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Original Article

Antioxidant Activity of Fractions from *Garcinia hombroniana* Pierre Leaves Extracts

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ABSTRACT

Introduction: Radicals were compounds that generated in normal metabolism and create cell damage. A significant increase of free radical and decreased radical elimination can lead to oxidative stress. Oxidative stress plays an important role in the development of many diseases. Enhanced supply of antioxidants will help prevent the morbidity of many diseases. *Garcinia hombroniana* Pierre has potency as an antioxidant, but study to evaluate the active fractions as an antioxidant has not been done. **Objective:** The objective of the study was to evaluate antioxidant activity of fractions separated from ethyl acetate (EtOAc) and methanol (MeOH) extract of *Garcinia hombroniana* leaves and to obtain active fractions to facilitate finding a pure antioxidant compound. **Methods:** The extract was fractionated using column chromatography, while antioxidant activity assay was conducted *in vitro* using spectrophotometric methods with DPPH and FRAP method. **Results:** EtOAc extract of *G. hombroniana* leaves yielded EA-8 with radical scavenging percentage 32.67% (10 ppm, with DPPH method) and EA-11 with antioxidant activity percentage 25.73% (10 ppm, with FRAP method) as the most active fraction from EtOAc extract, while MeOH extract yielded M-3 with radical scavenging percentage 37.42% (10 ppm, with DPPH method) and 26.70% (10 ppm, with FRAP method) as the most active fraction from MeOH extract **Conclusion:** Most active fractions has good antioxidant activity, worthy for further study to isolate antioxidant compound which is responsible for antioxidant activity. However, the percentage of radical scavenging or antioxidant activity of all active fractions were smaller than quercetin as a positive control.

Key words: Column chromatography, Free radicals, Spectrophotometric thin layer chromatography.

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INTRODUCTION

A balance between the rate of free radical production and its elimination is very important. Excessive free radical production can be harmful to the body. Radicals were compounds with free electrons that generated in normal metabolism and create cell damage thus have an adverse effect on the cellular metabolism.[1] A Significant increase of free radical and decreased radical elimination of cells can lead to oxidative stress. Oxidative stress plays an important role in the development of vascular complications in type 2 diabetes, the aging process, cancer and many diseases.[2,3] Antioxidants can inhibit the formation of free radicals, reduce free radicals, and increase the defense of enzymatic antioxidants. Enhanced supply of antioxidants will help prevent the morbidity of many diseases.[4]

The antioxidant compounds in many plants perform as a radical scavenger and could converting radicals into low-reactivity compound.[5] Indonesia has a lot of plants potentially to be prepared as an antioxidant agent. One of the genus in Clusiacease family is *Garcinia*, which native to regions of Northeast Australia, Asia, Southern Africa and West Polynesia.[6] Many species of *Garcinia* have been studied for their activity and phytochemical content and among many species of *Garcinia*, the scientific data of *Garcinia hombroniana* is still rare compared to other *Garcinia* species.

The earlier studies show the leaves of *G. hombroniana* contain tannins, terpenoids, alkaloids, phenols, flavonoids, and saponins.[7,8] Previous studies have demonstrated the antioxidant activity of ethyl acetate (EtOAc) and methanol (MeOH) extract from the leaves of *G. hombroniana*,[9] but study to obtain the active fraction as an antioxidant has not been done. The objective of the study was to evaluate antioxidant activity of fractions separated from EtOAc and MeOH extract of *G. hombroniana* leaves and to obtain active fractions to facilitate finding a pure antioxidant compound.

MATERIALS AND METHODS

Materials

EtOAc and MeOH extract from *G. hombroniana* Pierre leaves which extracted by maceration were obtained from Laboratory of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, University of Indonesia. The leaves of *G. hombro-*

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niana were identified by Dr. Joeni Setijo Rahajoe, collected in Bogor, Indonesia. A voucher specimen has been saved in Laboratory of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Indonesia. Analytical grade ethyl acetate (EtOAc), n-hexane, and methanol (MeOH), technical grade n-hexanex, EtOAc, and MeOH which have been distilled, silica gel 70-230 mesh (Merck), Thin Layer Chromatography Plate (Merck). Quercetin (Sigma Aldrich, India), DPPH (1, 1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, Germany), TPTZ (2,4,6-tripyridyl-s-triazine) (Sigma Aldrich, Switzerland), FeCl3, HCl, sodium acetate, and acetic acid (glacial) (Merck, Germany) were used.

