Research Article

The Potency of Cinnamon Bark Oil (*Cinnamomum burmannii* (Nees & T. Nees) Blume from 5 Regions in Indonesia as Anti-Inflammatory

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ABSTRACT

Introduction: An alternative anti-inflammatory with milder side effects is needed. One of them can be sourced from plants such as *Cinnamomum spp*. Indonesia is the largest producer of cinnamon bark (*Cinnamomum burmannii* (Nees & T. Nees) Blume) in the world. Thus, research is needed to increase the benefits and database of *C. burmannii* bark, especially as an anti-inflammatory. This study aims to determine the metabolite profile of the essential oil of *C. burmannii* bark (CBO) from 5 regions in Indonesia as an anti-inflammatory. **Methods**: The location of the sampling was carried out by fulfilling 3 criteria, namely the difference in the island, province, and the area that produces the bark of *C. burmannii*. Analysis was performed using GC-MS Agilent 7890B GC and 5977B MSD. A total of 40 male rats (*Rattus novergicus*) Wistar strain were divided into 8 groups, each group consisted of 5 rats. Group 1 as negative control treated with 1% CMC-Na solution, group 2 as positive control treated with ibuprofen at a dose of 27 mg/kg BW, Group 3, 4, 5, 6, 7, and 8 treated with CBO from 5 locations. After 30 min of treatment, experimental animals were injected left paw with carrageenan1%/100µl/paw. Measurements of left paw swelling were carried out at 1, 2, 3, and 4 hours and the animals were sacrificed. Edema inhibition percentage, number of inflammatory cells, and TNF-α expression were analyzed. **Result**: The results of this study indicate that CBO from 5 regions in Indonesia has anti-inflammatory properties. **Conclusion**: There was no significant difference in anti-inflammatory activity CBO from 4 regions, namely from Kerinci, Lombok Timur, Karanganyar, and Gorontalo areas.

Key words: Anti-inflammatory, Cinnamon bark Oil, Metabolite profile, Essential oil.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are usually used to reduce inflammation. NSAIDs are effective in reducing inflammation but have side effects such as gastrointestinal bleeding, increased blood pressure, increased risk of blockages leading to heart failure, clotting, and death of the heart muscle. Therefore, alternative anti-inflammatory drugs are needed with milder side effects[1]. One of them can be sourced from plants such as *Cinnamomum* spp. Trans-cinnamaldehyde which is the main component of *Cinnamomum* spp. has been studied to have anti-inflammatory effect[2,3]. Indonesia is the largest producer of cinnamon bark (*Cinnamomum burmannii* (Nees & T. Nees) Blume) in the world[4]. Therefore, research is needed to increase the benefits and complete the cinnamon bark database, especially as an anti-inflammatory.

The pharmacological activity of a drug preparation with active ingredients from plants such as cinnamon bark is influenced by its active compounds, namely secondary metabolites (metabolites). Cinnamon bark essential oil (CBO) which is composed of hundreds or even thousands of volatile components is very suitable to be analyzed using GC-MS which is suitable for the determination of volatile compounds because of its separation efficiency and sensitive detection[5,6]. Metabolites are synthesized by plants in limited quantities to defend themselves from their habitats and do not play an important role in primary metabolic processes. Metabolites are the end products of all cellular processes and the direct result of the activity of enzymes and proteins. Metabolites are more proximal to phenotype or disease than genetic or proteomic information[7]. Research on cinnamon bark essential oil from various places of plant origin in Indonesia needs to be carried out to determine differences in the number and types of metabolites associated with anti-inflammatory activity.

The main regulatory factor in the inflammatory response is the NF-kB family of transcription factors. This family of proteins is present in almost all human cells. In inflammatory cells such as macrophages and lymphocytes, NF-kB is activated upon cell stimulation by pro-inflammatory stimuli. Its activation causes the transcription of many different genes involved in the inflammatory response, including cytokines such as TNF-α. Inhibition of NF-kB is a target in the development of anti-inflammatory drugs[8]. Inflammation is a normal protective response caused by infectious and non-infectious agents and cell damage. The inflammatory response induces increased permeability of the endothelial cell layer and the entry of leukocytes into the interstitium and the release of cytokines such as interleukins and tumor necrosis factor-α (TNF-α). It also stimulates the activity of several enzymes (oxygenase, nitric oxide synthase, peroxidase, etc.), as well as arachidonic acid metabolism[9]. Signs of inflammation, in general, are swelling, pain, redness, heat, and loss of function (*functio laesa*). The etiology

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of inflammation is non-infectious factors, physical causes: burns, frostbite, physical wounds, foreign bodies, trauma, radiation. Chemical causes: glucose, fatty acids, toxins, alcohol, carrageenan. Biological causes: damaged cells. Causes due to infection: bacteria, viruses, and other microorganisms[10].

