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Bioactivity of Essential Oils from Leaves of *Dryobalanops lanceolata*, *Cinnamomum burmannii*, *Cananga odorata*, and *Scorodocarpus borneensis*

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Abstract

This research aimed to examine the bioactivity of essential oils. The essential oils obtained from the leaves of *Dryobalanops lanceolata*, *Cinnamomum burmannii*, *Cananga odorata*, and *Scorodocarpus borneensis* by steam distillation method. This research used antioxidant and antimicrobial test. The antioxidant activity was assayed by DPPH (1,1-diphenyl-2-picrylhydrazyl) and using ascorbic acid as positive control. The antimicrobial properties of the pure essential oils were determined using agar diffusion method. Two different microorganisms were used in this study were *Staphylococcus aureus* and *Candida albicans*. The zone of inhibition and activity index were measured and compared against a known synthetic standard. The yields of essential oils of *D. lanceolata*, *C. burmannii*, *C. odorata* and *S. borneensis* obtained in the present study were 0.12%, 1.01%, 0.04%, and 0.39%, respectively. The extracts inhibited all tested microorganism and susceptible against *S. aureus*. Only *C. odorata* has no inhibition against *C. albicans*. *D. lanceolata* and *C. odorata* have the largest inhibition zone against *S. aureus* (49.3 and 49 mm). The best inhibition zone was shown by the *S. borneensis* (52.7 mm) against *C. albicans*. The essential oils of *D. lanceolata* and *C. burmannii* also have potency to inhibit the free radicals at concentration 25 – 100 ppm.

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Keywords: Essential oil; Steam distillation; Antioxidant; *Staphylococcus aureus*; *Candida albicans*.

1. Introduction

It is well known that medicinal and aromatic plants containing active compounds are able to inhibit microbial growth and act as antimicrobial agents. The bioactivities of plant oils have demonstrated great influences and used

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for various purposes including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (Lis-Balchin, 1997; Buchbauer, 2000; Mercier et al., 2005).

A complex mixture of volatile molecules that are produced by the secondary metabolism of aromatic plants were composed formed essential oils (Faleiro, 2011). Plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots are obtained the aromatic oily (Van de Braak & Leitjen, 1999).

Essential oils have been shown to possess antimicrobial properties (Prabuseenivasan et al., 2006). The medicines were complementary used by many essential oils for bacterial and fungal infections including boils, acne, gingivitis, and vaginal candidiasis. Some numerous books and articles in the popular press said that essential oils have been recommended for use as home remedies for treatment of vaginal candidiasis.

Cinnamomum burmannii is a shrub or a small tree, commonly known as Indonesian cassia, Batavia cassia, and Padang cassia, and is a member of the Lauraceae family. The plant is distributed in Southeast Asia and is cultivated in parts of Indonesia and Philippines, the plant possess oblong-elliptical, 4-14 cm long, glossy green, oppositely arranged leaves and an ovoid 1 cm long fruit. The dried bark of the plant is found in the market in the form of rolls and quills, which is used for cooking and flavoring (Tan, 2005).

Cananga odorata belong to the Annonaceae family, with 125 genera and 2050 species. To date, the Cananga genus consists of two species of plant, namely, *C. odorata* and *C. latifolia*. *C. odorata* is a perennial tropical tree which grows natively in South-East Asia countries (Saedi & Crawford, 2006). *C. odorata* has a variety of medicinal properties and traditional uses. In Indonesia, ylang-ylang oil is used to enhance euphoria feel during sex and also reduce sexual anxiety (Holt, 1999) and in Indian the leaves of *C. odorata* is also believed to relieve itchiness by direct topical application and also to treat dandruff (Jain & Srivastava, 2005).

Scorodocarpus borneensis Becc. (the Olacaceae family) is a tall tree growing naturally on the island of Borneo and on the Malay Peninsula, which has been named by natives as "wood garlic" due to its strong garlic-like smell. This garlic smell is present in the leaves, flowers and fruit. The fallen fruit has a hard outer nutshell, and is similar in shape and size to a walnut (Burkill, 1935). The root of *S. borneensis* are made into decoctions to be taken orally to cure hemorrhoids (Mohammad et al., 2012).

