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Chemical composition and bioactivity of essential oil from the leaves of *Scorodocarpus borneensis* Becc. (Olacaceae) grown in Indonesia

HARLINDA KUSPRADINI[1*], SAAT EGRA[1], INDAH WULANDARI[1] and AGMI SINTA PUTRI[1]

The essential oil of *Scorodocarpus borneensis* Becc. (Olacaceae) was obtained from its leaves by steam distillation. In this study, *S. borneensis* leaves yielded a clear yellowish essential oil. Its chemical composition was analyzed by GC-MS. Six chemical compounds were identified, and most components of the essential oil of *S. borneensis* leaves were sulfur-containing compounds, such as trisulfide, dimethyl, methyl (methylsulfinyl) methyl sulfide, 2,4,6-trithiaheptane-2,2-dioxide, and methane, (methylsulfinyl) (methylthio). The major compounds are 2,4,6-trithiaheptane-2,2-dioxide (43.35%), and methyl (methylsulfinyl) methyl sulfide (34.03%). Anti-microbial properties were determined using the agar diffusion method. Four different microorganisms were used in this study: *Streptococcus sobrinus*, *S. mutans*, *Staphylococcus aureus* and *Candida albicans*. The zone of inhibition and activity index were measured and compared against a known synthetic standard. The essential oil showed strong activity against all tested microorganisms. Anti-oxidant activity was assayed with 1,1-diphenyl-2-picrylhydrazyl (DPPH) with ascorbic acid as a positive control. The essential oil has potency to inhibit free radicals at concentrations of 25-1,000 µg/mL. The results indicated that *S. borneensis* leaves oil is a good, new natural anti-microbial agent for oral pathogens.

Keywords: *Scorodocarpus borneensis* Becc., Olacaceae, essential oil, distillation, agar diffusion, DPPH, GC/MS, sulfur-containing compounds, anti-oxidant activity, anti-microbial agent, oral pathogens, *Streptococcus sobrinus*, *S. mutans*, *Staphylococcus aureus, Candida albicans*

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INTRODUCTION

Scorodocarpus borneensis Becc. (Olacaceae) is a tall tree that grows naturally on the island of Borneo and in the Malay Peninsula. The local people refer to it as "wood garlic" due to its strong garlic odor. The aroma of garlic is present in the leaves, flowers and fruits. The falling fruit has a rugged hard skin and is similar in shape and size to a walnut (Burkill 1935). The older leaves are used as a seasoning rather than consumed as a vegetable (Hoe & Siong 1999). The decoctions of *S. borneensis* roots are taken orally to cure hemorrhoids (Mohammad *et al.* 2012). In Sarawak, the leaves and bark are boiled and drunk to treat leprosy and diabetes (Lim *et al*. 2012).

Sesquiterpenscodopin and scorodocarpines are found in the fruits of *S. borneensis* and hemi-synthetic sesquiterpene and cadalene-β-carboxylic acid are found in its bark (Wiart *et al.* 2001). In previous studies, other compounds have been isolated and identified from the fruits of *S. borneensis*, including sulfur-containing compounds, such as 2,4,5-trithiahexane, 2,4,5,7-tetrathiaoctane, 2,4,5,7-tetrathiaoctane 2,2-dioxide, and 2,4,57-tetrathiaoctane 4,4-dioxide (Kubota *et al.* 1999*a, b*; Lim *et al.* 1998). Kubota & Kobayashi (1994)[9] found that components from the fruits of *S. borneensis* were useful as a natural preservative. Most components in the fruits of *S. borneensis* were sulfur-containing compounds, such as methyl methylthiomethyl disulfide and bis(methylthiomethyl) disulfide.

Many higher and aromatic plant-derived medicines used in folk medicinal systems have been reported as agents used to treat infectious diseases, and a number of these medicines have been investigated for their efficacy against oral pathogens. Essential oils from several plant species, namely: *Cinnamomum zeylanicum* Blume (Lauraceae), *Citrus aurantiifolia* (Christm.) Swingle (Rutaceae), *Lippia graveolens* Kunth (Verbenaceae), and *Origanum vulgare* L. (Lamiaceae) can control microorganisms related to oral bacteria (Miller *et al.* 2015).

To the best of our knowledge, no reports on the chemical compounds of essential oil from the leaves of *S. borneensis* have been published so far and little is known about the anti-microbial activity against oral pathogens or the effects on dental plaque formation *in vitro*. Thus, in the present study, essential oil was distilled from plant leaves collected in East Kalimantan, and oil composition was analyzed by gas chromatography mass spectrometry (GC/MS). Furthermore, its anti-microbial activity against four oral pathogens and anti-oxidant activities were assayed.

