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In vitro and *In vivo* Anti-inflammatory Activities of *Coptosapelta flavescens* Korth Root's Methanol Extract

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ABSTRACT

Coptosapelta flavescens Korth is a liana plant of the Rubiaceae family known in East Kalimantan as Akar Tambolekar or Akar Mereng. Its anti-inflammatory activity has not been scientifically proven *in vitro* or *in vivo*. The current study aimed to prove the anti-inflammatory activity of *C. flavescens* root (CFR) methanol extract *in vitro* and *in vivo*. The anti-inflammatory activity of CFR methanol extract was evaluated *in vitro* by examining its ability to stabilize the membrane of hypotonicity-induced red blood cells (RBC), and *in vivo* study showed the effect on carrageenan-induced inflammation on the left leg of Wistar rats following its oral administration at 1 hour before, during, and after carrageenan induction. Result of *in vitro* study revealed that the EC₅₀ of CFR methanol extract was 1.905 ± 0.119 mg/ml, lower than that of indomethacin (10.288 ± 0.212 mg/ml). *In vivo* study showed that CFR methanol extract at doses of 600 and 1200 mg/kg administered 1 hour before and during carrageenan induction exhibited anti-inflammatory activity, but only the CFR methanol extract showed anti-inflammatory activity at a dose of 1200 mg/kg when administered 1 hour after carrageenan induction. CFR methanol extract exhibited anti-inflammatory activity both *in vitro* and *in vivo*.

INTRODUCTION

Inflammation is a physiological response that protects the body from tissue injury. Acute inflammation, with exudation of fluid and plasma proteins as its main features, occurs very rapidly, and the process can last for few or several minutes to several days. Chronic inflammation occurs when the acute inflammatory process occurs repeatedly or continuously, with the process lasting for several weeks to months and even years (Paramita *et al.*, 2017). Although inflammation is a physiological process within the body, it can manifest as symptoms such as severe pain, rheumatoid arthritis, and asthma. Standard anti-inflammatory drugs are used to alleviate these symptoms, such as *non-steroidal* anti-inflammatory drugs (NSAIDs) and *corticosteroids*. NSAIDs and *corticosteroids*

inhibit the enzymes *cyclooxygenase* (COX) and *phospholipase* A₂ (PLA₂), respectively (Oyekachukwu *et al.*, 2017). Despite their benefits, these drugs have some side effects. Nonselective NSAIDs inhibit not only COX₂ but also COX₁, triggering the inhibition of *prostacyclin* and *prostaglandin* E₂ (PGE₂). In the stomach, *prostacyclin* and PGE₂ protect gastric mucosa against exposure to stomach acid. The side effects of long-term use of NSAIDs include nausea, vomiting, peptic ulcers, and gastric bleeding (Paramita *et al.*, 2017). Selective COX inhibitors also cause side effects, such as increased risk of heart attack and stroke (Oyekachukwu *et al.*, 2017). The disadvantage of *corticosteroids* is the occurrence of resistance to corticosteroids (Vazquez-Tello *et al.*, 2013). Given the various side effects of NSAIDs and *corticosteroids*, there is a need for alternative anti-inflammatory drugs with minimum adverse effect, especially those derived from natural ingredients. In Indonesia, particularly Kalimantan, various plants with potential anti-inflammatory activity are still unexplored, including the roots of *Coptosapelta flavescens* Korth (Ismail *et al.*, 2012; RIMU, 2015).

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4 *C. flavescens* is a liana plant of the Rubiaceae family. In East Kalimantan, it is known as Akar Tambolekar or Akar Merung. Its root is used as a medicine to treat a cough, shortness of breath (Darusman, 2004), and toothache (Mulyoutami *et al.*, 2009). In Vietnam, it is used for treating rheumatism and healing wounds. Its saponin content was reported to show anti-inflammatory activity against carrageenan-induced inflammation in rat legs (Tran and Tran, 2010). Its anti-inflammatory activity can be evaluated by its ability to inhibit carrageenan-induced release of histamine, serotonin, and bradykinin in the early phase and prostaglandins in the late phase of inflammation (Zhang *et al.*, 2013), as well as by its ability to prevent or treat inflammation following its administration before, during, or after carrageenan induction. However, there is no scientific data on evaluation of its anti-inflammatory activity through its ability to inhibit lysis and increase the stability of the lysosome membrane, which is analogous to the Red Blood Cells (RBC) membrane.

