

**ANTIOXIDANT ACTIVITY AND LIPOXYGENASE INHIBITORY ASSAY WITH TOTAL FLAVONOID CONTENT OF *GARCINIA LATERIFLORA* BLUME LEAVES EXTRACT**

SISMITA AVELIA, MARISTA GILANG MAULDINA, BERNA ELYA\*

Department of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Indonesia, Jakarta, Indonesia.

Email: [berna.elya@gmail.com](mailto:berna.elya@gmail.com)

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**ABSTRACT**

**Objective:** *Garcinia lateriflora* Blume has been reported to have antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl method with methanol, ethyl acetate, and n-hexane leaves extract inhibitory concentration 50% (IC<sub>50</sub>) levels of 6.18, 8.03, and 156.8 µg/mL, respectively. Meanwhile, there has been no literature regarding *G. lateriflora* Blume's lipoxygenase inhibition activity. The aim of this study was to determine the potential antioxidant activity and lipoxygenase inhibition activity of three leaf extracts of *G. lateriflora* Blume.

**Methods:** These study test methods involved an assessment of antioxidant activity using the ferric reducing antioxidant power method, an assessment of lipoxygenase inhibition activity through the *in vitro* method, and a qualitative analysis of flavonoid and total flavonoid content using thin-layer chromatography and the AlCl<sub>3</sub> colorimetric method to reveal the most active extract.

**Results:** Based on the assessment for methanol, ethyl acetate, and n-hexane, the results showed that the effective concentration 50% levels of the antioxidant activity of *G. lateriflora* Blume leaves extract were 9.567, 16.555, and 50.550 µg/mL, respectively. Furthermore, the IC<sub>50</sub> levels of the lipoxygenase inhibition activity were 0.693, 0.793, and 1.316 µg/mL, respectively. The most active extract for both of the tests was methanol extract, which has a total flavonoid content of 6.298 mg quercetin equivalents/g.

**Conclusions:** Based on the test results, it can be concluded that *G. lateriflora* Blume leaves extracts exhibit antioxidant and lipoxygenase inhibition activities, with methanol extract as the most active extract, containing more flavonoid than the other two extracts.

**Keywords:** Antioxidant, Ferric reducing antioxidant power, *Garcinia lateriflora* Blume, Flavonoid, Lipoxygenase.

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**INTRODUCTION**

Plants are beneficial and known to have many bioactive compounds that are useful for treating a variety of diseases. One of these compounds is flavonoid, which exerts a significant effect as an anti-inflammatory and antioxidant that can reduce free radicals [1]. One of the plants that exhibit significant antioxidant activity and lipoxygenase inhibition activity is a species of *Garcinia*. *Garcinia* is the largest genus within the Clusiaceae family, having approximately 400 species, and it is widespread in Asia, Africa, South America, and Polynesia. *Garcinia* plants are rich in secondary metabolites, including triterpenes, flavonoids, xanthenes, and phloroglucinols, which have pharmacological uses and produce anti-inflammatory, antibacterial, antiviral, anti-HIV, antidepressant, and antioxidant effects [2]. *Garcinia lateriflora* Blume is a species of the genus *Garcinia* that demonstrates antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl method to reveal the inhibitory concentration 50% (IC<sub>50</sub>) levels of methanol, ethyl acetate, and n-hexane extracts, resulting in 6.18, 8.03, and 156.8 mg/mL, respectively [3]. In addition, another *in vitro* method for analyzing antioxidant activity is the ferric reducing antioxidant power (FRAP) method. The results of this study demonstrate that the most active extract is methanol.

Some species of the genus *Garcinia* have also been shown to have anti-inflammatory effects through the inhibition of lipoxygenase. For example, methanol extract of the bark and leaves of *Garcinia nervosa* has inhibition values of 62.54% and 86.53%, respectively, with a final concentration of 100 µg/mL [4]; furthermore, garcinol, a khalkon compound of *G. indica*, inhibits 5-lipoxygenase with an IC<sub>50</sub> value of 0.3 mM [5]. As for *G. lateriflora* Blume, there is no prior literature regarding its lipoxygenase inhibitory activity. This study was conducted using the FRAP method

to obtain additional scientific data regarding antioxidant activity and to determine the lipoxygenase inhibition potential of *G. lateriflora* Blume leaves extract. The flavonoid content of each extract was determined qualitatively through using thin-layer chromatography (TLC). The total flavonoid content was then quantitatively determined using AlCl<sub>3</sub> methods to determine the most active extract.

