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Anti-inflammatory activity study and secondary metabolites detection in *Coptosapelta Flavescens* korth root's water extract

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Abstract

Background: The roots of *Coptosapelta flavescens* Korth (CFR) are traditionally used to treat toothache and rheumatism by boiling. CFR methanol extract has been scientifically proven to have anti-inflammatory activity, while the CFR water extract has not yet been known to have the anti-inflammatory effect or content of secondary metabolites. The aim of this study was to test the anti-inflammatory activity and detect secondary metabolites of CFR water extract by Wistar rats' red blood cell (RBC) membrane stabilizing method and color reactions respectively.

Method: RBC solution added by several concentrations of CFR water extract was induced with hyposaline solution. The supernatants obtained after centrifugation were measured for their absorbance with 560 nm wavelength. The EC_{50} of CFR water extracts was compared to Indomethacin using t-tests. The secondary metabolites tested were polyphenols, saponins, terpenoids and anthraquinone.

Results: EC50 of CFR water extract (3.68 ± 0.14) mg /ml was smaller than EC50 of Indomethacin (11.13 ± 0.52) mg / ml, indicating that the membrane stabilization effect of CFR water extract is stronger than Indomethacin. The secondary metabolites detected positively were polyphenols (tannins) and saponins.

Conclusion: CFR water extract has a stronger anti-inflammatory effect than Indomethacin, and contains polyphenols (tannins) and saponins.

Keywords: *Coptosapelta flavescens* Korth root, water extract, cell membrane stabilization, anti-inflammatory, secondary metabolites

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INTRODUCTION

Coptosapelta flavescens Korth roots (CFR) are traditionally used to treat toothache (Mulyoutami et al. 2009), rheumatism (Tran and Tran 2010), usually by boiling. CFR's methanol extract (MECFR) has been shown to have an anti-inflammatory effect (Kosala et al. 2018) while there have been no data from CFR water extract (WECFR) anti-inflammatory activity yet. The pharmacological effects of a medicinal plant are related to their content of secondary metabolites (Sahidin 2012). Therefore, the aim of this study was to detect the secondary metabolites content and to test the anti-inflammatory activity of WECFR by Wistar rats' red blood cell (RBC) membrane stabilization method (Anosike et al. 2012). If WECFR can stabilize RBC membranes, it can be interpreted that it inhibits phospholipase A2 (PLA2) release, which is a precursor of inflammatory mediators (Yoon and Baek 2005) and therefore, has anti-inflammatory effects.

METHODS

Preparation of WECFR

Coptosapelta flavescens Korth roots which were obtained from Paser District, East Kalimantan, were identified by taxonomists from the Faculty of Forestry, Mulawarman University. CFR dry powder weighed 300 grams, macerated with aquabidest 1.5 liters for 5 days while being stirred. After the filtrate was filtered with

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Table 1. Percentage of Inflammatory Protection on hyposaline-induced RBC membrane

Cons. Code	Percent Inflammation Protection of Indomethacin & WECFR on hyposaline-induced RBC membrane							
	Indometacin				WECFR			
	Cons. (mg/ml)	Mean	±	SEM	Cons. (mg/ml)	Mean	±	SEM
1	0.34	3.08	±	0.41	0.31	20.29	±	0.93
2	0.68	7.74	±	0.84	0.78	31.53	±	0.85
3	1.69	15.26	±	0.61	1.56	41.44	±	0.27
4	3.38	18.70	±	0.64	3.13	48.65	±	0.78
5	6.76	31.56	±	1.38	6.25	65.09	±	1.53
	EC50	11.13*	±	0.52	EC50	3.68*	±	0.14

n = 3, data was analyzed with t-test, *p<0.05

flannel cloth, it was put into a 50 ℃ oven for a week until a thick mass was formed. The mass was then weighed to calculate its yield. It was then stored in a refrigerator 4 ℃ before use.

In vitro Membrane Stability Assay

Preparation of Red Blood Cell (RBC) suspension

Wistar rat's blood which had been given anti-coagulant was centrifuged at 3000 rpm for 10 minutes; RBC was washed 3 times with isosaline (0.85%, pH 7.2) and the blood suspension was diluted to 10% v/v with isosaline.