In the countries, where infectious diseases are rife and resources limited, such challenge can assume overwhelming proportions, hence the resurgence in the use of herbal preparations to treat diseases (El-Mahmood, 2008). Plants and their essential oils are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, includes Faleiro (2001), Patricia (2004), Masango (2005), Tajuddin et al. (2012) and Prabuseenivasan et al. (2006). The antimicrobial activity of the essential oil is economically important considering the availability of herbal material for the production of natural antimicrobial products and for its use in traditional medicine.

Therefore, plants evaluations in antimicrobial and prevention antioxidant properties are important majorly in managing diseases by those that can accept their innumerable values for alternative therapy.

Although several studies have determined the biological activities of the *C. burmannii* and *C. odorata*, little information has been conducted the essential oil from their leaves. To the best of our knowledge, no report on the biological studies of essential oil from leaves of *D. lanceolata* and *S.boornensis* has been published so far. Thus, in the present study, the essential oil was extracted from leaves plants collected in East Kalimantan and its antimicrobial as well as antioxidant activities were determined.

2. Materials and methods

2.1. Materials and chemicals

Leaves of aromatic plants were collected from Botanical Garden of Mulawarman University, East Kalimantan, Indonesia, in March 2015. *Cinnamomum burmannii*, *Cananga odorata*, *Dryobalanops lanceolata*, and *Scrodocarpus borneensis* were the selected aromatic plants. The plants were dried and prepared for 1 day. Anhydrous sodium sulphate, glucose, and nutrient broth were obtained from Merck (Darmstadt, Germany). DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Other chemicals were commercially available.

2.2. Steam distillation method

The essential oils were collected by the steam distillation method. The steam distillation method adopted from Wong et al. (2014) with slight modification. The leaves were packed into the kettle sitting on a perforated plate above the boiling water. The essential oils were volatilized with boiling water at temperature 100°C for 2 hours or more. After the steam distillation process, the oil will collected and separated used the separatory funnel which can be used to separate the immiscible liquids of two layers such as oil and water. The oils which have higher density than water will sink at the bottom, but some of it did not sink, it was at the top and stuck to the separatory funnel. Wait to the water and oil separated and formed two layers and oil was collected. The process of separation will do several times until no oil was left in the separatory funnel and can be separate anymore. Na₂SO₄ was used to adsorb the residual of water that fused with the oil. The quantity of the oil was determined according to the yield.

2.3. Antimicrobial assay

Antimicrobial assays were done by the agar diffusion method (Singh et al, 2002) with slight modification. The microorganisms used in this study included *Staphylococcus aureus* and *Candida albicans*. All materials were sterilized by autoclaving at 15 lbs pressure (121°C) for 15 min (Sandhyarani et al., 2014). Microbes were inoculated in nutrient agar (NA). 5 ml sterile media were poured into sterile petridishes with 5 cm of diameter. After the media were altered to solidify, 25 µl of suspension microbial were inoculated by sterile swab and spread all over the surface of the media plates three times (Kunchana et al., 2014). Wells of 6 mm diameter were bored in solidified media using sterile cork borer. Add 20 µl of the pure essential oils into the well. Chlorhexidine was a synthetic standard as antibiotic for positive control at the concentration of 10µg/well and acetone as negative control. All the plates were incubated at 37°C for 24-48 hours. After incubation, the plates were observed for formation of clear inhibition zone around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well. All test were performed in duplicate. The diameter of inhibition zones were measured in mm taken with a ruler (Kusuma et al., 2014). Activity index was calculated for all essential oils (Munazir et al., 2012).

The yield of essential oils were calculated using the formula (Mercy et al., 2015).

$$\% \text{ Yield} = \frac{\text{Weight of oil (g)}}{\text{Weight of leaves taken (g)}} \times 100$$

The zone of inhibition for each sample was measured in mm. From this the Activity Index (AI) was calculated for all essential oils using the following formula (2) (Munazir et al, 2012).

$$A.I = \frac{\text{Mean zone of inhibition of each solvent extract}}{\text{Zone of inhibition obtained for standard}}$$

2.4. Antioxidant assay

The antioxidant activity of essential oils was determined by DPPH radical scavenging method (Shimizu et al., 2001; Politeo et al., 2006) with slight modification. The samples were dissolved in methanol (concentration of stock solution were 25, 50, and 100 ppm). Ascorbic acid was used as positive control. Absorbance measured at 517 nm using Shimadzu UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). Percent inhibition of the DPPH radical was calculated by all samples.