**METHODS****Antioxidant activity with FRAP method**

Measurements of antioxidant activity using the FRAP method are depicted in Table 1.

The mixture solution was incubated for 30 minutes at 37°C, and then, the absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 596 nm. The percentage of the capacity was calculated using the following equation:

$$\% \text{ capacity} = (1 - T_s) \times 100\%$$

In this equation, T<sub>s</sub> is transmittance, absorbance is -log T<sub>s</sub>, and A<sub>s</sub> is the absorbance of the standard/sample solution minus the absorbance of the control solution. The effective concentration 50% (EC<sub>50</sub>) levels were calculated using the linear regression equation (Microsoft Office Excel) and the non-linear equation (GraphPad Prism 7), and the concentration of the sample was represented by the x-axis while the percent capacity was represented by the y-axis.

**Lipoxygenase enzyme inhibition assay**

For the lipoxygenase inhibition assay, we optimized a pH borate buffer solution, stop solution, and enzyme and substrate concentrations. The measurement of lipoxygenase activity was performed using a

UV-Vis spectrophotometer instrument following the optimization. The conditions included the borate buffer (0.2 M, pH 8.5), linoleic acid as the substrate (900  $\mu$ M), lipoxygenase (5,000 units/mL), and methanol as a solvent stop. The inhibition test results of lipoxygenase activity are demonstrated in Table 2.

The inhibition percentage of the lipoxygenase inhibition value was calculated using the following equation:

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

In this equation, A is the absorbance of the blank solution (with the enzyme), B is the absorbance of the control blank solution (without the enzyme), C is the absorbance of standard/sample solution (with the enzyme), and D is the absorbance of the control standard/sample solution (without the enzyme). The  $IC_{50}$  value was determined through the use of the linear regression equation (Microsoft Office Excel), and the x-axis demonstrates the concentration of the sample while the y-axis demonstrates the inhibition percentage.

#### TLC analysis

An analysis by TLC was conducted to determine that *G. lateriflora* leaves extract have the highest, qualitative flavonoid content. The eluent used after the optimization of the mobile phase was n-hexane-ethyl acetate (6:4) for the n-hexane extract, toluene-ethyl acetate-formic acid (61:30:9) for the ethyl acetate extract, and dichloromethane-acetone-water (10:7:1) for the methanol extract. A plate of silica gel 60 F<sub>245</sub> was used as the stationary phase. In this TLC analysis, quercetin was used as a standard solution, which was treated similarly to the extract. The distance covered by the standard solution (the retention factor of standard) was then compared to the distance travelled by each extract (the retention factor of sample) after the plate was sprayed with an AlCl<sub>3</sub> 5% reagent at UV 254 and 366 nm.

#### Determination of the total flavonoid content

For the total flavonoid content, 0.5 mL of the standard and sample solutions were reacted with 1.5 mL of methanol for pro analysis, 0.1 mL AlCl<sub>3</sub> 10%, 0.1 mL 1M sodium acetate solution, and 2.8 mL of distilled water. Mix all the solution and the mixture solution was incubated for 30 minutes at room temperature. The absorption was measured using

a UV-Vis spectrophotometer at 435 nm. The calibration curve of the quercetin was required to obtain the linear regression equation so that the level of flavonoid in the sample could be calculated in the quercetin equivalent (QE) with the following equation:

$$\text{Content of total flavonoids} = \frac{C \times V \times FP}{\text{Sample weight}}$$

In this equation, C represents the concentration obtained from the calibration curve (x) ( $\mu$ g/mL), V is the total volume of the test solution (mL), FP is the dilution factor, and the weight of the sample is represented in grams.

## RESULTS AND DISCUSSION

Baicalein was used as the positive control to ensure that the testing method was performed and used properly. Baicalein works by binding to the iron element (Fe<sup>3+</sup>) on its trihydroxy, and it undergoes oxidation to slow or prevent the oxidation of the other molecules. Baicalein also acts as an inhibitor suppressing the reaction between lipoxygenase and linoleic acid. The inhibition of lipoxygenase caused by the presence of a phenolic compound, such as flavonoid, works as a reductive inhibitor. Baicalein is a reductive inhibitor, based on its catechol group, so it can be assumed that the catechol binding of the iron leads to a reduction of the iron active site while baicalein is oxidized into its quinone [6].