The aim of this study was to examine *in vitro* effect of *C. flavescens* root (CFR) methanol extract on hypotonicity-induced rat RBC membrane and *in vivo* effect of CFR methanol extract on carrageenan-induced inflammation in rat's left leg following CFR administration at one hour before, during, and one hour after induction.

MATERIALS AND METHODS

Preparing of CFR methanol extract

Samples of CFR were collected from Paser District, East Kalimantan and identified by the Dendrology and Forest Ecology Laboratory, Faculty of Forestry, Mulawarman University. From simplicia processing up to both *in vitro* and *in vivo* anti-inflammatory activity test, all were conducted in Pharmacology Laboratory, Faculty of Medicine, Mulawarman University.

Ground and dried CFR simplicia (300 g) was macerated in 1.5 liters of proanalytic methanol solution for 5 days while being shaken, after filtering methanol was added to the residue for 3 days while being stirred and this was repeated until a clear filtrate was obtained. All filtrate was collected and evaporated using a 50°C vacuum rotavapor until only a viscous mass remained. It was then inserted into a desiccator containing blue silica gel in an oven at 50°C to form a dry extract with a water content of <10%. The dried extract was then weighed and the yield was calculated and demonstrated by weight percentage (w/w) of the dried CFR extract weight and CFR simplicia powder weight. The dried extract was stored in the refrigerator at 4°C until before its use.

In vitro membrane stability assay

Preparation of Red Blood Cell (RBC) suspension

Blood isolated from rat was mixed with an anti-coagulant. Blood was centrifuged at 3000 rpm for 10 min and the cells were washed three times with isosaline (0.85%, pH 7.2). The blood volume was reproduced to 10% v/v suspension with isosaline.

Hemolysis induced by hypotonic fluids

Mixture of 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 CFR extract at various concentrations (0.55; 1.125; 2.75; 5.5; 10.8 and 21.5 mg/ml)

and standard drug indomethacin of various concentrations (2.75; 5.5; 13.5; 27.0; 54.0 and 108.0 mg/ml) and controls (aquabides replacing hyposaline to produce 100% haemolysis) were incubated at 56°C for 30 min in a water bath and each centrifuged at 3000 rpm for 10 min. The supernatant was measured using a spectrophotometer at a wavelength of 560 nm. Percentage of membrane stability protection was calculated by the formula: % protection = 100 - [(Optical density of test sample/optical density of control) × 100] (Oyedapo *et al.*, 2010; Anosike *et al.*, 2012). After obtaining the dose curve and percentage of cell membrane protection, the extracts' EC₅₀ was calculated and compared with standard drug (Chandrappa *et al.*, 2012).

In vivo anti-inflammatory activity assay

Animal

7 Male Wistar rat weighed 200-300 g obtained from Pharmacology Laboratory, Faculty of Medicine, Mulawarman University, kept at room temperature in a 12 hours dark/12 hours light condition, fed and drank *ad libitum*. Ethical Clearance was obtained from the Ethics Committee of the Faculty of Medicine Mulawarman University.

Carrageenan induction

Anti-inflammatory activity of CFR methanol extract was tested by carrageenan induction on rat's soles, the volume of edema occurring was measured using a plethysmometer (Ugo Basile, Italy). Rats fasted for 18 hours before treatment, only water was given *ad libitum*. Rats were grouped into 3 experiments with each experiment consisting of 5 groups with 5 rats in each group (calculated using Federer formula) (Dande *et al.*, 2010). Group 1 was a negative control (NC) in which the mice were given only 0.5% Carboxy Methyl Cellulose (CMC) solution; group 2 was positive control given Indomethacin 20 mg/kg BW; groups 3, 4 and 5 were given a solution of CFR methanol extract at dose 300, 600 and 1200 mg/kg BW. All test materials were dissolved in 0.5% CMC and 1 ml was given orally to each rat. One hour before carrageenan induction, the left foot's volume of all rats was measured.

1) *Administration of CFR methanol extract one hour before carrageenan induction*: before carrageenan injection, rat's left foot was measured after the administration of the test material. One hour later Carrageenan 0.1% in 0.1 ml normal saline was injected subcutaneously on the rat's left foot, then the volume of edema occurring at the rat's sole was measured using a plethysmometer. Afterward, hourly measurements of edema volume on the rat's sole were done up to six hours after inflammation induction.

