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A Comparative Analysis of Essential Oils from Three Species of *Cinnamomum* Growing Wild in East Kalimantan

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Abstract. The *Cinnamomum* genus is a plant of the Lauraceae family. This plant is widely spread on the island of Borneo. In this study, the essential oils of plant leaves originating from the *Cinnamomum* genus were tested for their antimicrobial activity. The types of plants are *Cinnamomum burmannii*, *Cinnamomum parthenoxylon*, and *Cinnamomum inners*. The essential oils derived from the leaves of *Cinnamomum* genus which grown in East Kalimantan were obtained by steam distillation. The yield of 0.6% 1.64%, and 0.01% of essential oil were obtained from *C. burmannii*, *C. parthenoxylon*, and *C. inners*, respectively. The oils were examined by gas chromatography to determine their major constituents. The major constituents of *C. burmannii* were eugenol (85.03%), *C. parthenoxylon* were safrole (55.38%), while the major constituents of the *C. inners* were Cyclobutal (1,2,3,4) dicyclooctene, hexadecahydro (17.94%). The essential oils were also examined for their antimicrobial activities on yeasts and bacteria. The microbial used were *Streptococcus mutans*, *Streptococcus sobrinus*, *Candida albicans*, and *Staphylococcus aureus*. Two methods (agar diffusion and broth microdilution) were used to assess the antimicrobial activity. The Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentration (MBC) were determined in every case. All types of plants *Cinnamomum* genus in this study has the potential in inhibiting the growth of *Streptococcus mutans*, *Streptococcus sobrinus*, *Candida albicans*, and *Staphylococcus aureus*.

Keywords: *Antimicrobial, Cinnamomum, Essential oil, leaves, Lauraceae.*

1. Introduction

Lauraceae is a tropical plant that is widely available in Indonesia [1]. This plant is widely used for various purposes, such as ironwood (*Eusideroxylon zwageri*) for building materials, *Cinnamomum burmannii* for spices, avocado or *Persea americana* as fruits and so on [2]. Since ancient times, indigenous people of Borneo also utilize plants in the treatment of various diseases in the daily life and one of them from genus *Cinnamomum* [3].

Traditionally, *C. burmannii* is used to treat flatulence, nausea, diarrhea, colds, flu, fever, and rheumatism [4]. On the other hand *C. inners* is also used as a traditional medicine to treat headaches and respiratory disorders [5]. *C. inners* is popular plant as evergreen tree with family name Lauraceae. Generally the traditional ethnic used as a traditional medicine to relieve appetite problem, breathing,



headaches, and several illness causing bacteria symptom [6]. *C. parthenoxylon* is also spread in the area of North Buton, Southeast Sulawesi with the name kondongia. So far, people only use *C. parthenoxylon* wood as a raw material for making boats and building houses, while the wood bark is used to eradicate ticks [7]. Jia et al (2009) reported that the polyphenol content found in *C. parthenoxylon* stem extract has the ability to reduce glucose levels tested in diabetic mice [8]. Chinese people use the stems, leaves, and fruit of *C. parthenoxylon* as traditional medicine (Traditional Chinese Medicine), among others, dysentery, rheumatism / rheumatoid arthritis, and pertussis (whooping cough) [9].

Currently the use of *Cinnamomum* or cinnamon is still limited to the bark of the wood, although the typical aroma of cinnamon can also be found in the leaves. On the basis of the above, it is necessary to research the materials other than bark of the plant species of the different *Cinnamomum* genus to find out the chemical content profile and its potential utilization as an antimicrobial.

The purpose of this study was to measure the essential oil yield of three types of aromatic plants from the *Cinnamomum* genus grown in East Kalimantan by the method of extracting the water vapor of the leaf and to know the antimicrobial activity of the essential oil using the agar and staining diffusion method. It is expected that this research can provide scientific information about the potential of East Kalimantan aromatic plants that can be useful as antimicrobial for humans.