#### Antioxidant activity with FRAP method

The FRAP method is a convenient method for assessing antioxidant activity by reducing Fe (III) to Fe (II) and through complexation with 2,4,6-tripyridyl-s-triazine as a chromogenic ligand, resulting in an intensive blue color [7]. The samples act as antioxidants in the FRAP method if the sample has a redox potential lower than the redox potential of Fe (III)/Fe (II) (0.77 V). Therefore, the sample would have the power to reduce Fe (III) to Fe (II), and these samples would undergo oxidation [8]. In using the FRAP method, the increase of absorbance is proportional to the increase of the antioxidant capacity in the reduction of Fe<sup>3+</sup> ions [9].

Based on this test, and using baicalein as a positive control, methanol, ethyl acetate, and n-hexane leaves extract of *G. lateriflora* demonstrates antioxidant activity with EC<sub>50</sub> values of 1.165, 9.567, 16.555, and 50.550  $\mu$ g/mL, respectively. The test also demonstrates that the EC<sub>50</sub> values of the aforementioned materials can be represented by the following: baicalein > methanol extract > ethyl acetate extract > n-hexane extract. The most active extract is methanol extract (Table 3).

#### Lipoxygenase inhibition assay

The inhibition activity of lipoxygenase is proportional to the concentration of the extract used in the test. The higher concentration of the extract used, the higher the lipoxygenase inhibition activity is as well; this means that inhibition will decrease the product of reaction between the substrate and lipoxygenase in the form of hydroperoxyoctadecadienoic

**Table 1: Composition of the solution for the antioxidant activity test using the FRAP method**

Material	Volume ( $\mu$ L)	
	Standard/sample	Standard/sample control
FRAP solution	3.800	3.800
Baicalein/extract	200	-
Ethanol pro analysis	-	200

FRAP: Ferric reducing antioxidant power

**Table 2: Composition of the solution for the lipoxygenase inhibition assay**

Material	Volume ( $\mu$ L)			
	Blank	Blank control	Standard/sample	Standard/sample control
Borate buffer (0.2 M, pH 8.5)	1.700	2.000	1.690	1.990
Baicalein/extract solution	-	-	10	10
Linoleic acid 900 $\mu$ M	1.000	1.000	1.000	1.000
The mixture solution was incubated for 10 minutes at 25°C				
Lipoxygenase solution 10,000 unit/mL	300	-	300	-
The mixture solution was incubated for 15 minutes at 25°C				
Stop solution (methanol pro analysis)	1.000	1.000	1.000	1.000
Total volume	4.000	4.000	4.000	4.000

Hydroperoxyoctadecadienoic acid absorbance was measured using a UV-Vis spectrophotometer at 235 nm

**Table 3: The EC<sub>50</sub> values of the antioxidant activity of baicalein and *G. lateriflora* leaves extract**

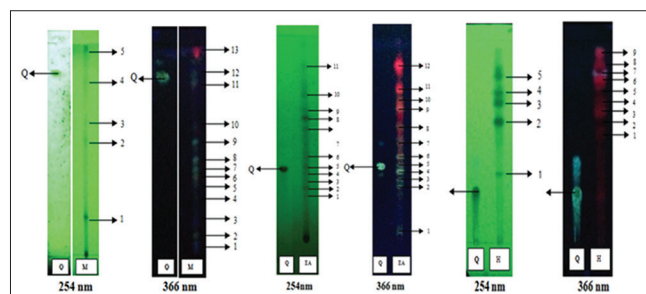
Material	EC <sub>50</sub> value (µg/mL)	Regression equation	R <sup>2</sup>
Baicalein	1.165	y=27.75x+17.66	0.992
Methanol leaves extract	9.567	y=3.324x+18.20	0.995
Ethyl acetate leaves extract	16.555	y=2.293x+12.04	0.991
N-hexane leaves extract	50.550	$y = \frac{100}{1 + 10^{((1.704 - x) * 1.075)}}$	0.993

