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Analysis of chemical composition and its analgesic and anti-inflammatory activity of essential oil of sintoc bark (*Cinnamomum sintoc* bl.) using in vivo methods

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ABSTRACT

Sintoc bark (*Cinnamomum sintoc* Bl) belongs to Lauraceae (the laurel family). It has been used empirically for a treatment for swelling caused by insects' bites. In this study, the research examined the analgesic activity and anti-inflammation of essential oil of sintocbark using in vivo methods. The mechanism of anti-inflammation was predicted using molecular docking against COX-2. Essential oil of *sintoc* bark was collected by distilling through steam distillation, and then analyzed by GC-MS. Analgesic and anti-inflammatory activity was examined by *in vivo*, which were conducted by writhing and carrageenan-induced methods, respectively. The findings showed that the tested sintoc bark oils contained 36 components of essential oil with eugenol (38.38 %) as a major compound. In the *in vivo* experiments, sintoc bark oils with doses 0.005 mL, 0.010 mL, and 0.020 mL/20g body weight significantly ($p<0.05$) reduced the number of writhing of mice when compared to negative control group. All of doses of sintoc bark oils gave significantly affect (confidence level 99 %) compare to negative control. Sintoc oil with dose 0.2 ml/200g had the strongest inhibition compare to positive control (indometasin 10 mg/kg body weight). The molecular docking results indicated that the compounds of aryl propanoid were generally potential to inhibit COX-2.

INTRODUCTION

Inflammation is a mechanism of the body's defense caused by the tissue response to such detrimental effects-both local or inside the body (Nathan, 2002). The effects can be devastating to physics, chemistry, bacteria, parasites and so forth. Physical damage can be caused by high temperature, light and radiation, including foreign objects inside the organ, or any causes that prompt devastating effects. Strong acids, strong bases and toxins immerse in chemical causes. Pathogenic bacteria of *Streptococcus*, *Staphylococcus* and *Pneumococcus* genus, for instance, are also devastating (Khansari*et al.*, 2009). Inflammatory reaction can be observed from clinical symptoms around affected tissue, such as increase in heat (calor), reddish spots indication (rubor), pains (dolor) and swelling (tumor) along with itching. It is followed by the changes in structural tissues that lead to loss of functions. Those damaged cells release inflammatory mediators, such as, histamine, bradykinin, serotonin, prostaglandins, and leukotrienes. The process of inflammation causes a change in blood flow, an increased permeability of blood vessels, or a damage of tissue through an activation and migration of leukocytes by synthesizing reactive oxygen derivatives and synthesis of inflammatory mediators locally. Oxidative stress caused by free radicals potentially have physiological or biochemical effects in metabolic disorders that lead to the death of cells. It suggests that antioxidants play a role to heal an inflammation. A synthesis of inflammatory mediator is inducted by phospholipase, cyclooxygenase (COX) and lipooksigenase (LOX) enzymes (Gilroy *et al.*, 1999). Arachidonic acid in cell membranes will be esterified into phospholipids, while the others are in the form of complex lipids. In prostaglandins biosynthesis, arachidonic acid will be released from lipid storage cells byacyl hydrolase.

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Two strands of arachidonic acid metabolisms are COX flow which produces prostaglandins and thromboxane, and LOX-5 flow which produces leukotrienes (Vendramini-Costa and Carvalho, 2012). Research on anti-inflammation has also conducted in some other *Cinnamomum* genus like essential oil of *C. insularimontanum* fruit that has an anti-inflammatory activity in vitro by inhibiting NF-kappa B as well as effectively reducing edema inducted in mice's ear at dose of 0.3 mg for each ear that reduces edema of 83% (Wiart, 2006).It proves that there is a potentiality of a plant belonging to *Cinnamomum* genus to be an anti-inflammatory drug. In Indonesia, the only simplicia immersed in *Cinnamomum* genus that has been listed in Indonesian Herbal Pharmacopoeia is *C. burmani* Ness ex Bl., while the others have not been examined much, including sintoc bark (*C. sintoc* Bl.) (Health, 2008).

Sintoc (*C. sintoc* Bl.) is a plant that grows in Indonesia, Malaysia and Thailand with a woody stem extending involved in Lauraceae tribe. Empirically, sintoc is utilized as outer or inner medicines. It is used to treat worms in the belly, amoebic dysentery and swelling (inflammation). Instead of Indonesia, research on chemical contents of sintoc bark has also been conducted in other countries. Jantan et al. examines chemical contents in sintoc bark form Malayan peninsula by means of gas chromatography with a mass spectroscopy (Jantan *et al*., 2005).They concluded that the chemical contents in it are safrole 23.4%, 13.5% murolen, along with such adequate amounts of eugenol, linalool, germakren, kadinen, terpinol, and the other terpenes.

