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Antiinflammatory Activity of Decoction of Lia Berueng (*Zingiber zerumbet*) Rhizomes, a Herbal Medicine using by Kutai Sub-Ethnic, Eastern of Kalimantan, Indonesia

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ABSTRACT

The research of decoction of lia berueng (*Zingiber zerumbet*) rhizomes, a herbal medicine using by Kutai sub ethnic, West Kalimantan, Indonesia, as the inhibitor of the *cyclooxygenase (COX*) enzyme *in vitro* has been done. *In vitro* anti-inflammatory activity of COX-1 and COX-2 enzymes used *TMPD (N,N,N',N'-tetramethyl-p-phenylediamine*) by spectrophotometry method. The results showed that the decoction of lia berueng rhizomes has anti-inflammatory activity with IC_{50} value of 22.72 ppm against COX-1 and 186.47 ppm against COX-2. Subsequently, phenolic compounds are considered to have a role as an inhibitor of COX enzyme. The total of phenolic contents was assayed using the Folin Ciocalteu method. The result showed that the total phenolic content of lia berueng rhizomes decoction was 44.60mg GAE/g.

INTRODUCTION

Kutai sub ethnic is a part of Dayak ethnic who lived at Kutai area, West Kalimantan, Indonesia. As other ethnics in Indonesia, generally, they used herbal for treatment disturbances of healthy condition. Traditionally, Kutai sub ethnics used lia berueng (*Zingiber zerumbet*) rhizome as anti-inflammatory agent (Noorcahyati, 2012). According to Somchit and Shukriyah (2003), lia berueng rhizomes had anti-inflammatory activity equivalent to mefenamic acid in rats, whilst Keong, et al, 2010, stated that lia berueng rhizome content *zerumbon* as chemical compound which have anti-inflammatory activity. Zerumbon was a monocyclic sesquiterpene. It is potential for chemopreventive agent besides anti-inflammatory agent. Sreevani, et al., 2013, concluded that the polyphenolic compounds in lia berueng rhizomes are compounds which have responsibility to anti-inflammatory activity.

The research aims to determine the anti-inflammatory activity of the lia berueng rhizome through the mechanism of COX enzymes inhibition, *in vitro*, as well as determine levels of total phenolics compounds to illustrate anti-inflammatory activity of lia berueng rhizomes as one of the traditional Kutai sub ethnic herbs for inflammatory

MATERIALS AND METHODS

Materials

Rhizome of Lia berueng are collected at Loleng Village, Kota Bangun Regency, Kutai Kartanegara, Eastern of Kalimantan at the rainy season between August – September. The rhizome were dried in shade of sunlight and then powdered. The other materials were kit reagents of colorimetric COX (ovine) inhibitor screening assay NO. 760111 (Cayman Chemical Co), Folin Ciocalteu reagent, and gallic acid.

Equipments

The equipments used in this study were Mettler Toledo analytical balance, a drip pan, freeze dryer Telstar, UV-Visible Spectrophotometer Specord 200, micropipette Socorex Switzerland, vortex, 96-wells plate Cayman Chemical, TC MRX microplate reader, and some glassware commonly used in the laboratory.

Materials

The materials used in this study were Lia Beruerng Rhizome (*Zingiber zerumbet* L. (J.E,Smith), distilled water, DMSO, COX colorimetric kit (Ovine) inhibitor screening assay No. 760 111 (Cayman Chemical Comp.), Folin-Ciocalteu reagent and Na_2CO_3 solution.

Methods

Sample Preparation

Samples of Lia Berueng rhizome were collected from Loleng Village, Kutai in East Kalimantan and were cleansed and processed. Then they were cut into small pieces and dried indirectly from the sun to obtain crude drugs, which were then extracted

Extraction Processing

A total of 167.03 grams of Lia berueng rhizome was heated in 2 L of water for 30 minutes at the temperature of 90^{o}C and dried using a freeze dryer to obtain dry extract.

