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Antibacterial Potency and Physicochemical Profiles of *Eucalyptus pellita* Leaf Waste Essential Oil from PT Surya Hutani Jaya, East Kalimantan

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Abstract

Eucalyptus is one of the fast growing species plants, currently used in the pulp and paper industry. The leaves of *Eucalyptus* are known as forest harvesting waste. This research aim to examine the antibacterial potency and the physicochemical profiles of *Eucalyptus pellita* leaves waste essential oils grown in PT Surya Hutani Jaya Site 32, East Kalimantan named EP B077. The essential oils distilled using water and steam distillation methods. Analysis of physicochemical profiles from this oil included yield, colour, refractive index, specific gravity, and chemical compositions by GC-MS. Antibacterial activity assayed by agar diffusion method against *Staphylococcus epidermidis, Streptoccoccus sobrinus* and *Streptoccoccus mutans* with slight modification. The results showed that EP B077 had the yellow colour, yield oils was 0.44%, 1.471 of refractive index, and 0.8706 of specific gravity. Chemical components of EP B077 oils were dominated by *α-Pinene (40.36%),* 1-β-Pinene (31.75%), Cyclohexene, 1-methyl-4-(1-methylethenyl)- (9.64%), Trans(β)-Caryophyllene (4.29%) and Eucalyptol (1,8-Cineole) (3.88%). This EP B077 oils was active to inhibit bacteria against *S. epidermidis* and *S. sobrinus* range from 9-15 mm, also against *S.mutans* ranging from 9-14 mm. Based on the results, this *Eucalyptus pellita* leaves named EP B077 had potential to develop as a new source of essential oil and antibacterial agents.

Keywords: Eucalyptus pellita; essential oils; antibacterial; α-Pinene; 1-β-Pinene.

INTRODUCTION

Globalization and the strengthening of developing economies caused a shift in forest products from west to east. Traditional forest areas were North America, Western Europe and Japan. These regions are losing their importance as consumers and producers and are being overtaken by developing countries such as China, India, Brazil and Indonesia. Wood is also supplied by industrial plantations located in, for example, Australia, the southern part of the United States, New Zealand, Asia and South America (McEwan et al., 2020). Depending on local conditions and market demands, different species are used in plantation forestry. The dominant genera are Pinus, Eucalyptus, Populus and Acacia. The species selected for a particular site must have an acceptable growth rate for plantation forestry to be viable, and the wood properties must be acceptable for the market they serve. The current expansion of Eucalyptus and Acacia plantations is largely related to global demand for pulp and paper.

Eucalyptus was known as fast growing species tree, also the main species in PT Surya Hutani Jaya of Sinarmas Forestry. This species has many benefits and economic value, where the wood uses for pulp and paper, furniture, plywood, also for charcoal industries (Kartiko et al., 2021). The leaves part of eucalyptus known as a forest harvesting waste and useless.

Eucalyptus pellita (red mahogany) is a species native to the tropics of northern Queensland, Papua New Guinea and Irian Jaya (Indonesia), mainly in moist areas such as gentle slopes, riverbanks and alluvial plains, with an annual rainfall of 900-2200mm (Hii et al., 2017; Kartiko et al., 2021). *E. pellita* has traditionally been cultivated in Southeast Asia as a source of fiber, especially for pulp production. It has also been identified as a potential species to complement native hardwoods in the production of solid wood and veneer, and is used in flooring, panels, panelling and general construction (Hii et al., 2017).

Several studies of *E. pellita* have been reported this species has large biological activities, especially the leave parts known as forest harvesting waste. Leaves part of *Eucalyptus* had the potential against disease such as cardiovascular, influenza, cancer, and other respiratory infections, also had a many bioactive compounds including phenolic and essential oils (Limam et al., 2020). The ministry of Health and Welfare, Japan were involved the natural compounds of *eucalyptus* leaves extracts in their food industry as additive in natural food and prevention of their various ailments (Miguel et al., 2018). Furthermore, essential oils of *eucalyptus* used in various factories and ingredient such as cosmetic, pharmaceuticals, perfume, alternative medicine, food industry also had the functional use such as antiseptic, deodorant, disinfectant, astringent, anesthetic and ets (Bachir & Benali, 2012; Limam et al., 2020).

This research aims to examine the antibacterial potency and the physicochemical profiles of *Eucalyptus pellita* leaves waste essential oils grown in PT Surya Hutani Jaya Site 32 as Industrial Plantation Forest (HTI), East Kalimantan named EP B077. We also evaluate the potential antibacterial activity against *Staphylococcus epidermidis*, *Streptococcus sobrinus* and *Streptococcus mutans*.

