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Characteristics of *Cymbopogon Nardus* L. Extract with Variations in Leaf Withering Time

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Abstract. The focus of this study was to evaluate the effect of variation level of withering leaf on the bioactive compounds of fragrant lemongrass *(Cymbopogon Nardus L.)* as antioxidant and antibacterial agents. The study was conducted with 4 levels of leaf withering times, namely: fresh, 24, 48, and 72 hours. The observed parameters were color, pH, phytochemical content, free radical trapping capacity (DPPH) and antibacterial activity. Antibacterial tests were done against *Staphylococcus aureus* and *Escherichia coli.* The data obtained were analyzed using analysis of variance (ANOVA) and further test DMRT (Duncan Multiple Range Test). The phytochemical content of the extracts from all withering times were as follows: tannins, alkaloid, flavonoid, and steroid. The best results from this study were the 2 days of withering time which have value for pH (4), color L (52), a* (60), b* (-14), and antioxidant activity (44.37%). The extract had antibacterial activity, showed by inhibitory zone 11.53±2.09 mm against *S aureus* and 20.35±1.13 mm against *E Coli* respectively for MIC 250-500 µg/mL dan MKC 300-600 µg/mL

INTRODUCTION

Citronella (*Cymbopogon nardus L.*) is one type of plant that has the potential to produce essential oils. This plant belongs to the class of grasses from the Gramineae family which in the world trade of essential oils, citronella is known as java citronella. Citronella essential oil which is the result of secondary metabolites can be obtained from the leaves and stems of the plant [1]. The need for essential oils is increasing every year along with the increasing development of modern industries such as the perfume, cosmetic, food, pharmaceutical, aromatherapy and drug industries [2].

In world trade, there are two types of citronella oil, namely the Sri Lankan type and the Javanese type. The Sri Lankan type, also called lenabatu, comes from the Cymbopogon nardus Rendle plant. The Java type called Mahapengiri comes from Java citronellal. The Mahapengiri type has shorter and larger leaves than lenabatu. In addition, the quality of the oil is better because it has higher levels of geraniol and citronellal. The use of fragrant citronella Mahapengiri is because the yield produced is more than other types of citronellas that the yield of the lenabatu variety is between 0.4 – 0.5% and Mahapengiri has a higher yield, which ranges from 0.7 – 1.6% [3].

The citronella plant produces oil in all parts of the plant, however, in this study only one part of the citronella plant was used, namely the leaf. In the citronella leaves, more oil is produced than other parts of the citronella plant. According to [4] that overall, all parts of the plant contain oil, but the leaves contain the maximum amount of oil. In addition, the oil from other parts is of lower quality.

Antioxidants and antibacterial have the potential to be developed in the pharmaceutical, beauty and food preservation industries. Antioxidants are thought to have anticancer effects [5]. The next important step that must be

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taken so that citronella can be trusted to function as an antioxidant and antibacterial is to determine the toxicity, antioxidant activity, and antibacterial activity of citronella leaf extract as an initial screening test for the activity of chemical compounds in citronella leaves.

The flexibility of using citronella is still limited considering that the content of bioactive compounds in plants is usually very small based on its wet weight. For this reason, it is necessary to extract citronella leaves to facilitate its use. According to [6] Extraction of bioactive components from plant cell materials must pay attention to two important things, namely the characteristics of the bioactive components of plant cell and the extraction method used. For this reason, the factors related to the isolation of the bioactive content of the citronella leaf also play an important role in order to be efficient while maintaining its activity.

Citronella leaves are usually distilled while they are still fresh, but when the number of harvests is large, the freshly cut or pruned clumps cannot be distilled immediately, so that some of the clumps of harvest are spread out in the field or left buried in a room, which causes the clump to become half dry. or wither. This stockpiling is often carried out due to obstacles in the distillery which has a capacity that is not large or also due to the absence of a refinery that can accommodate large quantities.

Like the nature of agricultural products in general, citronella leaves are perishable. This property is due to the relatively high-water content of the material. According to [7] Citronnella's leaves have a water content of 74,70%. As the time after harvesting increases, the water content in the leaves also decreases. Withering too long can reduce the water content and oil content because the higher the temperature of the material, the more water evaporates and along with the evaporation of the essential oil.

