

Identification of Potential Antioxidants from Leaves of *Eucalyptus grandis* PT Toba Pulp Lestari, Tbk.

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Abstract: Eucalyptus plants are one type of essential oil that is fast growing and is also known as a plant that can survive in the dry season and has a deep root system. This essential oil is widely used in various fields, such as for pharmaceuticals, cosmetics and the food industry both as antioxidants and antibacterial. This study aims to identification of potential antioxidants and the main active compounds from leaves of *Eucalyptus grandis* PT. Toba Pulp Lestari, Tbk. Extraction was carried out using solvents, methanol, ethanol and dichloromethane. The antioxidant potential test was determined by the DPPH method (1,1 diphenyl picrylhydrazyl). The variation of sample volume 20; 30; 40; 50; 60 μ L. Antioxidant activity with IC50 *Eucalyptus grandis* leaf extract was 5,349 μ g / mL. Eucalyptus leaf extract is categorized as providing weak antioxidant activity. The main active compound found is Sineol. The peak area was observed at 52% at retention time 13.605. The other compounds obtained in this leaf were α pinene, β pinene, 1,3,7-Octatriene, 3-Ethylpentane, Paracymene, and terpinene.

1 INTRODUCTION

Eucalyptus leaves can produce essential oils or better known in trade as eucalyptus oil. Essential oils or essential oils are produced by aromatic plants originating from shoots, flowers, leaves, stems, seeds, fruits, roots, wood, and bark (Teixeira et al., 2012). Essential oils are natural compounds that are volatile and very complex with strong odors. Essential oils have bactericidal, fungicidal and insecticidal properties (Filipitsova et al., 2017). There are more than 60 compounds contained in essential oils (Bakkali et al., 2008). Eucalyptus plants (Myrtaceae) have various species, such as *E. camadulensis*, *E. grandis*, *E. pellita*, *E. tereticornis*, and *E. Torreliana*.

Eucalyptus plants are fast growing plants, have many benefits, both in terms of stem, branches, and leaves. PT Toba Pulp Lestari Tbk has developed eucalyptus plants to be used as paper raw materials. However, what is still used from Eucalyptus plants

is still in the wood and branches, while the leaves have not been processed further.

The eucalyptus species is an aromatic plant that has antimicrobial and antioxidant properties from the essential oils produced. The essential oils produced are used in pharmaceutical and cosmetic products (Ait-Ouazzou et al., 2011; Santos et al., 2011). Eucalyptus leaves can be processed with several extraction techniques, such as hydro-distillation and steam extraction (Zhao et al., 2014; Singh et al., 2016). Analysis of the composition of essential oils can be analyzed by the gas chromatography method (Burt, 2004).

Previous researcher (Abdul-Majeed, 2013) University of Baghdad carried out distillation using the Stahl Tool, proving that eucalyptus oil contained large levels of cineol which reached 72.71%. By conducting research on the same species, (Cheng, 2008) distilled the Stahl Tool on *Eucalyptus europhylla* plants obtained 58.34% cineol levels which was far more than steam distillation.

Lack of utilization of eucalyptus leaves in the area of PT. Toba Pulp Lestari which has the potential to be used as raw material for making essential oils or better known as tradable eucalyptus oil, it is necessary to do research on the composition, antioxidant potential, and cytotoxic activity on *Eucalyptus grandis* leaves. The main oil composition that determines the quality of quality is based on the levels of cineol contained in it. (Copper et al., 1991). The greater the content of cineol in oil, the better the quality of eucalyptus oil produced.

2 MATERIALS AND METHODS

2.1 Materials

The main material for this research is *Eucalyptus grandis*. The solvent used in the distillation process is aquadest. The reagents used in the DPPH test are 2,2-diphenyl-1-pikrilhydrazil (DPPH) and methanol. The instrumentation used for sampling samples included the 1800 Shimadzu UV-Vis spectrometer.

2.2 Preparation of Sample

The fresh leaves obtained are immediately separated from the stem. Then the leaves are chopped using a cutter and scissors to produce chopped ingredients with a length of ± 0.5-1.0 cm.

Plant identification has been carried out in the HERBARIUM MEDANENSE (MEDA) Laboratory in the Department of Biology, University of North Sumatra. Samples in the form of: Fresh leaves in a single branch between 10-15 cm from the top.

2.3 Sample Extraction

Eucalyptus leaves dried for 24 hours at room temperature. Then the sample is weighed as much as 150 grams and inserted into a 1000 mL size flask. Adding aquabides to taste is then connected to a Stahl distiller, and boiled for ± 5-6 hours at ± 100 ° C to produce oil and distillation ends when the distillate is clear. The essential oil obtained is accommodated in the Erlenmeyer glass. The distillate obtained is a mixture of oil and water. Then the oil layer was added to CaCl₂ anhydrous to bind water which might still be mixed with essential oils, the oil layer was decanted and put into vial bottles, stored in a coolant in a bottle and tightly closed. Then extracted samples are stored in glass bottles for further analysis.

