Pharmacogn J. 2017; 9(2): 257-266
A multifaceted peer reviewed journal in the field of Pharmacognosy
www.phcogj.com | www.phcog.net

Original Article

Antioxidant Activity and Lipoxygenase Enzyme Inhibition Assay with Total Flavonoid Assay of *Garcinia porrecta* Laness. Stem Bark Extracts

Amalia Cipta Sari, Berna Elya*, Katrin

ABSTRACT

Introduction: The genus *Garcinia* which is rich of secondary metabolites, mainly flavonoids, have known to have antioxidant and anti-inflammatory activity through the inhibition of lipoxygenase. There isn't found literature indicating research on inhibition of lipoxygenase activity been done in this plant. The purpose of this study is to obtain the data and determine the potential antioxidant activity, and inhibition of lipoxygenase activity of *Garcinia porrecta* Laness. stem bark extracts. **Methods:** This research is included FRAP (Ferric Reducing Antioxidant Power) method antioxidant assay, *in vitro* lipoxygenase inhibition assay, flavonoids qualitative analysis by thin layer chromatography, and total flavonoids assay in the most active extract. **Results:** The results showed the methanol, ethyl acetate and n-hexane extracts of G. porrecta Laness. stem bark using FRAP method, has antioxidant activity with EC50 values respectively 1.33; 4.97; and 19.96 µg/mL and lipoxygenase inhibition activity with IC50 values 0.23; 0.52; and 4.87 µg/mL. The most active extract in the both assay is methanol extract which has total flavonoids of 5.66 mg QE/g (quercetin equivalent). **Conclusion:** The results from the study show extracts of the stem bark of *G. porrecta* Laness. has antioxidant activity and potential for lipoxygenase inhibition.

Key words: Antioxidant, Flavonoid, FRAP, *Garcinia porrecta* Laness, Lipoxygenase.

Amalia Cipta Sari, Berna Elya*, Katrin
Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Kampus Baru UI Depok, 16424, Depok, INDONESIA.

Correspondence

Berna Elya

Faculty of Pharmacy, Universitas Indonesia, Gedung A Rumpun Ilmu Kesehatan Lantai 1, Kampus UI, Depok, Jawa Barat – 16424, INDONESIA.

Phone: +62 21 727 0031

E-mail: berna.elya@gmail.com

History
- Submission Date: 21-12-2016;
- Review completed: 05-01-2017;
- Accepted Date: 16-01-2017.

DOI : 10.5530/pj.2017.2.44

Article Available online
http://www.phcogj.com/v9/i2s

INTRODUCTION

Free radicals are atoms or molecules that are unstable (one electron or more without a partner), so as to obtain an electron pair, free radicals tend to look for other molecules and atoms of lead compounds that are not normal and starts a chain reaction in the body.[1] One example of the kind of free radicals in the body is a reactive oxygen species (ROS) and reactive nitrogen species (RNS, such as nitric oxide, NO) generated by the enzyme NO synthase (NOS) and NAD (P) H oxidase isoform. The beneficial effects of ROS / RNS (eg superoxide radicals and nitric oxide) occurs at concentrations of low/moderate and involves a physiological role in the cellular response to *noxia*, such as in the defense against infectious agents, in function of a number of cellular signaling pathways, and the induction of mitogenic response , Excess production of ROS (arising either from mitochondrial electron transport chain or excessive stimulation of NAD (P) H) produces oxidative stress, a process that became the mediator of the damage to cell structures, including lipids in cell membranes, proteins, and DNA. As a result of oxidative stress that accumulates can lead to degenerative diseases such as Alzheimer's, cancer and body triggers cell death (apoptosis) faster.[2] One way to deal with free radicals is with antioxidants. Antioxidants are compounds that can slow or prevent damage caused by free radicals by dampening the activity of free radicals or break the chain reaction of oxidation caused by free radicals.[3]

One of the effects of free radicals is the process of induction of cytokines, inflammatory mediators in the body, which causes an inflammatory response occurs.[4] Inflammation is a protective response the body's normal when there is tissue injury which involves a variety of physiological processes in the body such as the activation of the enzyme inflammatory, inflammatory mediator release, the movement of white blood cells through the capillaries into areas of inflammation, cell migration, and the restoration of damaged tissue.[5] Lipoxygenase (LOX) is one enzyme that has a role in inflammation, especially in the biochemical processes of leukotrienes. Leukotrienes are the main regulator of allergic reactions and inflammation. Currently, lipoxygenase inhibitors become a potentially important agent that shows significant anti-inflammatory activity.[6,7] Flavonoids like baicalein and apigenin have been known to be useful as inhibitors of lipoxygenase *in vitro*.[8]

Garcinia is the largest genus of the family Clusiaceae which has about 400 species is widespread in Asia, Africa, South America, and the Polynesian Islands. Garcinia contains many secondary metabolites, mainly triterpenes, flavonoids, xanthones, and phloroglucinol that have pharmacological activities such as anticancer, anti-inflammatory, antibacterial, antiviral, anti-HIV, antidepressants, and antioxidants.[9] One species that grows in Indonesia Garcinia, *Garcinia porrecta* Laness at which previous studies have shown that stem bark of the plant contains several kinds of compounds such as porsanton and dulsisan-