MATERIALS AND METHODS

Plant material

The essential oil was distilled from cinnamon bark from 5 regions aged between 10 and 12 years with a stem circumference of 60 to 80 cm, namely from Karanganyar Regency on the slopes of Mount Lawu in the middle of Java Island, Hulu Sungai Selatan Regency on the slopes of the Meratus Mountains on the Kalimantan Island, Lombok Timur Regency on the island of Lombok, Kerinci Regency on the slopes of Mount Kerinci on the island of Sumatra, and Gorontalo Regency on the island of Sulawesi. Plant determination was carried out in the laboratory of the Indonesian Institute of Sciences in Purwodadi (LIPI), Jawa Timur. Determination was done by using samples of leaves, flowers, and fruit taken directly from the harvested plant. *C. burmannii* bark was the inner bark without an outer cork layer with a Loss on Drying equal to or less than 10%. Cinnamon bark used has met the standards of the Indonesian Herbal Pharmacopoeia[11].

Chemicals

Trans-Cinnamaldehyde compounds ≥ 98% (Merck), dichloromethane (Merck), Distilled water, NaCl, Na_2SO_4, Carrageenan (Merck), Ibuprofen, TNF-α monoclonal antibody E-AB-22159 Elabscience Biotechnology Inc., ketamine, xylazine base 20 mg/ml brand Xyla, 0.9% sodium chloride Intravenous Infusion B.P. Otsu-NS brand, formaldehyde buffer.

Experimental animal

The experimental animals were male rats (*Rattus novergicus*) Wistar strain with the criteria weighing 180-220 g, in good health (active movement, clean hair, and clear eyes), aged 3 months. Rats were obtained from the Pharmacology Laboratory, Faculty of Medicine, Universitas Airlangga. The research was conducted after obtaining an Ethical Clearance Certificate from the Faculty of Veterinary Medicine, Airlangga University No: 2.KE.075.05.2019.

Essential oil distillation

Cinnamon bark was powdered and sieved with mess 8. 3 kg of cinnamon bark powder was distilled on an electric bath using 5 liters of water. The boiler equipped with a water and powder separator was assembled with a condenser, ensuring there was no leakage and good circulation. The distillation process was carried out from the first drop of distillate to 6 hours and carried out 3 replications. The essential oil was separated from the aqueous phase using a separator flask. Each liter of water phase added 15 grams of NaCl. The oil phase had been separated from the water phase. The collected essential oils are purified by adding Na_2SO_4 to remove the remaining water droplets.

Instrument

GC-MS (Agilent Technologies 7890B Network GC System, 5977B MSD), Moisture Balance Azcet 50, Plethysmometer model 7140 UGO Basile S.R.I.Italy, Tissue processor, microtome, and other tools for staining Hematoxylin-Eosin (HE) and immunohistochemistry (IHK).

Metabolite profiles of CBO

The metabolite profile of CBO was determined using GC-MS Agilent 7890B GC and 5977B MSD interpretation of mass spectra using the National Institute Standard and Technology (NIST) database. Samples were diluted with dichloromethane (10 mg/mL) and 1.0 µl was injected for analysis. The identification of the components was carried out by matching the recorded mass spectra with the mass spectrum standard from the National Institute of Standards and Technology Library (NIST05.LIB). Quantitative analysis of each volatile oil component (expressed as a percentage of area) was carried out by calculating the peak area. Calculated as the average value of 2 samples. GC-MS analysis conditions as seen in Table 1[12].

In vivo anti-inflammatory activity test

A total of 40 male rats were used throughout this study housed under conditions of optimum light, temperature, humidity, and light (27^{0}C ± 2^{0}C, 65–75% humidity and 12 h light-dark cycle), food, and water provided ad libitum. Rats were divided into 8 groups; each group consist of 5 rats. Each rat's left leg was given a permanent ink mark right on the lateral malleolus. Observation begins with all animals measuring paw volume as volume t = 0. Group 1 as negative control treated with 1% CMC-Na solution, group 2 as positive control treated with ibuprofen at a dose of 27 mg/kg, Group 3, 4, 5, 6, 7, and 8 treated with CBO from 5 locations at a dose of 60 mg/kg BW in 1% CMC-Na. After 30 min of treatment, experimental animals were injected with carrageenan1%/100µl/paw (carrier 0.9% sterile NaCl). Measurements of paw swelling were carried out at 1,2, 3, and 4 hours using a plethysmometer[13,14]. After measuring the volume of edema, the animals were sacrificed by injecting ketamine and xylazine. The left paw was cut preserved in formaldehyde buffer liquid as material for HE and IHC preparations[15].

Histological examination

The number of inflammatory cells was counted using the HE technique. The paw tissue was fixed in neutral buffered formalin, then immersed in paraffin, sliced (3-5 m), and stained using hematoxylin and eosin. Histopathological examination to determine the number of inflammatory cells in paw tissue by two anatomical pathologists. Inflammatory cells were counted in five visual fields at 400x magnification. All examinations were carried out using a Nikon H600L

Table 1. GC-MS analysis condition of cinnamon bark essential oil.

