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Antioxidant Activity Of Lemongrass (*Cympogon nardus* L.) Hydrosol With Various Extraction Time

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Abstract

Lemongrass are well known in the community as a cooking spice, cultivated for their essential oil, and used as raw material for perfume and medicine. Hydrosol is a by-product of essential oil extraction. The purpose of this study was to determine the antioxidant activity of the lemongrass hydrosol and to determine the effect or difference of extraction time on the antioxidant activity of the lemongrass hydrosol. The research method used is experimental. Determination of antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhidrazil) method with a UV-Vis Spectrophotometer. Variations in extraction time were 1 hour, 2 hours and 3 hours. The results of the antioxidant activity test on lemongrass hydrosol were shown by the IC_{50} values at extraction times of 1 hour, 2 hours and 3 hours respectively 11.16 ± 2.51 ppm, 9.32 ± 1.63 ppm and 2.12 ± 0.72 ppm. It was concluded that there was no significant difference in the variation of distillation extraction time with the antioxidant activity of lemongrass hydrosols.

Key words: *antioxidant, extraction, hydrosol, lemongrass*

1. Introduction

The lemongrass plant (*Cymbopogon sp*.) is quite well known by the public, as a cooking spice. The lemongrass plant has more than one species, one of which is fragrant lemongrass. Traditionally lemongrass has been known to be efficacious in treating sore throat, colitis, stomach ulcers, diarrhea, mouthwash, stomach ache. Lemongrass extract also has properties as an antioxidant [1].

In this study conducted research onby-products or hydrosols from the extraction process on lemongrass (*Cymbopogon nardus* L.) stems. The method used was water distillation. The test method used to test antioxidant activity is the DPPH method. The samples used were distinguished by the collection time of 1, 2 and 3 hours after distillation.

The purpose of this study was to determine the antioxidant activity of lemongrass hydrosols and to determine the effect or difference of extraction time on the antioxidant activity of lemongrass hydrosols.

Lemongrass plants are cultivated for their essential oil, have high economic value, and are used as raw materials for perfumes and medicines [2]. The process of making essential oils / essential oils can be done by distillation / distillation method which is generally done by steam distillation method. From the results of steam distillation, two products will be produced, namely the main product is essential oil and a by-product is hydrosol [3].

Hydrosol is a by-product of essential oil extraction, where at the beginning of the extraction there are mixed essential oils, so the color that is formed is yellow to slightly clear [4]. Previous research providing information that the distillation residue of lemongrass (*Cymbopogon nardus*) contains antioxidant activity with an IC_{50} value of 189.905 ppm which can be categorized as moderate antioxidant intensity [1]. While the antioxidant activity of lemongrass essential oil is very strong with an IC_{50} result of 47.58 ppm [5].

One of the factors that can affect the IC_{50} value or the antioxidant activity of hydrosols is the length of extraction time. According to a study by Erminawati et al (2021), there were differences in antioxidant activity in lemongrass (*Cymbopogon citratus*) essential oil extracted by hydro-distillation (direct distillation) at 3, 4, and 5 hours.

Based on the background that has been described, the researcher is interested in conducting research on testing the antioxidant activity of lemongrass hydrosol (*Cymbopogon nardus* L.) with variations in extraction time using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The DPPH method is a method for determining antioxidant activity in samples by looking at their ability to ward off free radicals of 2,2-diphenyl-1-picrylhydrazyl compound [6].

According to previous research about antioxidant potential of the ethanol extract of lemongrass distillation residue (*Cymbopogon nardus* L.), it is known that the total phenol and antioxidant activity (IC_{50}). In this study the antioxidant test used the DPPH method with ERD concentrations (ethanol extract of distillation residues of lemongrass leaves) 50 ppm, 100 pm, 150 ppm, 200 ppm, and 250 ppm using quercetin reference solution with concentrations of 10 pm, 20 ppm, 30 ppm, 40 ppm and 50 ppm showed good antioxidant activity because it was lower than 200 ppm [1].

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The second comparative study was measured the antioxidant activity and inhibition of the three essential oils and their mixtures. In this study, the antioxidant activity test of the essential oils of the three samples used the DPPH method with concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. Based on the antioxidant activity tests that have been carried out, the strongest IC_{50} values were obtained for lemongrass oil and nutmeg oil at 3.80 ppm and 3.34 ppm [5].

2. Method

2.1. Plant Material

The material studied was fragrant lemongrass (*Cymbopogon nardus* L.) the part studied is the stem. Samples were taken from Sambirambe Village, Kalijambe, Sragen, Central Java.

