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Evaluation of Antibacterial Activity and Physico-Chemical Profiles of *Eucalyptus pellita* Essential Oil from East Kalimantan

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ABSTRACT
Eucalyptus is one of the plants used in the pulp and paper industry, and their leaves are known as forest harvesting waste that potential to produce essential oils. This research aimed to examine the physicochemical profiles of *Eucalyptus pellita* leaf essential oils grown in East Kalimantan and their potential antibacterial activity. The essential oils distilled using water and steam distillation methods. Analysis of physicochemical profiles from this oil included yield, colour, refractive index, solubility in alcohol, and chemical compositions by GC-MS. Antibacterial activity assayed by agar diffusion method against *Streptococcus sobrinus* and *S. mutans*. The results showed that *E. pellita* oil's physicochemical profile was 0.86% of yield, 1.465 of refractive index, and solubility in alcohol was 1:1. Chemical components contained in *E. pellita* oil dominated by β-pinene (33.49%), patchouli alcohol (13.77%), 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-ene (9.81%), eucalyptol (1,8-Cineole) (6.7%), and trans-β-caryophyllene (6.7%). This oil was active to inhibit all bacterias' growth in a range of 14.78-22.33 mm against *S. sobrinus* and ranging from 14.00 mm to 52.00 mm against *S. mutans*. This forest harvesting waste potential to develop as a new source of essential oil (eucalyptol oil).

Keywords: *Antibacterial, Physico-chemical, Eucalyptus pellita, East Kalimantan.*

1. INTRODUCTION

Eucalyptus is a fast-growing tree that has excellent benefits and economic value. This plant belongs to a family of Myrtaceae, a medium-size to large tree, up to 40 m in height and 1 m in diameter at breast height, has a straight trunk to about a half of the tree height and a large, heavily branched crown. [2]. It prospects for Industrial Plantation Forest (HTI), where the wood uses are essential raw materials for pulp and paper, building construction, plywood, furniture, and suitable firewood and charcoal industries [1,3]. At the same time, the leaves part is known as a forest harvesting waste and useless.

Eucalyptus pellita (red mahogany) is a species native to tropical north Queensland, Papua New Guinea and Irian Jaya (Indonesia), where it is mainly found on moist sites such as gentle slopes, creek banks and alluvial plains, with an annual rainfall of 900-2200 mm [4]. *E. pellita* has traditionally been grown in South-East Asia as a fibre source, particularly for pulp production. It has also been identified as a potential species to complement native hardwoods for solid wood and veneer production and used for flooring, cladding, panelling, and general construction [4, 5].

Several works have been reported that *E. pellita* has many potential biological activities, especially their leave parts. The leaf extract contained allelopathic, and it's possible as bioherbicide [6]. This plant's stem wood and bark had high phenolic and flavonoid contents indicating the potential antioxidative properties [7]. Its essential oil potential as an antibacterial agent against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus subtilis* [8]. Another species of genus Eucalyptus also produced essential oil and have been investigated their activities such as *E. globulus* [9], *E. camaldulensis* [10], *E. odorata* [11] as antibacterial and antifungal activities, *E. tereticornis,* and *E. deglupta* as repellent activity against *Culex quinquefasciatus* mosquito [12], etc. The present

study aimed to investigate the physicochemical of essential oil from *E. pellita* leaves, which grow in East Kalimantan, and evaluate the potential antibacterial activity against *Streptococcus sobrinus* and *S. mutans*.

2. MATERIAL AND METHODS

2.1. Plant Material

The leaves of *E. pellita* were collected from PT. Surya Hutani Jaya, Sebulu, Kutai, East Kalimantan, Indonesia. The sample was prepared to air-dried for 24 h, maintaining the Integrity of the Specifications.

2.2. Extraction of essential oil

The essential oil of *E. pellita* was obtained from 4000 g air-dried leaves by water and steam distillation [13,14], for four h. The pure essential oil was collected in a sealed vial and expressed in percentage of yield [15].