Condition GC-MS	Explanation
Column	Agilent column 19091S-433UI HP-5ms Ultra Inert-60°C-325°C (350°C)
Column dimention	30 m x 250 µm x 0.25 µm
run time	30 min
initial oven temperature	100°C
hold time	2 min
post-run	0°C
program rate	5°C value 200°C
hold time	8 min
equilibration time	0.1 min
max temperature	325°C
front injector syringe size	10 µl
injection volume	1 µl
front ss He	in split mode
heater	300°C
pressure	10,523 psi
total flow	14 ml / min
split ratio	10: 1
out MSD initial	100°C
pressure	10,523 *psi*
flow	1 ml / min
average velocity	37,293 cm / sec
hold time	1.3407 min

Table 2. Semi-quantitative Immunoreactive Score (IRS) scale.

X	Y
Score 0 : No positive cells Score	Score 0 : No color reaction
Score 1 : Positive cells less than 10%	Score 1: Weak yellowish color intensity
Score 2 : Positive cells range from 11% - 50%	Score 2: Medium color intensity (brown)
Score 3 : Positive cells range from 51% - 80%	Score 3 : Strong color intensity (dark brown
Score 4: Positive cells more than 80%	

Table 3. The specification of CBO from 5 regions.

No	Origin area	Essential oil levels (%)	Organoleptic	Specific grafity	Refractive Index	trans-Cinnamaldehyde levels (%)
A	Karanganyar	0.6556 ± 0.0560	Distinctive smell	1.0055 ± 0.0007	1.5527 ± 0.0041	40.260 ± 0.240
B	Hulu Sungai Selatan	0.5533 ± 0.0689	Smell more fragrant	1.0033 ± 0.0011	1.5573 ± 0.0041	37.235 ± 0.686
C	Lombok Timur	0.7078 ± 0.0379	Stronger distinctive smell	1.0041 ± 0.0011	1.5627 ± 0.0041	45.715 ± 0.841
D	Kerinci	0.8011 ± 0.0472	Strongest distinctive smell	1.0512 ± 0.0015	1.5713 ± 0.0041	43.990 ± 3.932
E	Gorontalo	0.5800 ± 0.0801	bau khas	1.0272 ± 0.0036	1.5637 ± 0.0041	28.820 ± 0.453

light microscope equipped with a 300 megapixel DS Fi2 digital camera and the Nikkon Image System image processing software.

Examination of TNF-α expression by IHC

General immunohistochemistry protocol consists of four main steps: fixation, antigen retrieval to increase the availability of TNF-α detection, blocking to minimize pesky background signals, and antibody labeling and visualization The staining process was first carried out before examining TNF-α expression under a light microscope by the researcher and two anatomical pathologists. This histopathological examination was intended to determine the expression of TNF-α in the healing areas of the paw tissue. The TNF-α expression in each sample was assessed semiquantitatively according to the modified Remmele method, where the Remmele scale Index (Immuno Reactive Score/IRS) is the result of multiplying the percentage score of immunoreactive cells with the color intensity score on the immunoreactive cells (Table 2). The data for each sample is the average IRS value observed at five different fields of view at 1000x magnification. All of these examinations used Nikon H600L light microscope, equipped with a 300-megapixel DSFi2 digital camera and the Nikkon Image System image processingsoftware[16].

The IRS semiquantitative scale is the result of multiplying the positive cell percentage score (X) and the color reaction intensity score (Y), IRS = (X x Y).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical evaluation anti-inflammatory activity testing method was carried out by one-way analysis of variance (ANOVA). Multiple comparisons one-way ANOVA analysis on the percentage of edema inhibition at a dose of 60 mg/kg BW was conducted to determine the difference in the anti-inflammatory effects of essential oils from 5 regions. Statistical significance was expressed as $P < 0.05$. Data were analyzed using an independent-sample t-test from the software product SPSS 24.0. The data obtained were analyzed with the Shapiro-Wilk test to determine the normality of data and analyzed with the Levene test to determine the homogeneity of data. If the data is normally distributed and homogenous, it is followed by an ANOVA test with a confidence level of 95%. Kruskal Wallis statistical test was performed to analyze differences in the number of inflammatory cells and TNF-α expression.

RESULT

The specification of CBO from 5 regions has a bright yellow color that was more yellow than the color of the marker compound trans-cinnamaldehyde (Figure 1). The specific gravity is more than 1.00 with the highest specific gravity in essential oils from the Kerinci Regency. The highest trans-cinnamaldehyde levels were from Lombok Timur.

Secondary metabolites produced by plants are not directly utilized, unlike primary metabolites which play a direct role in growth and development. Ecophysiological factors can radically modify the number and type of metabolites despite genetic limitations[17]. The pharmacological activity of the plant is the resultant effect of the activity of all metabolites.

This study used *C. burmannii* bark from 5 regions: Karanganyar, Hulu Sungai Selatan, Lombok Timur, Kerinci and Gorontalo. The first stage was an *in vivo* test of the anti-inflammatory effect of CBO from 5 regions at a dose of 60 mg/kg BW with paw test and histopathological analysis. Data on the percentage of edema inhibition in the paw test, data on the number of inflammatory cells in paw tissue using the Hematosilin-Eosin (HE) technique, and TNF- expression using the Immunohistochemical (IHC) technique.