Cite this Article: Sari AC, Elya B, Katrin. Antioxidant Activity and Lipoxygenase Enzyme Inhibition Assay with Total Flavonoid Assay of *Garcinia porrecta* Laness. Stem Bark Extracts. Pharmacognosy Journal. 2017;9(2):257-66.

ton which is a compound class and compounds porlanosterol xanthones. Testing the antioxidant activity in extracts of *G. porrecta* Laness. already been done by the DPPH (2,2-diphenyl-1-picrylhydrazyl) *radical scavenger* which showed significant antioxidant activity (IC_{50} <50 µg mL^{-1}).[10] Until now, this has never been tested inhibition of lipoxygenase enzyme activity and antioxidant activity test, especially with methods FRAP *(Ferric Reducing Antioxidant Power*) of the plant *G. porrecta* Laness.

It encourages the study of the antioxidant activity of *G. porrecta* Laness. stem bark extracts *in vitro* with FRAP method and on the inhibition of lipoxygenase enzyme activity which is one of the inflammatory factors. Extracts with antioxidant activity and inhibition of lipoxygenase and analyzed the content of flavonoids qualitatively by thin layer chromatography. Extract the most active in the antioxidant activity and inhibition of lipoxygenase assay will be set on total flavonoids assay with $AlCl_3$ quantitative method.

MATERIALS AND METHOD

Chemicals

The materials used in this research is distilled water, demineralised water, ethanol pro analysis (Merck), methanol pro analysis (Merck), ethyl acetate (Merck), n-hexane (Merck), dichlormethane, acetone, toluene, formic acid, enzymes lipoxygenase from soybeans (Sigma-Aldrich L37935), linoleic acid (Sigma-Aldrich), boric acid (Merck), sodium hydroxide (Mallinckrodt), sodium acetate, TPTZ (2,4,6-tripyridyl-s-triazine) (Sigma-Aldrich), iron (III) chloride (Merck), hydrochloric acid (Merck), glacial acetic acid, aluminum (III) chloride, and sodium acetate.

Material Comparative

Baicalein (Sigma-Aldrich) as a positive control of the test the antioxidant activity with FRAP and test methods lipoxygenase inhibition activity. Quercetin (Sigma-Aldrich) as a positive control on a thin layer chromatography and the assay methods flavonoids $AlCl_3$.

Equipment

Equipment used in this research include an analytical balance (Sartorius 7), digital scales (ACIS, Japan), *vortex mixer* (Wisemix), pH meter (Eutech Instruments, France), refrigerator (Sharp), *freezer* (Sharp), *a water bath* (Imperial IV Water Bath Lab-Line), condenser, 10-100 mL micropipette (Corning), 100-1000 mL micropipette (Finpipette), incubator (Gemmyco), sonicator (Wiseclean), UV-Vis spectrophotometer (PG instruments Ltd. T80), quartz cuvette (Merck, Germany), *chamber,* room thermometer, test tube rack and glass tools used in laboratories in general.

Work Stages

Stages of the work done in this study started with the preparation of the methanol extract, ethyl acetate and n-hexane bark of *Garcinia porrecta* Laness., the antioxidant activity FRAP method and the inhibitory activity of lipoxygenase done on the third step in order to obtain an extract that has the highest activity. The extract containing flavonoids will be assayed quantitatively by the method of $AlCl_3$.

Antioxidant Activity Test FRAP Ferric Reducing Antioxidant Power) Method

FRAP method procedure of antioxidant activity assay is in Table 1. Having obtained the data, calculated the percentage of capacity reduction of Fe ions^{3+} by the positive control baicalein/extract the FRAP solution. The percentage of capacity can be calculated using the formula

% Capacity = (1 - Ts) x 100%

Ts = Transmittan

As = - log Ts

As = Absorbance positive control/extract - The absorbance of the solution FRAP

EC_{50} of samples was calculated using the equation of nonlinear regression with aid analysis software GraphPad PRISM® version 7, the concentration of the sample is transformed into a logarithm as the x-axis and y-axis percent capacity.

Lipoxygenase Inhibition Activity Test

Optimization tests were done prior the sample test. Each procedure is described in Table 2 through 5. Optimization of Enzyme concentration was described in Table 11 . Inhibition of lipoxygenase activity by the extract of methanol, ethyl acetate, n-hexane *Garcinia porrecta* Laness. stem bark and baicalein (Table 6) can be known from the value of % inhibition calculated using the following equation.

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

Note:

A = Absorbance reference solution with enzyme

B = Absorbance reference solution without enzymes

C = Absorbance sample solution with the enzyme

D = Absorbance of the sample solution without enzymes

Value IC_{50} samples were determined by the equation of nonlinear regression with the help of analysis software GraphPad Prism version 7, the x-axis shows the concentration of the sample that has been transformed into a logarithm and the y-axis shows the % inhibition.