3. Results and Discussion

The most popular method for essential oil extraction process was steam distillation (Masango, 2005). The essential oil from the raw materials was extracted by the hot steam that applied in this method. The final of steam distillation process will be condensed and separated into the liquid form that were the mixture of oil and steam. The oils was less dense than water and could be separated using proper method and instruments (Tajjudin et al., 2012).

In this study, the essential oil were obtained from steam distillation of leaves part of *D. lanceolata*, *C. burmannii*, *C. odorata*, and *S. borneensis*. The yields of essential oils varied with plant species. The result of the yields are presented in Table 1.

Table 1. Yield of Essential Oils

No	Sample	Sample Weight (kg)	Moisture Factor	Content of Oils (g)	Yield (%)
1	<i>D. lanceolata</i>	4.15	0.51	2.54	0.12
2	<i>C. burmannii</i>	4.75	0.56	26.88	1.01
3	<i>C. odorata</i>	5.1	0.28	0.56	0.04
4	<i>S. borneensis</i>	4	0.41	6.52	0.39

All essential oils extracted from the leaves produced a clear, yellow liquid (data not shown). *C. burmannii* appears to be richer in oil (1.01 %) than the other plant species, while *C. odorata* was poorer (0.04%).

In this study, the cinnamomun oil yield was higher compared to that obtained by Rowaan. (1936) and Cheng et al. (1992). Rowaan investigated the presence of essential oil in the leaves of *C. burmannii* and found that the leaves of *C. burmannii* possess essential oil (0.4%). Cheng et al. examined the essential oil of the leaves of *C. burmannii* obtained by steam distillation. The yield of the oil was between 0.54% and 0.85%. Our results indicated that the *C. burmannii* grown in East Kalimantan had oil yields higher than previously reported.

Table 2 summarises the antimicrobial properties of the four essential oils (*D. lanceolata*, *C. burmannii*, *C. odorata*, and *S. borneensis*). Microorganisms susceptibility to the essential oils, as determined by the agar diffusion method, showed that oils with the highest inhibitory effects produced inhibition zones of 52.7 mm diameter.

Table 2. Antimicrobial Activity of Pure Essential Oils

Bacterial Strains	Sample	Zone of Inhibition expressed in mm	Zone of Inhibition of Standard drug expressed in mm	Activity Index
	<i>D. lanceolata</i>	49.3 ± 1.41		2.85
<i>Staphylococcus aureus</i>	<i>C. burmannii</i>	18.5 ± 1.13	17.3 ± 0.00	1.07
	<i>C. odorata</i>	49 ± 1.41		2.83
	<i>S. borneensis</i>	15.3 ± 1.41		0.88
	<i>D. lanceolata</i>	18.7 ± 1.41		0.89
<i>Candida albicans</i>	<i>C. burmannii</i>	10.7 ± 1.41	21 ± 0.00	0.51
	<i>C. odorata</i>	-		-
	<i>S. borneensis</i>	52.7 ± 0.49		2.51

Remarks: ZI, zone of inhibitions; AI, activity index.

Among all oils analyzed in this work, the essential oil of *D. lanceolata* (49.3 mm) was the most effective as an antimicrobial agent against *S. aureus* followed by Cananga oil (49 mm) and Cinnamomum oil (18.5 mm). Scorodocarpus oil showed the least antimicrobial activity against *S. aureus* (15.3 mm) but showed highest activity against *C. albicans* (52.7 mm). The order of antimicrobial activity against *C. albicans* was determined as *S.*

borneensis > *D. lanceolata* > *C. burmannii* > *C. odorata*. Their values, in fact, ranged from 10.7 ± 1.41 mm to 52.7 ± 0.49 mm and only *S. borneensis* was the higher than that of positive control (21 ± 0.00 mm). *S. aureus* very commonly causes infections in humans. The population of *S. aureus* found on the skin and in the nose and throat as a commensal organism (Ryu et al., 2014). However, one early study reported that *S. aureus* isolated from the mouth with malignant disease (Jackson, 2000) and the oral cavity and periodontal pocket of patients with chronic periodontitis (Loberto et al., 2004).