Chemometric analysis of essential oil metabolites and their relative levels from 5 regions was carried out through dendrogram analysis to determine the relationship between *C. burmannii* from various regions. There are 83 metabolite compounds from 5 regions, two of them can be seen in Figures 2 and 3 and Table 4. The results of this study were: *C. burmannii* from 5 regions was divided into 2 clusters, the first cluster was from Kerinci and Lombok Timur, the second cluster was from Karanganyar, Hulu Sungai Selatan, and Gorontalo areas. The second cluster consists of two sub-clusters, namely the Karanganyar and Hulu Sungai Selatan sub-clusters and the Gorontalo sub-cluster as seen in figure 4.

Edema in carrageenan-injected paws has generally reached a maximum of 2.5-3.5 hours after injection. Administration of essential oils has been shown to reduce maximal edema volume, reduce the number of inflammatory cells and TNF-α expression at doses of 60 mg/kg BW. The effect of edema inhibition on CBO administration from the 5 regions was not significantly different from one another except CBO from Hulu Sungai Selatan which were significantly different from CBO from Kerinci. The highest TNF-α expression was in the negative control group. Improvements in the histopathological image of rat paw tissue preparations were found in the group that received ibuprofen/ positive control, the group that was given CBO, and the group that was given the marker compound trans-Cinnamaldehyde. The results of this study showed that the CBO could inhibit the increase in edema volume, reduce the number of inflammatory cells, and the expression

Table 4. GC-MS analysis of the composition of CBO from Hulu Sungai Selatan Regency and KerinciRegency.

No.	Metabolite Compound	Hulu Sungai Selatan		Kerinci	
		%	STDEV	%	STDEV
1	α-Pinene	1.810	0.0141		
2	Champhene	0.340	0.0000		
3	Benzaldehyde	1.375	0.0212	1.320	0.0424
4	β-Pinene	0.735	0.0071		
5	O-Cymene	0.290	0.0000		
6	D-Limonene	1.470	0.0000		
7	Eucalyptol	3.715	0.0354	1.420	0.0566
8	Linalool	2.040	0.0283	6.700	0.6081
9	2 methyl Benzofuran	0.225	0.0071		
10	1-methyl-1H-Pyrrolo (2,3-b)pyridine	0.225	0.0071		
11	Benzenepropanal	1.290	0.0141	3.945	0.1344
12	endo-Borneol	0.335	0.0071	0.435	0.0071
13	Terpinen-4-0l	1.465	0.0354	0.610	0.0141
14	α-Terpineol	1.465	0.0212	1.210	0.0424
15	(E)-Cinnamaldehyde	37.235	0.6859	43.990	3.9315
16	3-Phenylpropanol	0.230	0.0141		
17	Bornyl acetate	0.720	0.0141	1.300	0
18	(E) 3-phenyl-2-Propen-1-0l	0.255	0.0071	0.375	0.0071
19	Hydrocinamic acid			0.280	0.0707
20	2-Carene	0.310	0.0000	0.380	0
21	Eugenol			0.670	0.0141
22	Octahydro-1,7a-dimethyl-5-(1-methylethyl)-,(1S-(1.α.,2.α.,3a.β.,4.α.,5.α.,7a.β.,85*))-1,2,4-Metheno-1H-indene	0.745	0.0071	0.355	0.0354
23	Ylangene	0.365	0.0071	1.095	0.0071
24	Copaene	6.350	0.0424	3.095	0.1202
25	Aromadendrene	0.485	0.0071	0.395	0.0071
26	trans-α-Bergamotene	3.990	0.0566	0.305	0.0071
27	Caryophyllene	2.545	0.0071	6.305	0.3041
28	Coumarin	1.665	0.0071		
29	cinamyl ester Acetic acid	5.460	0.0141	2.080	0.099
30	1a,2,3,4,4a,5,6,7b-oktahydro-1,1,4,7-tetramethyl-, (1aR-(1a.α.,4.α.,4a.β.,7b.α.))-1H-Cycloprop(e)azulene			0.630	0.0141
31	Isoledene			0.125	0.1768
32	Humulene	0.645	0.0071	1.000	0.1273
33	γ-Muurolene	4.420	0.2828	1.555	0.0495
34	(1R,4R,4aS,8aR)-4,7-Dimethyl-1-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene			0.665	0.0212
35	α- Muurolene	3.720	0.0566	3.565	0.0212
36	1-(1,5-dimethyl-4-hexenyl)-4-methyl-Benzene			1.195	0.5162
37	(1R,4aS,8aR)-1-Isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene	1.105	0.0212		
38	Epizonaren	0.870	0.0141		
39	decahydro-4a-methyl-1-methylene-7-(1-methylethenyl-,(4aR-(4a.α.,7α.,8a.β.)) Naphthalene	0.345	0.0071	0.305	0.0212
40	1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methyllethyl)-(1.α.,4a.β.,8a.α.)-(+/-.)-Naphthalene			0.340	0.0849
41	6 -ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-Cyclohexene	0.210	0.0000		
42	β-Bisabolene	0.290	0.0141	0.665	0.0495
43	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1S-cis)-Naphthalene	5.445	0.0778	3.485	0.0919
44	Cubenene	0.775	0.0495	0.110	0.1556
45	α-Calacorene	0.980	0.0141		
46	Caryophyllene alcohol	0.850	0.0000	2.600	0.0849
47	Caryophyllenyl oxide			0.440	0.0283
48	Tetradecanal	0.470	0.0000	0.530	0.0424
49	1-ethyl-5-methyl-Pyrazole-4-carboxaldehyde			0.145	0.2051
50	2-Acetyl-6-methoxynaphthalene	0.235	0.0071	0.135	0.1909
51	τ-Cadinol			0.690	0.0283
52	Methyl 5 -acetyl-2-methoxybenzoate			0.340	0.0283
53	Di-epi-1,10-cubenol			0.325	0.0212
54	τ-Muurolol	0.485	0.6859		
55	Copaene	0.440	0.0849		
56	α-Cadinol	0.550	0.0141	0.690	0.0283
57	cis-,α.-Co*paene-8-ol			0.335	0.0354
58	Alloaromadendrene oxide-(1)			0.310	0.0283
59	(2S)-2-((1R,3aR,4R,5S,7aS)-1,7a-Dimethyloctahydro-1H-1,2,4-(Epimethanetriyl)inden-5-yl)propan-1-ol	0.195	0.0071		
60	4-Isopropyl-6-methyl-1-methylene-1,2,3,4-tetrahydronaphthalene	0.210	0.0000	0.095	0.1344