Determination of Total Flavonoids with Colorimetric Method of AlCl3 to Quercetin and *G. porrecta* Laness. Stem Bark Extract

The test procedure involves reacting methanol extract at a concentration of 6000 mg / mL 0.5 ml, then added 1.5 mL of methanol, 0.1 ml of a solution of $AlCl_3$, a 10% 0.1 ml, and 2.8 ml aquadest. The mixture was incubated for 30 min at room temperature. Uptake is measured using UV-VIS spectrophotometry at a wavelength of 435 nm. Quercetin was used as a positive control and treated similarly to extract and quercetin calibration curves made with varying concentrations. The linear regression equation quercetin produced can be used to calculate the concentration of flavonoids contained in the sample. Controls for quercetin and extracts made by replacing the solution of $AlCl_3$ 10% with distilled water and treated the same as quercetin and samples. If needed all the prepared solution was filtered with filter paper before measuring absorbance. Levels of flavonoids total calculated in quercetin equivalent (QE), with the formula:

RESULTS AND DISCUSSION

Test Antioxidant Activity Method FRAP

Based on testing FRAP, extracts methanol, ethyl acetate, and n-hexane *G. porrecta* Laness. stem bark shows the same thing with baicalein of increased extract concentrations proportional to the increase in antioxidant capacity. Data Values of EC_{50} tests of antioxidant activity methods FRAP was described in Table 7. Meanwhile, data values of EC_{50} n-hexane, ethyl acetate and methanol extract tests of antioxidant activity methods FRAP was described in Table 8-10. When compared with the antioxidant capacity baicalein reaching 50% at a concentration of 1.16

mg / mL, then either extract methanol, ethyl acetate and n-hexane from the *G. porrecta* Laness. stem bark showed lower antioxidant activity with FRAP method. Based on the classification,[11] the methanol extract, ethyl acetate and n-hexane is classified as a very powerful antioxidant.

The antioxidant activity could have been influenced by the presence of hydroxyl groups, such as those found in the phenolic compounds, flavonoids, and tannins. The antioxidant activity depends on the number and position of hydroxyl contained in the compound.[12] The antioxidant activity of the extracts of methanol and ethyl acetate allegedly influenced by the presence of flavonoids compounds in both the extract. The content of flavonoids in the extract will be analyzed further using thin layer chromatography. Flavonoids in the extract are expected to be a reductant/electron donor from the -OH group at his disposal to reduce the Feions^{3+} to Fe2+.

In the n-hexane extract, antioxidant activity is also quite active although it is lower than both the other extracts. The antioxidant activity of n-hexane extracts suspected because of the non-polar compounds such as terpenoids interested in the n-hexane extract of *G. porrecta* Laness. stem bark.

Lipoxygenase Inhibition Activity Test

Inhibition of Lipoxygenase Activity of Baicalein Positive Control

Based on the previous testing, known baicalein have IC_{50} values of 0.0012 mM (0.32 mg / mL)[13] as a competitive inhibitor of linoleic acid and 22.5 µM (6.08 mcg / mL) as a lipoxygenase inhibitor *in vitro*. In this test, the IC_{50} values obtained from baicalein of 0.25 mg / mL was obtained by a linear equation as the data in Table 12. The difference results obtained presumably because testing procedures are different from previous studies because in this study the optimization tests were used to generate maximum data.

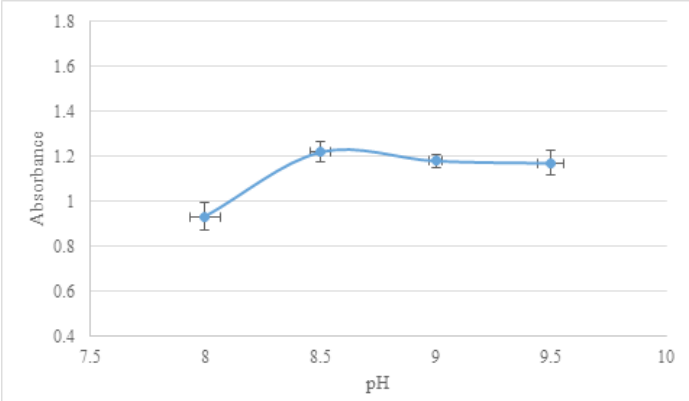

Figure 1: Curve relationship borate buffer pH 0.2 M with the uptake.

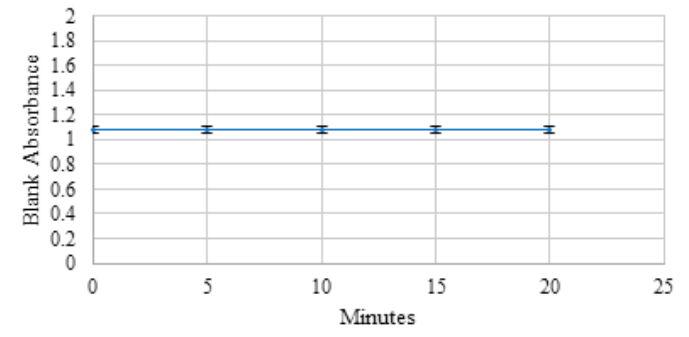

Figure 2: Curve relationship downtime solution stop the uptake.