Fractions Pooling using Column Chromatography and Analytical Thin Layer Chromatography (TLC)

EtOAc and MeOH extracts of *G. hombroniana* leaves were fractionated by column chromatography with wet packing method. Silica gel 70-230 mesh used as the stationary phase while the mobile phase used a mix of solvents (EtOAc, n-Hx, and MeOH) with increasing polarity. Solvent gradient system was used in elution process. Thin layer chromatography is used to identify fractions with the same chromatogram pattern. The fraction with similar chromatogram pattern then merged and checked its antioxidant with DPPH and FRAP method.

Antioxidant Activity Assay

DPPH Method

The antioxidant activity test method is a DPPH free radical scavenging method adopted from Bobo-García *et al* with slight modification.[10] The sample solution was piped 20 µL and inserted into the microplate well. Each solution was added 180 µL of 150 µmol / L DPPH solution. The control solution consisted of 20 µL methanol and 180 µL DPPH solution of 150 µmol / L, while the blank solution consisted of 200 µL methanol pro analysis. The solution is shaken for 60 sec and then incubated at room temperature in the dark room for 40 min. Absorbance was measured at 516 nm in the microplate reader (VersaMax). The percent DPPH scavenging was calculated using:

$$\#\ \text{DPPH Scavenging} = \left[1 - \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}}\right] \times 100$$

FRAP Method

The antioxidant activity test using FRAP reagent, with the procedure adopted from Pereira *in vitro* with slight modification.[11] The sample solution was piped 30µL, inserted into a microplate well, followed by additional of 270µL FRAP reagent, then incubated and the absorbance was measured. A mixture of 30µL methanol and 270µL FRAP reagents was used as blank.

The percent antioxidant activity was calculated using:

$$\%\ \text{Antioxidant Activity} = \left[A_{sample} - A_{blank}\right] \times 100$$

RESULTS AND DISCUSSION

This study was performed to evaluate antioxidant activity of fractions separated from EtOAc and MeOH extract of *G.hombroniana* leaves. EtOAc extract yielded 14 fractions from its fractionation, while MeOH extract yielded 12 fractions (Table 1 and 2). Fractions were evaluated its antioxidant activity using microplate reader (VersaMax). *in vitro* studies of fractions demonstrated that all samples have antioxidant activity. All EtOAc fractions and MeOH fractions were tested for their antioxidant activity by DPPH and FRAP method. The test concentration of all fractions used in this test was 10 ppm.

Table 1: Fractions from EtOAc extract.

Fraction	Eluent of column
EA-1	*n-Hx/EtOAc* = 9:1
EA-2	*n-Hx/EtOAc* = 8:2
EA-3	*n-Hx/EtOAc* = 8:2
EA-4	*n-Hx/EtOAc* = 8:2
EA-5	*n-Hx/EtOAc* = 8:2 – 7:3
EA-6	*n-Hx/EtOAc* = 7:3
EA-7	*n-Hx/EtOAc* = 7:3 – 6:4
EA-8	*n-Hx/EtOAc* = 5:5
EA-9	*n-Hx/EtOAc* = 4:6 – 3:7
EA-10	*n-Hx/EtOAc* = 2:8 – EtOAc/MeOH = 9:1
EA-11	EtOAc/MeOH = 9:1 – 8:2
EA-12	EtOAc/MeOH = 7:3
EA-13	EtOAc/MeOH = 6:4 – 2:8
EA-14	EtOAc/MeOH = 2:8 – 0:10

Description: EA = fraction from EtOAc extract, n-Hx = n-Hexane, EtOAc = ethyl acetate, MeOH = methanol.

Table 2: Fractions from MeOH extract.