Research on the activity of essential oil of *sintoc* bark from Malaysia shows that the essential oil has an in vitro anti-inflammatory activity by antagonizing LOX and *platelet activating factor* (PAF), while in vivo, it inhibits edema in mice's ear induced by tetradekanoilphorbol acetate (TPA) (Jantan *et al*., 2005b). According to the mention above, the laboratory examination was conducted due to investigating an anti-inflammatory activity of essential oil of *sintoc* bark through an inhibition of COX-2 enzyme. The research was aimed at finding out the mechanism of essential oil in sintoc bark towards an inhibition of COX-2 enzyme.

MATERIAL AND METHOD

A laboratory experimental research is conducted through two steps; the first step is distilling essential oil of sintoc bark by applying steam distillation method and analyzing the essential oil with GC-MS, then determining the level of essential oil and optical rotation. The second step is examining analgesic and anti-inflammatory activity *in vivo* using writhing and carrageenan-induced methods, respectively.

Tools

To analyze the essential oil with *Gas Chromatography-Mass Spectrometry* method and examine the inhibitory activity of COX-2, the tools used are microplat, CO_2incubator (Sanyo), Laminar Air Flow (LAF) (Jeio Tech), *High Speed Refrigerated Centrifuge* (Union 32 R), otoklaf (Memert), cells counter (*Improved Double Nubauer depth 0,1 mm*) microscope (Niken Eclipse 80) and microplate reader (Benchmark).

Plant Material

Sintoc barks from AM were collected in November 2009 from Yogyakarta district. Specimens were identified by the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran and voucher specimens was submitted to the Herbarium Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. *Sintoc* barks (1 kg) was dried in air for 14 days at room temperature to produce dry samples, each weighing 0.5 kg of barks prepared for the distillation.

Animals

The animals in this experiment were used Swiss albino mice weighing between 15-35g for evaluation of analgesic activity. These animals were divided into five different groups each containing six animals. Male rats of Wistar strains weighing 200-250 grams each was used for antiinflammatory experiment. Food was obtained 12 hours prior to drug administration till completion of experiment. All experimental procedures for animal use have been approved by the Ethical Committee of Experimental Animals.

Physicochemical Analysis

Determining Density

Determining essential oil density was carried out by utilizing a pycnometer. A certain amount of essential oil was incorporated into the pycnometer with its weight and volume measured, and then weighed it using an analytical balance. Essential oil density could be observed from the reduction of essential oil weight in pycnometer with the empty pycnometer weight.

Determining Optical Rotation

Essential oil of *sintoc* barks (1 mL) was dissolved into n-hexane until it reached 10 mL, and homogenized afterwards. The mixture was put into 100 mm polarimeter tube and then put under the checker tool between the polarizer and the analyzer. The analyzer was slowly rotated and viewed through a telescope until it obtained the same light intensity of illumination. The rotation direction was determined whether it was dextro (+) if clockwise, or levo (-) if counter clockwise. The analyzer was rotated in accordance with the rotation direction until the position between two fields can be viewed clearly and sharply. Degrees on a position of middle-light and middle-dark were written down.

Determining Refractive Index

The determination of refractive index was performed by applying an abbe refractometer. The tool was installed sufficiently, thus the sodium light illuminated. Prior using it, a prism on

refractometer was cleansed by acetone as the oil to be tested was dripped into the prism by turning a screw on the prism, hence the prism could be slightly opened, and then the screw was shut completely. The slide was driven back and forth until it formed such a gas/ribbon colour vividly and divided into two parts: bright and dark.

Determining Viscosity

Viscosity determination of essential oil of *sintoc* bark was carried out by using an Otswald viscometer. The essential oil was poured into the viscometer through the end point of the tube with a large diameter. Previously, the viscometer was placed on stative in the bottom of the tube and soakedit in water to keep the temperature stable as 25 ^{o}C. Furthermore, the essential oil was sucked by a pipette ball through the end point of the tube with a small diameter, until it passed the upper limit. The time required for the essential oil to flow down from the upper limit was measured afterwards. At this point, viscosity was calculated by the formula below:

$$\eta 0 = (d_0 \times t_0)/ (dw \times tw) \times \eta w$$

ηo= oil viscosity
ηw= water viscosity
do= oil density
do= water density
to= the time limit for oil to flow down from the upper limit to the lower limit
ta = the time limit for water to flow down from the upper limit to the lower limit

