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Research Article
Chemical Composition, Antibacterial and Antioxidant Activities of Essential Oils of *Dryobalanops lanceolata* Burck. Leaf

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Abstract

Background and Objective: *Dryobalanops lanceolata* (*D. lanceolata*) Burck is one of the plant species from *Dipterocarpaceae* family which have several medicinal purposes such as mouth ulcers, abscesses, boils and cold sores. The stem barks of *D. lanceolata* have antibacterial and cytotoxic properties against human breast cancer. While, the tree produce a clear yellow-aromatic resin, scientific support for the essential oil from this plant and its uses is still limited. This research was aimed to obtain the essential oil from leaves of *D. lanceolata* and to evaluate the antibacterial activities as well as antioxidant activity by *in vitro* research. **Materials and Methods:** The essential oil from *D. lanceolata* leaves was characterized by gas chromatography mass spectrometry (GC-MS). This oil was obtained by steam distillation and presented eugenol (28.73%), gamma-terpinene (15.60%), 2-beta-Pinene (9.80%) and 1-Limonene (8.09%) as the major compounds. The antibacterial activity and antioxidant effect of essential oil from the leaves of *D. lanceolata* were determined. The antibacterial activities of *D. lanceolata* oil were observed *in vitro* on *Streptococcus sobrinus* (*S. sobrinus*) and *Streptococcus mutans* (*S. mutans*) strains. The oil was investigated against two standard references using agar well diffusion method. The antioxidant activity was assayed by DPPH (1,1-Diphenyl-2-picryhydrazyl) and using ascorbic acid as positive control. **Results:** The yield of *Dryobalanops lanceolata* oil obtained in the present study was 0.12%. The oil was active against *S. sobrinus* and *S. mutans*. The *Dryobalanops lanceolata* oil also has potency to inhibit the free radicals at concentration 1.5625-25 ppm, which the IC_{50} was 14.28 ppm. **Conclusion:** These results demonstrate that *Dryobalanops lanceolata* oil has high antimicrobial activity for bacterial that cause dental caries disease and are antioxidant.

Key words: *Dryobalanops lanceolata*, essential oil, *Streptococcus mutans*, *Streptococcus sobrinus*, eugenol

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Essential oils are volatile oils, which derived from leaves, stems, flowers or twigs of plants that usually carry flavor or taste. The content of typically aroma or fragrance of crops usually characterizes that the essential oil of a plant that is biologically active as an antioxidant and antimicrobial[1]. In general, the function of essential oils is as a perfume but the essential oil can also be used as an ingredient for the treatment of various diseases such as asthma, headache and cough[2]. Several studies have been proved that the essential oil contained compounds that could scavenged free radical and inhibit the growth of bacteria and fungi. Essential oils are derived from aromatic plants like cloves[3], cinnamon[4,5], ginger[6], turmeric and other spices potentially as antimicrobial agents against microbial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and the fungus *Candida albicans*, even also function as a natural insecticide[7]. The role of essential oils as antimicrobials can be developed more widely into antimicrobial preparations such as disinfectants as well as antiseptics.

Dryobalanops lanceolata Burck. was an endemic plant of Kalimantan but common and widespread in Sabah, which has been named by vernacular as "Kapur paji". The genera of *Dryobalanops* were have large trees, shade tolerant, fast growing and found in clay-rich and sandy soils[8,9]. Wibowo *et al.*[10] investigated that the oligostilbenoid derivatives from stem bark of *D. lanceolata* have an antibacterial activity against *Staphylococcus aureus*, *S. epidermidis* and *S. xylosus*. Our previous study[11] investigated that *Dryobalanops lanceolata* oil from leaf has the potency as an antimicrobial agent against *S. aureus* and *Candida albicans*.

To the best of our knowledge, no report on the antibacterial activity of essential oil from leaves of *D. lanceolata* against dental caries pathogen (*Streptococcus sobrinus* and *Streptococcus mutans*) has been published so far. Thus, in the present study, the essential oil was extracted from leaves plant collected in East Kalimantan and its antibacterial as well as antioxidant activities were determined.

MATERIALS AND METHODS

The study was conducted between April and November, 2014. The study was carried out at the Forest Products Chemistry Laboratory, Mulawarman University, Samarinda, East Kalimantan, Indonesia.

Materials and chemicals: Leaves of *D. lanceolata* were collected from Education Forest of Mulawarman University, East Kalimantan, Indonesia. The leaves were dried and prepared for 1 day. Anhydrous sodium sulphate, glucose and nutrient broth were obtained from Merck (Darmstad, Germany). DPPH (1,1-Diphenyl-2-picrylhydrazyl) was purchased from Wako Pure Chemical Industries, Ltd (Japan). Other chemicals were commercially available.