Fraction	Eluent of column
M-1	*n-Hx/EtOAc* = 8:2
M-2	*n-Hx/EtOAc* = 7:3 – 2:8
M-3	*n-Hx/EtOAc* = 2:8 – EtOAc/MeOH = 9:1
M-4	EtOAc/MeOH = 9:1 – 8:2
M-5	EtOAc/MeOH = 7:3
M-6	EtOAc/MeOH = 6:4 – 5:5
M-7	EtOAc/MeOH = 5:5 – 3:7
M-8	EtOAc/MeOH = 2:8 – 5:5
M-9	EtOAc/MeOH = 4:6
M-10	EtOAc/MeOH = 0:10
M-11	EtOAc/MeOH = 0:10
M-12	EtOAc/MeOH = 0:10

Description: M = fraction from MeOH extract, n-Hx = n-Hexane, EtOAc = ethyl acetate, MeOH = methanol.

The antioxidant activity test results with DPPH method can be seen in Table 3 and 4, showed that the eighth fraction of EtOAc extract (EA-8) was the most active fraction as antioxidant compared with other EtOAc fractions, while the third fraction of MeOH extract (M-3) showed the highest activity as antioxidant compared with other MeOH fractions. Based on the antioxidant test using DPPH method, it can be seen that potential antioxidant activity between M-3 and EA-8 not much different.

The antioxidant activity test results with FRAP method can be seen in Table 5 and 6, showed that EA-11 is the fraction with the best antioxidant activity compared to other EtOAc fraction, whereas EA-8 has the second best antioxidant activity. M-3 is the fraction with the best antioxidant activity compared to other MeOH fractions. The highest percentage of antioxidant activity was demonstrated by M-3. Antioxidant activity of active fractions may be due to the content of its electron donor compounds, hydrogen donor compounds or the possibility of synergistic effects among the compounds so as to provide antioxidant activity.[12,13] Phyto-

Table 3: Antioxidant Activity of Fractions from EtOAc extract by DPPH method.

Fraction	Percent Inhibition (%) ± SD
EA-1	19.34 % ± 0.67
EA-2	17.19 % ± 0.19
EA-3	18.90 % ± 0.29
EA-4	18.69 % ± 0.25
EA-5	17.97 % ± 0.51
EA-6	21.72 % ± 0.16
EA-7	23.11 % ± 0.67
EA-8	32.67 % ± 1.09
EA-9	25.65 % ± 0.58
EA-10	32.61 % ± 2.44
EA-11	32.28 % ± 1.43
EA-12	31.29 % ± 1.92
EA-13	23.55 % ± 0.16
EA-14	18.74 % ± 0.29

Data are mean ± SD or % ± SD for triplicate measurements.

Table 4: Antioxidant Activity of Fractions from MeOH Extract by DPPH method.

Fraction	Percent Inhibition (%) ± SD
M-1	32.76 % ± 0.25
M-2	17.97 % ± 0.19
M-3	37.42 % ± 0.67
M-4	26.29 % ± 0.42
M-5	22.44 % ± 0.92
M-6	34.66 % ± 0.57
M-7	18.13 % ± 1.59
M-8	18.19 % ± 0.58
M-9	19.02 % ± 1.10
M-10	20.01 % ± 0.79
M-11	25.54 % ± 0.57
M-12	19.79 % ± 0.19

Data are mean ± SD or % ± SD for triplicate measurements.

constituents belonging to *Garcinia hombroniana* leaves, such as flavonoid may play a role in antioxidant activity. Flavonoids have antioxidant property, caused by the main structure such catechol structure, double bonds, and hydroxyl groups. Capability of flavonoid to chelate free radicals by donating electron or hydrogen atoms indicates its activity as a powerful antioxidant.[14]

The standard used in the antioxidant activity test is quercetin. Antioxidant activity of quercetin using DPPH showed that radical scavenging percentage of quercetin in 10 ppm is 99.17 % while using FRAP reagent showed that antioxidant activity percentage of quercetin in 10 ppm is 63.90 % (Table 7). Quercetin, used as a positive or comparative standard in antioxidant activity trials, showed better antioxidant activity compared with all active fractions. The antioxidant activity of quercetin is due to the presence of hydroxyl groups, double bonds, carbonyl groups and aromatic rings with hydroxyl groups, which play a role in its ability as an antioxidant.[15]

Table 5: Antioxidant Activity of Fractions from EtOAc extract (FRAP method).