2.4 Characterization

2.4.1 Analysis of GC-MS

The Specifications Instrument GC-MS QP 2010S Shimadzu, using Column 5MS with type of ion source Electron Impact, Injector Temperature: 300°C, Carrier Temperature: 50°C, Carrier: Helium, Gas flow rate of carrier: 1.0 mL / min, Temperature oven: 50°C for 5 minutes then 240°C for 7 minutes., Ionization electron: 70 ev.

The solution of each 1 µL standard cineol series was inserted into the syringer to be injected into the GCMS. Only the conditions adjusted to the conditions of each piece of equipment and then observed Mass Chromatogram data generated interpreted data. Obtained data then in Perform calculations to get the calibration curve and do the determination of levels through the equation.

2.4.2 Analysis of Antioxidant Potential

Analysis of Antioxidant Potential in Essential Oils with Ultraviolet-Visible Spectrophotometry Method (UV-Vis).

3 RESULTS AND DISCUSSION

Antioxidant test of eucalyptus oil used DPPH method. The results can be seen in Table 1. The antioxidant activity test showed that eucalyptus oil produced in this study showed IC₅₀ value = 5.349. This results shows that the eucalyptus oil produced has a weak antioxidant value.

Table 1: Antioxidant test of *Eucalyptus grandis* oil.

Volume sample (µL)	Repetition	ADPPH	A _{sample}	I (%)
20	1	0.643	0.303	52.87
	2		0.303	52.87
	3		0.303	52.87
30	1		0.216	66.40
	2		0.216	66.40
	3		0.216	66.40
40	1		0.232	63.91
	2		0.232	63.91
	3		0.232	63.91
50	1		0.163	74.65
	2		0.163	74.65
	3		0.163	74.65
60	1	0.159	75.27	
	2	0.159	75.27	
	3	0.158	75.42	

Table 1 shows that volume of sample was used 20 until 60 μ l. The repeat the experiment carried out 3 times. The wavelength of DPPH was 0,643. The absorbant value of sample decreases with increasing sample volume (0.303 – 0.158) This result is in accordance with the results obtained by molyneux (2004) . This causes that % inhibition to increase. By using the IC50 calculation formula, an IC50 value of 5,349 was obtained. Based on the IC50 values obtained indicate that the eucalyptus oil produced has a weak antioxidant value (Ait-Ouazzou, 2011) . DPPH is a purple organic nitrogen radical that is purple. The presence of a radical-reducing compound will reduce DPPH radicals by donating hydrogen atoms to form diphenyl picrilhydrazine (non-radical) compounds which can be characterized by changes from DPPH radical purple to yellow (picril group) (Molyneux, 2004). This process can be seen in Figure 1.

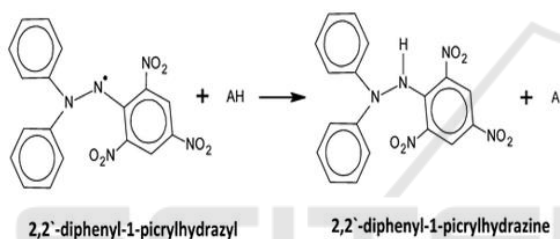


Figure 1: DPPH reaction.

The analysis of GCMS (Figure 2) shown of the sineol compound content in the peak area of 52% and a retention time of 13,605.

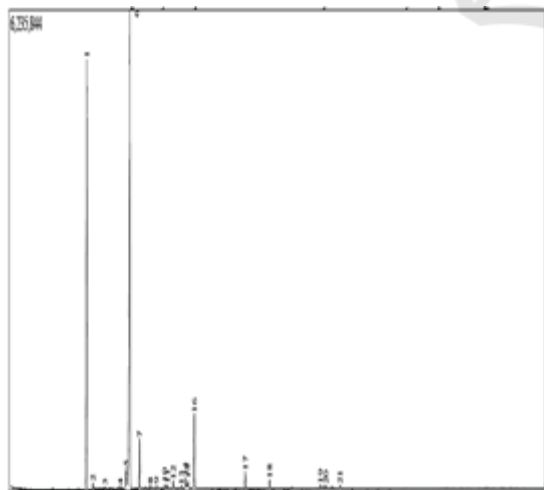


Figure 2: The Chromatogram of Eucalyptus oil.

Figure 2 shown that the main active compound found is Sineol. The peak area was observed at 52% at retention time 13.605. The other compounds

obtained in this leaf were α pinene, β pinene, 1,3,7-Octatriene, 3-Ethylpentane, Paracymene, and terpinene.

4 CONCLUSIONS

Eucalyptus oil as a result of *E. grandis* leaves was carried out by antioxidant testing using the DPPH method (1.1 diphenyl picrylhydrazyl). Antioxidant test results showed that *E. grandis* oil had weak antioxidant activity. Sineol is the main active compound in Eucalyptus oil (the peak area 52% and RT 13.605). The other compounds obtained in this leaf were α pinene, β pinene, 1,3,7-Octatriene, 3-Ethylpentane, Paracymene, and terpinene.

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