Figure 1. Differences in the color of CBO from 5 regions; Description of Region: A. Karanganyar, B. Hulu Sungai Selatan, C. Lombok Timur, D. Kerinci, E. Gorontalo, CD. Trans-Cinnamaldehyde (marker).

Figure 2. Secondary metabolite components of CBO from Hulu Sungai Selatan Regency using GC-MS.

Figure 3. Secondary metabolite components of CBO from Kerinci Regency using GC-MS.

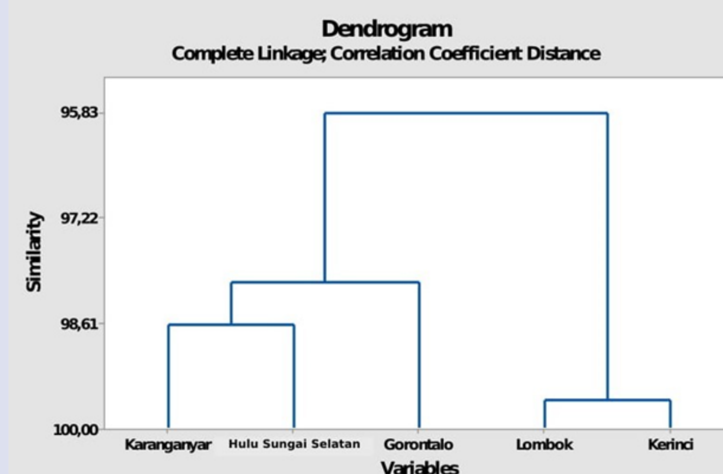

Figure 4. CBO dendrogram from 5 regions.

Table 5. Mean of amount of inflammatory cells in each group.

Groups	Amount of Inflammatory Cells / 5 Field of View
Kerinci*	143.00± 82.14
Karanganyar*	484.00±218.9
Hulu Sungai Selatan*	412.75±140.01
Ibuprofen 27 mg/kg BW	129.00± 64.92
Lombok Timur*	421.00± 122.07
Gorontalo*	450.25± 131.76
Trans-Cinnamaldehyde30 mg/kg BW	438,00± 205.95
Negative control	625.50±199.64

*cinnamon bark oil 60 mg/kg BW

Table 6. TNF-α value calculation.

Groups	Score
Kerinci*	8.73 ± 2.32
Karanganyar *	10.53± 2.04
Hulu Sungai Selatan*	8.13 ± 3.30
Ibuprofen 27 mg/kg BW	9.13± 1.25
Lombok Timur*	11.4 ± 1.04
Gorontalo *	9.7 ± 1.67
Trans Cinamaldehyde	12.00 ± 0.00
Negative control	12.00 ± 0.00

