

Antioxidant Activity and Lipoyxygenase Enzyme Inhibitory Assay with Total Flavonoids Content from *Garcinia hombroniana* Pierre Stem Bark Extract

Amanda Listiyani, Berna Elya*, Nuraini Puspitasari

ABSTRACT

Introduction: *Garcinia* has been known as a rich source of xanthenes, flavonoids, and phenols. The aim of this research is to obtain data of antioxidant activity and to observe potential inhibition of lipoyxygenase activity that most active from methanolic, ethyl acetate and n-hexane extracts with total flavonoids content from most active extracts from the bark of *Garcinia hombroniana* Pierre. **Methods:** The antioxidant activity was measured using the ferric reducing antioxidant power (FRAP), the anti-inflammatory assay was measured using inhibition of lipoyxygenase activity test, qualitative analysis of flavonoids using thin layer chromatography, and total flavonoids content was measured using AlCl_3 colorimetric method. **Results:** The results showed that the ethyl acetate extract from *G. hombroniana* Pierre stem bark as the most active extract for antioxidant and lipoyxygenase inhibition activity with EC_{50} and IC_{50} value consecutively 15.34 $\mu\text{g/ml}$; 0.26 $\mu\text{g/ml}$. Total flavonoids content of ethyl acetate is 7.430 mg QE/g extract. The results of this study showed bark extract *Garcinia hombroniana* Pierre has antioxidant activity and potent to inhibit lipoyxygenase activity. **Conclusion:** Based on the research for methanolic, ethyl acetate and n-hexane extract, it can be concluded that the ethyl acetate extract of *G. hombroniana* Pierre as the most active extract for antioxidant and lipoyxygenase inhibition activity.

Key words: Antioxidant, *Garcinia hombroniana* Pierre, Inflammation, Lipoyxygenase, Total flavonoids content.

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INTRODUCTION

Garcinia is a plant that rich source of secondary metabolites, especially xanthenes, flavonoids, and polyphenols.¹ A wide variety of biological and pharmacological activity have been reported such as anti-HIV, anti-cancer, antioxidant, anti-tuberculosis, anti-fungal, antibacterial, anti-inflammatory, and anti-Alzheimer² Anti-inflammatory activities showed in the presence of 12-lipoyxygenase inhibition by α -mangostin from *Garcinia mangostana* with IC_{50} was 0.58 μM .³ *Garcinia hombroniana* Pierre is a native plant from Sumatra. At this time, *G. hombroniana* Pierre has been cultivated in Bogor Botanical Garden, West Java. Some constituents were identified as lupeol acetate, β -sitosterol, xanthenes, leucodin, betulin, betulinic acid, and stearic acid⁴ that may be used for treatments.

The previous study provides that *G. hombroniana* Pierre stem bark extract has antioxidant activity (ethyl acetate extract 5579.8 ± 117.77 ; methanol extract 4709.6 ± 88.0 ; n-hexane extract <2000 mol TE /g), anti-cholinesterase, and anti-bacterial. Traditionally, this plant also used as anti-inflammatory. Total flavonoids content of *G. hombroniana* Pierre was 3317.6 ± 131.0 QE (*quercetin* equivalent)/g extract.² However, few studies that discuss anti-inflammatory and antioxidant activity using *Ferric reducing antioxidant power* (FRAP) method of *G. hombroniana* Pierre. Therefore, further research to determine the inhibi-

tion of lipoyxygenase activity and antioxidant activity using FRAP. Furthermore, the phytochemical screening using thin layer chromatography (TLC) and total flavonoids contents at the most active extracts also discussed in this study.

MATERIAL AND METHODS

Antioxidant Activity Assay using FRAP Method

FRAP assay was done according to the method described by Benzie and Strain (1996)⁵ with some modifications. Working solution of FRAP was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich) in 40 mM HCl and 220 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (10:1:1). 3.8 ml FRAP reagent solution reacted with 0.2 ml baicalein solution or sample, then incubated for 30 minutes at 37°C. 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 absorbance of the product was then measured at 595 nm. The percentage of capacity can be calculated using % capacity calculation. The analysis was done in triplicate. The result was expressed as EC_{50} ($\mu\text{g/ml}$), and obtained by using Microsoft Office Excel or GraphPad Prism 7.

