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Original Article

Antioxidant Activity and Lipoxygenase Enzyme Inhibition Assay with Total Flavonoid Content from Garcinia hombroniana Pierre Leaves

Shinta Marlin, Berna Elya*, Katrin

ABSTRACT

Objective: *Garcinia hombroniana* Pierre leaves extract have been known to contain flavonoid, but it has not been known yet for its antioxidant activity and inhibition of lipoxygenase activity. This study aims to determine antioxidant activity and inhibition of lipoxygenase activity of *G. hombroniana* leaves extract. **Method:** Antioxidant activity tested by using FRAP (Ferric Reducing Antioxidant Power) method and inhibition of lipoxygenase activity using baicalein as the positive control. Total flavonoid assay is also quantitatively done by $AlCl_3$ colorimetric method on the most active extract using quercetin as the positive control. **Results:** The test result showed that the n-hexane, ethyl acetate and methanol extract of *G. hombroniana* Pierre leaves have antioxidant activity which showed by EC_{50} value consecutively are 36.260; 2.969; and 7.416 µg/mL, and also can inhibit lipoxygenase activity which showed by IC_{50} value consecutively are 2.052; 0.134; and 1.314 µg/mL. Ethyl acetate extract of *G. hombroniana* Pierre leaves has the most active antioxidant activity and inhibition of lipoxygenase activity. Total flavonoid content of ethyl acetate extract of *G. hombroniana* Pierre leaves is 42.004 mg QE/g sample. **Conclusion:** Garcinia hombroniana Pierre leaves extract has antioxidant activity and can inhibit lipoxygenase activity.

Key words: Antioxidant, Antiinflammation, Flavonoid, *Garcinia hombroniana* Pierre, FRAP, lipoxygenase.

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INTRODUCTION

Plants contain various chemical compounds such as phenolic, flavonoid, carotenoid, steroid, and thiol compound that may help protect cells from oxidative stress damage and reduce the risk of chronic disease. Flavonoid such as catechin and epicatechin can be used for the activity of antioxidant and anti-inflammatory.[1]

Garcinia hombroniana Pierre are in Indonesia, one of them in the Bogor Botanical Garden, but has not been much research on this plant. People familiar with *G. hombroniana* Pierre as mangosteen family of plants that are ornamental plants, food crops, and as a traditional medicine for itchy.[2] Previous research has been conducted on the bark of *G. hombroniana* Pierre which showed that the ethyl acetate extract had the highest content of flavonoid and antioxidant activity EC_{50} values obtained by FRAP method is 5579.8 ± 117.7 mol TE *(Trolox Equivalent)*/g.[2]

Garcinia hombroniana Pierre leaves extract have been known to contain flavonoid,[3] but it has not been known yet for its antioxidant activity and inhibition of lipoxygenase activity. This study aims to determine antioxidant activity tested by using FRAP (*Ferric Reducing Antioxidant Power*) method and inhibition of lipoxygenase activity of *G. hombroniana* leaves extract. This research is expected to provide information on the strength of antioxidant activity and inhibition of lipoxygenase activity in the most active extract of n-hexane, ethyl acetate, and methanol leaves of *G. hombroniana* Pierre as a source of antioxidant and anti-inflammatory.

MATERIALS AND METHODS

Antioxidant Activity Assay

For the antioxidant activity assay, 3.8 mL FRAP reagent solution reacted with 0.2 ml baicalein solution or sample, then incubated for 30 minutes at 37°C. The solution absorbance was measured at 595 nm. For sample or standard control, 3.8 mL FRAP reagent solution reacted with 0.2 ml ethanol pro analysis, then treated the same as well as the sample or standard.

The percentage of capacity can be calculated using this equation:

% Capacity = (1 - Ts) x 100%

Ts = Transmittan

As = - log Ts

As = Absorbance of standard or sample solution – Absorbance of reference solution

EC_{50} value gained by using Microsoft Office Excel and GraphPad Prism 7.