Determining of Ester Value

The essential oil was weighed two times to five times, and then dissolved in 5 mL of neutral ethanol p. Free acid in the oil was neutralized by KOH-ethanol 0,1 N using an indicator of 1 mL phenolphthalein. Subsequently, added 25 mL of KOH-ethanol 0,4 N and refluxed it on a water bath for 1 hour 30 minutes and cooled eventually. The excess of KOH-ethanol was retritrated by HCl 0, 5 N using 1 mL of phenolphthalein indicator. Afterwards, conducted a blank titration by making a sample containing 5 mL of neutral ethanol p, and then added 25 mL N. In subsequent, both KOH-ethanol 0,4 N are differentiated, after that the solution was refluxed for about 1 hour 30 minutes, then cooled and titrated by HCl 0,5 titration which was equal as the bases needed to soap the esters. Ester value were calculated by the following formula:

$$\frac{(b-a)\ x\ N\ \ HCl\ \ x\ \ 56{,}1}{W} x100\,\%$$

a = volume (HCl) of required
b = volume (HCl) of required forms
w = weight (g) of the substances utilized

Determining of Solubility in Ethanol

Ethanol solution was made from various concentrations (50%, 60%, 65%, 70%, 75%, 80%, 90%, dan 95%). Essential oil (0.1 mL) was inserted into a lid, and then slowly added a small amount of ethanol into it, started from the lowest concentrations. The ethanol volume added was written down when the clear solution had been obtained before the volume reached 1 mL. If the volume reached 1 mL, but the mixture was not clear yet, the other higher concentrations could be used.

Isolation of essential oil

Dry samples (1 kg barks) on steam-distillation in Monaco Lembang, West Java, for 3 hours to isolate the essential oil fraction. Oil stored at -20 ° C after the addition of sodium sulfate.

Analytical Condition

Essential oils were analyzed on QP5050A GC/MS-(Shimadzu) equipped with a fused silica capillary DB-5ms 30 mm x 0.25 nm, 0.25 μ m, carrier gas helium 95.3 kPa, flow rate 1.7 ml/ minutes. Temperature has been setup with the program as follows: 60 ° C for 5 minutes and then 250 ° C to 10 ° C / min, ending with 5 minutes at 300 ° C. Port Injector and detector temperature was 250 ° C and 280 ° C, respectively. The sample is injected by split and split ratio 1:20. MS operating conditions were: interface temperature 240 ° C; electron impact ionization at 70 eV by scanning the mass range (m / z) of 40-350 daltons with a sampling rate of 1.0 scans / s. Identification of compounds was done by searching on a computer using a digital library of mass spectral data by a Class-5000 software and by comparison of retention indices and mass spectra library authentic(Adams, 1995; Ausloos *et al.*, 1999; Babushok *et al.*, 2007; Strehmel *et al.*, 2008), relative to the C8-C20 and C21-C40 n-alkane series (Sigma) (Mijin *et al.*, 1999) in temperature-programmed run.

Analgesic Activity Test

Acetic acid induced writhing method was employed for evaluation of analgesic activity(Mishra, Ghosh, Kumar, & Panda, 2011). For inducing, acetic acid 1% (v/v) solution was used, while solution of aspirin (dose-65 mg/kg/10ml) was prepared in normal saline water. The solution of sintoc oil was achieved with mixing sintoc oil into PGA 10 %.

The animals were divided in each group five as mention below:

Negative Control: PGA 10 %
Positive Control: Acetosal 65 mg/kg/10ml
Sintoc bark oils I: Doses 0.005 mL/20 g
Sintoc bark oilsII: Doses 0.010 mL/20g
Sintoc bark oilsIII : Dosis 0,020 mL/20g

The sintoc bark oils and control drugs were administered orally. The writhing was induced by intraperitonial (i.p) injection of 1% acetic acid in volume of 0.1 ml/20gbody weight after 60 minutes. The writhing episodes were observed every 5 min in a 60 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted. The data was analyzed by ANOVA statistics. The percent protection at each dose-level was achieved by calculating using formula as follows (Subarnas & Wagner, 2000):

% protection= % mean writing of acetosal / % mean writing of sintoc bark oils × 100