Procedures

Steam distillation method: The essential oil was collected by the steam distillation method. The steam distillation method adopted from Wong *et al.*[12] with slight modification (kettle were used for distillation process). The leaves were packed into the kettle sitting on a perforated plate above the boiling water. The essential oil was volatilized with boiling water at temperature 100°C for 2 h or more. After the steam distillation process, the oil was 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. Sodium sulfate (Na_2SO_4) was used to adsorb the residual of water that fused with the oil. The quantity of the oil was determined according to the yield. The yield of essential oil was calculated using the equation[13]:

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

Gas chromatography-mass spectrometry (GC-MS) analysis: The essential oil was analysed by GC-MS (Shimadzu-QP-5050A). Column: HP-5MS, 60 m×250 μm ID×0.25 μm film thickness. Temperature program: from 70-290°C (40 min) at 15°C min^{-1}. Injection temperature: 290°C. The injection port temperature was 290°C and that of the detector was 250°C. Injection mode: split (50:1). Inlet pressure: 18.03 psi. The carrier gas was helium with a flow rate of 1 mL min^{-1}. The mass spectrometer conditions were as follows: Ionization voltage 70 eV. The MS source temperature at 250°C, MS quadruple temperature at 150°C, interface temperature at 290°C, electron ionization mass spectra were acquired over the mass range 40-800 m/z. Compounds were identified using their MS

data compared to those from the NIST and Willey Mass Spectral Library and published mass spectra.

Antibacterial assays: Antibacterial assays were done by the agar diffusion method[14] with slight modification (the different size of petri dish diameter and volume of media agar were used in this study). The microorganisms used in this study included *Streptococcus sobrinus* and *Streptococcus mutans*. All materials were sterilized by auto claving at 15 lbs pressure (121°C) for 15 min[15]. Microbes were inoculated in nutrient agar (NA). About 5-7 mL sterile media were poured into sterile petri dishes with 6 cm of diameter. After the media were altered to solidify, 25 µL of suspension microbial were inoculated by sterile swab and spreaded all over the surface of the media plates three times[16]. The wells were bored in solidified media using sterile cork borer with 6 mm diameter. The final concentrations used in this test were 100% (pure of essential oil), 10 and 1% which diluted in 40% ethanol. Twenty microliter of the sample were adding into the well. Chlorhexidine and chloramphenicol were a synthetic standard as antibiotic for positive control at the concentration of 10 µg/well and 40% ethanol as negative control. All the plates were incubated at 37°C for 18-24 h. 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[17]. Activity index of sample was calculated using following equation[18]:

$$\text{Activity index } (\%) = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Antioxidant assay: The effect of essential oil on the DPPH radical was determined by DPPH radical scavenging method according to the method of Amiri[19]. The samples were dissolved in methanol (concentration of stock solution were 25, 12.5, 6.25, 3.125 and 1.5625 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 the sample. The 50% inhibition (IC_{50}) of antioxidant activity was calculated as the concentrations of samples that inhibited 50% of scavenging activity of DPPH radicals.

Data analysis: The mean results of the percentage inhibition of antioxidant against concentrations was plotted using the Microsoft Excel computer program, which also gives the regression equations. The regression equations were used to calculate IC_{50}.

RESULTS

In this study, the essential oil of *D. lanceolata* leaves was obtained by steam distillation method. The steam distillation yielded clear and yellowish essential oil (Fig. 1). The oil from *D. lanceolata* leaves isolated by steam distillation method obtained yield and yellow refractive index 1.340 and 0.12%, respectively.

The components of *D. lanceolata* oil have been evaluated by GC-MS. The compounds were identified comparing by retention time, molecular formula, molecular weight and area (Table 1). In the essential oil from *D. lanceolata* leaf oil, 22 components were identified, which were dominated by monoterpene hydrocarbons. The main constituents of the sample were Eugenol, g-Terpinene, 2-β-Pinene and 1-Limonene (28.30, 15.60, 9.80 and 8.09%, respectively). According to these results, eugenol was mainly present in *D. lanceolata* essential oils. The structure of the major bioactive compound is presented in Fig. 2.

The anti-bacterial activity of *D. lanceolata* essential oils against *S. mutans* and *S. sobrinus* were summarized in Table 2. The antimicrobial potential of the *D. lanceolata* essential oils was evaluated according to their zone of inhibition against *S. mutans* and *S. sobrinus* and the results (zone of inhibition) were compared with the activity of the standards, chlorhexidine and chloramphenicol (10 mg mL^{-1}). The zone of inhibition above 6 mm in diameter was taken as positive result. Generally, the tested organisms were sensitive to *D. lanceolata* essential oils with varying magnitudes. When the activity index is considered (Table 1), antibacterial effect of *D. lanceolata* leaf oil at concentration 1, 10 and 100% present in the following order: *S. sobrinus*>*S. mutans*. It can be seen that 100% of *D. lanceolata* oil possess a higher antibacterial effect than chlorhexidine and chloramphenicol (at concentration 10 µg/well) against *S. sobrinus* and *S. mutans*.