Inhibition of Lipoxygenase Activity Assay of Methanol, Ethyl Acetate, and N-Hexane *G. porrecta* Laness. Stem Bark Extract

Based on inhibition of lipoxygenase activity assay that has been made to extract methanol, ethyl acetate, and n-hexane bark *G. porrecta* Laness. IC_{50} values obtained successively by 0.23 ug / ml; 0.52 ug / ml; and 4.87 ug / ml, can be seen in Table 13 to 15. The test results showed that the extract has the most active inhibition of lipoxygenase activity is methanol extract. When compared with the positive control baicalein which reaches 50% inhibition at a concentration of 0.25 mg / mL, then either extract ethyl acetate and n-hexane from the bark of *G. porrecta* Laness. showed inhibition of lipoxygenase activity is lower than baicalein. The methanol extract stem *G. porrecta* Laness. have a better activity than the positive control baicalein in inhibiting lipoxygenase can be seen from the IC_{50} values at lower concentrations.

The inhibition of lipoxygenase activity is proportional to the concentration of the extract used in the test. The higher the concentration of the extract is used, the higher the lipoxygenase inhibitory activity as well, so that the product of a reaction between the substrate and lipoxygenase in the form of HpODE will decrease. It is characterized by decreasing absorption of the sample minus the control samples to the increased concentration of the extract.

Determination of Total Flavonoids Level of Methanol Extract from *G. porrecta* Laness. Stem Bark.

Based on testing by the FRAP method antioxidant activity and inhibition of lipoxygenase activity, it is obtained methanol extract as the most active extract. Towards the methanol extract of the stem bark of *G. porrecta Laness.* then performed the assay method flavonoid $AlCl_3$ using UV-Vis spectrophotometer. The results of the optimization maximum wavelength show that the greatest uptake is at a wavelength of 435 nm. Therefore, for the assay positive control quercetin and methanol extract of the

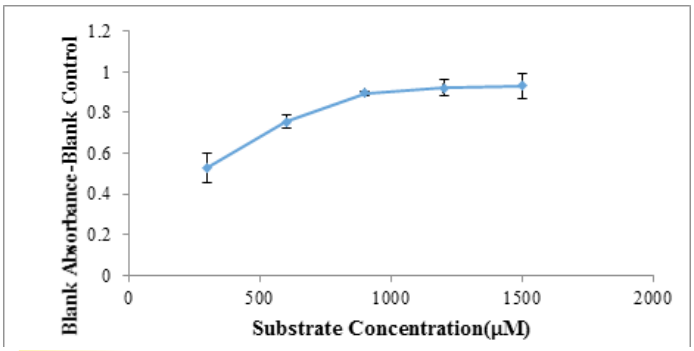

Figure 3: Curve relations substrate concentration by absorbance.

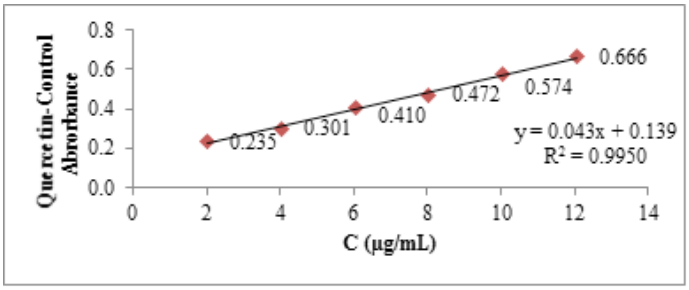

Figure 4: The calibration curve of quercetin.

Table 1: Composition of the solution to test the antioxidant activity methods FRAP

Material	Volume (mL)	
	Standard/Sample	Control Standard/ Sample
Solution FRAP	3800	3800
Baicalein / Extract	200	-
Ethanol	-	200
were incubated for 30 minutes at a temperature of 37°C and measured absorbance at 595 nm λ		

Table 2: Composition of the mixture on the optimization of pH buffer solution boric

Material	Volume (mL)	
	Blank	control Blank
solution buffer borate pH 8 , 5; 9.0; 9.5	1700	2000
600 µM linoleic acid / mL	1000	1000
incubated 25°C for 10 minutes		
lipoxygenase 10000 units / mL	300	-
incubated 25°C for 15 min		
stop solution (methanol pro analysis)	1000	1000
Volume end	4000	4000
Measured absorbance at λ 234 nm		

Table 3: Composition of the enzyme reaction stopping time optimization of

Material	Volume (mL)	
	blank	blank Control
0.2 M borate buffer pH 8.5	1700	2000
linoleic acid600 µM	1000	1000
were incubated 25°C for 10 minutes		
lipoxygenase 10000 units / mL	300	-
vortexed then incubation 25°C for 15 min		
stop solution (methanol pro analysis)	1000	1000
Volume end	4000	4000
Measured λ absorbance at 234 nm at time 0; 5; 10; 15; and 20 minutes		