Plant bioactive compounds are very sensitive to environmental influences such as pH, temperature, and light. The drying of roselle flowers resulted in a decrease in the antibacterial power of the material with the equation Y = -22.93x + 122.8 [8]. In addition, the content of bioactive compounds in the leaves also tends to decrease because it is volatile.

Distillation is a process of physically separating a mixture of two or more products that have different boiling points, by first boiling the components that have low boiling points separated from the mixture or can also be defined as the separation of the components of a mixture of two type of liquid or more based on the difference in vapor pressure of each of these compounds. The purpose of the distillation process is to obtain essential oils from aromatic plants that have etheric content that is difficult to extract under normal environmental conditions. In this study, the characterization of the citronella distillate was carried out at various times of leaf withering.

MATERIAL AND METHODS

The main ingredients used in this study were citronella leaves which were purchased at the Kedung Randu cultivation garden, Purwokerto. The test bacteria used were *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922., Nutrient Agar (NA) solid media, Nutrient Broth (NB) liquid media for bacterial culture propagation and maintenance.

The Extraction of *Cymbopogon nardus* L

The study begins with the preparation of tools and materials to be used in the study, followed by the extraction of citronella using the steam distillation method. The manufacturing process is citronella leaves are placed in a boiling flask and soaked with water solvent, then the top destilator and the contents of the water in the bottom destilator. Then heated desiccator and water in the distillation tank into steam. Steam passes through a pile of citronella leaves and carries the essential oil contained in the citronella leaves. The steam carrying citronella essential oil is then cooled using cooling water from the cooling water reservoir. The cooled steam turns into condensate. Cooling water from the condenser is returned to the cooling water reservoir naturally. The cooling water temperature required for the condensation process is 25°C – 30°C.

Determination of the phytochemical content of the extract

Qualitative determination of phytochemical content was carried out to determine the presence or absence of groups of active compounds contained in rosella flower petal extract, including phenolics, alkaloids, flavonoids, steroids, and tannins.

Analysis of phenolic compounds. A total of 1 mL of the sample was dripped on the spot plate and 10% NaOH was added. A red color is formed which indicates a positive test for the presence of hydroquinone phenolic compounds.

Steroid and Triterpenoid Compounds. A total of 1 mg of the dried sample was dissolved in 2 mL of chloroform. Then 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid were added to the solution. The solution was shaken slowly and left for a few minutes. A positive test is indicated by the formation of a green color (steroid compound) and a red or purple color (triterpenoids).

Alkaloids. A total of 1 mL of the sample was shaken with 10 drops of 2M H_2SO_4 and the acid layer was separated into another test tube. This acid layer is dripped on a drip plate and Mayer, Wagner and Dragendorf reagents are added which will cause white, brown and red-orange color deposits, respectively.

Tannin Compounds. A total of 1 gram of the sample was put into a beaker then added 12 mL of hot water and boiled for 15 minutes and then filtered. The filtrate was added a few mL of 1% $FeCl_3$ solution. The appearance of a dark blue or blackish green color indicates a positive tannin compound

Flavonoid Compounds. A total of 1 mL or 1 g of the sample was put into a beaker then added 100 mL of hot water and boiled for 5 minutes after which it was filtered, and the filtrate (10 mL) was added 0.5 g of magnesium powder 2 mL of alcohol chlorhydrate (a mixture of 37% HCl and ethanol). 95% with the same volume) and 20 mL of amyl alcohol. Then shaken vigorously. The formation of red, yellow and orange colors on the amyl alcohol layer indicates the presence of flavonoids.

Antioxidant activity

Testing of antioxidant activity was carried out using the Ferric Thiocyanate method [9]. A total of 0.6 mL of the extract was dissolved in 0.12 mL of 98% ethanol and 2.88 mL of a 2.51% solution of linoleic acid in ethanol. 9 mL of 40 mM phosphate buffer (pH 7) was added. The mixture was incubated in the dark at 40°C for 3 days. After incubation 0.1 mL of the solution was taken and 9.7 mL of 75% ethanol was added: 0.1 mL ammonium thiocyanate 30% and 0.1 mL 20 mM Ferrous chloride in 3.5 HCl. After incubation for 3 min, absorbance was measured at 500 nm. The degree of oxidation is measured by calculating the ratio of absorbance to blank (not with sample extract). So, the antioxidant activity is one that is reduced by the degree of oxidation. Antioxidant activity is expressed by the IC50 value, is the concentration of the extract that can inhibit the oxidation reaction up to 50%

Antibacterial activity

Determination of KHM and KBM values**.** Determination of the value of MIC (minimum inhibitory concentration) and MBC (minimum killing concentration) was carried out by the dilution method [9][10]. 1 mL of extract with various concentrations (1000 ppm to 15,000 ppm) was mixed with 1 mL of NB media containing the test bacteria. Each was put in a test tube, then incubated at 37°C for 24 hours. The concentration of the extract with no bacterial growth (clear) was visually described as the MIC value. The clear extract concentration was mixed with NA medium and poured into a petri dish, then incubated at 37°C for 24 hours. The MBC value was determined based on the smallest extract concentration where there was no bacterial colony growth in the media.