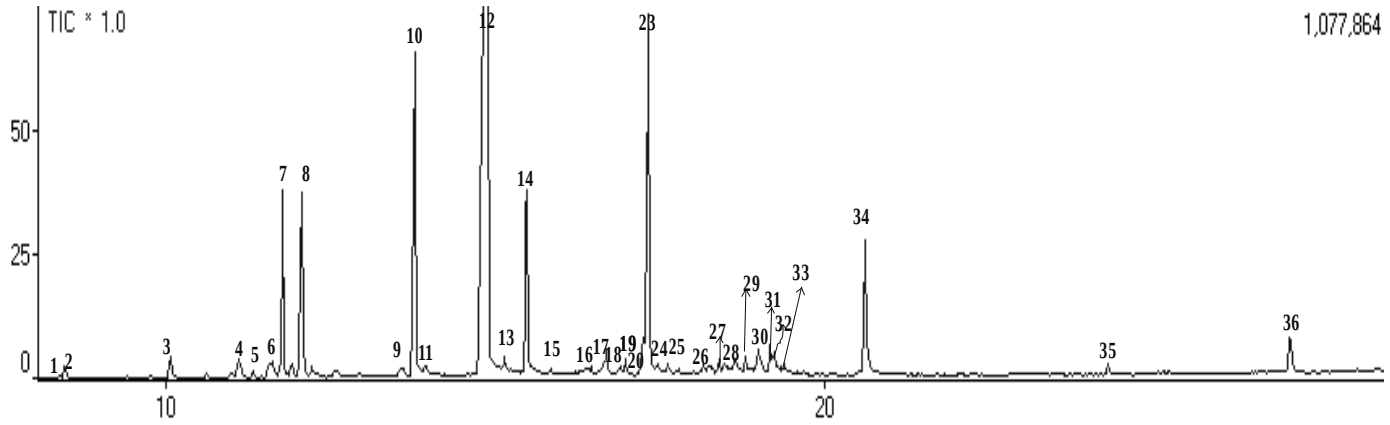

Fig. 1: Total ion chromatogram of sintoc bark essential oils.

Table 1: Psychical characteristics, chemical characteristics and the level of essential oil of *sintoc* bark.

Evaluation Parameters	Results
Level of Essential Oils	1.1%
Organoleptic	
• Coulor	Green yellowish
• Smell	Strong
• Taste	Spicy
Density	0.964
Refraction index	1,4986 µD (20°C)
Viscosity	3,66cp (at room temperature)
EsterValue	17.96
Solubility in ethanol	10 drops in etanol 50%

Table 2: The composition of essential oil in sintoc bark.

No. Peak	Retention Time	LRI exp[b]	LRI Ref[a]	Name Compounds	% Area Under Curve
	8.392	1028	1031	L-Limonene	0.08
	8.473	1033	1039	1,8-sineol	0.27
	10.083	1095	1098	L-linalool	0.47
	11.129	1140	1146	Isopulegol	0.72
	11.583	1146	1143	Camphor	0.41
	11.628	1162	1165	Borneol	0.48
	11.781	1174	1177	4-terpineol	4.26
	12.077	1191	1189	α- terpineol	4.40
	13.613	1275	1285	bornyl acetate	0.31
	13.785	1287	1285	Safrole	10.17
	13.958	1291	1290	Tymol	0.25
	14.894	1358	1356	Eugennol	38.38
	15.152	1372	1376	α–Copaene	0.47
	15.485	1400	1401	Methyleugenol	4.14
	15.855	1412	1408	Trans-caryophylene	0.06
	16.403	1420	1418	β- caryophylene	0.06
	16.463	1428	1439	Aromadendrene	0.17
	16.643	1444	1477	γ- murolene	0.17
	16.698	1450	1483	α -curcumene	0.50
	16.903	1486	1499	α-murolene	0.16
	17.217	1508	1503	gernacrene	0.22
	17.254	1518	1524	δ-cadinene	0.78
	17.343	1520	1520	myristicin	13.54
	17.634	1540	1542	α-calocorene	0.28
	17.803	1575	1576	Spatulenol	0.07
	18.161	1582	1581	Caryophylene oxide	0.20
	18.243	1587	1583	Globulol	0.20
	18.292	1591	1590	Viridiphlorol	0.14
	18.648	1620	-	Isomyrisiticin	1.14
	19.005	1638	1640	δ-cadinol	1.63
	19.182	1648	1653	alfa-cadinol	0.98
	19.256	1665	1691	Junipher camphor	0.69
	19.342	1700	-	Eugenic acid	0.24
	20.622	1760	1762	Benzyl benzoate	4.66
	24.316	1857	-	Derivative eugenol	0.28
	27.073	2176	-	Hexadecanoic acid	1.00

a LRI reference in Adams (1995) with DB5 column
b LRI experiment with DB5-MS column

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using a carrageenan-induced paw edema test methods(Subarnas & Wagner, 2000). The animals were divided into control and test groups. Each group contained of 5 rats. The rats were injected of 0.1 ml of 1 % carrageenan into the subplantar tissue of the right hindpaw of each ratsas 30 min after the i. p. administration of the test compound or control vehicle. The paw edema was achieved before and 1 to 5 hours after the carrageenan administration using a plethysmometer. Doses of sintoc bark oils were administered at 0.05; 0.10 and 0.20 mL/200g body weight of rat.