MATERIALS AND METHODS

Leaves of *Scorodocarpus borneensis* were collected from the Botanical Garden of Mulawarman University, East Kalimantan, Indonesia. The leaves were dried and prepared in one day. Glucose, nutrient broth and anhydrous sodium sulfate were obtained from Merck (Darmstad, Germany). DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Wako Crude Chemical Industries, Ltd., Japan. Other chemicals were commercially available. The plant name was verified as the accepted name of the species in the genus *Scorodocarpus* (Olacaceae) at www.theplantlist.org. A

voucher specimen was deposited in the Dendrology Laboratorium of Mulawarman University.

Steam distillation method. Steam distillation is used widely in isolating liquids from natural sources. The leaves into the kettle are placed on a hollow plate over boiling water. The essential oil is evaporated for 4 hours or more. The oils were collected and separated using a separatory funnel after the steam distillation process. Oil and water will separate and form two layers. Oil that has a higher density of air will sink to the bottom while some oil does not sink and will be trapped at the top of the separation funnel. The process of separating and collecting oil is repeated several times until no oil is left in the separation funnel and nothing else can be obtained. Anhydrous sodium sulfate was used to adsorb the residual water that fused with the oil. The quantity of oil was determined according to the yield (Kuspradini *et al*. 2016).

The percent and yield of essential oil was calculated by following formula (Siddiqui *et al.* 2006):

%Yield = [Weight of oil (grams)/Weight of sample taken (grams)] x 100%

Anti-microbial assay. Anti-microbial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial and anti-fungal activities of the plant samples. Nutrient agar for microbial cultures were prepared according to the manufacturer's instructions.

Anti-microbial assays were conducted using the agar diffusion method with modification (Donaldson *et al*. 2005). The microorganisms used in this study were *Streptococcus sobrinus, S. mutans, Staphylococcus aureus,* and *Candida albicans*. All materials were sterilized by autoclaving at 15 lbs per square inch pressure (121ºC) for 15 min. Microbes were inoculated in nutrient agar (NA). Approximately 5 ml of sterile media was poured into 5 cm-diameter petri dishes. After the media solidified, 25 µl of microbial suspension was inoculated using a sterile swab and was spread over the surface of the media three times. Next, 6 mm-diameter wells were bored into the solidified media using sterile cork borer. Serial dilutions of *S. borneensis* essential oil were prepared with 40% ethanol. The crude essential oils were diluted to serial dilutions at the following concentration 1, 1/10, and 1/100 (v/v). Twenty microliters of each essential oil concentration were added into the wells. Chlorhexidine (10 µg per well), a standard synthetic antibiotic, was used as a positive control, and 40% ethanol was used as a negative control. All plates were incubated at 37˚C for 24-48 hours. After incubation, the petri dishes were observed for formation of a clear inhibition zone around the well, indicating the presence of anti-bacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well. All tests were performed in duplicates. AI was calculated using the formula adopted from Arya *et al*. (2010):

AI = Inhibition zone of sample/Inhibition zone of standard

Anti-oxidant assay.The anti-oxidant activity of the essential oil was determined by the DPPH radical scavenging method (Bachrouch *et al.* 2015) with modification. Five hundred microlitres of the sample in methanol solution weres added to 500 µL of a methanolic DPPH solution with final concentration at 25, 50, 100, 250, 500, 1,000 µg/mL. Ascorbic acid was used as a positive control. The measurement of absorbance was made against a blank prepared for each concentration at 517 nm using a Shimadzu UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The anti-radical activity (three replicates per treatment) was expressed as IC_{50} (µg/mL), the concentration required to cause a 50% DPPH inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$SA\% = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound); A_{sample} is the absorbance of the test samples. Samples were analyzed in triplicates.

Gas chromatography-mass spectrometry (GC/MS) analysis. The essential oil was analyzed by GC-MS (Shimadzu-QP-5050A) with the following setting: column - HP-5 MS, 60 m x 250 µm ID x 0.25µm film thickness; temperature program - from 70 to 290°C (40 minutes) at 15°C.minute^{-1}, and injection temperature - 290°C. The injection port temperature was 290°C, and the detector temperature was 250°C. The injection mode was split (50:1), and the inlet pressure was 18.03 psi. The carrier gas was helium with a flow rate of 1 ml.minute^{-1}. The mass spectrometer conditions were as follows: ionization voltage: 70 eV; MS source temperature at 250°C; MS quadruple temperature at 150°C; interface temperature at 290°C and electron ionization mass spectra were acquired over the mass range of 40-800 m/z.