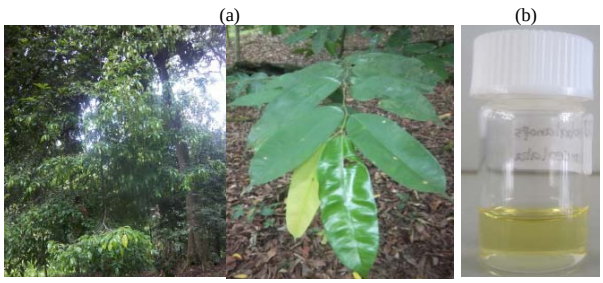

Fig. 1(a-b): (a) Tree and leaves of *D. lanceolata* and (b) Color of essential oil of *D. lanceolata*

Table 1: Chemical constituents of the essential oil of *D. lanceolata* by GC-MS

No	Retention time (min)	Name of compounds	Molecular formula	Molecular weight	Area (%)
1	5.74	α-Thujene	$C_{10}H_{16}$	136.23	4.82
2	5.88	g-Terpinene			15.60
3	6.09	α-Pinene			0.35
4	6.32	Sabinene			7.19
5	6.39	β-Myrcene			1.41
6	6.45	2-β-Pinene			9.80
7	6.73	1-Phellandrene			2.09
8	6.89	α-Terpinene			0.80
9	7.04	p-Cymene	$C_{10}H_{14}$	134.22	1.77
10	7.10	1-Limonene	$C_{10}H_{16}$	136.23	8.09
11	7.15	β-Phellandrene			3.71
12	7.52	g-Terpinene			0.80
13	8.04	α-Terpinolele			0.32
14	8.17	Linalool	$C_{10}H_{18}O$	154.25	0.17
15	8.81	4-isopropyl-1-methyl-2-cyclohexen-1-ol			0.19
16	10.02	4-Terpineol			7.95
17	10.30	g-Terpinene	$C_{10}H_{16}$	136.23	0.92
18	14.30	Eugenol	$C_{10}H_{12}O_2$	164.20	28.73
19	15.61	Caryophyllene	$C_{15}H_{24}$	204.35	2.41
20	16.44	Humulene			0.33
21	17.23	E, E-α-Farnesene			0.61
22	19.60	Patchoulane	$C_{15}H_{26}$	206.37	1.94

Table 2: Antibacterial activity of essential oil of *D. lanceolata*

		Zone of inhibition (mm)		
Bacteria	Sample	100%	10%	1%
S. mutans	*D. lanceolata* oil	30.83±2.59	12.67±0.00	7.00±0.00
	Chlorhexidine	15.11±0.19		
	Activity index	2.04	0.84	0.46
	Chloramphenicol		29.67±2.03	
	Activity index	1.04	0.43	0.24
	40% ethanol		0.00±0.00	
S. sobrinus	*D. lanceolata* oil	37.67±4.24	14.67±1.41	7.67±0.94
	Chlorhexidine		16.33±0.33	
	Activity index	2.31	0.89	0.47
	Chloramphenicol		26.78±2.52	
	Activity index	1.41	0.55	0.29
	40% ethanol		0.00±0.00	

Data are means of three measurements±SD

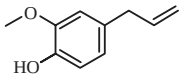

Fig. 2: Structure of the Eugenol, major compounds of *D. lanceolata* essential oil

A highly significant decrease of the DPPH radical concentration due to the scavenging activity of each oil concentration and standards as illustrated in Fig. 3. The activity to scavenge DPPH radical increases significantly with increasing oil concentration had been found. Antioxidant activity of the sample was determined by concentration of sample that could scavenge 50% free radical (IC_{50}) and the sample showed strong antioxidant activity on DPPH. The oil had a good radical scavenging (2.71-70.56%). Highest

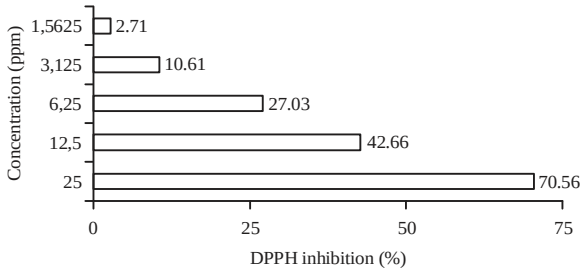

Fig. 3: Free radical scavenging activity of *D. lanceolata* essential oil

Scavenging activity was measured using the DPPH radical assay, Data are means of three measurements±SD

percentage shown in concentration 25 ppm was 70.56% and it was inhibited the 50% DPPH radicals (IC_{50}) in concentration 14.28 ppm.