Table 4: Composition of the optimization of the concentration of lipoxygenase

Material	Volume (mL)	
	Blank	Blank Control
0.2 M borate buffer pH 8.5	1700	2000
600 µM linoleic acid / mL	1000	1000
incubated 25 ° C for 10 minutes		
lipoxygenase (2500; 5000; 7500 and 10000 units / mL)	300	-
incubated 25 ° C for 15 min		
the solution stop (methanol pro analysis)	1000	1000
Volume end	4000	4000
Measured absorbance at λ 234 nm		

Table 5: Composition of mix optimization the concentration of linoleic acid substrate

Material	Volume (mL)	
	blank	blank Control
0.2 M borate buffer pH 8.5	1700	2000
linoleic acid (300; 600; 900; 1200 and 1500 µM / mL)	1000	1000
incubated 25°C for 10 minutes		
lipoxygenase 10000 units / mL	300	-
vortexed then incubation in 25°C for 15 min		
the solution stop (methanol pro analysis)	1000	1000
Volume end	4000	4000
Measured absorbance at λ 234 nm		

Table 6: Composition of the lipoxygenase inhibition test by baicalein or extract

Material	Volume (mL)			
	Blank	Blank Control	Standard /Sample	Standard Control/ Sample
0.2 M borate buffer pH 8.5	in 1700	1690 1990		2000
solution baicalein / extract	-		10	10
linoleic acid 900 µM / mL	to 1000	1000	1000	1000
was incubated 25 ° C for 10 minutes				
lipoxygenase 10000 units / mL			300-300	-
vortexed then incubate 25 ° C for 5 minutes				
solution stop (Methanol pro analysis)	1000 1000 1000			1000
Volume end	4000 4000 4000			4000
Measured by absorbance at 234 nm λ				

bark of *G. porrecta* Laness. use a maximum wavelength of 435 nm. In testing the use of quercetin as a positive control and quercetin calibration curve was made to determine the linear regression equation. The linear regression equation of quercetin obtained value of 0,0431x + y = 0.1394. By testing methanol extract 6012 mg / mL, absorption generated after deducting the control sample of 0.286. Based on calculations using the linear regression equation quercetin, obtained value x (extract concentration) of 3.40 mg / mL. Levels of total flavonoids calculated using equivalent levels of quercetin and obtained flavonoid (quercetin equivalents / g) of 5.66 mgQE / g. From the results obtained, the levels of flavonoids in the methanol extract of the bark of *Garcinia porrecta* Laness which have antioxidant activity and inhibition of lipoxygenase enzymes highest is only 0.56%. Suspected of secondary metabolites such as group xanthones,[10] triterpene and phloroglucinol provide a synergistic effect with compounds flavonoids in antioxidant activity and inhibition of enzyme lipoxygenase.[9]

Table 7: Table of data values EC_{50} baicalein on tests of antioxidant activity methods FRAP

Early C (µg/mL)	FinalC (µg/mL)	S	Mean S	SD	CV (%)	KS	Mean KS	S-KS	Ts	(1-Ts) x100	Linear Regression	EC_{50} (µg/mL)
		0.214				0.054						
10.16	0.51	0.212	0.215	0.0031	1.42	0.046	0.052	0.162	0.688	31.18		
		0.218				0.057						
		0.275				0.054						
15.24	0.76	0.277	0.276	0.0012	0.41	0.046	0.052	0.224	0.597	40.29		
		0.277				0.057						
		0.312				0.054						
20.32	1.01	0.321	0.317	0.0045	1.42	0.046	0.052	0.264	0.544	45.59		
		0.317				0.057					y = 27.75x + 17.66	1.16
		0.376				0.054						
25.40	1.27	0.376	0.377	0.0012	0.30	0.046	0.052	0.324	0.474	52.61	R^2 = 0.9918	
		0.378				0.057						
		0.437				0.054						
30.48	1.52	0.425	0.433	0.0067	1.54	0.046	0.052	0.380	0.416	58.34		
		0.436				0.057						
		0.549				0.054						
35.56	1.78	0.552	0.551	0.0021	0.37	0.046	0.052	0.499	0.317	68.30		
		0.553				0.057						

C = Concentration; S = Baicalein Absorbance; KS = Control Baicalein Absorbance; Ts = Transmittant

Table 8: Data of EC50 value of n-hexane *Garcinia porrecta* Laness. stem bark extract antioxidant assay FRAP method

Early C (µg/mL)	FinalC (µg/mL)	S	Mean S	SD	CV (%)	KS	Mean KS	S-KS	Ts	(1-Ts) x100	Non-Linear Regression	EC_{50} (µg/mL)
		0.239				0.021						
200.72	10.03	0.282	0.266	0.0235	8.84	0.038	0.035	0.231	0.58	41.25		
		0.277				0.046						
		0.336				0.021						
401.44	20.07	0.339	0.338	0.0021	0.61	0.038	0.035	0.303	0.49	50.26		
		0.34				0.046						
		0.391				0.021						
602.16	30.10	0.351	0.378	0.0240	6.34	0.038	0.035	0.343	0.45	54.67	$y=0.70x + 34.318$ $R^2=0.9860$	
		0.394				0.046					19.96	
		0.455				0.021						
802.88	40.14	0.458	0.454	0.0040	0.89	0.038	0.035	0.419	0.38	61.92		
		0.45				0.046						
		0.53				0.021						
1003.60	50.18	0.529	0.528	0.0026	0.50	0.038	0.035	0.493	0.32	67.86		
		0.525				0.046						
		0.706				0.021						
1204.32	60.21	0.711	0.710	0.0045	0.63	0.038	0.035	0.675	0.21	78.89		
		0.715				0.046						