MATERIALS AND METHODS

Plant material

The leaves of EP B077 were collected from PT Surya Hutani Jaya Site 32, Sebulu District, Kutai Kartanegara, East Kalimantan. The samples were washed thoroughly with water to remove the extraporeneous and prepared to air-dried about 2 days in the laboratory with air conditioned (A.C.) set for 20-25ºC.

Figure 1. *Eucalyptus pellita* B077 by author on PT Surya Hutani Jaya Site 32.

Procedures

- ***Distillation of essential oil***

The essential oils of EP B077 were collected by the water and steam distillation method with slight modification (Kartiko et al., 2021). About 8000 g air-dried leaves were distilled for 5 hours, then the oils were collected and separated using a separatory funnel. Furthermore, MgSO4 was added to the oils. The pure essential oils were sealed in a vial bottle and expressed in percentage of yield. The yield of essential oil was calculated using the following formula (Limam etal., 2020):

$$\% \, yield \, of \, oil = \frac{Weight \, of \, oil}{Weight \, of \, dried \, leaves} x \, 100$$

- ***Phsycochemical characteristics***

The Physicochemical properties of EP B077 essential oils were characterized by color, specific gravity, and refractive index. The color of essential oils was determined visually (Kartiko et al., 2021). The specific gravity was determined using a pycnometer (Boukhatem et al., 2020); and the refractive index was determined using a hand refractometer (Kartiko et al., 2021).

- ***Gas Chromatography-Mass Spectrometry Analysis***

The volatile composition of EP B077 essential oils was analyzed by GC-MC (Ultra Shimadzu-QP-2010), with an RTX5 column (30 m x 250 µm ID) with slight modification (Julianus Sohilait, 2016), with the following program: from 70ºC to 250ºC at 25.71ºC.minute^{-1}, injection temperature 250ºC, the detector temperature was 250ºC, split ratio 200, the inlet pressure was 98.3 kpa, carries gas helium, and flow rate of 3ml.minute^{-1}. The composition was reported as the peak area percentage and identified by comparing mass spectra with the reference mass spectra in NIST databases and literature.

Antibacterial activity testing

Antibacterial activity of EP B077 essential oils were test using agar diffusion method with slight modification (Kuspradini et al., 2018) also using pathogenic test bacteria, namely *Staphylococcus epidermidis*, *Streptococcus sobrinus* and *Streptococcus mutans*. About 20 ml aliquots of sterile media were transferred to Petri dishes and allowed to solidify. The sterile cork borer about 7 mm used to cut the sterile media and 20 µl acetone solution containing 1.25%-20% essential oils were added to the well. Chloramphenicol (CHP) was used as a positive control at the concentration of 10µg/20 µl in each well. The Petri dishes were incubated in the dark for 24 hours at 30-32ºC. The inhibition zone around the well were measured and presented in mm (Kartiko et al. 2021).

RESULTS AND DISCUSSION

The results of percentage yield of EP B077 essential oils from the water and steam distillation process was 0.44%. The other physicochemical profiles of EP B077 essential oils included colour, specific gravity and refractive index are presented in Table 1.

Table 1. Physicochemical properties of EP B077 essential oils.

No	Variables	Information
1.	Colour	Yellow
2.	Refractive index	1.471
3.	Spesific gravity	0.8706

Previously study about E. pellita leaf from PT. Surya Hutani Jaya reported that the oil content was 0.86% using water and steam distillation system (Kartiko et al., 2021). Other studies, reported the oil content of E. pellita leaf from several Indian regions was range from 0.26-0,44% using hydrodistillation system (Aldoghaim, Flematti & Hammer 2018; Kuspradini et al. 2019). Season, location, climate, soil type, leafage, fertility regime, drying technique for plant material, and oil extraction technique are just a few of the site-specific edaphic elements that can have a significant impact on oil output (Kartiko et al., 2021).

Table 2. Chemical composition of EP B077 essential oils by GC-MS analysis.