The inhibition percentage of edema was achieved by calculating using equation as follows:

$$\% \text{ Inhibition} = \frac{\text{edema of indomethacin - \% edema of sintoc bark oils}}{\text{\% edema of indomethacin}} \times 100$$

Statistical analysis

Data were analyzed using an ANOVA, and the significance of difference was calculated according to the Newman Keuls.

RESULTS

The Contents of Essential oil Ki Lemo

The result of testing physical and chemical characteristics of essential oil of sintoc bark (*C.* sintoc Bl.) is shown in Table 1. According to the table, it can be seen that the value of essential oil of sintoc bark density is 0,964 g/cm^3. It is in the range of essential oil density which is between 0.696-1.18g/cm^3(Guenther, 1990).

The Compositions of Essential Oil of Sintoc Bark

The compositions of essential oil of sintoc bark were shown by Figure 1. On the figure, it can be seen that the greatest peak is at no. 12 (eugenol), while none of monoterpena compounds stand out in the compositions of essential oil of sintoc bark. Eugenol was major compound (38.38 %), respectively followed by myristicin (13.54 %), safrole (10.17 %), metileuegunol (4.14 %). The complete data can be seen in Table 2. The contents of 4-terpinol (4,2 %) and α-terpineol (4.4 %) from sintoc bark are not as great as the contents of aryl propanoid compound derivatives.

Analgesic Activity of Essential Oil of *Sintok*

Examining the analgesic activity of essential oil of *sintoc* barkhad been done by 0.7% PGA-induced writhing method, and its further effectiveness was compared to aspirin 65 mg/kg. Essential oils with doses 0.005 mL, 0.010 mL, and 0.020 mL/20g of sintoc bark significantly ($p<0.05$) reduced the number of writhing of mice when compared to negative control group. The result of examination is shown in Table 3. The writhing was observed for 60 minutes. In this study, PGA 10% was used as negative control and solution of asetosal as positive control. As shown Table 3, Doses of sintoc bark oils were 0.005 mL, 0,010 mL, dan 0,020 mL/20g body weight. Based on Table 3 and Fig. 2, all doses of sintoc bark oils of administered decreased the writhing numbers of mice.

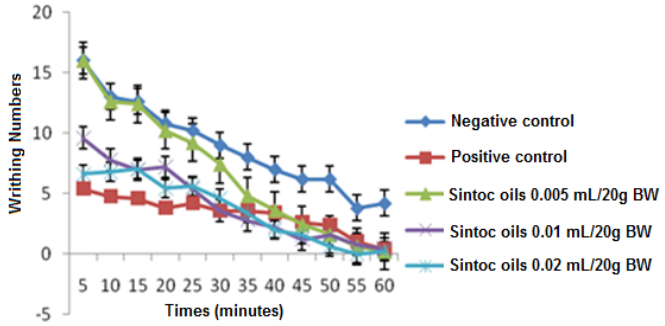

Fig. 2: Effect of sintoc bark oils onthe acetic acid - induced writhing response in mice.

Dose of 0.005 mL/ 20 g body weight provided significantly affect ($p< 0.01$) compare than negative control since 35 minutes, while dose of 0.005 ml/20 g body weight contributed significantly affect each minute to ten and to five. The results showed a dose-dependent increase in the decreasing of writhing of mice as compared to the positive control (acetosal 65 mg/kg body weight). It was very interesting to note that sintoc oil was more selective for the analgesic activity as demonstrated by the high writhing inhibition percentage of mice in the control. The percentage of pain protection and effectiveness therapy could be calculated based on the data of mention above by comparing the effects of acetosal. The results were showed in Table 4 and Fig. 3. Table 4 and Fig. 3 showed that a dose-dependent increase in the increasing of protection capacity of pain by induced acetic acid 0.7 % as compared to the control. In addition, a dose-dependent of sintoc bark oils increased more increase of therapy effectiveness compare than acetosal 65 mg/kg body weight. Statistics analysis results showed that there were significantly different belong treatment at $p<0.01$. It was mean that all of the doses of sintoc bark oils gave significantly effect compare than negative control (PGA 10 %).