C = Concentration; S = Baicalein Absorbance; KS = Control Baicalein Absorbance; Ts = Transmittant

Table 9: Data of EC50 value of ethyl acetate *Garcinia porrecta* Laness. stem bark extract antioxidant assay FRAP method

Early C (µg/mL)	FinalC (µg/mL)	S	Mean S	SD	CV (%)	KS	Mean KS	S-KS	Ts	(1-Ts) x100	Non-Linear Regression	EC_{50} (µg/mL)
		0.261				0.032						
51.43	2.57	0.265	0.264	0.0031	1.15	0.040	0.037	0.227	0.593	40.66		
		0.267				0.041						
		0.334				0.032						
102.87	5.14	0.332	0.332	0.0020	0.60	0.040	0.037	0.295	0.507	49.22		
		0.330				0.041						
		0.395				0.032						
154.30	7.71	0.397	0.396	0.0015	0.38	0.040	0.037	0.359	0.437	56.25	$y=\frac{100}{(1+10^{(4.97-x)0.694})}$ R^2=0.9631	4.97
		0.398				0.041						
		0.431				0.032						
205.74	10.28	0.432	0.432	0.0010	0.23	0.040	0.037	0.395	0.403	59.67		
		0.433				0.041						
		0.516				0.032						
257.17	12.89	0.518	0.518	0.0025	0.48	0.040	0.037	0.481	0.330	66.93		
		0.521				0.041						
		0.582				0.032						
308.61	15.43	0.584	0.584	0.0020	0.34	0.040	0.037	0.547	0.284	71.57		
		0.586				0.041						

C = Concentration; S = Baicalein Absorbance; KS = Control Baicalein Absorbance; Ts = Transmittant

Table 10: Data of EC_{50} value of methanol *Garcinia porrecta* Laness. stem bark extract antioxidant assay FRAP method

Early C (µg/mL)	FinalC (µg/mL)	S	Mean S	SD	CV (%)	KS	Mean KS	S-KS	Ts	(1-Ts) x100	Non-Linear Regression	EC_{50} (µg/mL)
		0.223				0.021						
10.01	0.50	0.225	0,224	0.0015	0.68	0.046	0.035	0.189	0.64	35.33		
		0.226				0.039						
		0.270				0.021						
20.03	1.00	0.272	0.272	0.0020	0.73	0.046	0.035	0.236	0.58	42.01	$y=\frac{100}{(1+10^{(0.12-x)0.728})}$	
		0.274				0.039						
		0.349				0.021						
30.04	1.50	0.350	0.352	0.0049	1.40	0.046	0.035	0.317	0.48	51.80	R^2=0.9639	1.33
		0.358				0.039						
		0.403				0.021						
40.05	2.00	0.399	0.4023	0.0031	0.76	0.046	0.035	0.367	0.43	57.04		
		0.405				0.039						
		0.439				0.021						
50.07	2.50	0.441	0.432	0.0127	2.94	0.046	0.035	0.397	0.40	59.94		
		0.418				0.039						
		0.524				0.021						
60.08	3.00	0.526	0.523	0.0031	0.58	0.046	0.035	0.488	0.32	67.49		
		0.520				0.039						

C = Concentration; S = Baicalein Absorbance; KS = Control Baicalein Absorbance; Ts = Transmittant

Table 11: Table of data optimization enzyme concentration,

the concentration of the enzyme (units / mL)	Uptake Blank (a)	Average Uptake blank (a)	SD	CV (%)	Uptake control Blanko (b)	a-b
2500	0.773 0.786 0.910	0.823	0.0756	9.19	0.471	0.352
5000	1.243 1.247 1.184	1.225	0.0353	2.88	0.474	0.751
7500	1.333 1.389 1.301	1.341	0.0445	3.32	0.471	0.870
10000	1.687 1.740 1.591	1.673	0.0755	4.51	0.471	1.202

Table 12: Table of data values IC50 baicalein at lipoxygenase inhibition activity test

B (a)	KB (b)	B-KB (ab)	mean B-KB	C early (ug / mL)	C final (ug / mL)	S	mean S (c)	SD	CV (%)	KS (d)	S-KS (cd)%	Inhibition	Linear Regression	IC_{50} (ug /ml)
1.793	0.491	1.302	1.296	41.6	0.104	2.145 2.140 2.195	2.160	0.0304	1.40	1.174	0.986	23.90	y = 176.7x + 5.799 R^2 = 0.9910	0.25
				52.0	0.130	2.076 2.050 1.974	2.033	0.0530	2.60	1.119	0.914	29.43		
1.788	0.497	1.291		62.4	0.156	1.990 2.025 1.986	2.000	0.0215	1.07	1.150	0.850	34.37		
				72.8	0.182	1.868 1.888 1.946	1.901	0.0405	2.13	1.093	0.808	37.66		
1.794	0.500	1.294		83.2	0.208	1.886 1.941 1.872	1.900	0.0365	1.92	1.131	0.769	40.67		
				114.4	0.286	1.526 1.473 1.506	1.502	0.0268	1.78	0.947	0.555	57.19		