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Lipoxygenase Enzyme Inhibition Assay

Preliminary test consist of optimization of borate buffer (pH 8; 8.5; 9; 9.5), stop solution (1 ml of methanol), enzyme concentration (2500; 5000; 7500; and 10000 units/ml) and substrate concentration (300; 600; 900; 1200; 1500 μ M). The lipoxygenase assay was performed by reacting 10 ml of baicalein (Sigma-Aldrich) or sample solution (with various concentration), 1690 μ l of 0.2 M borate buffer (pH 8.5), 1000 μ l of a linoleic acid substrate (Sigma-Aldrich) (900 μ M). Incubated for 10 min at 25°C. 300 μ l of lipoxygenase solution (5000 units/ml) was added and incubated 15 min at 25°C. 1000 μ l of stop solution added and measured its absorbance using UV-Vis spectrophotometer at 235 nm. Analysis was done in triplicate

Inhibition of the activity of lipoxygenase 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

The result was expressed as IC_{50} (μ g/mL), and obtained by using Microsoft Office Excel or GraphPad Prism 7.

Phytochemical Screening using Thin Layer Chromatography

Thin Layer Chromatography analysis for methanolic, ethyl acetate, and n-hexane extract using mobile phase that has been optimized. Stationary phase using silica gel 60 F_{254} plates. Mobile phase for methanolic extract is ethyl acetate-formic acid (4:0.2), toluene-ethyl acetate-formic acid (61:30:9) for extract ethyl acetate and n-hexane-ethyl acetate (6:4) for extract n-hexane. each extract solution was spotted on a TLC plate. TLC plates which had been eluted dried and sprayed with $AlCl_3$ 5% to analyze the presence of flavonoids compounds. The plates were examined under UV light at a wavelength of 254 nm and 366 nm. Quercetin is used as the reference standard that treated similarly to extract. Determined the Rf between quercetin and sample.

Determination of Total Flavonoids Content (TFC)

Total flavonoid content was measured using the modified method adapted from Chang *et al.*⁶ The most active extract in antioxidant activity and inhibition of lipoxygenase highest measured the total flavonoids content. Prepared samples (0.5 ml) by reacting 1.5 ml of methanol 95% (v/v), 0.1 ml of 10% aluminum chloride (w/v), 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water. The mixture samples were incubated at a temperature of $\pm 25^\circ C$ for 30 min. The absorbance was read at wavelength 435 nm. The analysis was done in triplicate. Quercetin is used as a standard with the same treatment as the samples for the calibration curve. The total flavonoid content was reported as total quercetin equivalent per g extract (mg QE/ g extract).

RESULT

Antioxidant Activity Assay using FRAP Method

FRAP method is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri (2-pyridyl) -s triazine (TPTZ), forming intense blue color of the complex Fe^{2+} -TPTZ that measured in maximum absorbance at 595 nm.⁷ This reaction depends on pH (optimum pH was 3.6).⁸ FRAP methods performed at acidic pH conditions (3.6) for an iron solubility and the most important is to facilitate the transfer of

electrons, thereby increasing the redox potential.⁹ FRAP methods using reagents tripyridyl triazine (TPTZ) as an iron-binding ligand. FRAP test is a method that is simple, fast, and cheap enough and does not require special equipment.¹⁰ Antioxidant capacity was expressed as EC_{50} . EC_{50} is a concentration of sample or standard that can exhibit 50 % of FRAP capacity. From the experiments, data showed the EC_{50} of baicalein (positive control), ethyl acetate extract, methanolic extract, and n-hexane extract respectively 1.16; 15.34; 27.21, and 110.9 μ g/ ml. Ethyl acetate as the most active extract because has the lowest EC_{50} value but less potent than baicalein as a positive control. Sequentially, that has the highest antioxidant activity then was baicalein > ethyl acetate extracts > methanolic extract > n-hexane extract.

Lipoxygenase Enzyme Inhibition Assay

From the preliminary test obtained data for optimum conditions for lipoxygenase enzyme inhibition assay, there was 8.5 for optimum pH, 1 ml of methanol for stop solution, 5000 U/ml for enzyme concentration, and 900 μ M for substrate concentration. The result showed that IC_{50} of baicalein, methanolic extract, ethyl acetate extract, n-hexane extract respectively 0.24; 0.26; 0.95; 5.09 μ g/ml. Ethyl acetate as the most active extract because has the lowest IC_{50} value but less potent than baicalein as a positive control. The previous study reported that *Garcinia* contains xanthenes and benzophenone derivative, and rich in flavonoids that have anti-inflammatory activity.¹¹

Phytochemical Screening using Thin Layer Chromatography

Analysis using thin layer chromatography was used for screening the presence of flavonoids. The results showed that ethyl acetate and methanolic extract contains flavonoid that proved by yellow color in silica gel plates at 366 nm while n-hexane doesn't. The yellow color in ethyl acetate extract is more and intensive than methanolic extract. The result corresponded in antioxidant activity and lipoxygenase inhibition assay that state the ethyl acetate as the most active extracts. The result can be seen in Figure 1, 2 and 3.

