima Miq. Methanol Extract

From the fractionation using column chromatography, there were ten fractions (fractions A, B, C, D, E, F, G, H, I, and J). The highest percentage of the weight was the fraction H (53.87%).

Inhibition Zone Test for Fractions

The result of the inhibition zone diameter test from fractions of *G. latissima* Miq. stem bark methanol extract was showed on Table 1.

From the table above showed that the largest fraction inhibition zone diameter was fraction G against *B. subtilis* (7.83 ± 0.46 mm) and *S. aureus* (7.43 ± 0.15 mm). Fraction D, E, F and J did not give inhibition zone diameter against *B. subtilis* and *S. aureus.* Fraction A and B did not give inhibition zone diameter against *B. subtilis.* Fraction H and I did not give inhibition zone diameter against *S. aureus.*

The antibiotic as positive control were erythromycin 15 µg for *B. subtilis* and gentamycin 10 µg for *S. aureus.*

The results of the minimum inhibition concentration of fraction against *B. subtilis* and *S. aureus* was showed on Table 2. The lowest of minimum inhibition concentration against *B. subtilis* was 2500 ppm (fraction C, D, G, and H) and against *S. aureus* was 1250 ppm (fraction G).

Antioxidant Activity Test of Fractions with DPPH Method

Antioxidant activity test of fractions at concentration of 100 µg mL up took at a wavelength of 516 nm. Data of antioxidant activity test of fractions can be seen in Table 3. From the table showed that the fraction of G was the most active fraction which has the highest % inhibition (93,39%). So that further testing on fraction G by making some series of concentration to get the value of IC50.

The relationship between concentration and percentage inhibition of fraction G was showed in Figure 1. From six points of fraction G concentration was obtained linear equation y=9.7532x+0.2464 (r=0.992) with x was concentration and y was % inhibition. The antioxidant activity test of fraction G obtained that IC50 value of 5.10 µg/mL. It can also be defined that an antiradical concentration of 5.10 µg/mL was required in the fraction G to decrease the initial concentration of DPPH by 50%[7].

Antioxidant grouping by Blois states that samples with IC50 or EC50 values below 50 µg/mL were very potent antioxidants, 50-100 µg/mL was a potent antioxidant, 101-150 µg/mL was a moderate antioxidant, and more than 150 µg/mL was a weak antioxidant [8]. Based on these clusters, the G fraction can be said to have very potent antioxidant activity, and its antioxidant strength was still one group with the antioxidant power of ascorbic acid. IC50 ascorbic acid and IC50 were tested and the IC50 ascorbic acid value was 3.02 µg / mL. The high activity of this antioxidant was related to the content of phenolic compounds contained in the sample[9].

Antioxidant Activity Test of Fractions with FRAP Method

The FRAP method used a microplate reader that was affected by sample volume and composition. To eliminate that effect requires a calibration process[10]. Because in this study using Versamax microplate reader that did not have pathlength (correction factor), it needs to be normalized so that the data obtained was proportional to the test data using Uv-Vis Kuvet spectrophotometer.

The FRAP method was incorporated into the method of colorimetry based on the ability of antioxidants to reduce Fe^{3+} ions to Fe^{2+} in the presence of 2,4,6-tripyridyl-s-triazine (TPTZ) to form intensive blue Fe^{2+} TPTZ complexes at low pH as measured in absorbance Maximum of 593 nm[11]. The reduction reaction is indicated by a solution of Fe^{3+} ions and an extract which was initially clear yellowish color when mixed with FRAP solution, the mixture of the solution becomes blue, and after incubation, the blue color becomes intensified. The more intense the blue color that is formed, the higher the absorbance was measured. The use of acidic pH (3.6) in this test was to maintain the stability of TPTZ reagents, as well as irradiated iron in low water at neutral pH. The pH of the acid is also essential to facilitate the speed of the reduction of Fe^{3+} ions[12].

Ammonium ferrous sulfate was one of the reducing agents used as a standard on the antioxidant activity test by the FRAP method. In horizontal photometry such as a spectrophotometer, the distance traveled through the absorbent material was determined by the physical dimension of the cuvette, which was 1 cm. In vertical photometry, as in the microplate reader, the distance traveled through the absorbing material depends on the volume in the well, so adjustment and accuracy using a micropipette were necessary[13].

Ammonium ferric sulfate was measured by absorbance using microplate reader method and spectrophotometry Uv-Vis kuvet method, from the test will get the gradient from linear regression equation of each method, so that can be determined path-length correction or correction factor which will be used to normalize the test result data with microplate Reader[10]. The result of the test using micro-plate reader method got the gradient value which was known from the linear regression that was 7,912.9 and spectrophotometry method of UV-Vis kuvet got gradient value 10,884, so it got path-length correction 0.73 cm (7912,9 / 10884).

From the test results obtained FeEAC value of the fractions as in Table 4.

The G fraction is the most active fraction having the highest FeEAC value of 1189 µmol FeE/g fraction.

Phytochemical Screening of The Most Active Fraction as Antioxidant

Flavonoids test showed a positive result, where the fraction G after sprayed $AlCl_3$ changed color to yellow under UV

at 366 nm. Flavonoid compounds had a strong ability to capture free radicals[14]. Flavonoids can protect unsaturated fatty acids on phospholipid membranes by donating hydrogen atoms to absorb free radicals. The hydroxyl groups present in the A and or the B ring of flavonoids can increase the antioxidant capacity of the flavonoids, especially the hydroxyl groups in ortho positions in C3 'and C4'[8]. Also, the fraction G also showed the positive result of having tannin compound characterized by the formation of sediment in a solution of fraction G which was given Pb acetate solution, and the formation of white precipitate in a solution of G fraction which was given 10% gelatin solution.

CONCLUSION

The results obtained reveal that the fraction G of stem bark of *G. latissima* Miq. methanol extract possessed the highest antibacterial activity against *B. subtilis* and *S. aureus* and highest antioxidant activity. The fraction G contained flavonoids and tannins.

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AVAILABILITY OF DATA AND MATERIALS

Not applicable since all the data supporting the results reported are in this manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

CONSENT FOR PUBLICATION

Not applicable since our manuscript does not contain any individual person's data in any form.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This information is not relevant since our study does involve neither animals nor human.

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