C. albicans are the most commonly found fungi which colonizes in the oral cavity (Brawner & Cutler, 1989; Fotos et al, 1992). Wearing removable complete dentures, fixed and removable orthodontic appliances, dry mouth, high-sugar diet, and poor oral hygiene were several local oral factors which can increase the oral Candida carriage changed to pathogenic form and caused Candida-associated buccal lesions (Vazquez & Sobel, 1992). Khanpayeh et al. (2014) reported that *C. albicans* colonies found in patient's mouth which isolated from saliva in patients with fixed and removable orthodontic appliances.

In this study, chlorhexidine (a synthetic standard) was used as antibiotic for positive control (10µg/well). Chlorhexidine is widely used in endodontics as an irrigant and intracanal medicaments (Athanasiadis et al., 2007).

The main chemical components of *D. lanceolata* from stem bark are malaysianol B (Wibowo et al., 2012), hopeaphenol (Ito et al., 1997), stenophyllol A (Ohyama et al., 1998), nepalensinol B (Yamada, 2006), vaticanol B and C (Tanaka et al., 2000), upunaphenol D (Ito et al., 2005), and flexuosol A (Li et al., 1998) to which the antimicrobial activity might be attributed. A study conducted by Wibowo et al. (2013) explored the antimicrobial activity of *D. lanceolata* stem bark and found it effective against a wide variety of bacteria. *S. borneensis* has the chemical components such as sesquiterpen scodopin and scorodocarpienes A-C were found in the fruit and hemisynthetic sesquiterpene, cadalene-b-carboxylic acid from bark (Wiarta, 2001). The antimicrobial activity of essential oil of leaves of *D. lanceolata* and *S. boornensis* against *S. aureus* and *C. albicans* is reported for the first time in this study.

Several studies have shown that cinnamon had strong and consistent inhibitory effects against various pathogens. Antimicrobial activity of *C. burmannii* has been proved by a study conducted by Chandurkar et al. (2014); Pratiwi et al. (2015), but there is still a few information about the leaves oil. The trans-Cinnamaldehyde and eugenol was found in the essential oil of *C. burmannii* leaves oil (Rowaan, 1936; Wang et al., 2009). The trans-Cinnamaldehyde showed antibacterial activity and promote inhibition of growth for all planktonic microorganisms extended spectrum β -lactamases positive tested (Pinto et al, 2013). Eugenol as a phenolic compound with well-known antimicrobial activity against many strains of microorganisms was tested. Eugenol shows excellent effects against all strains using both methods, agar diffusion and agar dilution (Jirovetz et al., 2006). The main chemical components of *C. burmannii* oil might be attributed the antimicrobial activity.

Components of *C. odorata* oil were found to contain more abundant of monoterpene essential oil such as myrcene, -pinene, and terpinen-4-ol and hexanol (Zollo et al., 1998). These compound is useful for have proved the antimicrobial potential. Myrcene, the hydrocarbonated monoterpene tested was not able to inhibit the microorganisms of *S. aureus* and *C. albicans* (Gallucci et al., 2010). The relative inactivity of hydrocarbons like myrcene is associated with its low aqueous solubility, while the formation of hydrogen bonding is found to be associated with high antimicrobial activity. However, oxygenated terpenoids show characteristics and distinct activity pattern towards microorganisms. Moreover, the essential oils constituents that contain alcohols possess higher activity than the corresponding carbonyl compounds (Zygodlo & Juliani, 2000). Pinene has been previously shown active against many organisms (Bakkali et al., 2008; Jiang et al., 2011). According to previous report, α -Pinene showed higher antimicrobial activity than the essential oil with a diameter of zones of inhibition. The antioxidant and antimicrobial properties of the essential oil may be attributed to the synergistic effects of its diverse major and minor components (Dai et al., 2013). The essential oil of *Melaleuca alternifolia* (tea tree oil) consists largely of cyclic monoterpenes of which about 50% are oxygenated and about 50% are hydrocarbons. It exhibits a broad-spectrum antimicrobial activity that can be principally attributed to terpinen-4-ol (Southwell et al., 1993). Even though earlier studies have reported better antimicrobial activity for *C. odorata* from bark and leaf extract (Rahman et al., 2005; Indrakumar et al., 2012; Kusuma et al., 2014), in our study cananga oil from leaves showed no activity against *C. albicans*. Differences were noticed in the antibacterial activities. These attributes are linked to the differences in the chemical components of the plants extract.

