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Total Flavonoid Content of Lemongrass Leaf (*Cymbogoncitratus* (DC.) Stapf) Extract and Antioxidant Activity with Frap

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Abstract

Lemongrass (*Cymbogoncitratus* (DC.) Stapf) leaves contain alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids. Flavonoids act as antioxidants as they can reduce free radicals. This study aims to determine the total flavonoid content, antioxidant activity, and IC_{50} value of lemongrass leaf extract (*Cymbogoncitratus* (DC.) Stapf). Extracts were made by maceration using 96% ethanol as solvent. Testing of total flavonoid content with the $AlCl_3$ method using UV-Vis spectrophotometry was carried out three times. The antioxidant activity test used the FRAP (ferric reducing antioxidant power) method on extracts containing 100, 200, 300, 400, and 500 ppm. The test results showed that the leaf extract of citronella (*Cymbogoncitratus* (DC.) Stapf) had a total flavonoid content of 22.60 mg QE/g extract. Furthermore, there was antioxidant activity in the leaf extract of lemongrass (*Cymbogoncitratus* (DC.) Stapf indicated by the formation of a blue color purplish when reacted with FRAP solution, and IC50 extract value was 71.59 ppm and included in the category of strong antioxidants.

Keywords: *Antioxidant Activity; Lemongrass Leaf Extract (Cymbogoncitratus (DC.) Stapf); Total Flavonoid Content*

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INTRODUCTION

Indonesia is a country with a tropical climate where there are various kinds of plants that have therapeutic activity.[1] The use of herbal medicines from plants increases disease prevention efforts as the side effects tend to be harmless and can be minimized.[2] One of the wild plants widely used by the community is lemongrass (*Cymbogon citratus* (Dc.) Stapf). The extract of lemongrass leaf (*Cymbopogon citratus* (DC.) Stapf) contains several vegetable constituents, namely essential oils.[3] Lemongrass (*Cymbopogon citratus* (DC.) Stapf) can empirically be used as a medicine for headaches, coughs, stomach pains, diarrhea, body warmers, fever reducers, and mosquito repellents.[4] It contains saponins, tannins, alkaloids, and flavonoids. Flavonoids are secondary metabolites often found in various green plants so they can be found in every plant extract. Flavonoids are found in many green plants, so they can be found in every plant extract. Flavonoids contribute to

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pigment production and belong to the water-soluble polyphenol family.[5] Flavonoids can counteract free radicals by liberating hydrogen atoms from their hydroxyl groups; thus, flavonoids are called antioxidants.[6]

Antioxidants are compounds that can scavenge free radicals. Free radicals are generated due to several factors, such as smoke, dust, pollution, and consuming fast food that is not balanced between carbohydrates, proteins, and fats.[7] When free radical compounds meet other molecules, they can form new free radicals, resulting in chain reactions. This kind of reaction will continue and will stop if antioxidants reduce the reactivity.[8] Antioxidant compounds will donate one electron to unstable free radicals so that these free radicals can be neutralized and no longer interfere with body metabolism. Total flavonoid content can be measured by identifying the absorbance value at a certain wavelength based on the Lambert-Beer principle of each herbal plant using a UV-Vis spectrophotometer.[9]

The method used to determine total flavonoids is the $AlCl_3$ method, using UV-Vis spectrophotometry as flavonoids contain a conjugated aromatic system so that they show strong absorption bands in the ultraviolet and visible spectrum regions. The antioxidant activity test uses the FRAP (*Ferric Reducing Antioxidant Power*), which is used to determine the antioxidant activity of a material based on the ability of antioxidant compounds to reduce Fe^{3+} ions (*ferri-tripyridyl-trazine*) to Fe^{2+} (*ferro-tripyridyl-trazine*).[3] In this study, the total flavonoid content of the lemongrass leaf extract (*Cymbogon citratus* (DC.) Stapf) will be tested using the UV-Vis spectrophotometry method, and the antioxidant activity will be tested by using the FRAP method.