Lipoxygenase Enzyme Inhibition Assay

For the lipoxygenase enzyme inhibition assay, first to be optimized pH borate buffer solution, stop solution, enzyme concentration, and substrate concentration. Test of inhibition of lipoxygenase activity carried out by reacting 10 mL of baicalein or sample solution (various concentration) with 1690 mL of 0.2

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M borate buffer pH 8.5 and 1000 mL of linoleic acid solution (900 µM). The mixture solution was then incubated for 10 minutes at 25°C. 300 mL of lipoxygenase solution (5000 units/mL) was added and incubated another 15 minutes at 25°C. 1000 mL of stop solution is added and measured its absorbance using UV-Vis spectrophotometer at 235 nm.

Inhibition of the activity of lipoxygenase can be known from the value of the inhibition percentage that was calculated using the following equation:

$$\%\ \text{lipoxygenase inhibition} = \frac{(A-B)-(C-D)}{(A-B)} \times 100\ \%$$

A = Absorbance of reference solution with enzyme

B = Absorbance of reference solution without enzyme

C = Absorbance of standard or sample solution with enzyme

D = Absorbance of standard or sample solution without enzyme

IC_{50} value gained by using Microsoft Office Excel and GraphPad Prism 7.

Thin Layer Chromatography (TLC) Analysis

Analysis by thin layer chromatography (TLC) was conducted to determine the leaves extract of *Garcinia hombroniana* that have the highest content of flavonoid qualitatively. Eluent used after optimization of the mobile phase is ethyl acetate-formic acid (40:1) for methanol extract, toluene-ethyl acetate-formic acid (61:30:9) for ethyl acetate extract and n-hexane-ethyl acetate (6:4) for n-hexane extract. The plate of silica gel 60 F_{245} is used as the stationary phase.

In this TLC analysis, quercetin is used as a standard solution that is treated similarly to the extract. The distance covered by the standard solution (Rf standard) is then compared to the distance traveled by each extract (Rf sample).

Determination of the total flavonoid content (TFC)

For total flavonoid content, 0.5 mL standard or sample solution was reacted with 1.5 mL methanol pro analysis; 0.1 mL $AlCl_3$ 10%; 0.1 mL 1M sodium acetate solution; and 2.8 mL distilled water. The mixture solution was incubated for 30 min at room temperature. Absorption was measured by using UV-VIS spectrophotometer at 435 nm. The calibration curve of quercetin was required to obtain the linear regression equation so that the level of flavonoid in the sample can be calculated.

RESULTS

Antioxidant Activity Assay

Test of antioxidant activity using baicalein as the positive control. The test result obtained EC_{50} value of baicalein is 1.165 µg/mL (Table 1). The test result of extract n-hexane, ethyl acetate and methanol leaves of *G. hombroniana* obtained EC50 values consecutively are 36.260; 2.969; and 7.416 µg/mL (Table 2).

Lipoxygenase Enzyme Inhibition Assay

Lipoxygenase inhibition test is done by using baicalein as a positive control. The test result obtained IC_{50} value of baicalein is 0.250 µg/mL (Table 3). The test result of extract n-hexane, ethyl acetate and methanol leaves of *G. hombroniana* obtained IC50 values consecutively are 2.052; 0.134; and 1.314 µg/mL (Table 4).

Thin Layer Chromatography (TLC) Analysis

Analysis with TLC (Thin Layer Chromatography) was conducted to determine the presence of flavonoid in the extract n-hexane, ethyl acetate and methanol leaves of *G. hombroniana* qualitatively. The TLC result showed that there aren't any flavonoid in the n-hexane and methanol extract, (Figure 1 and 3) but there is flavonoid in the ethyl acetate extract of the leaves of *G. hombroniana* (Figure 2).

Analysis by TLC (Thin Layer Chromatography) gave the result that ethyl acetate extract of the leaves of *G. hombroniana* has the highest content of flavonoid. Then the ethyl acetate extract of the leaves of *G. hombroniana* undergoes the determination of total flavonoid content by $AlCl_3$ colorimetric method.