Anti-Inflammatory Activity Test

Anti-inflammatory test was conducted using the colorimetric method of TPM listed in the catalog kit of colorimetric COX (Ovine) inhibitor screening assay No. 760111. Total of 160µL of buffer Tris-HCl and 10µL heme was incorporated into 2 wells as background wells. Tris-HCl buffer 150mL, 10mL heme, and 10µL enzyme were included in the 2 wells as 100% initial activity wells. Tris-HCl buffer 150mL, 10mL of heme, the enzyme 10mL, and 10mL of the test sample with a concentration of 20 ppm, 40 ppm, 80 ppm, and 160 ppm were put into the wells inhibitors. 10mL of solvent was added to the 100% initial activity wells and background wells. The plate was shaken for a few seconds and incubated for 5 minutes at 25^{o}C. Colorimetric substrate solution of 20mL and 20mL arachidonic acid was incorporated into all wells being used. The plate was shaken gently for a few seconds and incubated for 5 minutes at 25°C, and then performed an absorbance reading at (λ) 590 nm using a microplate reader.

Background absorbance value of wells was 100% initial activity, and the average value of each sample in the well inhibitors was taken and then calculated as follows (Cayman Chem. Comp., 2013).

$$a = A_{100\%\ initial\ wells} - A_{Background\ wells}$$

$$b = A_{Inhibitor\ wells} - A_{Background\ wells}$$

$$\% \text{ Inhibition} = \left(\frac{a-b}{a}\right) \times 100\%$$

Data inhibition percentage of the activity of COX-1 and COX-2 at each concentration of test samples was analyzed using linear regression analysis to determine the IC_{50} (50% Inhibition Concentration).

Assay of Total Phenolic Content

The assay was done by using the Folin-Ciocalteu method (Alfian and Susanti, 2012). Extract solution with a certain concentration of 300µL was put into a test tube, then added 1.5mL of Folin-Ciocalteu reagent (1:10). The mixture was homogenized and allowed to stand for 3 minutes, and then added 1.2mL of 7.5% Na_2CO_3 solution, then the mixture was incubated in the dark for 30 minutes. Absorbance was read at (λ) 765 nm with UV-Vis spectrophotometer

RESULTS AND DISCUSSION

Anti-inflammatory activity test of decoction of Lia Berueng rhizome against activity of Cyclooxygenase enzymes are represented in Table as showed bellow.

Tabel 1. Anti-inflammatory activity of decoction of Lia berueng rhizome against COX-1 and COX-2 enzymes

Concentration Of Extract (ppm)	COX-1		COX-2	
	% of inhibitory	IC_{50} (ppm)	% of inhibitory	IC_{50} (ppm)
10	37.50		2.52	
20	47.50		5.04	
40	62.50	22.72	16.81	186.47
80	77.50		28.57	
160	91.25		40.34	

The ratio of IC_{50} of COX-1 to COX -2 is 8.21. It means that the decoction of Lia Berueng Rhizome is more selective as inhibitory of COX-1 enzyme than to COX-2 enzyme.

Further testing of total phenol extract was determined because the phenolic compounds have anti-inflammatory capabilities as an agent through their ability to inhibit COX enzymes, so that these compounds are considered to responsible for anti-inflammatory activity of the enzyme COX, both COX-1 and COX-2.

The test was performed by using the Folin-Ciocalteu method. The principle of this method is the formation of a blue colored complex compounds that can be measured at wavelength of 765 nm (Alfian and Susanti, 2012). This reagent oxidizes the phenol group of phenolic compounds to form a colored complex. This reaction can only occur in alkaline conditions. The greater the concentration of phenolic compounds in a material, the more oxidation occurs to the phenol group of phenolic compounds, so that the complex formed will be more and more and the blue solution formed will be more concentrated. The test results of total phenolic content of the extract showed tiwai onion bulb extract contains phenolic compounds as much as 16.78 mg GAE/g sample.

CONCLUSION

Based on the research that has been done, it is known that extract of Lia berueng rhizome (*Zingiber zerumbet*) (L.) Smith rhizome have anti-inflammatory activity against the COX enzyme. This extract works more by blocking enzymes COX-1 than COX-2 enzyme. In addition, it is known that the activity of the extract turned out to contain phenolic compounds as much as 16.78mg GAE/g sample.

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