Clearance inhibitory zone**.** Twenty milliliters of sterile NA media were inoculated with 20 µL of fresh culture aged 24 hours in NB medium, shaken evenly then poured into sterile petri dishes and allowed to freeze. A total of 10 µL of rosella flower petal extract was dripped into a 6 mm paper disc, then the disc paper was placed in a petri dish containing a solid agar medium. Then the plates were incubated for 24 hours at 37°C. Observations were made by measuring the clear zone around the paper disc with a caliper which indicated the amount of antibacterial activity.

RESULT AND DISCUSSION

The qualitative composition of *Cymbopogon nardus* L as seen in Table1. The phytochemical content of the extracts from all withering times were as follows: tannins, alkaloid, flavonoid, and steroid. None of extract content with phenolic compounds. It is agreed with [11] stated that Cymbopogon have antifungal properties because of presence of secondary metabolites such as tannins, terpenes, alkaloids and flavonoids.

In extraction, the polarity of the solvent plays an important role. The polarity of the solvent is determined by the dielectric constant. The dielectric constants of water, ethanol, ethyl acetate and hexane are as follows: 80.20: 24.30; 6.02;1.89. so, the polarity of bioactive compound on extract is liked the polarity of water [12]. Tannins are phenolic compounds that tend to dissolve in water, so they tend to be polar. The tannin test showed that the tannins contained in the citronella extract were marked with a dark blue color in the citronella essential oil. Tannins function as secondary antioxidants [13]. Examination of polyphenols was negative in citronella extract.

TABLE 1 Determination of the phytochemical content of the extract

Compound	0	1 day	2 days	3 days
Phenolic	-	-	-	-
Tannin	+	+	+-	+
Flavonoid	+	+	+	+
Steroid	-	-	-	-
Alkaloid	++	++	+	+

Notes: (+) negative contains secondary metabolites, (-) negative contains secondary metabolites

The content of flavonoids in lemongrass extract with withering time of 0, 1, 2 and 3 days. Flavonoids are polar compounds because they have a number of hydroxyl groups. Flavonoids are soluble in polar solvents. The addition of concentrated hydrochloric acid during the flavonoid test serves to protonate flavonoids to form flavonoid salts. After addition of magnesium powder, positive results were indicated by a change in the color of the solution to red, yellow, orange, on the amyl layer [14].

Antibacterial activity was expressed by the diameter of the clear zone generated around the disc. Diameter <6 mm indicates the extract is inactive, while diameter >6 mm, the extract is classified as having antibacterial activity [15]. The difference in activity on bacterial growth that was tested was caused by differences in the phytochemical content in the extracts. The antibacterial activity of the extract used in this in vitro test can be higher if the active compound of the extract can be purified.

The major chemical constituents are geraniol, citral, citronellal, and citronellol [16]. Studies have demonstrated the antiviral [17], antibacterial, and antifungal activities [18] of this oil. In general, Gram-positive bacteria are more sensitive than Gram-negative bacteria [19]. So that the resulting clear zone against *S. aureus* is greater than against *E coli*. The Efficiency of *C. nardus* essential oil as an antibacterial agent was indicated by the association of citronellol and citronellal [20]. Essential oils act as antibacterial by interfering with the process of forming membranes or cell walls so that they are not formed or formed imperfectly. Essential oils that are active as antibacterial generally contain hydroxyl (-OH) and carbonyl functional groups [21].