Hemolysis induced by hypotonic fluids

The test material containing 1 ml of phosphate buffer [pH 7.4, 0.15 M], 2 ml hyposaline [0.36%], 0.5 ml RBC suspension [10% v / v] with 0.5 ml of WECFR at various concentrations (2.5; 6.25; 12.5; 25; and 50 mg / ml) and standard drug indomethacin of various concentrations (2.75; 5.5; 13.5; 27.0 and 54.0 mg / ml) and controls (aquabides instead of hyposaline to produce 100% haemolysis) were incubated at 56° C for 30 minutes in water bath and each centrifuged at 3000 rpm for 10 min. The supernatant was measured at a wavelength of 560 nm using a spectrophotometer. The percentage of membrane stability protection was calculated by the following formula (Anosike et al. 2012, Oyedapo et al. 2010):

$$\%\ protection = [100 - \frac{\text{Optical density of test sample}}{\text{Optical density of control}}] \times 100$$

After obtaining the curve of doses vs cell membrane protection percentage, the extracts' EC_{50} was calculated and compared with standard drug (Chandrappa et al. 2013).

Secondary Metabolites Identification in WECFR by Color Reactions

Polyphenols, Terpenoids, Anthraquinone, Saponin and Tanin were detected using color reactions methods (Kosala 2015).

RESULTS

Table 1 shows that WECFR's EC50 is smaller than Indomethacin's EC50. This showed that the WECFR protective effect on hyposaline-induced RBC membranes was greater than Indomethacin, indicating that WECFR's anti-inflammatory activity was stronger than Indomethacin.

Table 2. Secondary metabolites in WECFR

Secondary metabolites	Identification
Polyphenols	+
Terpenoids	-
Anthraquinon	-
Saponin	+
Tannin	+

Table 2 shows the content of secondary metabolites in WECFR including Polyphenols, Tanins and Saponins.

DISCUSSION

Tissue and the lysosome membrane injury are caused by leukocyte infiltration during the inflammatory response, which is the body's defense mechanism against inflammatory activity (Kumar et al. 2011). These leukocytes release the contents of their lysosomes, such as bactericidal enzymes or proteases, which cause further tissue damage and inflammation (Paramita et al. 2017). Tissue or lysosome membrane injury triggers the release of phospholipase A2 (PLA2) (Umapathy et al. 2010) which mediates the hydrolysis of phospholipids to lysophospholipids and free fatty acids, such as arachidonic acid. The arachidonic acid pathway forms prostaglandins and leukotrienes, while the lysophospholipid pathway forms platelet activating factor (PAF) (Meyer et al. 2005). These two phospholipid metabolites are precursors of inflammatory mediators. Inhibition of PLA2 can cause inhibition of COX or LOX and ultimately inhibit the inflammatory process (Umapathy et al. 2010). The release of lysosome contents is also induced by Ca^{2+} influx. Stabilizing the lysosome membrane will prevent Ca^{2+} influx and subsequently the release of the lysosome contents, so that it can prevent tissue damage and exacerbation of the inflammatory response (Yoon and Baek 2005).

The RBC membrane has the same characteristics as the lysosome membrane, thus the RBC hemolysis inhibition method is used to measure the anti-inflammatory activity of medicinal ingredients and plant extracts. RBC which is exposed to harmful substances (such as hypotonic media, heat, methyl salicylate or phenylhydrazine) produces membrane lysis accompanied by hemolysis and hemoglobin oxidation. Hypotonic hemolysis is associated with excessive intracellular fluid accumulation, which results in RBC membrane rupture. Injury to the RBC membrane increases cell susceptibility to secondary damage

through lipid peroxidation which is induced by free radicals and PLA2 release. Membrane stabilization prevents protein leakage and serum fluid into RBC during periods of increased permeability caused by inflammatory mediators (Anosike et al. 2012).

In this study, WECFR stabilized the RBC membrane. This showed that WECFR can prevent the RBC membrane lysis so that it can potentially prevent the release of lytic enzymes such as PLA2 and active inflammatory mediators. The effects of membrane stabilization from WECFR can also inhibit the release of PLA2 enzymes, which play an important role in the inflammatory process; and prevent Ca^{2+} influx, so that the release of lysosome contents is inhibited. Therefore, WECFR is a potential therapeutic agent for treating inflammatory diseases (Yoon and Baek 2005).

This study also showed that the strength of WECFR's anti-inflammatory activity was stronger than Indomethacin, and weaker than CFR's methanol extract (MECFR) (Kosala et al. 2018). This is related to the content of their secondary metabolites. WECFR contains polyphenols (tanin) and saponins (**Table 2**), while MECFR contains polyphenols, saponins, terpenoids and anthraquinone (Kosala 2015). In accordance with the nature of the solvent, it can be explained that organic solvents such as methanol can attract all types of secondary metabolites, both polar and nonpolar (Sahidin 2012), while water solvents can only attract polar secondary metabolites. Terpenoids and anthraquinone in MECFR may also have anti-inflammatory effects, thus increasing the strength of the MECFR's anti-inflammatory effects above WECFR.

CONCLUSION

CFR water extract has a stronger anti-inflammatory effect than Indomethacin. The Secondary metabolites in WECFR are polyphenols (tannins) and saponins.

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