2. Materials and Methods

2.1. Plant materials and chemicals

Leaves medicinal plants were collected from KRUS (Samarinda Educational Forest), East Kalimantan, Indonesia. The selected plants were: *Cinnamomun burmannii*, *Cinnamomun parthenoxylon*, and *Cinnamomun inners*. The plants were identified by a taxonomist and confirmed by references. The plant materials were destilate for 6 h to obtain the essensial oil with distillation tool. Antimicrobial assay used nutrient broth from Difco (Detroit, MI, USA). Other chemicals were of HPLC grade or the highest purity commercially available.

2.2. Steam distillation method

The essential oil was discovered by means of the steam distillation process [10]. The distillation process is carried out by inserting 1-3 kg of leaves into a distillation flask on a perforated plate. The essential oil is evaporated using boiling water at a temperature of 100oC for 6-10 hours until the water and essential oil will mix. Water and oil are separated using a separating funnel. The essential oil has a higher density so it will sink at the bottom and vice versa if the density is lower it will appear at the top of the water and stick to the separating funnel. The separation process usually lasts for 15 minutes. Wait for 15 minutes until two layers are completely formed and the oil collects. In the final stage, Na2SO4 is used to adsorb the remaining water which combines with the oil.

2.3. Antimicrobial assay

The diffusion method using nutrient agar for bacteria and fungi was adopted from [10]. This method was used to observe the in vitro antimicrobial activity of essential oils against oral pathogens. The microorganisms used in this study were *Streptococcus mutans*, *Streptococcus sobrinus*, and *Staphylococcus aureus* (bacteria) and *Candida albicans* (fungi). In the test, the concentrations used were 100% (pure oil), 10%, and 1% which were diluted in 40% ethanol. As a positive control, two standard antibiotics, namely Chlorhexidine for bacteria and Chloramphenicol for fungi were selected. Ethanol 40% was presented as a negative control. The antimicrobial activity value was determined after incubation at 32oC for 18-24 hours. Next, the diameter of the inhibition zone was measured and all experiments were carried out in triplicate [11].

3. Results and Discussion

The three species of *Cinnamomum* essential oils exhibit different properties. The highest yield value of the three *Cinnamomum* species is produced from the *Cinnamomum burmanii* species with a value of

1.64%. The refractive index varies from 1.364 to 1.527. The colors produced on each of the volatile oils in this study were either colorless or colorless in *C. inners*, pale yellow on *C. parthenoxylon* and yellow on *C. burmannii*. The result of GCMS analysis on the three essential oils of *Cinnamomum* plant species grown in Kalimantan shows different chemical profiles in Table 1.

Table 1. Percentage of major compounds from essential oil

No	Essential Oil	Major compound	(%)
1	<i>Cinnamomum burmannii</i>	Eugenol	85.03
2	<i>Cinnamomum parthenoxylon</i>	Safrole	55.38
3	<i>Cinnamomum inners</i>	Cyclobutal (1,2,3,4) dicyclooctene, hexadecahydro	17.94

Cinnamomum burmannii has the highest chemical component of eugenol 85.03%. *C. burmannii* leaves contain a lot of eugenol and contained little amount of cinnamaldehyde [12]. *C. parthenoxylon* contains 55.38% safrole, the chemical components contained in *C. parthenoxylon* oil are safrole, eugenol, and cinnamic aldehyde. Essential oils of *C. inners* leaves in this study contained 17.94% of the chemical components cyclobutal (1,2,3,4) dicyclooctene, hexadecahydro. This is in contrast to the results of research conducted by [13], where the essential oil of *C. inners* leaves obtained from the water distillation method has the main chemical component (E) -caryophyllene (20.38%).

Results of antimicrobial assay on 3 types of bacteria and 1 type of fungus showed different results. All essential oil samples of the three types of *Cinnamomum* have the potential to inhibit the growth of these 4 microbes. Table 2 shows that in all three species have different capabilities to inhibit the growth of each bacterium. The highest inhibition was found in *C. inners* against *S. mutans* bacteria of 27.00 ± 0.42 at $24.9 \mu\text{g} / \text{well}$. However *C. burmannii* has the best inhibitory ability against all four microbes to the lowest concentration. The different concentrations used for each type of *Cinnamomum* are based on the different types of essential oils owned by each plant.