Peak	R.Time[a]	Compounds	MF[b]	MW[c](g/mol)	% Area
1.	3.961	*α-Pinene*	C10H16	136	40.36
2.	4.141	Camphene	C10H16	136	1.75
3.	4.492	1-β-Pinene	C10H16	136	31.75
4.	4.566	*β-Myrcene*	C10H16	136	0.58
5.	5.074	Benzene,methyl(1-methylethyl)-	C10H14	134	1.27
6.	5.132	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	C10H16	136	9.64
7.	5.180	Eucalyptol (1,8-Cineole)	C10H18O	154	3.88
8.	7.323	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	C10H18O	154	0.34
9.	7.529	3-Cyclohexene-1-methanol, α, α, 4-trimethyl-	C10H18O	154	2.78
10.	9.111	Kaur-16-ene	C20H32	272	0.48
11.	10.817	Trans(β)-Caryophyllene	C15H24	204	4.29
12.	11.082	(+)-Aromadendrene	C15H24	204	0.36
13.	11.280	α-Humulene	C15H24	204	0.47
14.	12.962	Spathulenol	C15H24O	220	0.68
15.	13.017	(-)-Caryophyllene oxide	C15H24O	220	1.38

Remarks: [a]R. Time (Retention Time), [b]MF (Molecular Formula), [c]MW (Molecular Weight).

The essential oils of EP B077 rich in monoterpenes group, such as α-Pinene, 1-β-Pinene, Cyclohexene, 1-methyl-4-(1- methylethenyl)-, Trans(β)-Caryophyllene and Eucalyptol (1,8-Cineole). It is different from (Aldoghaim et al., 2018), the chemical elements of E. pellita from Western Ghats of Karnataka, India had the highest percentage of eucalyptol (1,8- cineole), about >40%, although β-pinene was also the primary component (9.07%). E. pellita leaf oil from West Kalimantan contained β-pinene (20.88%) higher than 1,8-cineol (0.72%). Other research also reported that 1,8-cineol was not found in the oil from Cuba and also investigated in a similar location found 1,8-cineol content in the oil (Aleksic Sabo & Knezevic, 2019; Aoki et al., 2019; Hou et al., 2019). It might occur because of environmental factors such as location, rainfall intensity, and nutrients in the soil that influence essential oil composition.

The pure essential oil could inhibit the growth of all tested bacterias, and the decrease of this activity was observed when diluting the essential oil in 40% ethanol. The inhibition zone diameter of essential oil compared with chloramphenicol (CHP) as positive controls are shown in Table 3.

Table 3. Inhibition zone and activity indices of EP B077 essential oils against antibacterial.

Bacteria	Doses (%)	Inhibition Zone (mm)	Activity Index (%)
Staphylococcus epidermidis	CHP 5%	25±0.19	100
	1.25	9±0.19	35
	2.5	11±0.19	43
	5	12±0.38	49
	10	13±0.69	55
	20	15±0.38	61
Streptococcus sobrinus	CHP 5%	29±3.51	100
	1.25	9±0.19	29
	2.5	10±0.58	34
	5	11±0.19	39
	10	12±0.51	42
	20	15±0.38	51
Streptococcus mutans	CHP 5%	28±0.38	100
	1.25	9±0.51	33
	2.5	11±0.38	41
	5	12±0.00	44
	10	13±0.51	47
	20	14±0.38	51

*Results are reported as the mean ± SD of three experiments.

This pure oil showed the best inhibition against S. epidermidis and S. sobrinus was 15 mm, while against S. mutans was 14 mm. The essential oils of EP B077 was intermediate to inhibit the bacterial. E. pellita oil was intermediate to inhibit E. coli and S. aureus' growth (El Atki et al., 2019; Guimarães et al., 2019; Khammassi et al., 2022; Mutlu-Ingok et al., 2020). The presence of a monoterpene group, including α-Pinene and 1-β-Pinene, was predicted as an inhibitor agent against tested bacteria's growth. The chemical constituents contained in the essential oil of E. globulus such as 1,8-cineole, citronellal, citronellol, citronelil acetate, ρ-cymene, limonene, linalol, β-pinene, γ-terpinene, and α- terpineol were the potential chemical compound as antibacterial agents (Álvarez-Martínez et al., 2021; Dawood et al.,2021; Insuan & Chahomchuen, 2020; Rafiei et al., 2022).

CONCLUSIONS

In this study, *Eucalyptus pellita* leaves named EP B077 had potential to develop as a new source of essential oil and antibacterial agents.

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***Authors' Contributions*:** In this research, Wartomo was designed the research, supervised all process and wrote the manuscript. Farida Aryani was observing and taking research samples. Muhammad Fikri Hernandi controlled the samples and preparation of materials. Erna Rositah supervised research data analysis and the manuscript. Sri Ngapiyatun controlled research data analysis and manuscript writing. Nur Maulida Sari supervised all research data analysis and manuscript writing.

***Competing Interests*:** The authors declare that there are no competing interests.

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