The potential antioxidant activity of the essential oils was determined by the scavenging activity of the stable free radical DPPH. The DPPH radical-scavenging activities of the 4 essential oils and of references are shown in Fig. 1.

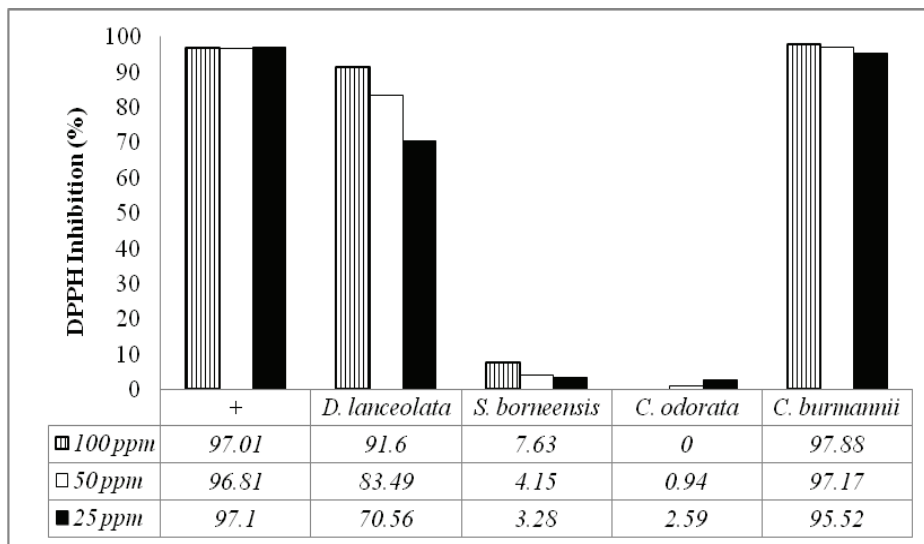


Fig. 1. Antioxidant Activity of Essential oils

As shown in Figure 1, not all tested samples exhibited a good radical scavenging activity. Only *D. lanceolata* and *C. burmannii* exhibited a good radical scavenging activity with varied degrees. The order of antioxidant activity at 100 ppm was determined as *C. burmannii* > *D. lanceolata* > *S. borneensis* > *C. odorata*.

The highest percentage of DPPH radical scavenging activity (98%) were exhibited by the 100 ppm µg/ml essential oil of *C. burmannii*. Their values in different concentration (25 – 100 ppm) were higher than that of ascorbic acid (97 %). Thus, *C. burmannii* leaves's oil had high potential DPPH radical scavenging activity. It might exhibits the antioxidant activity that can be principally attributed to trans-Cinnamaldehyde and eugenol as a major compound of *C. burmannii* leaves's oil (Rowaan, 1936; Wang et al., 2009). Cinnamon oil was tested with a rapid and simple TLC screening based on decolorization of either DPPH radical (DPPH-TLC) corresponded to authentic volatile compounds of cinnamaldehyde, eugenol and methyl-eugenol exhibited remarkable antioxidant activity (El-Baroty et al., 2010).

All the tested oils have been investigated for their antimicrobial and antioxidant activities. This study proved that the essential oils from leaves of aromatic plants have potential to inhibited the growth of *S. aureus* and *C. albicans*. The largest inhibition zones against *S. aureus* were shown by the *D. lanceolata* and *C. odorata*. The *S. borneensis* oil showed the best antimicrobial activity against *C. albicans*. In this context, *D. lanceolata* leaves, gave interesting results, being the best performing essential oil in terms of both antimicrobial activity and ability to neutralize free radicals. Future study may be needed in order to characterize the chemical compound from these plants.

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