Determination of the total flavonoid content (TFC)

The standard solution with the various concentration of quercetin used to create a calibration curve[6] (Table 5). The absorbance of ethyl acetate extract of leaves of *G. hombroniana* plotted against the quercetin calibration curve and then calculated its total flavonoid content. The content of flavonoid in the sample expressed in QE *(quercetin Equivalent)*. QE is the equality number of milligram quercetin in 1 gram sample. The result obtained total flavonoid content of ethyl acetate extract of the leaves of *G. hombroniana* is 42.004 mg QE/g sample.

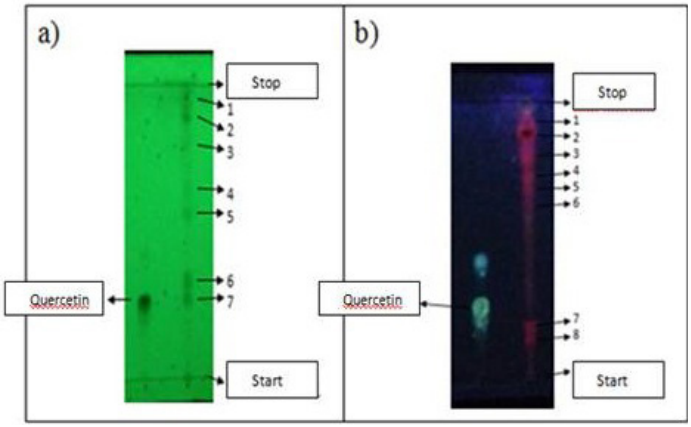

Figure 1: TLC result of n-hexane extract of the leaves of G. hombroniana at 254 nm (a) and 366 nm (b).

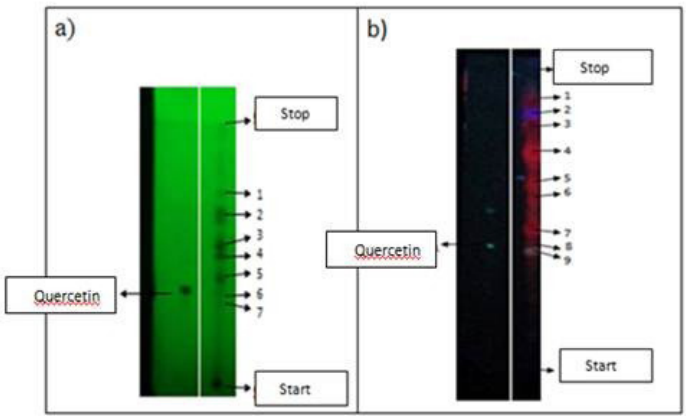

Figure 2: TLC result of ethyl acetate extract of the leaves of G. hombroniana at 254 nm (a) and 366 nm (b).

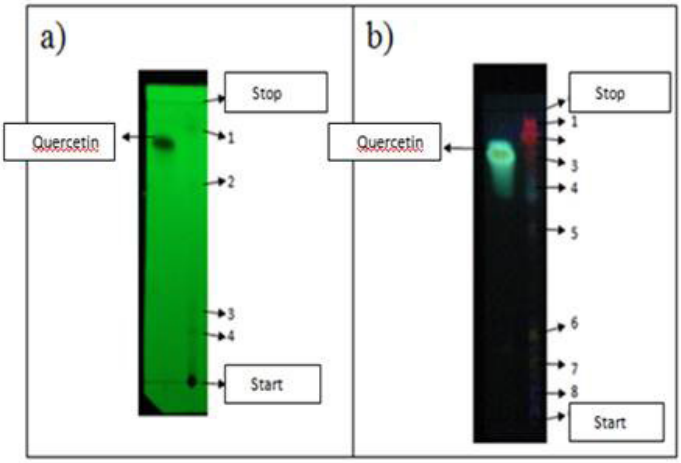

Figure 3: TLC result of methanol extract of the leaves of G. hombroniana at 254 nm (a) and 366 nm (b).