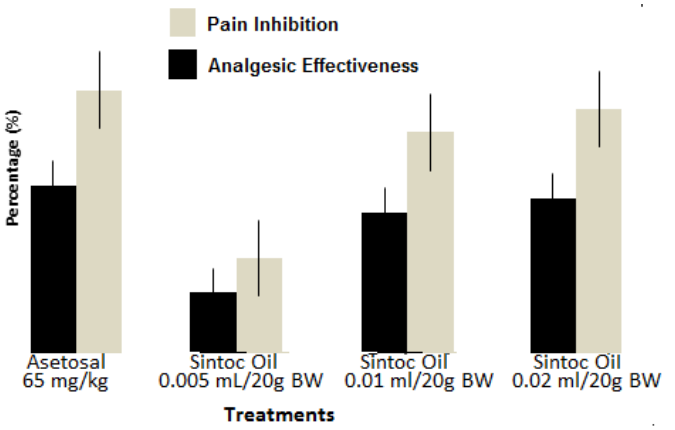

Fig. 3: Graph of pain protection percentage and effectiveness of essential oils sintoc bark.

Table 3: Effect of sintoc bark oils on the acetic acid-induced writhing response in mice over 60 min.

Treatment Groups	Average numbers of Writhing every 5 minutes in 60 minutes												Total
	5	10	15	20	25	30	35	40	45	50	55	60	
Control (-)	16	13,4	12,6	10,8	10,2	9	8	7	6,2	5	3,8	3,5	105
Acetosal 65mg/kg	5,4	4,8	4,6	3,8	4,2	3,6	3	2,9	2,4	2,1	1	0,4	38,2*
Sintoc bark oils I	16	12,6	12,4	10,2	9,2	7,4	4,8*	3,6	2,4	1,6	0,6	0,2	81*
Sintoc bark oils II	9,6	7,8*	7*	6,5*	5,4*	3,6*	2,8*	2,2*	1,2*	1,6*	0,8*	0,4*	48,9*
Sintoc bark oils III	7*	6,8*	6,5*	5,4*	5,0*	4,6*	3,4*	2*	1,6*	0,6*	0*	0,2*	43,1*

Tabel 4: Percentage of pain protection and effectiveness of essential oils sintoc barks.

Tratmen Groups	Doses (mL/20 g BW)	% Protection	% Effectiveness
Asetosal	65 mg/kg BW	63.62	100
Sintoc bark oils I	0.005	22.86	35.93
Sintoc bark oils II	0.01	53.43	83.98
Sintoc bark oils III	0.02	58.98	92.71

Table 5: Percentage of means average in evaluation of anti-inflammatory activity of sintoc bark oils.

Treatment Groups	Doses(mL/200g)	Percentage of means average of paw edema of rats (%) (in hours)				
		1	2	3	4	5
Control (-)	PGA	47.69	101.6	141.53	188.00	221.01
Indometasin	10 mg/kg	13.44	44.76	78.82	109.59	85.31
Sintoc bark oils I	0.05	25.66	91.96	130.95	110.02	96.73
Sintoc bark oils II	0.10	21.34	45.58	96.43	71.30	57.10
Sintoc bark oils III	0.20	13.01	39.83	86.31	55.83	35.06

Anti-inflammatory Effect of Sintoc bark oils

Sintoc bark oils were evaluated the anti-inflammation activity using Winter methods by inducing paw edema of male rats. As shown in Table 5 and Fig.4, all doses of sintoc bark oils weight inhibited plantar edema in the mice (induced by 1 % of carrageenan) significantly compare to negative control. In Fig.4, a dose-dependent of sintoc bark oils increased in decreasing paw edema of rats. The inhibition of edema percentage was clearly shown in Fig.4.

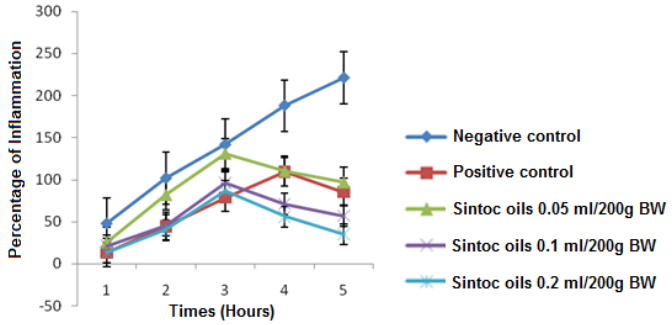

Fig. 4: Percentage of means of inflammation of paw edema of rats each treatments.