2.3. Physico-chemical characteristics

Physico-chemical properties of E. pellita essential oil were characterised by colour, refractive index, and solubility of alcohol. The colour of essential oil was determined visually [16]. The Refractive index was evaluated by a hand-refractometer [17]. The solubility of alcohol was assayed using absolute alcohol, and this test measures the volume of alcohol required to solubilise 1 volume of essential oil (v/v) [18].

2.4. Gass Chromatography-Mass Spectrometry Analysis

The volatile composition of *E. pellita* leaf oil was analysed by GC-MS (Ultra Shimadzu-QP-2010), with an RTX5 column (30 m x 250 μm ID), adopted by Sohilait and Kainama [19] with minor modification, with the following program: from 70^{o}C to 250^{o}C at 25.71 ^{o}C.minute^{-1}, injection temperature 250^{o}C, the detector temperature was 250^{o}C, split ratio 200, the inlet pressure was 98.3 kpa, carries gas helium, and flow rate of 3 ml.minute^{-1}. The composition was reported as the percentage peak area and identified by comparing mass spectra with the reference mass spectra in NIST databases and literature.

2.5. Antibacterial Assay

Antibacterial activity of tested essential oil was carried out by the agar diffusion method [20]. Two kinds of bacteria used in this study are *Streptococcus sobrinus* and *S. mutans*. This assay used three tested concentrations: pure oil (100%), 50%, and 25% diluted in 40% ethanol. The positive control was used containing chloramphenicol (CHP) and chlorhexidine (CHX) at a final concentration of 10 μg/well. The experiments were performed in triplicate and incubated for 18-24 h at 37^{o}C. The diameters of inhibition zones were measured and presented in mm [21].

3. RESULT AND DISCUSSIONS

The percentage yield of essential oil obtained from the water and steam distillation process of *E. pellita* leaves was 0.86%. Their other physicochemical profiles included colour, refractive index, and solubility in alcohol, are presented in Table 1.

Table 1. Physicochemical properties of *E. pellita* essential oil

Variables	Information
Colour	Greenish
Refractive index	1.465
Solubility in alcohol	1:1

Utalenavar [22] reported that the range of oil content of *E. pellita* leaf from several Indian regions was 0.26-0.44% using a hydrodistillation system. The oil yield from Yogyakarta was 0.124% [23], and from West Kalimantan was 0.89% [24]. In this case, the *E. pellita* from Kalimantan was rich in essential oils than another location. The environment may influence the oil yields markedly between seasons and concerning site-specific edaphic factors including season, location, climate, soil type, leafage, fertility regime, the method used for drying the plant material, and the method of oil extraction [25].

Kasmudjo [23] mentioned that the refractive index value of this oil was 1.464. Ratnaningsih et al. [26] investigated that *E. pellita* oil has the range of refractive index are 1.460-1.470. This study is also in line with her research that evaluated the solubility in alcohol (80%) of the essential oil from Riau with similar quality with *E. pellita* oil from East Kalimantan. Its physical and chemical properties could determine the quality of essential oil.

Table 2 gives the components of essential oil that were detected by GC-MS analysis. A total of 30 peaks were recorded in this study. The tested essential oil contained five major compounds that are β-pinene (33.49%), patchouli alcohol (13.77%), 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-ene (9.81%), eucalyptol (1,8-Cineole) (6.7%), and *trans*-β-caryophyllene (6.7%).