TABLE 2. Determination of antibacterial activity

Sample	Clearance zone (mm)		MIC (ppm)		MKC (ppm)	
	S aureus	*E coli*	*S aureus*	*E coli*	*S aureus*	*E coli*
0	10.53 ± 2.19^{b}	18.35 ± 3.13^{b}	400	500	500	600
1	11.70 ± 2.01^{a}	19.28 ± 3.30^{b}	250	400	300	400
2	11.53 ± 2.09^{a}	20.35 ± 1.13^{b}	250	300	300	400
3	10.70 ± 2.0^{b}	19.58 ± 2.30^{b}	300	350	350	400
blank	0.05 ± 0.05^{c}	0.02 ± 0.04^{c}	-	-	-	-

The results showed that the withering time treatment had a significant effect on color, pH. Antioxidant activity and antibacterial activity

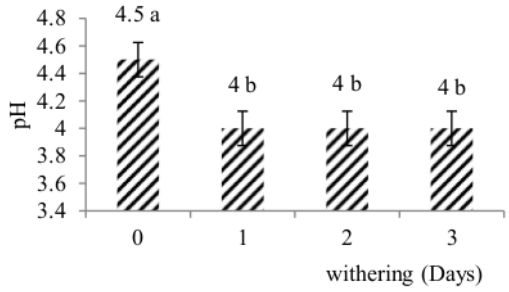

FIGURE 1. The influenced of withering time to pH value

In Figure 1 showed that the pH value of the extract looks to decrease until the withering time of 2 days and after that it rises again. Drying process preserve the sample from deterioration by microorganism and preserve its bioactive compound. It means until 2 days of withering time microorganism activities are increased [22]. The quality of the

essential oil produced can be affected by withering time or wind drying, steam pressure and the quality of the engine used [23].

Color measurement is often done with a color reader using the CIE hunter system, which is characterized by 3 parameters colors, namely the chromatic (Hue) colors of red and green which are written with notation a*, color intensity (chroma) of blue and yellow with notation b*, and brightness (lightness) with L* notation [24]. Color is an important parameter in determining oil quality. The intensity of the color is determined by how much or at least the content of certain color pigments in the oil. The color of the newly extracted essential oil is usually colorless or yellowish, but there are also reddish, green, and brown colors, depending on the type of plant extracted. If essential oils are left in contact with the air for a long time and exposed to sunlight, the oil can darken, the smell changes, over time thickens and finally resin is formed [25].

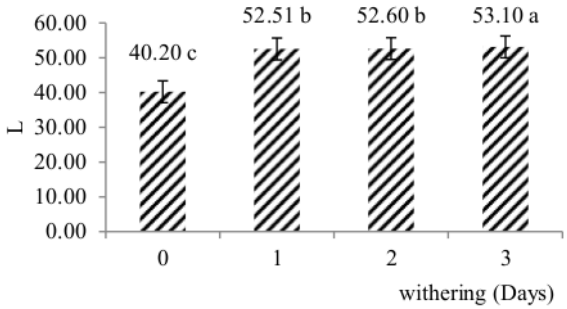

FIGURE 2. The influenced of withering time to L value (brightness)

As seen in Figure 2, L value increased with the increasing withering time. This happens because the longer the withering time, the lower the moisture content of the material. This causes bioactive compounds, especially volatile ones, to be more easily extracted because water as a binder has been reduced. After 2 days the withering time of the volatile compounds in the leaves has disappeared.

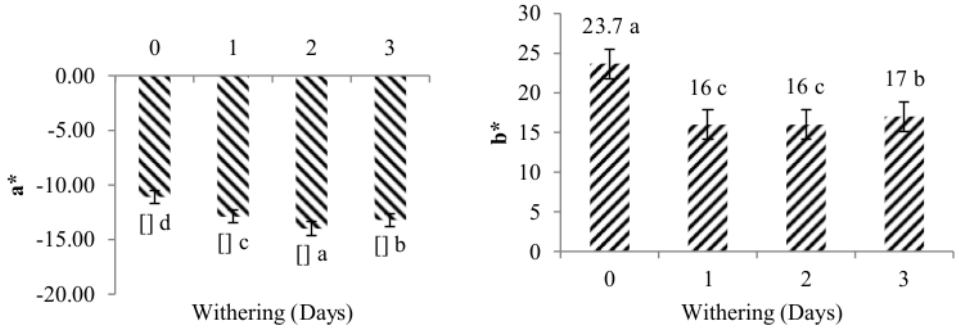

FIGURE 3. The influenced of withering time *to a** (a) and b* (b) value

CONCLUSION

Leaf withering time affects color, pH, antioxidant activity and antibacterial activity. The best result from this study was the 48 hours of withering time.

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