2.2. Tools & Materials

The equipment used in this study was a set of locally made steam distillation apparatus consisting of a distillation boiler, heater, condenser and condensation container to obtain essential oils and hydrosols, erlenmeyer and thermometer. While the analytical equipment used includes aluminum foil, stirring rod, 5 ml measuring cup, 10 ml measuring cup, 50 ml beaker glass, glassware, watch glass, cuvette, 5 ml measuring flask, 10 ml measuring flask, 50 ml measuring flask, 100 ml measuring flask, 1000 ml round bottom flask, analytical balance, volume pipette, dropper pipette, test tube rack, UV-VIS spectrophotometer, test tube, tissue, vortex.

The materials used in this study were distilled water and lemongrass (*Cymbopogon nardus* (L.) Rendle). The materials for analysis were ethanol pro-analyst and DPPH (2,2 diphenyl-1-pircrylhydrazil).

2.3. Distillation Extraction

Lemongrass hydrosol is obtained from the byproduct of the process of making lemongrass essential oil (*Cymbopogon nardus* (L.) Rendle). Fragrant lemongrass was reduced in size by ± 2 cm, then weighed as much as 200 grams. The lemongrass pieces are put into a 1000 ml round bottom flask, in the round bottom flask the lemongrass pieces are added with 750 ml distilled water or until all the simplicia is submerged. Distillation was carried out at 90^0C for 1 hour, 2 hours and 3

hours from the first drop. At the end of the distillation process, the distillate is separated to produce essential oil as the main product and hydrosol as a side product.

2.4. Research Procedures

The IC_{50} value was determined for DPPH (2,2 diphenyl-1-pircrylhydrazil) using the following procedure:

1) Preparation of test solutions

   A 100 ppm mains solution of lemongrass hydrosol was prepared by dissolving 1 ml of hydrosol with ethanol pa in a 10 ml volumetric flask. Then the 100 ppm mother liquor was made with various concentrations of 15, 20, 25, 30, 35, 40 ppm in a 5 ml volumetric flask diluted with ethanol solvent up to the boundary mark.

2) Preparation of DPPH solution

   A 100 ppm DPPH solution was prepared by weighing 0.01 g of DPPH, put it in a 100 ml volumetric flask and dissolved with ethanol pa up to the mark mark. Next, the absorption of the DPPH wavelength is measured. The wavelength range for measurements using the DPPH method is 400 nm - 700 nm [7].

3) Determination of Operating Time

   Operating time (OT) aims to determine the time required for the test solution to completely reduce the DPPH free radical after it reacts. Measurement during operating time (OT) is intended to minimize errors in terms of measuring DPPH radicals with test compounds. The operating time (OT) is determined based on the time when the absorbance value starts to stabilize or the difference in the absorbance value at each time interval starts to be small. The operating time results obtained for the test solution were 105 minutes marked with a fixed absorbance value.

4) Antioxidant Activity Test

   The extract was dissolved in pa ethanol and prepared in various concentrations, namely 15, 20, 25, 30, 35, and 40 ppm of 2 ml each. Into each solution, 4 ml of 10 ppm DPPH was added and incubated in the dark for 105 minutes and vortexed for 3 minutes. Next, the absorption was measured with a UV-Vis spectrophotometer at a maximum wavelength of 513.3 nm DPPH. As a blank used ethanol pa Calculation of percent antioxidant activity of DPPH used the following formula

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Radical inhibition (%) = (A blank-A sample)/(A blank) x 100%

A blank = 10 ppm DPPH radical absorption

A sample = 10 ppm DPPH radical absorption after being treated with the sample.

Antioxidant activity was determined using the IC_{50} value (50% Inhibition Concentration). IC_{50} is a number indicating the concentration of an extract that can inhibit the activity of a radical by 50%. The IC_{50} value of each sample concentration was calculated using the formula of the linear regression equation, which states the relationship between the concentration of the antioxidant fraction expressed as the x-axis and the % inhibition expressed as the y-axis from the measurement replication series.

3. Result and Discussion

3.1. Manufacture of lemongrass hydrosol

The manufacture of lemongrass hydrosol is carried out to separate the essential oil and lemongrass residue (Hydrosol) carried out in the distillation process where the hydrosol used is a solution mixed with simplicia in a round bottom flask. From the extraction results, the amount of solution obtained can be seen in Table 1.