2) *Administration of CFR methanol extract during carrageenan induction*: Shortly after injection of carrageenan, CFR methanol extract was administered and the rat's left foot volume was measured, followed by hourly measurements of edema volume of rat's sole up to six hours after inflammation induction.

3) *Administration of CFR methanol extract one hour after carrageenan induction*: CFR methanol extract was given One hour after carrageenan induction, then the volume of rat's left foot was measured followed by hourly measurements of edema volume of rat's sole up to six hours after inflammation induction.

Table 1: Percent of RBC membrane stability protection against hyposaline induction on the administration of CFR methanol extracts and Indomethacine.

Conc Code	Percent of Inflammation Protection of Indomethacine & EMCFR on RBC membrane induced with hyposaline							
	Indom				EMCFR			
	Conc (mg/ml)	Mean	±	SEM	Conc (mg/ml)	Mean	±	SEM
1	0.34	3.08	±	0.41	0.07	17.95*	±	1.31
2	0.68	7.74	±	0.84	0.14	19.65*	±	0.33
3	1.69	15.26	±	0.61	0.34	30.44*	±	2.02
4	3.38	18.70	±	0.64	0.68	38.22*	±	1.80
5	6.76	31.56	±	1.38	1.35	45.53*	±	1.68
6	13.51	65.49	±	1.09	2.70	56.91*	±	0.71
	EC ₅₀	10.29	±	0.21	EC ₅₀	1.90*	±	0.12

Indom = Indomethacine; EMCFR = *Coptosapelta Flavescens* root's methanol extract; n = 3; data was analyzed with t-test, p < 0.05, *shows data with significant difference.

After 24 hours, the last measurement was done. The percentage increase in edema volume was calculated using the following formula: $100 \times (V_1 - V_0) / V_0$ where V_1 is the rat's leg edema volume after carrageenan injection, V_0 is the rat's leg edema volume before carrageenan injection (George *et al.*, 2014; Shaikh *et al.*, 2016).

Statistical analysis

Data on membrane stabilization was mean ± Standard Error Mean (s.e.m.) of 3 repetitions, analyzed with t-test at 95% confidence level (p < 0.05).

Data on *in vivo* test was mean ± Standard Error Mean (s.e.m.) of 5 repetitions. Data were analyzed using Sigmaplot 12.5 with Analysis of Variance (ANOVA) and Tukey's test, at 95% confidence level (p < 0.05).

RESULTS

In vitro membrane stability assay

Data in Table 1 is the percentage of RBC membrane protection against hyposaline induction by CFR methanol extract and indomethacin. This data showed the higher the concentrations of CFR methanol extracts the greater is the % RBC membrane

protection. After calculation, EC₅₀ (concentration at which 50% protection occurs) was obtained. The lower the EC₅₀ the higher is the protection activity. EC₅₀ of CFR methanol extract was 1.90 ± 0.12 mg/ml, which was lower and significantly different than EC₅₀ of indomethacin at 10.29 ± 0.21 mg/ml. This showed that the anti-inflammatory activity through RBC membrane lysis protection is stronger on CFR methanol extracts compared to indomethacin.

In vivo anti-inflammatory activity assay

Table 2 shows the percentage of increase in the volume of rat's left foot edema after carrageenan induction in which CFR methanol extract was given orally One hour before induction. The data showed that CFR methanol extract at doses 300, 600 and 1200 mg/kg BW have anti-inflammatory activity. On 300 mg/kg BW of CFR methanol extract, anti-inflammatory activity was seen after the 2nd to the 5th hour but in the 6th hour, there was no anti-inflammatory activity anymore. CFR methanol extract at 600 mg/kg BW had anti-inflammatory activity after the 2nd hour to the 24th hour but not significant when compared with Indomethacin group (P > 0.05) whereas 1200 mg/kg BW had anti-inflammatory activity from 0 hours to the 24th hour at a significantly higher level than indomethacin group (P < 0.05).

Table 2: Percent increase in the volume of rat's foot edema when EMCFR was administered 1 hour before carrageenan induction.