The significant inhibition caused by a dose of 0.2 mL/kg body weight occurred from 1 to 5 hours after the administration of carrageenan compare then positive control (indometasin 10 mg/kg body weight). However, the doses of 0.05 dan 0.1 mL/200g body weight gave percentage inhibition less than positive control. The percentage of inhibition of inflammation could be measured from Table 4 and the data was shown in Table 6 and Fig. 5. Percentage inhibition of inflammation illustrated the capability of compounds tested to inhibit the formation of edema. This value could be determined though the edema scores of the compounds tested. The groups that obtained small edema percentage had high capability to inhibit of the edema. Tabel 6 showed that inhibition edema percentage of sintoc bark oils increased in line with the increasing of doses. It indicated that a dose-dependent increased more increasing the activity in edema percentage inhibition of sintoc bark oils that induced by carrageenan. In the Table 6, sintoc bark oils clearly appeared that sintoc oil with dose 0.2 ml/200g had the strongest inhibition compare to positive control (indometasin 10 mg/kg body weight). All of doses of sintoc bark oils gave significantly affect (confidence level 99 %) compare to negative control. However, the doses of 0.05 and 0.1 ml/200g body weight had less inhibition compare to positive control.

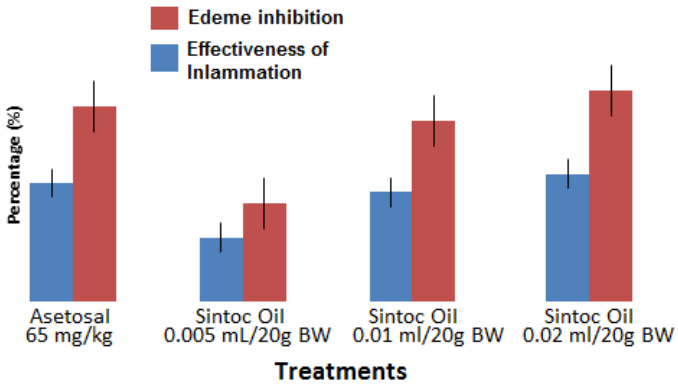

Fig. 5: Inhibition percentage of inflammation and effectiveness of anti-inflammation of sintoc bark oils.

Table 6: Percentage of Inhibition of Edema and Effectiveness of Anti-inflammatory of Sintoc bark oils.

Treatment Groups	Doses (mL/200g)	Inflammation Inhibition (%)	Effectiveness of Anti-inflammatory (%)
Indometasin	10 mg/kg	60.40	100
Sintoc bark oils I	0.05	32.67	50.09
Sintoc bark oils II	0.10	55.83	92.43
Sintoc bark oils III	0.20	65.35	108.18

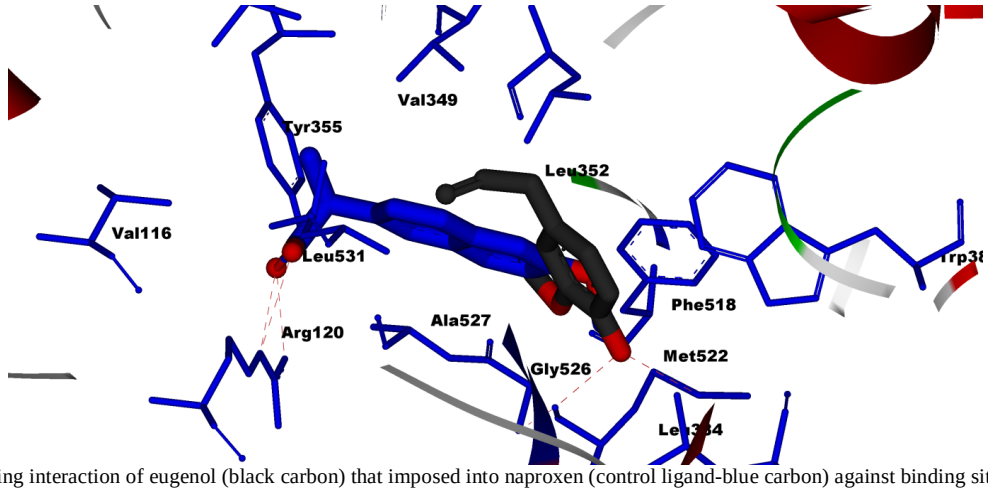

Fig. 6: Binding interaction of eugenol (black carbon) that imposed into naproxen (control ligand-blue carbon) against binding site of COX-2.

The Prediction of Essential Compounds Relational Interaction towards COX-2 through Molecular Docking

According to previous research (Chericoni, *et al.*, 2010; Thakur and Pitre, 2009), here we predicted that the mechanism of eugenol might acts against cyclooxygenase-2 target.