Statistical data analysis. The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Regression analysis with polynomial model of dose-response curve plotting between different concentration and percent inhibition were used to obtained the IC_{50} value. Microsoft Excel 2010 statistical package was used for all analyses.

RESULTS

Essential oil from the leaves of *S. borneensis* was clear and yellowish. The results of the yield and refractive index were 0.39% and 1.435, respectively. The GC-MS chromatogram of the essential oil of *S. borneensis* showed 6 peaks, indicating the presence of six compounds (Table 1). The chemical compounds were identified by comparing their retention time, molecular formula, molecular weight and area (Table 1).

The most abundant compounds identified in the essential oils were 2,4,6-trithiaheptane-2,2-dioxide, methyl (methylsulfinyl) methyl sulfide, and 1,5-heptadien-3-yne (Figure 1). GC-MS analysis revealed that the essential oil of *S. borneensis* is mainly composed of sulfur-containing compounds. Most components in the essential oil of *S. borneensis* leaves were sulfur-containing

compounds, and 35.99 and 47.5% of these compounds consisted of sulfides with two and three sulfur atoms, respectively.

Table 1. Chemical constituents of the essential oil of *Scorodocarpus borneensis* by GC-MS.

No.	Retention Time (min)	Compound names*	Molecular Formulas	Molecular Weights	Area (%)
1	6.45	Trisulfide, dimethyl	$C_2H_6S_3$	126.26	4.15
2	9.18	Methyl (methylsulfinyl) methyl sulfide	$C_3H_8OS_2$	124.22	34.03
3	14.56	1,5-Heptadien-3-yne	C_7H_8	92.14	10.50
4	18.51	2,4,6-trithiaheptane-2,2-dioxide	$C_4H_{10}S_3$	154.32	43.35
5	24.15	Furan, tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl)	$C_{10}H_{18}O$	154.25	6.01
6	27.09	Methane, (methylsulfinyl) (methylthio)	$C_3H_8OS_2$	124.225	1.96

*Compounds were identified using the NIST, Wiley Mass Spectral Library and the mass spectroscopy data analysis.

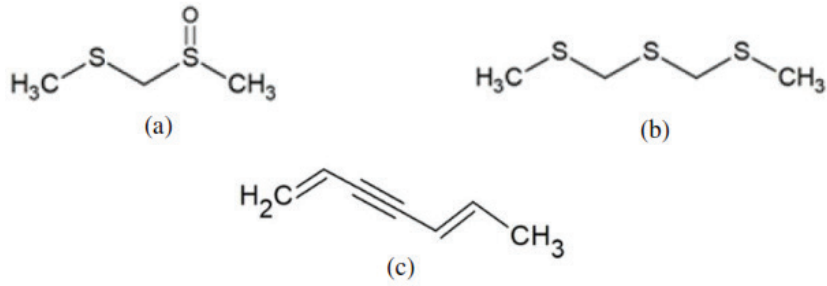

Figure 1. Major compounds identified in the essential oil of *Scorodocarpus borneensis*: (a) Methyl (methylsulfinyl) methyl sulfide; (b) 2,4,6-trithiaheptane, and (c) 1,5-Heptadien-3-yne.

In this study, four microbes were investigated in susceptibility tests with different concentrations of the essential oil. The essential oil showed better activity than the commercial antibiotics, chlorhexidine and chloramphenicol (Table 2). The essential oil was found to be highly effective at inhibiting the growth of all tested microbes.

Table 2. Anti-microbial activity of essential oil of *Scorodocarpus borneensis* against four microbes.

Sample concentration	Diameter of inhibition (mm)			
	Streptococcus sobrinus	*Streptococcus mutans*	*Candida albicans*	*Staphylococcus aureus*
CHX*	20.70±0.00	21.00±0.00	17.70±0.00	17.00±0.00
CMP*	28.70±0.00	31.30±0.00	17.30±0.00	23.30±0.00
1	51.57±0.94	53.67±0.94	52.67±0.47	52.89±0.51
1/10	50.33±0.00	51.67±0.47	51.33±0.47	51.78±1.01
1/100	18.00±0.94	18.50±0.24	18.17±0.71	15.67±0.58

Remarks : CHX= Chlorhexidine, CMP= Chloramphenicol, * at concentration (10 µg)