Time Interval (hour)	Percent increase in edema volume of rat's foot when EMCFR was given 1 hour before carrageenan induction														
	NC (%)			Indom (%)			EMCFR1 (%)			EMCFR2 (%)			EMCFR3 (%)		
	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m
0	36.98	±	2.39	19.37*	±	1.58	8.81**	±	0.50	11.39**	±	0.87	19.51*	±	1.52
1	44.44	±	2.62	21.03*	±	0.85	27.72**	±	1.23	22.67*	±	1.07	13.50**	±	0.42
2	55.37	±	2.10	28.13*	±	1.48	34.05*	±	1.42	28.64*	±	1.19	9.68**	±	0.26
3	59.52	±	2.66	25.75*	±	1.52	30.85*	±	1.36	25.97*	±	0.75	16.16**	±	1.25
4	53.84	±	3.23	19.09*	±	1.23	25.36*	±	0.81	18.45*	±	0.86	14.39*	±	1.09
5	44.96	±	3.69	14.43*	±	0.79	28.40**	±	0.64	11.97*	±	0.65	9.19*	±	0.58
6	45.53	±	2.94	9.65*	±	1.02	45.58*	±	1.66	9.33*	±	0.70	7.05*	±	0.44
24	22.97	±	2.68	3.87*	±	0.54	11.76**	±	1.04	2.66*	±	0.38	3.47*	±	0.43

NC = Negative Control = CMC 0.5%; Indom = Indomethacine 20 mg/kgBW; EMCFR1 = *Coptosapelta Flavescens* root's methanol extract 300 mg/kg BW; EMCFR2 = *Coptosapelta Flavescens* root's methanol extract 600 mg/kg BW; EMCFR3 = *Coptosapelta Flavescens* root's methanol extract 1200 mg/kg BW; n = 5 rats, Data was analyzed with ANOVA Tukey's test, p < 0.05 *shows significant difference compared to NC; **significant difference compared to NC and indomethacine.

Table 3: Percent increase in the volume of rat's leg edema when EMCFR was given during carrageenan induction.

Time Interval (hour)	Percent increase in edema volume of rat's leg when EMCFR was given during carrageenan induction														
	NC (%)			Indom (%)			EMCFR1 (%)			EMCFR2 (%)			EMCFR3 (%)		
	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m
0	20.70	±	2.17	21.43	±	3.24	15.93	±	0.85	16.33	±	0.26	16.14	±	0.95
1	30.58	±	2.77	26.13	±	3.27	19.89*	±	0.75	17.35**	±	0.77	15.83**	±	0.40
2	35.93	±	2.16	20.37*	±	2.97	23.26*	±	0.92	17.82*	±	0.79	4.21**	±	0.35
3	40.35	±	2.15	19.97*	±	4.61	28.20*	±	1.31	17.71*	±	1.49	15.21*	±	0.79
4	32.48	±	2.67	12.72*	±	2.73	23.83*	±	1.26	13.47*	±	1.57	13.10*	±	0.69
5	23.97	±	2.32	7.67*	±	1.46	20.29°	±	0.70	10.94*	±	1.39	8.71*	±	0.68
6	32.04	±	3.27	4.54*	±	0.69	37.88°	±	1.36	5.36*	±	1.01	6.25*	±	0.47
24	12.01	±	1.63	4.73*	±	1.39	14.22°	±	1.34	-0.43*	±	0.96	5.25*	±	0.36

NC = Negative Control = CMC 0.5%; Indom = Indomethacin 20 mg/kgBW; EMCFR1 = *Coptosapelta Flavescens* root's methanol extract 300 mg/kg BW; EMCFR2 = *Coptosapelta Flavescens* root's methanol extract 600 mg/kg BW; EMCFR3 = *Coptosapelta Flavescens* root's methanol extract 1200 mg/kg BW; n = 5 rats, Data was analyzed with ANOVA and Tukey's test, p < 0.05 *shows significant difference compared to NC; **shows significant difference compared with NC and indomethacin; °shows significant difference compared to Indomethacin.

Table 4: Percent increase in the volume of rat's leg edema when EMRCF was given 1 hour after carrageenan induction.