DISCUSSION

The most popular method for extraction process of essential oil was steam distillation which the raw materials were applied in this method. The final process of steam distillation was gain the liquid form (the mixture of oil and water) and it separated using proper method[20,21]. The range of the refractive index values indicated that the components were neither degraded nor polymerized and remained as mono or sesquiterpenoids and their derivatives[22]. Guenther[23] suggested that the refractive index is affected by the length of the carbon chain and the number of double bonds. If the carbon chain was longer, the refractive index would bigger but it will decrease with the addition of double bonds.

In general, essential oil components can be subdivided into two distinct groups of chemical constituents, the hydrocarbons which are made up almost exclusively of terpenes and the oxygenated compounds (oxygenated terpenoids). Studies showed that oxygenated terpenoids such as alcoholic and phenolic terpenes have more antimicrobial activity than the other constituents. Several reports on the antimicrobial activity of monoterpenes have shown that the number of double bonds in a structure and the acyclic, monocyclic and/or bicyclic structure have no significant influence on their activity, although higher inhibitory activity is seen in aromatic compounds such as carvacrol, thymol and eugenol. However, oxygenated terpenoids show characteristic and distinct activity patterns towards microorganisms, terpenoids that contain alcohols possess higher activity than the corresponding carbonyl compounds[24-26].

Previously study reported that the leaves of *D. lanceolata* contained monoterpenes such as β-Pinene, Limonene, β-Myrcene[27]. The monoterpenes and sesquiterpenes contained in *D. lanceolata* also found in the *D. aromaticum* leaves such as Camphene, α-Pinene, α-Copaene, β-Caryophyllene, α-Amorphene, Aromadendrene, α-Caryophyllene, γ-Cadinene, β-Selinene, Germacrene and Selina-3,7(11)-diene[28]. Hopeaphenol and vaticaphenol A were isolated from methanolic crude extract of *D. lanceolata*[29] and malaysianol B and C, stenophyllol, nepalensinol B, vaticanol B and C, upunaphenol D, flexuosol A, nepalensinol E, ε-viniferin, ampelopsin F and laevifonol found in the acetone extract of the stem bark of *D. lanceolata*[30,31]. Different chemical compounds in the leaf of *D. lanceolata* might be due to the preparation method of extract.

The degree of the antibacterial activities of the essential oil could be attributed to the hydrophobicity of the components. The hydrophobicity of essential oils might enable them to partition in the lipid component of bacterial cell membrane, rendering them permeable and leading to leakage of bacterial cell contents[32,33]. The GC-MS study revealed eugenol to be the major constituent of *D. lanceolata* oil. The antimicrobial and antioxidant capacity of *D. lanceolata* essential oil could be mainly related to the occurrence of eugenol as assessed by Chen *et al.*[34] and Politeo *et al.*[35]. Eugenol possesses various biological abilities, including antioxidant activity, antiinflammatory action, anticarminative, anti-spasmodic, antiparasitic activities, antibacterial and antiviral effects. In medicine, eugenol is used as an antiseptic and an anesthetic. Eugenol-producing dental materials are used in clinical dentistry[36,37].

The *D. lanceolata* oil has antioxidant activity against DPPH (1,1-Diphenyl-2-picryhydrazyl) as free radicals. DPPH is used to test the ability of the compound to act as a free radical trap or hydrogen donor and evaluate the antioxidant activity[38]. The antioxidant activity of essential oils could be attributed to their hydrogen donating ability. Antioxidant properties are very important in counteracting the deleterious role of free radicals in foods and biological systems[39]. The potential antioxidant activity of the essential oils was determined by the scavenging activity of the stable free radical DPPH.

CONCLUSION

In conclusion, essential oil of *D. lanceolata* leaves showed a good *in vitro* antioxidant and antibacterial activity against *S. sobrinus* and *S. mutans*. This activity could be attributed to the eugenol compounds present in this oil. This study proved that essential oil of *D. lanceolata* leaves can be used as an alternative anti-bacterial agent to prevent and treat dental caries diseases.

SIGNIFICANCE STATEMENTS

This study discovers the essential oil from the leaves of *Dryobalanops lanceolata* that can be beneficial for medicinal purposes. This study will help the researcher to uncover the critical areas of essential oil from *Dryobalanops lanceolata* that many researchers were not able to explore. Thus a new theory on the bioactivity of *Dryobalanops lanceolata* essential oil may be arrived at.

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