Estimation of the potential antimicrobial can be measured quantitatively. It can be measured by the activity index (AI) values by comparing the inhibition zone of sample to inhibition zone of respective standards. The AI against chlorhexidine showed that the essential oil of *S. borneensis* exhibited the strongest bactericidal activity at a concentration of 1 (crude essential oil, v/v) against *S. aureus*. The AI decreased in the following order: *S. aureus* > *C. albicans* > *S. mutans* > *S. sobrinus* (Figure 2a). Furthermore, the AI against chloramphenicol showed that the essential oil of *S. borneensis* exhibited the strongest bactericidal activity on crude essential oil (concentration 1, v/v) against *C. albicans*. The activity index of essential oil against chloramphenicol decreased in the following order: *C. albicans* > *S. aureus*> *S. sobrinus* > *S. mutans* (Figure 2b). Based on the activity index, the essential oil had the best activity index of 3.11 in *S. aureus.*

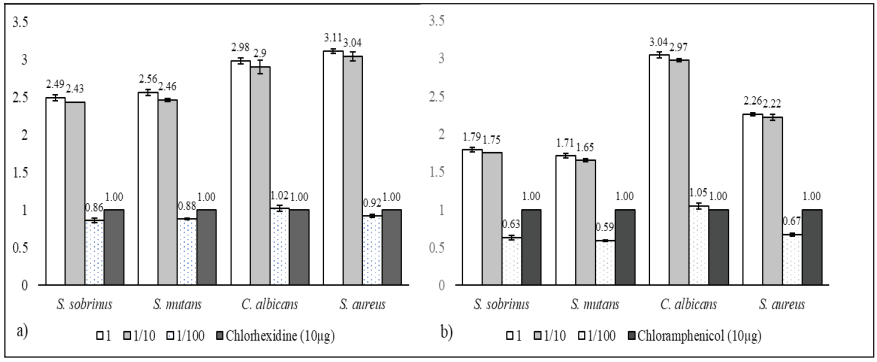

Figure 2. Comparison of essential oil and antibiotics activity index: (a) Chlorhexidine and (b) Chloramphenicol.

The essential oil of *S. borneensis* showed a dose-dependent inhibitory effect against radical scavenging DPPH with an IC_{50} value of 715.97 µg/mL. The essential oil did not have good radical scavenging compared to ascorbic acid (Table 3).

Table 3. Anti-oxidant activity of essential oil of *Scorodocarpus borneensis.*

No.	Samples	Percentages of Anti-oxidant Activity (%)						IC_{50} (µg/mL)
		1000 µg/mL	500 µg/mL	250 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	
1	Ascorbic acid*	-	-	-	97.01	96.81	97.1	-
2	*S. borneensis*	54.52	37.18	36.34	7.63	4.15	3.28	715.97

*There were no tests for concentrations of 250-1,000 µg/mL.

DISCUSSION

Steam distillation appears to be a good method for extracting the essential oil from leaves of *S. borneensis* as it results in good yield and is a simple technique. The advantage of this technique is the favored material distills at a temperature below 100°C. Therefore, the decomposition may be prevented if an unstable or excessively boiling essential ingredient is removed from the mixture. Because all gases mix, the two materials can be mixed in the evaporation and co-distil. Once the distillate is cooled, the desired components which are miscible, are separated from the water (Pavia 2011). In this study, we report the presence of a methyl (methylsulfinyl) methyl sulfide and 2,4,6-trithiaheptane-2,2-dioxide in the essential oil of *S. borneensis* leaves. Similar results have been obtained, but these compounds were found in another part of *S. borneensis* plant. Methyl (methylsulfinyl) methyl sulfide was the main component in the fruits of *S. borneensis*. This compound has natural preservative properties (Kubota & Kobayashi 1994). 2,4,6-Trithiaheptane-2,2-dioxide was previously isolated from the fruits of *S. borneensis*. Trisulfide, dimethyl or DMTS is a sulfur-based molecule found in garlic, onion, broccoli and similar plants, and it has been reported to act as a sulfur donor-type cyanide counter measure and key mediator of pollinator attraction in brood-site deceptive plants (Rockwood *et al.* 2016, Zito *et al.* 2014). 1,5-Heptadien-3-yne were fatty acid compounds that are also found in the flower fragrance of *Cypripedium tibeticum* (Li *et al.* 2006). Furan, tetrahydro-2,2-dimethyl-5-(1-methyl-1-propenyl) has a citrus odor and categorized as a flavoring agent (Burdock 2010). Methane, (methylsulfinyl) (methylthio) is also found in the essential oil of *Allium atroviolaceum* and *Sonchus arvensis* leaves (Lorigooini *et al.* 2014).