Table 1: Capacity percentage of baicalein against FRAP and EC_{50} value of baicalein

Sample	Sample Concentration (µg /mL)	Capacity percentage (%)	Standard Deviation (SD)	Coefficient of Variation (%)	Regression Equation	EC_{50} (µg / mL)
Baicalein	0.508	31.188	0.003	1.423	y = 17.6646 + 27.7559x R^2 = 0.992	1.165
	0.762	40.297	0.001	0.418		
	1.016	45.592	0.005	1.424		
	1.270	52.612	0.001	0.307		
	1.524	58.345	0.007	1.539		
	1.778	68.304	0.002	0.378		

Table 2: Capacity percentage and EC_{50} value of the leaves extract of G. hombroniana

Sample	Sample Concentration (µg / mL)	Capacity percentage (%)	Standard Deviation (SD)	Coefficient of Variation (%)	Regression Equation	EC_{50} (µg / mL)
n-hexane extract	19.984	27.334	0.011	5.371	$y = \frac{100}{1 + 10^{[(1{,}559 - x) * 1{,}611]}}$ R^2 = 0.9875	36.260
	24.980	37.483	0.005	1.916		
	29.976	40.434	0.007	2.347		
	39.968	52.902	0.008	1.963		
	44.964	60.250	0.010	2.115		
	49.960	62.330	0.004	0.837		
ethyl acetate extract	2.007	29.801	0.006	2.826	$y = \frac{100}{1 + 10^{[(0{,}4725 - x) * 2{,}138]}}$ R^2 = 0.9969	2.969
	2.509	41.070	0.005	1.543		
	3.011	50.834	0.005	1.200		
	3.513	60.463	0.010	2.010		
	4.014	64.600	0.007	1.285		
	4.516	70.669	0.027	4.512		
methanol extract	4.011	34.987	0.006	2.565	y=15,6115+4,6372x R^2 = 0.983	7.416
	5.014	37.339	0.004	1.752		
	6.017	44.110	0.004	1.407		
	7.020	49.067	0.004	1.376		
	8.022	51.434	0.008	2.390		
	9.025	58.089	0.006	1.482		

Table 3: Inhibition percentage and IC_{50} values of baicalein

Sample	Sample Concentration (µg / mL)	Inhibition Percentage (%)	Standard Deviation (SD)	Coefficient of Variation (%)	Regression equation	IC_{50} (µg / mL)
Baicalein	0.104	23.900	0.030	1.408	y = 5.7998 + 176.7657x R^2 = 0.991	0.250
	0.130	29.431	0.053	2.607		
	0.156	34.371	0.022	1.073		
	0.182	37.664	0.041	2.132		
	0.208	40.674	0.037	1.920		
	0.286	57.191	0.027	1.782		

Table 4: Inhibition percentage and IC_{50} value of the leaves extract of G. hombroniana

Sample	Sample Concentration (µg / mL)	Inhibition Percentage (%)	Standard Deviation (SD)	Coefficient of Variation (%)	Regression Equation	IC_{50} (µg / mL)
n-hexane extract	1.249	37.679	0.029	2.290	y=17.8651+15.6631x R^2 = 0.972	2.052
	1.499	39.561	0.007	0.531		
	1.998	47.222	0.003	0.261		
	2.498	61.290	0.045	4.214		
	3.497	75.045	0.014	2.618		
	3.997	77.240	0.025	4.624		
ethyl acetate extract	0.075	32.019	0.022	2.636	y=7.1961+319.3169x R^2 = 0.980	0.134
	0.100	40.431	0.014	1.773		
	0.126	45.426	0.019	2.654		
	0.151	52.261	0.021	3.138		
	0.176	66.194	0.005	0.790		
	0.201	71.241	0.019	3.072		
methanol extract	0.251	35.058	0.030	2.768	y=32.8619+13.0391x R^2 = 0.984	1.314
	0.501	39.962	0.051	5.004		
	1.254	49.555	0.010	0.713		
	1.504	54.271	0.011	0.795		
	1.755	55.241	0.043	3.289		
	2.006	57.882	0.004	0.274		