*G. lateriflora*: *Garcinia lateriflora*, EC<sub>50</sub>: Effective concentration 50%

**Table 4: The IC<sub>50</sub> values of the lipoxygenase inhibition of baicalein and *Garcinia lateriflora* leaves extract**

Material	IC <sub>50</sub> value (µg/mL)	Regression equation	R <sup>2</sup>
Baicalein	0.250	y=176.7x+5.799	0.991
Methanol leaves extract	0.693	y=26.64x+31.53	0.996
Ethyl acetate leaves extract	0.793	y=29.42x+25.94	0.991
N-hexane leaves extract	1.316	y=18.36x+25.83	0.990

*G. lateriflora*: *Garcinia lateriflora*, IC<sub>50</sub>: Inhibitory concentration 50%

**Fig. 1: TLC results of the three leaf extracts of *Garcinia lateriflora* at 254 and 366 nm (Q: Quercetin, M: Methanol extract, EA: Ethyl acetate extract, H: N-hexane extract)**

acid. This is characterized by the decrease of the absorbance of the baicalein or the extract of *G. lateriflora* minus the control of baicalein or the extract of *G. lateriflora* to increase the concentration of the extract.

Based on this test, and using baicalein as a positive control, methanol, ethyl acetate, and n-hexane leaves extract of *G. lateriflora* demonstrates lipoxygenase inhibition activity with IC<sub>50</sub> values of 0.250, 0.693, 0.793, and 1.316 µg/mL, respectively (Table 4). The test also shows that the IC<sub>50</sub> values of the aforementioned materials can be represented by the following: baicalein > methanol extract > ethyl acetate extract > n-hexane extract. The most active extract is methanol extract.

### Flavonoid

Antioxidant and lipoxygenase inhibition activities are presumably caused by polyhydroxyl compounds, such as xanthone, tannin, and flavonoid [10]. As a result, in this study, we want to determine the relationship between flavonoid content and antioxidant and lipoxygenase inhibition activities. The flavonoid compound in *G. lateriflora* leaves extract will presumably reduce Fe<sup>3+</sup> ions to Fe<sup>2+</sup> ions, based on its hydroxyl group. The positive results of flavonoid content are demonstrated with yellow, green, or blue fluorescence spots, and these results may become more intensive or change after they are sprayed with a spot spray reagent [11]. The TLC results demonstrate that there is no

flavonoid content in the n-hexane extract, but that flavonoid is present in the ethyl acetate and methanol extracts of *G. lateriflora* leaves. Flavonoid content in ethyl acetate and methanol extract is observed with yellow spots in a chromatogram at UV 366 nm. In methanol extract, there are 12 yellow spots, and in ethyl acetate extract, there are 10 yellow spots (Fig. 1). As a result, based on the chromatogram, the extract with the most flavonoid content is methanol extract.

### Total flavonoid content

Based on the antioxidant activity test, which employed the FRAP method, and the inhibition of lipoxygenase assay, methanol extract was determined to be the most active extract, containing the most flavonoid (which was qualitatively by TLC). The total flavonoid content of methanol extract of *G. lateriflora* was then measured using a UV-Vis spectrophotometer. A standard solution with various concentrations of quercetin was used to create a calibration curve. Based on this test, the linear regression equation for total flavonoid content is y=0.1393+0.0431x with R<sup>2</sup>=0.995. The absorbance of methanol extract of *G. lateriflora* was plotted against the quercetin calibration curve, and its total flavonoid content was subsequently calculated. The flavonoid content in the sample was expressed in the QE. QE is the equality number of milligrams of quercetin in a 1-gram sample. The result obtained for the total flavonoid content of methanol extract of *G. lateriflora* leaves is 6.298 mg (QE)/g.

### CONCLUSION

Methanol, ethyl acetate, and n-hexane extract of *G. lateriflora* Blume demonstrate antioxidant activity with the EC<sub>50</sub> values of 9.567, 16.555, and 50.550 mg/mL, respectively, as well as lipoxygenase inhibition activity with IC<sub>50</sub> values of 0.693, 0.793, and 1.316 mg/mL, respectively. Both of these tests demonstrate that the most active extract is methanol extract, which contains more flavonoid than the other extracts in terms of its total flavonoid content (6.298 mg (QE)/g).

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