Identification and Analysis of Potential Antioxidants from Leaves of *Eucalyptus robusta* PT. Toba Pulp Lestari, Tbk.

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Abstract: PT Toba Pulp Lestari Tbk is a global pulp producing company that develops forest industry concessions and stores a variety of underutilized biodiversity such as *Eucalyptus robusta* leaves which are widely found around the PT Toba Pulp Lestari Tbk forest area. *Eucalyptus robusta* leaves produce oil that is used as liniment, cough medicine, perfume, soap, detergent, disinfectant and pesticides. *Eucalyptus robusta* and supports its antioxidant potential. Eucalyptus leaves are first described with several solvents, such as methanol, ethanol and dichloromethane. The potential of antioxidants using the DPPH method on the 1800 Uv-Vis Spectrometer. The antioxidant activity test was carried out with the DPPH method (1.1 diphenyl pikrilhidrazil) with a variation of sample volume 20; 30; 40; 50; 60 µL. Eucalyptus leaf extract showed antioxidant activity with IC50 of 8,386 µg / mL. The Eucalyptus leaf extract is categorized as providing weak antioxidant activity. Analysis of active composition that can be used in eucalyptus leaves using the GCMS method. The main compounds contained were cineol 52%.

1 INTRODUCTION

Eucalyptus is a genus of the Myrtaceae family and has as many as 600 species and subspecies. Although native eucalyptus originated in Oceania, these tall green trees spread throughout the world and occupy an area estimated at between 16 and 19 million hectares (Bułkowskaet al., 2016). *Eucalyptus* sp. Plant. (Myrtaceae) has various species, namely *E. camadulensis, E. grandis, E. pellita, E. tereticornis,* and *E. torreliana*. Planting of *Eucalyptus* sp. Where most are found in Sumatra (Aceh, North Sumatra, Jambi) and Kalimantan (West Kalimantan, East Kalimantan and South Kalimantan) (Nair, 2000).

The *Eucalyptus robusta* species to be carried out in this study was produced by PT. Toba Pulp Lestari Tbk, which is one of the HTI companies located in Indonesia, especially the North Sumatra province which is the HTI with the largest area in North Sumatra, where the total area reaches 188,055 Ha. And the commodities mainly developed are eucalyptus plants (*Eucalyptus* spp). Eucalyptus belongs to fast-growing plants or better known as Fast Growing Species. One location in North Sumatra that has a high biodiversity composition is at PT Toba Pulp Lestari Tbk where TB has developed eucalyptus plants to be used as raw material for paper making but which is still utilized from eucalyptus plants still in wood and its branches lack of utilization of Eucalyptus leaves in the area Toba Pulp Lestari which actually has the potential to be used as raw material for making essential oils or better known as eucalyptus oil which can be traded as a precursor in chemical synthesis.

The effects of etiologically free radicals have been believed to be the cause of various chronic and aging diseases. This is because these reactive free radicals can attack various biomolecules. Antioxidants can come from natural or synthetic ingredients. Synthetic antioxidants include BHA (butyl hydroxyanisole) and BHT (butyl hydrozxytoluene). Antioxidants derived from natural ingredients include vitamin E (- tocopherol) and resveratrol. Both are natural phenolic antioxidants (Hart et al, 2003).

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Synthetic antioxidants have a harmful effect if consumed by humans. Synthetic antioxidants such as BHT (Butyl Hydroxy Toluene), TBHQ (Tertiary Butyl Hydroquinone) can increase the occurrence of carcinogenesis in humans (Amarowicz et al., 2000) and liver damage (Osawa & Namiki, 1981). Therefore, natural antioxidants are more recommended for human consumption. This fact encourages a lot of research done to look for natural ingredients that can be used as antioxidants (Rohman & Riyanto, 2005).

The working principle of GC-MS is based on differences in the polarity and molecular mass of samples that can be evaporated. Samples in the form of liquid or gas are directly injected into the injector, if the sample is solid, it must be dissolved in a solvent that can be evaporated. The flow of gas flowing will bring the evaporated sample to enter the column. The components in the sample will be separated based on the partition between the mobile phase (carrier gas) and stationary phase (column). The result is a gas molecule which will then be ionized in a mass spectrophotometer so that the gas molecule will experience fragmentation in the form of positive ions. Ions will have a specific ratio between the mass and the charge (Fowlis, 1998).