*CBO 60 mg/kg BW

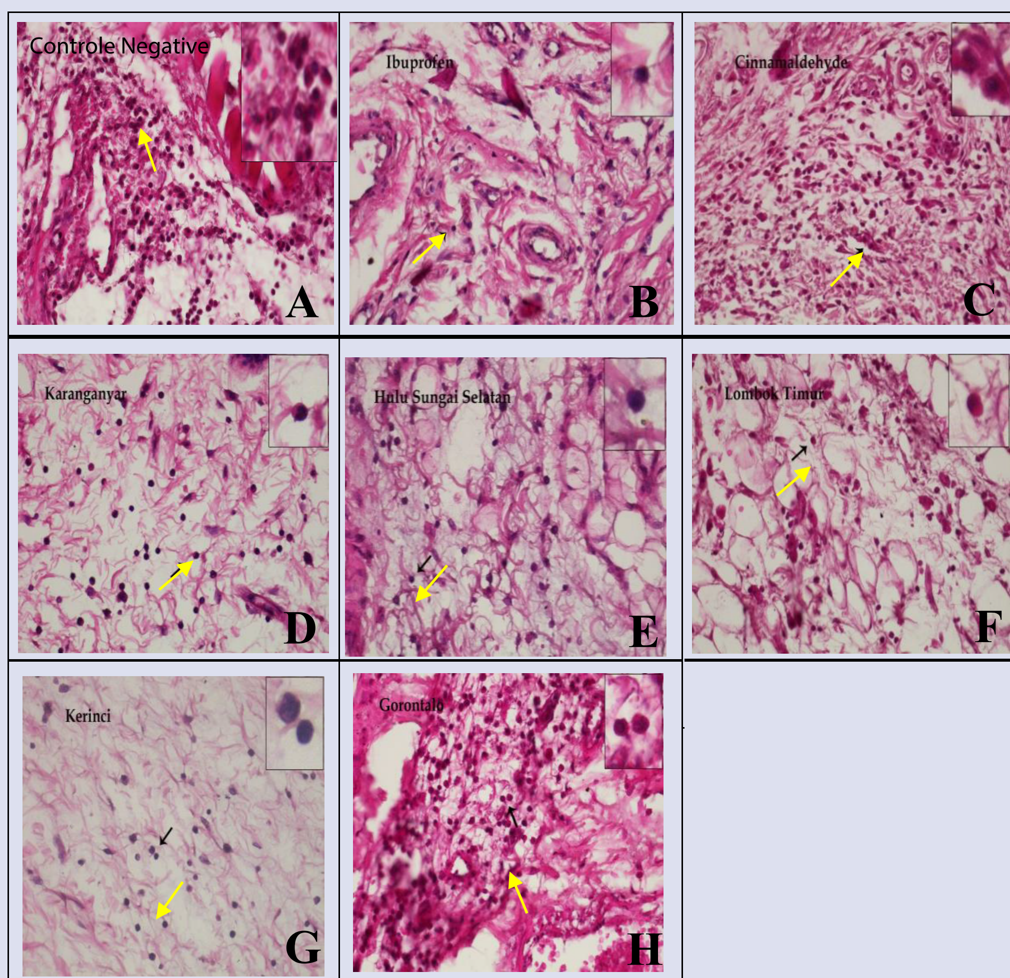

Figure 5. Photomicrographs of the histopathological analysis of paw tissue from rats treated with CMC Na as negative control(A); Ibuprofen27 mg/kg BW as the positive control (B); trans-cinnamaldehyde (C), CBO 60 mg/kg BW from Karanganyar (D), Hulu Sungai Selatan (E), Lombok Timur (F), Kerinci (G), Gorontalo (H) 400x magnification; Nikon H600L microscope; 300 megapixel DS Fi2 camera.