Time Interval (hour)	Percent increase in edema volume of rat's leg when EMACF was given 1 hour after carrageenan induction														
	NC (%)			Indom (%)			EMCFR1 (%)			EMCFR2 (%)			EMCFR3 (%)		
	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m	Mean	±	s.e.m
0	20.49	±	2.64	26.14	±	2.06	12.32*	±	1.55	21.36	±	1.28	18.95	±	0.65
1	24.86	±	3.13	35.45*	±	1.80	18.15	±	1.23	29.44	±	1.30	24.14	±	1.16
2	29.14	±	3.43	31.73	±	1.81	24.98	±	1.59	36.96	±	2.05	28.10	±	1.47
3	34.10	±	3.30	27.48	±	1.78	33.61	±	1.46	41.75*	±	2.45	26.00	±	1.59
4	25.93	±	3.05	19.00	±	1.51	25.76	±	1.46	31.20*	±	2.56	18.45	±	1.76
5	21.15	±	3.01	12.47	±	1.57	23.20*	±	1.48	27.05*	±	2.55	15.82	±	1.35
6	24.76	±	3.99	8.89*	±	0.91	40.29**	±	2.02	23.51*	±	2.02	13.30*	±	1.16
24	6.88	±	0.96	5.59	±	1.02	16.47**	±	0.98	5.21	±	0.56	8.96	±	0.99

NC = Negative Control = CMC 0.5%; Indom = Indomethacin 20 mg/kgBW; EMCFR1 = *Coptosapelta Flavescens* root's methanol extract 300 mg/kg BW; EMCFR2 = *Coptosapelta Flavescens* root's methanol extract 600 mg/kgBW; EMCFR3 = *Coptosapelta Flavescens* root's methanol extract 1200 mg/kgBW; n = 5 rats, Data was analyzed with ANOVA and Tukey's test, p < 0.05, *shows significant difference compared to NC; **shows significant difference compared with NC and indomethacin; °shows significant difference compared to Indomethacin.

Table 3 shows the percentage of increase in the volume of the rat's leg edema in which CFR methanol extract was given during carrageenan induction. The data showed that EMCFR at 300 mg/kg BW dose (EMCFR1) administered during carrageenan induction resulted in anti-inflammatory activity. However, it was not significant when compared with the Indomethacin group and after the 5th hour, the anti-inflammatory activity weakened. Anti-inflammatory activity of 600 mg/kg BW EMCFR2 between 1st to 24th hour is not significantly higher compared with Indomethacin group, while for EMCFR3 after the 2nd and the 3rd hour anti-inflammatory activity are significantly higher than Indomethacin group.

Table 4 shows the percentage of increase in the volume of the rat's leg edema which was being administered with

EMCFR at 1st hour after carrageenan induction. Data showed that EMCFR at doses 300 and 600 mg/kg BW do not have anti-inflammatory activity, while EMCFR at dose 1200 mg/kg BW has anti-inflammatory activity at 3rd to the 24th hour, similar with indomethacin group.

DISCUSSION

Leukocyte infiltration occurs during inflammatory response due to its role in defense against inflammation. These cells release their lysosomal contents, such as bactericidal enzymes and protease, which cause further tissue damage and inflammation. Injury to the cell membrane increases the cells' susceptibility to secondary damage by the free radicals produced by lipid peroxidation. As the RBC membrane is similar

in characteristics to the lysosome membrane, the inhibition of RBC hemolysis is used to measure the anti-inflammatory activity of medicinal substances, including plant extracts. Injury to lysosome membrane triggers the release of phospholipase A2 (PLA2) (Umapathy *et al.*, 2010) that mediates the hydrolysis of phospholipids into lysophospholipids and free fatty acids, such as arachidonic acid. Both phospholipids metabolites are precursors of inflammatory mediators. The arachidonic acid pathway forms prostaglandins and leukotriene, whereas lysophospholipids pathway forms platelet activating factor (PAF) (Meyer *et al.*, 2005). PLA2 inhibition may lead to the inhibition of COX or LOX and eventually the inflammatory process (Yoon and Baek, 2005). Stabilization of the lysosomal membrane prevents the lysis and subsequent release of cytoplasmic contents, averting tissue damage and exacerbation of inflammatory responses (Umapathy *et al.*, 2010).

In the present study, the CFR methanol extract exhibited higher ability to stabilize RBC membrane than indomethacin, indicating that the CFR methanol extract may prevent the lysis of the RBC membrane. Since the membranes of RBC and lysosome share similar properties, the membrane-stabilizing effect of the CFR methanol extract may also inhibit the release of the PLA2 enzyme, which plays an important role in inflammatory processes. Therefore, this extract could become a potential therapeutic agent to treat human inflammatory diseases (Yoon and Baek, 2005).