Table 5: Calibration curve of quercetin

Sample	Sample Concentration (µg / mL)	Absorption (A)	Standard Deviation (SD)	Coefficient of Variation (%)	Linear Regression Equation
Quercetin	2.012	0.235	0.006	2.009	y = 0.1393 + 0.0431x R^2 = 0.995
	4.024	0.301	0.002	0.403	
	6.036	0.410	0.003	0.612	
	8.048	0.472	0.013	2.186	
	10.060	0.574	0.019	2.873	
	12.072	0.666	0.002	0.213	

DISCUSSION

Antioxidant Activity Assay

Test of antioxidant activity using baicalein as the positive control to ensure that the testing method performed properly and can be used. Baicalein works by binding to the iron element and undergoes oxidation to slow or prevent the oxidation of other molecules.[4]

The capacity percentage of baicalein is better compared to the sample leaves extract of *G. hombroniana*. This may cause by baicalein is a purely positive control (there are no impurities or other compounds), whereas the leaves extract of *G. hombroniana* contains various compounds that attracted during the extraction process and not all of the compounds are reducing agents to FRAP. Ethyl acetate extract of leaves of *G. hombroniana* has the best antioxidant activity presumably because the ethyl acetate extract contains higher flavonoid than another extract.

Lipoxygenase Enzyme Inhibition Assay

Lipoxygenase inhibition test is done by using baicalein as a positive control to ensure that the testing method performed properly and can be used. Baicalein acts as an inhibitor which will inhibit the reaction between lipoxygenase and linoleic acid.

Inhibition of lipoxygenase caused by the presence of phenolic compounds such as flavonoid that work as a reductive inhibitor.[4] Ethyl acetate extract of leaves of *G. hombroniana* has the best inhibition activity presumably because the ethyl acetate extract contains flavonoid higher than another extract.

Thin Layer Chromatography (TLC) Analysis

Analysis of TLC using stationary phase silica gel 60 F_{254} and the mobile phase is different for each extract because the extract obtained by maceration process so that each has different polarity. Mobile phase n-hexane and ethyl acetate (6: 4) is used for n-hexane extract; mobile phase toluene, ethyl acetate and formic acid (61: 30: 9) is used for ethyl acetate extract; and mobile phase ethyl acetate and formic acid (40: 1) is used for methanol extract.

The TLC result showed that there aren't any flavonoid in the n-hexane and methanol extract, but there is flavonoid in the ethyl acetate extract of the leaves of *G. hombroniana*. The absence of flavonoid in the methanol extract may cause by the abundance of chlorophyll which causes most of the spot produced red color at 366 nm.[5]

Determination of the total flavonoid content (TFC)

The antioxidant activity and inhibition of lipoxygenase activity contained in the leaves extract of *G. hombroniana* presumably caused by the presence of flavonoid, but the result showed that the ethyl acetate extract of leaves of *G. hombroniana* has the low level of flavonoid. In the previous study, xanton was found at the twigs of *G.hombroniana*.[7] The antioxidant activity and inhibition of lipoxygenase activity in leaves extract of *G. hombroniana* may be caused by xanton and other compounds that have antioxidant activity and may inhibit lipoxygenase activity.

CONCLUSION

Based on the research that has been done, it can be concluded that the ethyl acetate extract of leaves of *Garcinia hombroniana* Pierre has the most active antioxidant activity and also the most active in inhibiting lipoxygenase activity. Total flavonoid content of ethyl acetate extract of the leaves of *Garcinia hombroniana* Pierre is 42.004 mg QE/g sample.

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CONFLICT OF INTEREST

None

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