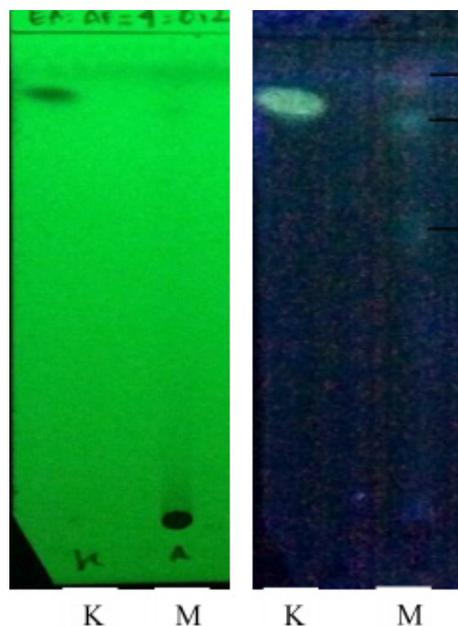


Figure 1: Spot of methanolic extract. K = quercetin; M = methanolic extract. Conditions: stationary phase silica gel F_{254} and the mobile phase ethyl acetate-formic acid (4: 0.2) at 254 nm UV light (a) and 366 nm (b) after being sprayed with $AlCl_3$ 5%.

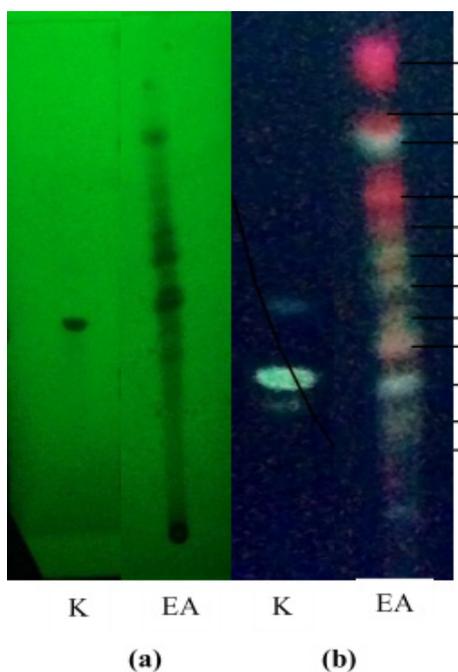


Figure 2: Spot of ethyl acetate extract. K = quercetin; EA = ethyl acetate extract. Conditions: stationary phase silica gel F_{254} and the mobile phase toluene-ethyl acetate-formic acid (61: 30: 9) at 254 nm UV light (a) and 366 nm (b) after being sprayed with $AlCl_3$ to 5%.



Figure 3: Spot of n-hexane extract. K = quercetin; NH = n-hexane extract. Conditions: stationary phase silica gel F_{254} and the mobile phase n-hexane-ethyl acetate (6: 4) at 254 nm UV light (a) and 366 nm (b) after being sprayed with $AlCl_3$ to 5%.

Determination of Total Flavonoids Content (TFC)

The total flavonoids contents were measured by using $AlCl_3$ colorimetric method for ethyl acetate extract. The principle of determination of the flavonoid content consists in the fact that aluminum chloride forms

stable acid complexes with the carbonyl group at C4 and hydroxyls at C3 (flavonols) and C5 in flavonols and flavones, besides forming labile acid complexes with hydroxyls in the ortho position in A or B rings of flavonoids.⁶ Obtained linear regression $0,0431x + y = 0.1394$ from calibration curve of quercetin, with $R^2=0.9957$ According to the formula¹² total flavonoids content of ethyl acetate extract is of 7.430 mg QE (quercetin equivalents / g extract).

CONCLUSION

Based on the research for methanolic, ethyl acetate and n-hexane extract, it can be concluded that the ethyl acetate extract of *G. hombroniana* Pierre as the most active extract for antioxidant and lipoxygenase inhibition activity with EC_{50} and IC_{50} value consecutively 15.34 $\mu\text{g/ml}$; 0.26 $\mu\text{g/ml}$. Total flavonoids content of ethyl acetate is 7.430 mg QE/g extract. However, there should be more research for ethyl acetate extract as the most active extract include isolation and characterization of compounds.

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CONFLICT OF INTEREST

No conflict of interest are declared.

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