B = blank; KB= bank control; C = concentration; S = baicalein absorbance; KS = baicalein control absorbance

Table 13: Table of data values IC_{50} n-hexane extract at lipoxygenase inhibition activity test

B	KB	B-KB	C early	C final	S	mean S	SD	CV	KS	mean KS	S-KS	% inhibition	Non-Linear Reg.	IC50
(a)	(b)	(a-b)	(µg/mL)	(µg/mL)		(c)		(%)		(d)	(c-d)			(µg/mL)
					1.068				0.684					
			1505.4	3.76	1.004	1.058	0.0507	4.79	0.684	0.684	0.374	46.99		
					1.105				0.684					
					0.986				0.605					
			2007.2	5.02	1.011	1.002	0.0144	1.44	0.605	0.605	0.397	49.89		
					1.011				0.605					
					1.059				0.689					
1.528	0.731	0.797	2509	6.27	1.186	1.109	0.0676	6.09	0.689	0.689	0.420	52.75	$y=\frac{100}{(1+10^{(0.68-x)0.629})}$ R^2=0.9766	4.87
					1.083				0.689					
					1.148				0.694					
			3010.8	7.52	1.141	1.144	0.0035	0.30	0.694	0.694	0.450	56.54		
					1.145				0.694					
					1.168				0.692					
			3512.6	8.78	1.165	1.163	0.0051	0.44	0.692	0.691	0.472	59.24		
					1.158				0.692					
					1.207				0.726					
			4014.4	10.03	1.221	1.221	0.0145	1.18	0.726	0.726	0.495	62.11		
					1.236				0.726					

B = blank; KB= bank control; C = concentration; S = baicalein absorbance; KS = baicalein control absorbance

Table 14: Table of data values IC_{50} ethyl acetate extract at lipoxygenase inhibition activity test

B	KB	B-KB	C early	C final	S	mean S	SD	CV	KS	mean KS	S-KS	% inhibition	Non-Linear Reg.	IC_{50}
(a)	(b)	(a-b)	(µg/mL)	(µg/mL)		(c)		(%)		(d)	(c-d)			(µg/mL)
					0.692				0.494					
			51.43	0.12	0.698	0.697	0.0046	0.65	0.494	0.494	0.203	31.13		
					0.701				0.494					
					0.757				0.506					
			102.87	0.25	0.729	0.736	0.0185	2.51	0.506	0.506	0.230	35.27		
					0.722				0.506					
					0.732				0.504					
1.304	0.652	0.652	154.30	0.38	0.807	0.777	0.0401	5.15	0.504	0.504	0.273	41.97	$y=\frac{100}{(1+10^{(-0.28-x)0.681})}$ R^2= 0.9439	0.52
					0.794				0.504					
					0.797				0.481					
			205.74	0.51	0.802	0.803	0.0071	0.88	0.481	0.481	0.322	49.43		
					0.811				0.481					
					0.868				0.526					
			257.17	0.64	0.887	0.880	0.0110	1.24	0.526	0.526	0.354	54.39		
					0.887				0.526					
					0.998				0.54					
			308.61	0.77	0.883	0.923	0.0650	7.04	0.54	0.54	0.383	58.74		
					0.888				0.54					

B = blank; KB= bank control; C = concentration; S = baicalein absorbance; KS = baicalein control absorbance

Table 15: Table of data values IC_{50} methanol extract at lipoxygenase inhibition activity test

B	KB	B-KB	C early	C final	S	mean S	SD	CV	KS	mean KS	S-KS	% inhibition	Non-Linear Reg.	IC_{50}
(a)	(b)	(a-b)	(µg/mL)	(µg/mL)		(c)		(%)		(d)	(c-d)			(µg/mL)
					0.632				0.465					
			50.07	0.12	0.636	0.638	0.0077	1.21	0.465	0.465	0.173	37.84		
					0.647				0.465					
					0.525				0.289					
			100.14	0.25	0.521	0.508	0.0260	5.13	0.289	0.289	0.219	47.81		
					0.478				0.289					
					1.080				0.769					
0.847	0.389	0.458	150.21	0.37	1.001	1.030	0.0434	4.22	0.769	0.769	0.261	56.99	$y=\frac{100}{(1+10^{(-0.63-x)1.328})}$	0.23
					1.009				0.769				R^2=0.9944	
					0.714				0.394					
			200.28	0.50	0.727	0.727	0.0140	1.92	0.394	0.394	0.333	72.85		
					0.742				0.394					
					1.104				0.761					
			250.35	0.62	1.115	1.133	0.0422	3.72	0.761	0.761	0.372	81.36		
					1.182				0.761					
					1.019				0.618					
			300.42	0.75	1.057	1.043	0.0213	2.05	0.618	0.618	0.425	92.94		
					1.055				0.618					

B = blank; KB= bank control; C = concentration; S = baicalein absorbance; KS = baicalein control absorbance

CONCLUSION

Based on the results of testing that has been done to extract methanol, ethyl acetate and n-hexane bark of *Garcinia porrecta* Laness., can be summed up as follows.

extracts of methanol, ethyl acetate and n-hexane bark of *Garcinia porrecta* Laness. have antioxidant activity with ECvalues$_{50}$ of 1.33 respectively; 4.97; and 19.96 ug/ml. Extracts with activity are the most active extract of methanol.