Scorodocarpus borneensis leaf oil has strong activity against all microbial tested in this study and this result agreed with Kuspradini *et al.* (2016) that screened the anti-microbial activity of several essential oils. It has been reported that the crude oil of *S. borneensis* leaves had anti-microbial activity against *S. aureus* and *C. albicans* with no comparisons of different concentrations. Compared to results from the previous study, the inhibition activity of *S. borneensis* essential oil against *S. aureus* is stronger in this study. This difference in activity is probably due to variations in the essential oil composition at different sample collection times. The biological and pharmacological activities of essential oils rely upon the species,

ecological factors and environmental conditions (Lahlou 2004). Based on the activity index (AI), the essential oil had good potency as an anti-microbial agent. The ranges of AI were 0.59-1.05, 1.65-3.04, 1.79-3.11 at different diluted concentration of 1, 1/10 and 1/100, respectively. Activity index of positive controls/antibiotics was 1. The AI value of more than 1 indicated the considerable role of sample. Interestingly, high activity indexes (AI > 1) were observed at essential oil concentration of 1/10 and 1. According to Hosamath (2011) and Awan *et al.* (2013), the activity index of the test substance above 0.5 was considered as significant activity and the more AI values evaluated the more significant the results. The anti-microbial activity of a given essential oil may depend on only one or two of the oil's major constituents. However, increasing evidence indicates that the inherent activity of essential oils may rely on not only the ratio of the main active constituents but also the interactions between them and the oil's minor constituents (Singh *et al.* 2014). In this study, most components of the essential oil of *S. borneensis* leaves were sulfur-containing compounds, with 93.49% consisting of sulfides with two or three sulfur atoms. Researchers have demonstrated that sulfur-containing compounds may be useful as anti-microbials (Naganawa *et al.* 1996, Lim *et al.* 1998, Kim *et al.* 2006). According to earlier studies (e.g. Kim *et al.* 2004), sulfides, especially those with three or more sulfur atoms, apparently possess potent anti-microbial activity. It has been suggested that the strong anti-microbial activity of *S. borneensis* leaf oil is due to the availability of its major constituent with three sulfur atoms: 2,4,6-trithiaheptane-2,2-dioxide.

The half maximal inhibitory concentration 50 (IC_{50}) were used to determine anti-oxidant capacity of sample compared to standard. Samples that had IC_{50} values lower than 50 µg/ml were very strong anti-oxidant, 50-100 µg/ mL were strong anti-oxidant, 101-150 µg/mL were medium anti-oxidant, while weak antioxidant with IC_{50} higher than 150 µg/ mL (Blois 1958). Based on this, the essential oil of *S. borneensis* could be classified as weak anti-oxidant with an IC_{50} of more than 150 µg/ mL. Researchers have demonstrated that sulfur-containing compounds may be useful as anti-oxidants (Dansette *et al.* 1990, Pappa *et al.* 2007). But, in many essential oils due to the absence of phenols, most of them exhibit no or low anti-oxidant activities (Sharopov *et al.* 2015). It can be assumed that the anti-oxidant activity of the tested essential oils as nonpolar extracts could be linked to their phenolic concentration. However, it is important to realize that in certain cases, anti-oxidants can be pro-oxidant and can stimulate free radical reactions. The methods of expressing anti-oxidant activity appear to be as varied as the methods of anti-oxidant measurement. The measurement of anti-oxidant activities, especially for anti-oxidants that are mixtures, multi-functional or acting in complex multi-phase systems, cannot be evaluated satisfactorily by a simple anti-oxidant test without considering the many variables influencing the results (Antolovich *et al.* 2002).

CONCLUSION

In this study, the leaves of *S. boornensis* can produce the essential oil with the strong anti-microbial activities against oral pathogens. To the best of our knowledge, no reports on the chemical compounds of essential oil from the leaves

of *S. borneensis* have been published so far. This is the first report that discusses the essential oil isolated from *S. borneensis* leaves, its anti-microbial, anti-oxidant and its chemical composition. The most components of the essential oil of *S. borneensis* leaves were sulfur-containing compounds, with 93.49% consisting of sulfides with two and/or three sulfur atoms. Therefore, this property could be used to develop new anti-microbial ingredients from *S. borneensis* leaf oil for oral or personal products.

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