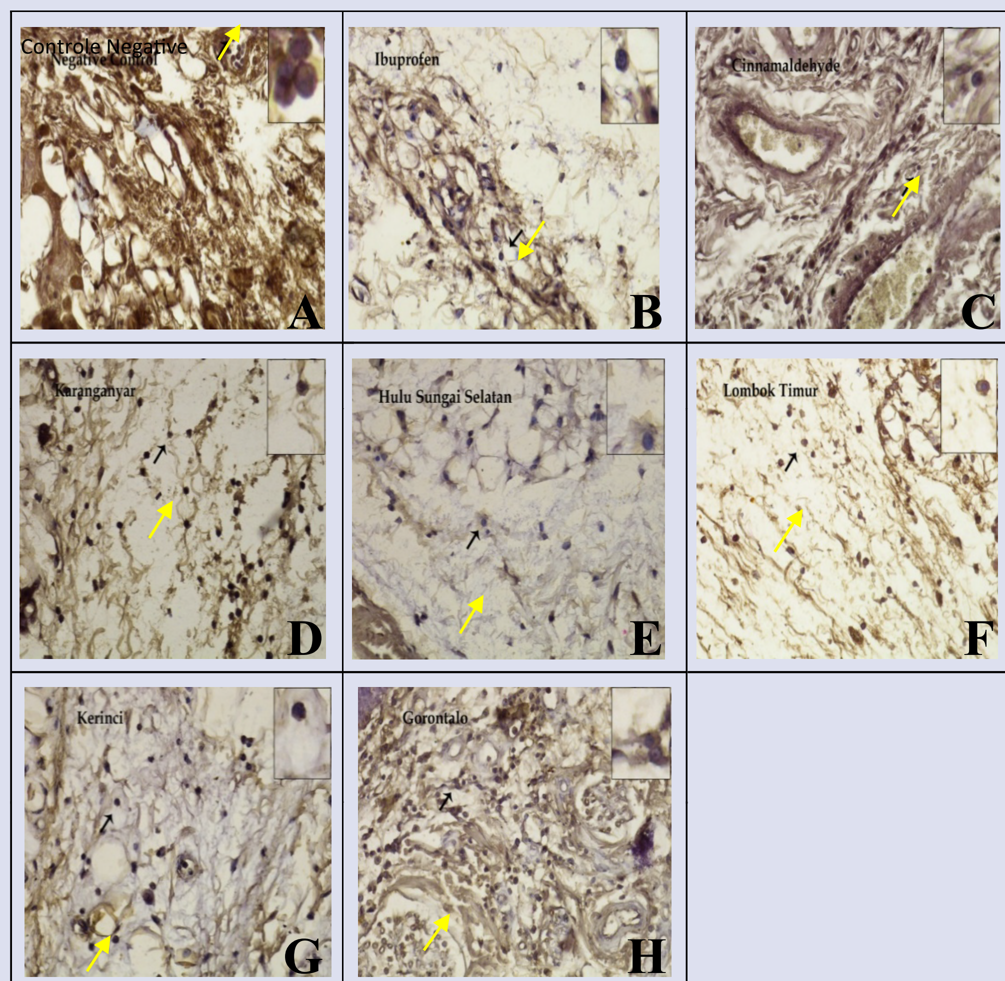

Figure 6. Comparison of TNF-α expression between treatments: with CMC Na as negative control (A); Ibuprofen 27 mg/kg BW as the positive control (B); trans-cinnamaldehyde (C), CBO 60 mg/kg BW from Karanganyar (D), Hulu Sungai Selatan (E), Lombok Timur (F), Kerinci (G), Gorontalo (H), (Inlet: shows TNF-α expression represented by chromogenic brown color in the nucleus and cytoplasm of macrophage cells) (immunohistochemical staining, 1000x magnification; Nikon H600L microscope; 300 megapixels DS Fi2 camera).

of TNF-α which indicates the inhibition of prostaglandin release. Decreased TNF-α expression can be used as an indicator of a decrease in the severity of inflammation.

Trans-Cinnamaldehyde, eucalyptol, linalool, benzenepropanal, endo borneol, terpinen-4-ol, α-terpineol, bornyl acetate, Ylangene, Copaene, Aromadendrene, trans-α-bergamotene, caryophyllene, cinamyl ester Acetic acid, Humulene, γ-Muurolene, α-Muurolene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl-,(4aR(4a.α.,7α.,8a.β.)) Naphthalene, β-Bisabolene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1S-cis)-Naphthalene, Cubenene, Tetradecanalare a chemical profile of cinnamon bark essential oil from Indonesia. It was found in every *C. burmannii* bark essential oil studied.

DISCUSSION

Metabolites function as protection against herbivores, bacteria, fungi, viruses, and competing plants. Other external factors such as light, temperature, groundwater, soil mineral composition, and salinity affect the number and types of metabolites[18]. Metabolites also function as a means of communication between plants, performing symbiosis with other microorganisms such as attracting materials for other organisms. Metabolites are also products that are an important part of the defense system against pathogens and disease attacks, repair wounds, anticipate the bad effects of ultraviolet light, climate change, and attack microorganisms[19]. Thus, because the function of metabolites is to defend themselves, as a means of communication between plants and other organisms and adapt to the environment, the number and type of metabolite components are influenced by the environmental conditions in which they grow.

The high light intensity can cause the leaves to dry out and fall easily. Low light intensity causes an unfavorable effect on growth because it affects the photosynthesis process. Low light intensity, will cause low temperature, high humidity, and low evaporation rate, this can be seen in the forest area of Haratai Loksado village in Hulu Sungai Selatan district. In this area, *C. burmannii* is planted in the middle of the forest so that sunlight is blocked from entering the cinnamon forest area. In Kerinci Regency, *C. burmannii* is planted intercropped with small plants such as chili plants, the sunlight is relatively unobstructed by other trees. Rainfall is one of the factors that determine humidity and temperature. The decrease in water availability can cause a decrease in the rate of photosynthesis and affect the rate of plant growth. *C. burmannii* plants require even rainfall throughout the year. Kerinci Regency has an ideal geographical position and even rainfall intensity throughout the year

Edema in carrageenan-injected paws has generally reached a maximum of 2.5-3.5 hours after injection. Administration of CBO has been shown to reduce maximal edema volume, reduce the number of inflammatory

cells and TNF-α expression at doses of 60 mg/kg BW. The effect of edema inhibition on CBO administration from the 5 regions was not significantly different from one another except for essential oils from Hulu Sungai Selatan which were significantly different from essential oils from Kerinci. The highest TNF-α expression was in the negative control group. Improvements in the histopathological image of rat paw tissue preparations were found in the group that received ibuprofen, the group that was given CBO, and the group that was given the marker compound trans-Cinnamaldehyde. The results of this study showed that CBO could inhibit the increase in edema volume, reduce the number of inflammatory cells, and the expression of TNF-α which indicates the inhibition of prostaglandin release. Decreased TNF-α expression can be used as an indicator of a decrease in the severity of inflammation.