The methanol extract, ethyl acetate and n-hexane bark of *Garcinia porrecta* Laness. has the lipoxygenase inhibitory activity with ICvalues$_{50}$ of 0.23 respectively; 0.52; and 4.87 mg / mL. Extracts with an activity of the most active are methanol extract.

Levels of total flavonoids of the most active extracts, namely methanol extract are equal to 5.66 mg QE / g*(quercetin* equivalent).

ACKNOWLEDGEMENT

Thanks to PITTA Grant University of Indonesia that funding this research.

CONFLICT OF INTEREST

None

ABBREVIATION USED

REFERENCES

1. Handajani A, Roosihermiatie B, Maryani H. Faktor-faktor yang Berhubungan dengan Pola Kematian pada Penyakit Degeneratif di Indonesia. Bulletin Penelitian Sistem Kesehatan. 2010;13(1):42-53.
2. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry Cell. 2007;39(1357-2725(Print)):44-84.
3. Jung HA, Ning SBao, Keller WJ, Mehta RG, Kinghorn AD. Antioxidant Xanthones from the Pericarp of Garcinia mangostana (mangosteen). Journal of Agricultural and Food Chemistry. 2006;54.
4. Corwin E J. Handbook of Pathophysiology (3rd Edition). Philadelphia: Lippincott Williams & Wilkins. 2008.
5. Kumar V, Bhat ZA, Kumar D, Khan NA, Chashoo IA. Evaluation of anti-inflammatory potential of leaf extracts of Skimmia anquetilia. Asian Pacific Journal of Tropical Biomedicine. 2012;2(8):627-30.
6. Roussaki et al. Bioorganic & Medicinal Chemistry Structural modifications of coumarin derivatives : Determination of antioxidant and lipoxygenase (LOX) inhibitory activity. Bioorganic and Medicinal Chemistry. 2014;22(23):6586-94.
7. Murray RK, Granner DK, Rodwell VW. Biokimia Harper Edisi 27. Jakarta: EGC Penerbit Buku Kedokteran. 2006
8. Deschamps JD, Kenyon VA, Holman TR. Baicalein is potent in vitro inhibitor against both reticulocyte 15-human and platelet 12-human lipoxygenases. Bioorganic and Medicinal Chemistry. 2006;14(12):4295-301.
9. Ritthiwigrom T, Laphookhieo S, Pyne SG. Chemical constituents and biological activities of Garcinia cowa Roxb. Maejo International Journal Od Science Ang Technology. 2013;7(2):212-31.
10. Kardono LBS, Hanafi M, Sherley G, Kosela S, Harrison LJ. Bioactive Constituent of Garcinia porrecta and G. parvifolia Grown Ni Indonesia. Pakistan Journal of Biological Sciences. 2006;9(3):483-6.
11. Fidrianny I, Aristya T, Hartati R. Antioxidant Capacities of Various Leaves Extracts from Three Species of Legumes and Correlation with Total Flavonoid, Phenolic, Carotenoid Content. International Journal of Pharmacognosy And Phytochemical Research. 2015;7(3):628-34.
12. Abdullah AR, Bakhari NA, Osman H. Study on the Relationship of the Phenolic, Flavonoids, and Tannin Content to the Antioxidant Activity of Garcinia atroviridis. University Journal of Applied Science. 2013;1(3):95-100.
13. Schomburg I, Schomburg D. Class 1 Oxidoreductases X_ EC 1. In Springer Handbook of Enzyme, Vol 25, Second Edition (Second Ed., pp. 593–595). Berlin: Springer. 2007

ABOUT AUTHORS

Amalia Cipta Sari Undergraduate Student from Faculty of Pharmacy, University of Indonesia. Enrolling Apothecary Program in Faculty of Pharmacy University of Indonesia.

Elya: Lecturer, Researcher, and Laboratory of Phytochemistry and Pharmacognos, Faculty of Pharmacy, University of Indonesia.

Katrin: Lecturer, Researcher, and Laboratory of Phytochemistry and Pharmacognos, Faculty of Pharmacy, University of Indonesia

Cite this Article: Sari AC, Elya B, Katrin. Antioxidant Activity and Lipoxygenase Enzyme Inhibition Assay with Total Flavonoid Assay of *Garcinia porrecta* Laness. Stem Bark Extracts. Pharmacognosy Journal, 2017;9(2):257-66.