According to Table 3, only alcohol compounds derivatives that had the ability to compete as the inhibitors of COX-2, while none of essential compounds was commonly able to compete with the control compounds of naproxen. In the table, 4-terpinol compound had the lowest binding energy, which means that it is the most competitive compound to be an inhibitor of COX-2, yet eugenol compound had a better interaction visually. Phenol part of eugenol (black carbon) forms hydrogen bound with Met522 and Gly 526 from COX-2, as well as phenol part of naproxen (blue carbon). Nevertheless, eugenol does not form electrostatic interaction in between carboxyl and ammonium ions as well as carboxyl groups of naproxen with ammonium ions of Arg 120. The description is shown in Figure 6.

Table 7: Molecular docking results of some components of sintoc bark oils

No	Name Compounds	Free Energy Binding (kcal/mol)	Ki (Inhibition Constant)
1	Naproksen (kontrol)	-7.55	40 nM
2	eugenol	-5.69	68.02 µM
3	L-linalool	-4.90	220 µM
4	Isopulegol	-4.89	246 µM
5	4-terpineol	-6.36	56.12 µM
6	α- terpineol	-5.20	93.5 µM
7	Safrol	-4.64	323 µM
8	1,8-sineol	-5.21	98.1 µM
9	metileugenol	-5.60	70 µM
10	miristisin	-5.58	80.74 µM
11	isomirisitisin	-4.88	234 µM

DISCUSSION

The result of analyzing essential oil in Gas Chromatography-Spectroscopy period (GC-MS)shows that the largest component of essential oil in sintoc bark is eugenol (38,38 %), followed by other compounds which have smaller percentages, such as *4-terpineol, 1-terpineol, camphor, methyl trans-isogenol, dan alpha-pinene.* Here we also showed that our results is significantly different from previous studies from other countries (Malaysia) (Jantan *et al.*, 2005). Jantan *et al.* (2005) declared that linalool (23.4%) and γ γ-muurolene (13.5%) were the major components of the bark oil and surprisingly eugenol has been not detected in sintoc bark oils. It might be caused that the geographic and seasonal factors may be important in determining the chemical composition (Muchtaridi *et al.*, 2014).

In the examination of pharmacology activity, it is proved that essential oil of sintoc bark has analgesic activity on stretching method. Analgesic activity is indicated by the decrease in the times of mice stretching induced by acetic acid 0.7 %. The decrease in the amount of stretching indicates that the essential oil can protect a pain caused by acetic acid.

The effect of inhibition edema was studied along a 60-min test, as shown in Figure 5. In addition, sintoc bark oils also provided anti-inflammatory activity, which is related to the previous evidence that essential oils that contained eugenol inhibits edema in rats (Chericoni*et al.*, 1994; Thakur andPitre, 2009). However, the anti-inflammatory activity seen weak, as it was observed only at a higher dose (0.2 mL/200g body weight). These results suggest that this oils might have effective ant-inflammation activity.

Sharma *et al.* (1994) propose that respective doses of 33 mg/kg eugenol and ginger oil given on the right knee and right foot of mice for 26 days induced by *Mycobacteiun tuberculosis* significantly decrease the inflammation. The research conducted by Cherironi *et al.* (2005) has also proved that essential oil and eugenol bark of *C. zeylanicum* Bl. in vitro inhibit an anti-inflammation induced by peroxynitrite and lipid peroxidation, which reduce 3-nitrotyrosin formation with IC_{50} 18,4 and 46,7 µg/mL respectively.

In addition, Thakcur and Pitre (2009) prove that eugenol form essential oil of basil (*Ocimum sanctum* L.),as much as 100 mg/kg of body weight, is able to inhibit an inflammation on the mice's foot induced by *keragenan* as much as 33 %. It is also supported by research that proposes the increase of COX-2 expression induced by tioasetamida (TA) can be inhibited by

eugenol (Yogalakshmi, Viswanathan, & Anuradha, 2010). Furthermore, Magalhaes *et al.* (2010) show that eugenol has an anti-inflammatory activity in vivo by inhibiting LPS in an injured lung, improving lung function. In line with the notion, bioactive compound of essential oil that is responsible for inhibiting COX-2 activity is apparently précised through molecular docking. It could be concluded that the compounds of aryl propanoid was generally potential to inhibit COX-2 operation. In previous study, molecular docking results has shown that isoeugenol can effectivelyinhibit cyclooxygenase and lipoxygenase enzymatic action. Docking results of isoeugenol against COX-2 (PDB ID: 4COX) shows that the molecule docked into the active site with similar orientation with indomethacin (Zarlaha *etal.*, 2014).

CONCLUSION

The essential oils of sintoc bark contained 1.1 % essential oils with eugenol (38.3 %) as major component. The essential oils of sintoc bark had analgesic an anti-anflammatory activities. In molecular prediction, eugeunol had better prediction, while none of essential compounds was commonly able to compete with the control compounds of naproxen

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