Lysosome lysis occurs during inflammation, releasing enzymes that produce various tissue damages. NSAIDs inhibit the release of lysosomal enzymes by stabilizing lysosomal membranes, preventing lysis. RBC exposure to harmful conditions or substances, such as hypotonic media, heat, methyl salicylate, or phenylhydrazine results in membrane rupture followed by hemolysis and oxidation of hemoglobin. Hypotonic hemolysis is associated with excessive accumulation of intracellular fluid, resulting in the rupture of RBC membrane. Injury to the RBC membrane increases the cells' susceptibility to secondary damage through free radical-induced lipid peroxidation and release of PLA2. Membrane stabilization prevents the leakage of serum proteins and fluids into the RBC during periods of increased permeability induced by inflammatory mediators (Anosike *et al.*, 2012). In this study, CFR methanol extract stabilized RBC membrane, potentially by preventing the release of lytic enzymes such as PLA2 and active inflammatory mediators.

In vivo anti-inflammatory activity of the CFR, methanol extract was examined by measuring the edema volume of carrageenan-induced rat legs. CFR methanol extracts orally administered One hour before carrageenan induction inhibited the release of inflammatory mediators and the action of the COX2 enzyme. However, its administration at low doses of 300 mg/kg BW after 5 hours post-induction showed no anti-inflammatory effect.

Acute inflammatory response induced by carrageenan injection occurs in two phases: early phase occurs immediately after carrageenan exposure and lasts for one hour, indicated by the release of histamine, serotonin, bradykinin, and a low amount of prostaglandin mediators. The late phase occurs One hour after carrageenan exposure due to infiltration of polymorphonuclear (PMN) leukocytes and the formation of advanced prostaglandins

(Zhang *et al.*, 2013). Suppression of the early phase may be associated with the inhibition of the release of early mediators, such as serotonin and histamine, whereas suppression of the second phase may be attributed to the inhibition of cyclooxygenase activity (Lande *et al.*, 2015).

Anti-inflammatory activity of CFR methanol extract at a dose of 1200 mg/kg BW administered One hour before carrageenan injection occurred in both early and late phases, suggesting its ability to prevent the release of histamine and serotonin, as well as the formation of prostaglandins by inhibiting the COX2 enzyme. CFR methanol extract at doses of 300 and 600 mg/kg BW administered One hour before carrageenan induction exhibited anti-inflammatory activity against the late phase of inflammation, showing its inhibitory effect on the formation of prostaglandins.

CFR methanol extract at doses of 600 and 1200 mg/kg BW administered during carrageenan induction suppressed the late phase of inflammation, possibly through COX2 inhibition.

However, only CFR methanol extract at a dose of 1200 mg/kg BW showed anti-inflammatory activity through COX2 inhibition when administered at One hour after carrageenan induction. Thus, the present study revealed that a high dose of CFR methanol extract at 1200 mg/kg BW is effective in preventing and treating carrageenan-induced inflammation *in vivo*.

Both *in vitro* and *in vivo* test results showed that CFR methanol extract exerts anti-inflammatory activity through the stabilization of RBC membrane, which is analogous to lysosomal membrane, suggesting its ability to prevent the lysosomal release of PLA2, histamine, serotonin, and bradykinin, as well as the formation of prostaglandin during the inflammatory response.

Anti-inflammatory activity of CFR methanol extract may be related to its secondary metabolite content. The CFR methanol extract has been known to contain saponins, polyphenols, terpenoids, steroids, and anthraquinones (Kosala, 2015).

Saponins consist of triterpenoids or steroidal aglycons and oligosaccharide substituents. The hydrophilic properties of its sugar portion and the lipophilic nature of its aglycon contribute to the amphiphilic or surfactant properties of saponins, which are responsible for their ability to form stable foams and form complexes with membrane steroids and lipids (Hassan *et al.*, 2012). A saponin from *Coptosapelta tomentosa* ethanol extract has been investigated and found to exhibit anti-inflammatory activity (Tran and Tran, 2010). Other researchers have also proven the anti-inflammatory activity of crude saponin extracts from other plants, such as *Sesbania sesban* (L.) leaf Merr (Dande *et al.*, 2010) and the Nigerian plants *Schwenkia americana* Linn whole plant, *Asparagus africanus* Lam rhizomes, *Dichrostachys cinerea* Linn leaf, *Ficus iteophylla* Miq stem, and *Indigofera pulchra* Willd leaf (Hassan *et al.*, 2012).