Table 2. Inhibition zone of essential oils againsts *S. sobrinus*, *S. mutans*, *S. aureus* and *C. albicans*

Essential Oil	Conc. $\mu\text{g}/\text{well}$	Inhibition zone (mm)							
		<i>S. sobrinus</i>		<i>S. mutans</i>		<i>S. aureus</i>		<i>C. albicans</i>	
<i>C. burmannii</i>	22.7	22.00	± 1.15	20.00	± 0.00	19.35	± 1.91	15.35	± 0.49
<i>C. parthenoxylon</i>	23.8	20.35	± 4.74	26.30	± 5.66	19.35	± 0.49	10.35	± 0.49
<i>C. inners</i>	24.9	19.50	± 2.12	27.00	± 0.42	11.85	± 0.21	13.65	± 0.49
Chloramphenicol	10	28.70	± 0.00	31.30	± 0.00	23.30	± 0.00	17.30	± 0.00
Chlorhexidine	10	20.70	± 0.00	21.00	± 0.00	17.00	± 0.00	17.70	± 0.00

* = $\mu\text{g}/\text{well}$

Eugenol and cinnamaldehyde which are the main content of *C. burmannii* have potential as antibacterial and antibiofilm. Based on research conducted by Dwijayanti (2011) [14], essential oil of bark of *C. burmannii* has antibacterial power to *S. mutans* with 23.7 ± 0.04 barrier and 5% MIC and MBC at 20%. Leaf ethanol extract and bark of *C. burmannii* were able to inhibit the growth of *E. coli* and *S. aureus* bacteria [15]. Hertiani et al (2010) [16] also reported that the essential oil mixture of *S. aromaticum* and bark *C. burmannii* was effective as an antibacterial and antibiofilm against *S. mutans* bacteria. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration are shown on the following table (Table 3).

Table 3. MIC and MBC values of essential oils againsts *S. sobrinus*, *S. mutans*, *S. aureus* and *C. albicans*

Essential oil	MIC ($\mu\text{g}/\text{mL}$)				MBC ($\mu\text{g}/\text{mL}$)			
	<i>S. sobrinus</i>	<i>S. mutans</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>S. sobrinus</i>	<i>S. mutans</i>	<i>S. aureus</i>	<i>C. albicans</i>

<i>C. burmanii</i>	312.5	312.5	312.5	312.5	>1250	>1250	>1250	>1250
<i>C. inners</i>	1250	625	312.5	1250	>1250	>1250	>1250	>1250
<i>C. parthenoxylon</i>	1250	1250	312.5	1250	>1250	>1250	>1250	>1250
<i>Chlorhexidine</i>	312.5	312.5	312.5	312.5	312.5	312.5	312.5	312.5

In the test by this staining method, the lowest MIC value (312.5 µg / mL) was shown in *C. burmannii* species in all microbes used. *C. burmanii* also contain coumarin, it has reported as a toxic constituents from steam bark, probably it has play rules of inhibition of the bacteria. The three essential oils of this type of *Cinnamomum* in this study are excellent in inhibiting *S. aureus*. Ethyl acetate and methanol extracts from *C. inners* leaf have antibacterial activity against *S. aureus* (MRSA) and *C. albicans* [6]. Research conducted by Ubonnuch et al (1998) [13] explains that the essential oil of *C. inners* leaves has antimicrobial activity against *S. aureus* and *C. albicans* with MIC values 0.312% and 0.078%. In conclusion, the yield value of the essential oil produced is *Cinnamomum parthenoxylon* > *Cinnamomum burmanii* > *Cinnamomum inners*. The profile of major chemical components contained in *Cinnamomum burmanii*, *Cinnamomum parthenoxylon* and *Cinnamomum inners* are eugenol, safrole and cyclobutal (1,2,3,4) dicyclooctene, hexadecahydro, respectively. The three-plant species of the *Cinnamomum* genus in this study have the potential to inhibit microbes that growth in the mouth, with each character.

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