Table1. Extraction Results

Extraction Time	Hydrosol & Essential Oil Mixture	Mix of Hydrosol & Plant
1 hour	115 mL	507 mL
2 hours	128 mL	420 mL
3 hours	197 mL	300 mL

3.2. Maximum Wavelength Determination

Maximum wavelength determination was carried out on DPPH and Ethanol pa solutions. Maximum wavelength is an important factor in analysis using UV-Vis spectrophotometry because optimum absorption is obtained in the form of the absorbance value of a compound being measured. Scanning is carried out at a wavelength of 400-700 nm. The maximum wavelength measurement results using 20 ppm DPPH, obtained an absorbance of 0.711 at a wavelength of 515 nm. The maximum wavelength value range of DPPH is 515 – 520 nm [7].

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3.3. Determination of Operating Time

Operating Time (OT) aims to determine the optimum time in reducing free radicals with DPPH solution to react completely. Measurement during operating time is intended to minimize errors in terms of measuring DPPH radicals with test compounds. Operating time is determined by measuring the absorption of the DPPH solution that has been added to the test compound. The solution was vortexed for 3 minutes before being measured with the aim of homogenizing the mixture so that it reacts optimally. Absorbance scanning was carried out using a UV-Vis spectrophotometer for 2 hours with an interval of 5 minutes. The operating time is determined when a stable absorbance is obtained, that is, there is no visible decrease in absorbance. The results showed that at 120 minutes, the absorbance of DPPH was relatively constant, so the antioxidant activity test was carried out at 120 minutes (2 hours).

3.4. Test Results of Lemongrass Hydrosol Antioxidant Activity

Lemongrass hydrosol was tested for its antioxidant activity using the DPPH (1,1 diphenyl - 2-picrylhidrazyl) method. The DPPH method is a method that can measure antioxidant activity quickly, simply and relatively easily. The antioxidant activity test using this method can be seen qualitatively by the loss of the purple color to yellow, this occurs due to the reduction of DPPH by antioxidants [8].

The intensity of this missing purple color was measured using a UV-Vis spectrophotometer, the wavelength in this study corresponds to the maximum wavelength range stated by Karcidin et al in 2020, namely 400-700 nm. Tests were carried out at 6 series of concentrations and reacted with DPPH free radicals in a ratio of 2 : 1 then incubated for 2 hours. The blank solution used is ethanol pa because ethanol pa is the solvent used in diluting DPPH and samples so the absorbance is not measured.

At each time variation tested, the absorbance was obtained and the % inhibition was calculated at each concentration. Percent inhibition is obtained from the difference between the absorbance of the control and the absorbance of the sample. Furthermore, to determine the free radical scavenging activity of DPPH, the IC_{50} parameter was used. Determination of IC_{50} from the extracted sample aims to determine the amount of extract content that can reduce the absorption intensity of DPPH free radicals by 50% [9].

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Calculation of the IC_{50} value for each concentration is calculated using a calibration curve to obtain a linear formula, namely y = ax + b. Calculations by linear regression where x is the concentration and y is the percent inhibition. The IC_{50} value was obtained from the x value after replacing y = 50. The IC_{50} value was calculated based on the percentage inhibition of the DPPH radicals from each replicated time variation. The IC_{50} values obtained indicate antioxidant activity in lemongrass hydrosols which can be seen in Table 2.

Table 2. Antioxidant Activity (IC_{50})

Time (hour)	IC_{50} value (ppm)			Average IC_{50} (ppm)
	I	II	III	
1	13.38	8.44	11.68	11.16 ± 2.51
2	7.57	9.597	10.79	9.32 ± 1.63
3	5.68	4.20	2.20	2.12 ± 0.72

The higher the concentration of the sample solution, the lower the absorbance. This is because the higher the concentration of the sample, the higher the content of antioxidants, so that the sample will inhibit more DPPH and the less DPPH remains, so the absorbance value gets smaller [10].

The presence of geraniol and mineral components contained in lemongrass essential oil is thought to act as an antioxidant and free radical scavenger. Based on variations in distillation time, it can be concluded that the length of distillation time can affect the antioxidant activity of lemongrass essential oil. This is because the longer the distillation time, the more heat received by the material to evaporate the oil cells and materials so that the amount of geraniol and minerals will be higher [11].

4. Conclusion

Based on the results of the research that has been done, it can be concluded that the antioxidant activity in lemongrass hydrosol in various extraction time (1, 2 and 3 hours) was very strong. The longer extraction time, the higher antioxidant activity of lemongrass hydrosol.

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