Fraction	Percent Inhibition (%) ± SD
EA-1	10.40 % ± 0.52
EA-2	5.70 % ± 1.28
EA-3	6.30 % ± 0.72
EA-4	6.20 % ± 0.62
EA-5	4.00 % ± 0.58
EA-6	7.77 % ± 0.46
EA-7	8.10 % ± 0.26
EA-8	22.37 % ± 0.67
EA-9	15.20 % ± 3.85
EA-10	22.10 % ± 1.13
EA-11	25.73 % ± 1.99
EA-12	18.90 % ± 1.68
EA-13	9.60 % ± 0.26
EA-14	3.80 % ± 0.35

Data are mean ± SD or % ± SD for triplicate measurements.

Table 6: Antioxidant Activity of Fractions from MeOH Extract (FRAP method).

Fraction	Percent Inhibition (%) ± SD
M-1	4.400 % ± 1.000
M-2	6.070 % ± 0.321
M-3	26.700 % ± 1.136
M-4	16.100 % ± 0.436
M-5	7.90 % ± 0.30
M-6	23.03 % ± 1.10
M-7	3.07 % ± 0.06
M-8	2.53 % ± 0.35
M-9	2.13 % ± 0.32
M-10	3.77 % ± 0.42
M-11	10.67 % ± 0.55
M-12	4.30 % ± 0.36

Data are mean ± SD or % ± SD for triplicate measurements

Table 7: Antioxidant Activity of Quercetin (10 µg/mL).

Method	Percent Inhibition (%) ± SD
DPPH	99.17 ± 0.70
FRAP	63.90 ± 0.60

Data are mean ± SD or % ± SD for triplicate measurements

CONCLUSION

The test result showed that most active fraction from the crude EtOAc extract are EA-8 with radical scavenging percentage 32.67% (10 ppm, DPPH method) and EA-11 with antioxidant activity percentage 25.73% (10 ppm, FRAP method), while most active fraction from the crude MeOH extract is M-3 with radical scavenging percentage 37.42%

(10 ppm, DPPH method) and antioxidant activity percentage 26.70% (10 ppm, FRAP method). The percentage of radical scavenging or antioxidant activity of all active fractions were smaller than quercetin as a positive control. Most active fractions have good antioxidant activity, worthy for further study to isolate antioxidant compound which is responsible for antioxidant activity.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ABBREVIATIONS

TLC: Thin Layer Chromatography; **DPPH:** 1, 1-diphenyl-2-picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **TPTZ:** 2,4,6-tripyridyl-s-triazine.

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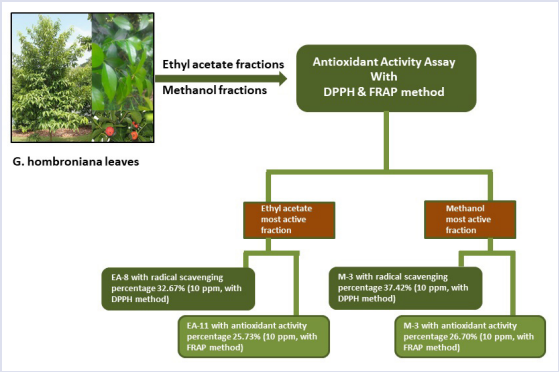
GRAPHICAL ABSTRACT



SUMMARY

- Fractionation of *G.hombroniana* leaves extract was conducted using column chromatography.
- Antioxidant activity assay of fractions was conducted *in vitro* using spectrophotometric methods with DPPH and FRAP method.
- Ethyl acetate extract of *G. hombroniana* leaves yielded EA-8 with radical scavenging percentage 32.67% (10 ppm, with DPPH method) and EA-11 with antioxidant activity percentage 25.73% (10 ppm, with FRAP method) as the most active fraction.
- Methanol extract yielded M-3 with radical scavenging percentage 37.42% (10 ppm, with DPPH method) and 26.70% (10 ppm, with FRAP method) as the most active fraction.

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