Table 2. Chemical Compositions of *E. pellita* Essential Oil by GC-MS Analysis

Peak	R. Time[a]	Compounds	MF[b]	MW[c] (g/mol)	% Area
1	3.174	β-Pinene	C10H16	136	33.49
2	3.469	*O*-cymene	C10H14	134	0.78
3	3.504	1,7,7-trimethyl-bicyclo[2.2.1]hept-2-ene	C10H16	136	9.81
4	3.534	Eucalyptol (1,8-cineole)	C10H18O	154	6.7
5	3.694	1-methyl-4-(1-methylethyl)-1,4-Cyclohexadiene	C10H16	136	0.34
6	3.889	1-methyl-4-(1-methylethylidene)-cyclohexene	C10H16	136	0.45
7	4.132	1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-ol	C10H18O	154	1.52
8	4.292	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1α,3α,5α)]-	C10H16O	152	0.9
9	4.422	2(10)-Pinen-3-one, (.+/-.)-	C10H14O	150	0.33
10	4.487	Borneol	C10H18O	154	1.14
11	4.530	3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	C10H18O	154	0.22
12	4.628	3-Cyclohexene-1-methanol, α, α 4-trimethyl-	C10H18O	154	2.53
13	4.830	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, formate, endo-	C11H18O2	182	0.23
14	5.773	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-	C15H24	204	0.27
15	5.809	β-Patchoulene	C15H24	204	0.82
16	6.022	*Trans*-β-caryophyllene	C15H24	204	6.7
17	6.077	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1s-(1α,4 α ,7α)]-	C15H24	204	3.33
18	6.148	Aromadendrene	C15H24	204	0.6
19	6.273	1,6-Methanonaphthalene, decahydro-1,4,8a-trimethyl-9-methylene-, [1S-(1α,4α,4Aβ,6α,8Aβ)]-	C15H24	204	2.17
20	6.302	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aβ,7α,7aα,7bα)]-	C15H24	204	0.26
21	6.355	1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexahydro-1,4,9,9-tetramethyl-	C15H24	204	1.77
22	6.518	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,4α,7α)]-	C15H24	204	1.29
23	6.565	δ-Guajene	C15H24	204	5.77
24	7.056	Spathulenol	C15H24O	202	0.61
25	7.109	(-)-Globulol	C15H26O	222	1.65
26	7.162	Epiglobulol	C15H26O	222	0.44
27	7.300	Cubenol	C15H26O	222	0.59
28	7.366	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1ar-(1aα,4β,4aβ,7α,7aβ,7bα)]-	C15H26O	222	0.26
29	7.457	1,1,4,7-Tetramethyldecahydro-1h-cyclopropa[e]azulen-4-ol #	C15H26O	222	1.24
30	7.579	Patchouli alcohol	C15H26O	222	13.77

Remarks: [a]R. Time (Retention time), [b]MF (Molecular formula), [c]MW (Molecular weight).

This oil-rich monoterpenes group, such as β-pinene, eucalyptol (1,8-Cineole), 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-ene, and sesquiterpenes are patchouli alcohol and *trans*-β-caryophyllene. For Eucalyptus species, 1,8-cineole was the main component; however, *E. pellita* oil from East Kalimantan could be classified as low cineole.

It is different from Utalenavar [22], 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%) [24]. Orwa et al. (2009) also reported that 1,8-cineol was not found in the oil from Cuba. However Proenza et al. (2017) investigated in a similar location found 1,8-cineol content in the oil. 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 (at 25% and 50%). The inhibition zone diameter of essential oil compared with chloramphenicol (CHP) and chlorhexidine (CHX) as positive controls are shown in Figure 1.

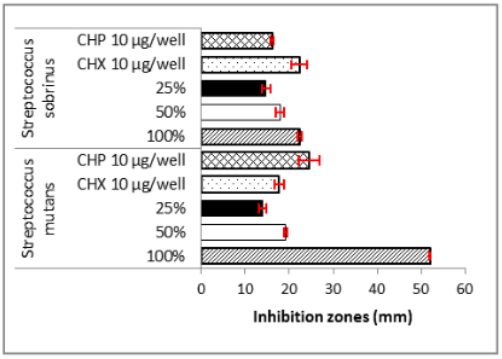

Figure 1 Antibacterial activity of *E. pellita* essential oil against *S. sobrinus* and *S. mutans*

This pure oil showed the best inhibition against *S. mutans* was >50 mm, while against *S. sobrinus* was >20 mm. *E. pellita* oil was intermediate to inhibit E. coli and S. aureus' growth [24]. The presence of a monoterpene group, including β-pinene and 1,8-cineol, 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 [27].

4. CONCLUSIONS

In this study, *E. pellita* leaves, known as forest harvesting waste, could produce essential oils. Its oil is very potential to develop in the microbiology industry. The oil was powerful to inhibit the growth of tested microorganisms causing dental caries (*S. sobrinus* and *S. mutans*). This present work proved that *E. pellita* leaf essential oil was a new natural source of antibacterial agents.

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