The principles of the Gas Chromatography - Mass Spectrometry tool are: Sample injection can be done manually or use an automatic sample taker through a rubber seal that can be closed again. The sample is evaporated on the portal part of the injection which is heated and subjected to condensation at the top of the column. The column can be a packed capillary column or column, which will be discussed in more depth. The mobile phase used to carry samples through the column is a gas - usually nitrogen or helium. The column is closed in an oven which can be set at a temperature between room temperature and approximately 400 ° C. The detector used is mass spectrometry (MS). The University of North Sumatra sample is inserted into the instrument source by heating it at the end of a sensor until it evaporates, aided by very hollow in the instrument If it is in the vapor phase, the analyte is bombarded with electron electrons produced by the rhenium or tungsten filaments, which are accelerated towards a positive target with an energy of 70 eV. Two types of systems are usually used to separate ions based on load comparisons of their mass (Watson, 2005).

2 MATERIALS AND METHODS

2.1 Materials

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

2.2 Preparation of Sample

Leaves are obtained from PT. Toba Pulp Lestari which is located on Jl. Indorayon Subdistrict Dolok Nauli, Toba Regency Samosir, North Sumatra. The selected leaves are leaves along with stems 5-10 cm from the top of the plant. Taking is done in the morning at 7-9 in the morning.

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 $CaCl_2$ 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: 300ºC, Carrier Temperature: 50ºC, Carrier: Helium, Gas flow rate of carrier: 1.0 mL / min, Temperature oven: 50ºC for 5 minutes then 240ºC for 7 minutes., Ionization electron: 70 ev.

The solution of each 1 µL standard sineol 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

3.1 Test Antioxidant Activity

The DPPH radical capture method is based on DPPH radical solution color changes due to the administration of compounds that are antioxidant. Antioxidant interactions with DPPH either electron transfer or hydrogen radicals at DPPH will neutralize the free radical character of DPPH. If all the electrons in the DPPH free radical are paired, then the color of the solution changes from dark purple to bright yellow. This color change will be observed in the form of a decrease in DPPH absorbance.

Max determination was carried out by scanning survey tests on the uv-vis spectrophotometer. This test produces max DPPH at 517 nm. After obtaining the max value then it is determined the reaction time between DPPH and the test sample. This test is carried out by reacting the DPPH with the test material sample then observing the change in absorbance from time to time to get a constant absorbance reading.

Shows that the radical capture reaction by the test material has occurred perfectly. *Eucalyptus robusta* extract until the 60th minute has not seen a constant reading, but the difference in absorbance between times is not too large, so the reaction time for *Eucalyptus robusta* extract is set to 60 minutes, while for the test material the methanol extract absorbance reading shows a constant value in minutes 25th, so that the reaction time for methanol extract is set to 25 minutes.

Even though some fluctuations in the concentration of the test material, this radical capture action shows an upward trend along with the increase in concentration. Positive control of quercetin indicates radical capture, this indicates that the test method carried out runs correctly.

Overall the results above show that the *Eucalyptus robusta* methanol extract has an IC50 value of 8,386g / mL of the test material which has weak antioxidant effectiveness, making it less effective as an antioxidant.

3.2 Analysis of Compound Content in *E. robusta*

Qualitative and quantitative analysis of compound content in *Eucalyptus pellita* using GC-MS instrumentation. The results of GC-MS Analysis were then carried out with data interpretation. The Qualitative Analysis results show that there are 17 (seventeen) compounds in the form of a chromatogram which has seventeen peaks.

The results of GC-MS analysis on *Eucalyptus robusta* leaf essential oil showed that the compounds in essential oils contained 17 (twelve) peaks of compounds contained with a large abundance of 52% cineol compounds from the results of the chromatogram produced by Gas Chromatography then broken down into fragmentation.

4 CONCLUSIONS

The *Eucalyptus robusta* test material has antioxidant activity with an IC50 value of 8,386 wherein the compound has weak antioxidant activity and GC-MS Analysis Results on *Eucalyptus robusta* leaf essential oil shows that the compound in the essential oil has 17 (twelve) peak compounds contained in the abundance of cineol compounds as much as 52%.

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