CONCLUSION

This study concludes that the metabolite profile of the *C. burmannii* bark essential oil is divided into two clusters, namely the first cluster in the Kerinci area and the Timur Lombok area. The second cluster is the Karanganyar area, the Hulu Sungai Selatan area, and the Gorontalo area. The dose of *C. burmannii* bark essential oil as an anti-inflammatory in rats (*Rattus novergicus*) Wistar strain was 60 mg/kg BW. There was no significant difference in anti-inflammatory activity from 4 regions, namely from Kerinci, Lombok Timur, Karanganyar, and Gorontalo areas. However, there is a significant difference in the anti-inflammatory activity of essential oils from the Hulu Sungai Selatan area with essential oils from the Kerinci area.

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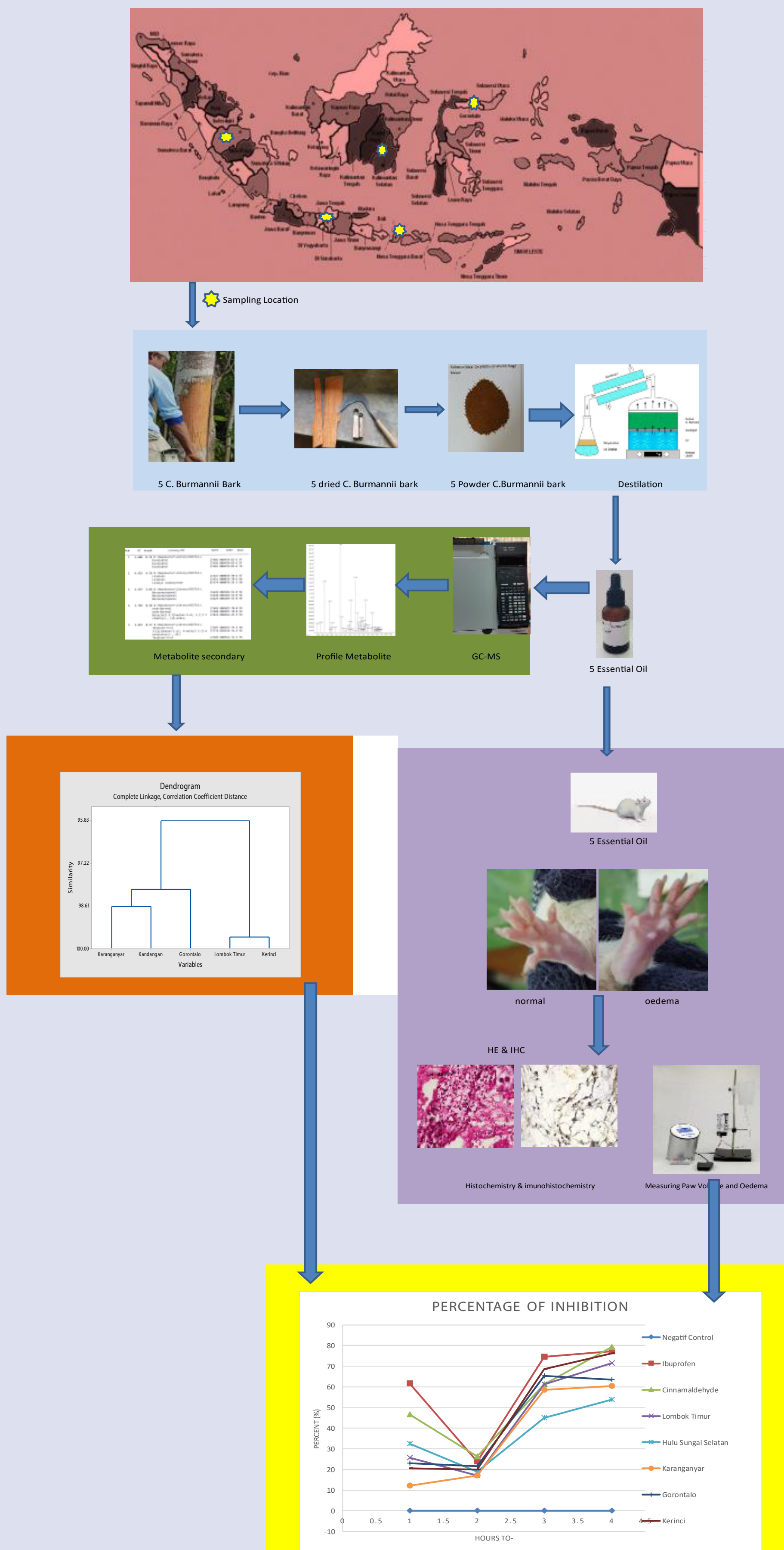
GRAPHICAL ABSTRACT



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