Polyphenols are divided into 4 major groups, namely flavonoids, stilbenes, lignans, and phenolic acids. Polyphenols from food materials have also been reported to have anti-inflammatory activity. Catechin and epigallocatechin gallate from green tea may inhibit COX2 expression induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), tumor promoters in rat skin, and COX activity in macrophages induced by lipopolysaccharides (LPS). Stilbene compound resveratrol may suppress carrageenan-induced edema on rat's foot through COX activity inhibition. Flavonoids

3 kaempferol, quercetin, morin, and myricetin inhibit 5-LOX activity (Yoon and Baek, 2005). Curcumin inhibits the peroxidase activity of COX and 5-LOX activity, blocking cPLA2, and thereby reducing COX2 and LOX expression (Jurenka, 2009).

Steroids from the gorgonian coral *Pinnigorgia sp.* reportedly inhibit the accumulation of pro-inflammatory proteins iNOS and COX-2 in RPS-64.7 macrophage cells induced by LPS (Su *et al.*, 2016).

Terpenoids have been reported to exhibit anti-inflammatory effects through NFκB-inhibiting mechanisms that play an important role in the regulation of immune and inflammatory responses (Heras *et al.*, 2009).

9 Moreover, anthraquinone from the roots of *C. flavescens* have been reported to show anti-protozoal activity (Houkong *et al.*, 2014)

Therefore, almost all secondary metabolites present in the CFR methanol extract (saponins, polyphenols, terpenoids, and steroids) have been reported to exhibit anti-inflammatory activities.

CONCLUSION

The methanol extract of the roots of *Coptosapelta flavescens* Korth has anti-inflammatory activity by stabilizing the lysosome membrane *in vitro*, and inhibits the release of inflammatory mediators of histamine, serotonin, and bradykinin and inhibits COX *in vivo*. Suggested follow-up studies will be to ascertain if its anti-inflammatory activity is via PLA2 inhibition.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

ABBREVIATION USED

CFR: *Coptosapelta flavescens* Roots;
RBC: Red Blood Cells;
NSAIDs: Non Steroid Anti Inflammatory Drugs;
COX: Cyclooxygenase;
LOX: Lipoygenase;
PLA2: Phospholipase A2;
PGE2: Prostaglandin E2;
EC: Effective Concentration;
NC: Negative Control;
CMC: Carboxy Methyl Cellulose;
BW: Body Weight.

REFERENCES

Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). DARU J Pharm Sci, 2012; 20:76.
Chandrapa CP, Govindappa M, Anil Kumar NV, Channabasava R. Invitro Anti-Inflammatory Activity of Carmona Retusa (Vahl.). World J of Pharm and Pharm Sci, 2013; 2(5):3991-3997.
Dande PR, Talekar VS, Chakraborty GS. Evaluation of crude saponins extract from leaves of *Sesbania sesban* (L.) Merr. for topical anti-inflammatory activity. Int. J. Res. Pharm. Sci, 2010; 1(3):296-299.

Darusman LK. FINAL TECHNICAL REPORT, The Potential of Medicinal Plants to Support Sustainable Forest Management: Ecological, Economic, and SOciocultura. I Aspects, Bogor Indonesia. 2004.

George A, Chinnappan S, Chintamaneni M, Kotak CV, Choudhary Y, Kueper T, Radhakrishnan AK. Anti-inflammatory effects of Polygonum minus (Huds) extract (Lineminus™) in *in-vitro* enzyme assays and carrageenan induced paw edema. BMC Complement and Altern Med, 2014; 14:355.

Hassan, HS, Sule MI, Musa AM, Musa KY, Abubakar MS, Hassan AS. Anti-Inflammatory Activity of Crude Saponin Extracts from Five Nigerian Medicinal Plants. Afr J Tradit Complement Altern Med, 2012; 9(2):250-255.

Heras BDL, Sonsoles H. Molecular Basis of the Anti-Inflammatory Effects of Terpenoids, Inflamm & Allergy-Drug Targets, 2009; 8 (1): 28-39(12),

Houkong K, Sawangjaroen N, Kongyen W, Rukachaisirikul V, Voravuthikunchai SP, Phongpaichit S. Anti-intestinal protozoan activities of 1-hydroxy-2 hydroxymethylanthraquinone from *Coptosapelta flavescens*. Asian Pac. J Trop Dis, 2014; 4(6):457-462.

Ismail S, Mujiono K, Taufik I, Razaki HA, Merry. Research on Local Ethnomedicine and Medicinal Knowledge Exploration in Indonesia: Community-Based in East Kalimantan. Agency for Health Research and Development, Ministry of Health, Jakarta 2012. [Text in Indonesia].

Jurenka JS. Anti-inflammatory Properties of Curcumin, a Major Constituent of Curcuma longa: A Review of Preclinical and Clinical Research. Altern Med Rev, 2009; 14(2):141-153.

Kosala. Uji Fitokimia dan Toksisitas Fraksi Ekstrak Akar Tambolekar (*Coptosapelta flavescens* Korth) Dengan Reaksi Warna dan *Brine Shrimp Lethality Test*. Molucca Medica Jurnal Kedokteran dan Kesehatan, 2015; 8(1) [Text in Indonesia].

Lande AA, Ambavade SD, Swami US, Adkar PP, Ambavade PD, Waghmare AB. Saponins isolated from roots of Chlorophytum borivilianum reduce acute and chronic inflammation and histone deacetylase. J Integr Med, 2015; 13(1):25-33.

Meyer MC, Rastogi P, Beckett CS, McHowat J. Phospholipase A2 Inhibitors as Potential Anti-Inflammatory Agents. Curr Pharm Des, 2005; 11(10).

Mulyoutami E, Rismawan R, Joshi L. Local knowledge and management of simpukng (forest gardens) among the Dayak people in East Kalimantan, Indonesia, Forest Ecology and Management. FORECO-11489, p 8. 2009.

Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. Int J Plant Physiol and Biochem, 2010; 2(4):46-51.

Oyekachukwu AR, Elijah JP, Eshu OV, Nwodo OFC. Anti-Inflammatory Effects of The Chloroform Extract of *Ammonia muricata* Leaves on Phospholipase A2 and Prostaglandin Synthase Activities. Transl Biomed, 2017; 8(4): 137.

Paramita S, Kosala K, Dzulkifli D, Saputri DI, Wijayanti E. Anti-inflammatory activities of ethnomedicinal plants from Dayak Abai in North Kalimantan, Indonesia. Biodiversitas, 2017; 18(4):1556-1561

RIMU [Research Institute of Mulawarman University]. Research on Local Ethnomedicine and Medicinal Knowledge Exploration in Indonesia: Community-Based in East and North Kalimantan. [Research Report]. Agency for Health Research and Development, Ministry of Health, Jakarta. 2015. [Text in Indonesia].

Shaikh RU, Pund MM, Gacche RN. Evaluation of anti-inflammatory activity of selected medicinal plants used in Indian traditional medication system *in vitro* as well as *in vivo*. J. of Trad and Complement Med, 2016; 6:355-361.

Su Y-D, Cheng C-H, Wen Z-H, Wu Y-C, Sung P-J. New anti-inflammatory sterols from a gorgonian *Pinnigorgia sp.* Bioorganic & Med Chem Lett, 2016; 26(13):3060-3063

Tran TVA, Tran H. Study Chemical Constituents in Anti Inflammatory Extracts of *Coptosapelta TomENTOSA*. Y Hoc TP. Ho Chi

Minh, 2010; 14(1):116–122

Umapathy E, Ndebia EJ, Meeme A, Adam B, Menziwa P, Nkeh-Chungag BN, Iputo JE. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J. Med Plants Res*, 2010; 4(9):789-795.

Vazquez-Tello A, Halwani R, Hamid Q, Al-Muhsen S. Glucocorticoid Receptor-Beta Up-Regulation and Steroid Resistance Induction by IL-17 and IL-23 Cytokine Stimulation in Peripheral Mononuclear Cells. *J Clin Immunol*, 2013; 33(2):466–478.

Yoon J-H, Baek SJ. Molecular Targets of Dietary Polyphenols with Anti-inflammatory Properties. *Yonsei Med J*, 2005; 46(5):585–596.

Zhang J, Wang H, Wang T, Chong Y, Yu P, Lu C, Xue Y, Fu F, Zhang L. Anti-inflammatory activity of Yanshu spraying agent in animal models. *Exp and Ther Med*, 2013; 5:73-76.

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