METHOD

Plastic bag, knife, scissors, label paper, oven (*Memmert)*, herb grinder, drop pipette, micro pipette, analytical balance (*Ohaus)*, test tube, test tube clamp, test tube rack, maceration jar, stirrer, spatula, 60 mesh sieve, filter paper, funnel, measuring cup, beaker, handscoon gloves, aluminum foil, rotary vacuum evaporator, tissue, cuvette, spectrophotometry UV-Vis (EMCLab), TLC chamber, Silica Gel 60 F TLC plate (Merck), UV lamp 366 nm, citronella leaf (*Cymbopogon citratus*), ethanol96%, potassium acetate 120mM (Merck), aquadest, quercetin (Sigma), $AlCl_3$(Merck, 98%), concentrated H_2SO_4(Merck, 95-97%), sodium acetate trihydrate (Merck 99-101%), acetic acid (Merck, 99.8%), TPTZ powder (Sigma), concentrated HCl (Merck), $FeCl_3.6H_2O$ (Merck), ascorbic acid (Merck 99-100.05%), oxalic acid1% (Merck), chloroform, concentrated ammonia (Merck), Dragendorf reagent, Mayer, Wagner, Lieberman Burchard, $FeCl_3$1%, ether, n-hexane, and ethyl acetate.

Lemongrass leaf samples were obtained from Sribit Village, Delanggu, Klaten, and then determined at the Biology Laboratory of Universitas Ahmad Dahlan. Lemongrass leaves were washed with running water, drained, dried in an oven, and ground by a herb grinder to become Simplicia powder before being extracted. Extraction was done by maceration method with 96% ethanol solvent for 3x24 hours. The filtrate was filtered and evaporated to obtain a thick extract. Furthermore, phytochemical analysis was carried out on the lemongrass leaf extract, including the test for alkaloids, flavonoids, saponins, tannins, and steroids/ triterpenoids by TLC (Thin Layer Chromatography). The total flavonoid content test in the extract was started by

determining the maximum wavelength of the quercetin standard solution, carried out by running the quercetin solution in the UV-Vis wavelength range of 370 - 450 nm. The determined wavelength was 400 nm. Furthermore, the standard curve of quercetin was made by making several concentrations, namely 10 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm. For each standard, 1 mL of quercetin was added with 1 mL of 2% $AlCl_3$ and 1 mL of 120 mM potassium acetate and incubated for 30 minutes. The absorbance was measured using UV-Vis spectrophotometry with a determined wavelength (400 nm). The total flavonoid content of lemongrass leaf extract was determined using the $AlCl_3$ method by dissolving 10 mg of extract in 10 mL of 96% ethanol. 1 mL of extract was added with 1 mL of 2% $AlCl_3$ solution and 1 mL of 120mM potassium acetate and incubated for 30 minutes at room temperature. The absorbance was determined by UV-Vis spectrophotometry. Three replications were made for each analysis, and the average absorbance was then identified

Determination of the antioxidant activity of lemongrass leaf extract started with making a FRAP solution by mixing 25 mL of acetate buffer solution, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3.6H_2O$ solution, then added with aquadest to 100 mL in a volumetric flask. Determination of the maximum wavelength of the FRAP solution was carried out by mixing 3 mL of FRAP reagent and 1 mL of distilled water and then measuring the absorption at a wavelength of 400-600 nm using UV-Vis spectrophotometry. Based on the result, the maximum wavelength was determined at 595 nm. Ascorbic acid was used as a standard antioxidant solution also as a positive control. A standard curve solution of 100 ppm was prepared by dissolving 10 mg of ascorbic acid dissolved in 1% oxalic acid to the 100 mL volumetric flask limit. Furthermore, the stock solution of 100 ppm was diluted into several concentrations, namely 10, 15, 20, 25, and 30 ppm. 1 mL of FRAP reagent was put and then added with 3 mL of ascorbic acid from each concentration, then homogenized and allowed to stand for 10 minutes at 37°C. The absorbance was then observed at a determined wavelength of 595 nm. The IC50 value of lemongrass leaf extract was determined by taking 50 mg extract, dissolved in 50 mL of 96% ethanol, obtained a concentration of 1000 ppm, and then diluted into several concentrations, namely 500, 400, 300, 200, and 100 ppm. 1 mL of FRAP reagent was put in, and 3 mL of sample were added from each concentration. It was homogenized and allowed to stand for 10 minutes at 37°C, then observed the absorbance at a determined wavelength of 595 nm.

RESULTS AND DISCUSSION

The results of the extraction of lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf) can be seen in table 1 below.

Table 1. The results of the extraction of lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf)

Powder weight (g)	Extract weight (g)	Yield (%) (w/w)
426	57.37	13.46

The yield of lemongrass leaf extract was 13.46% w/w. A good extract yield is indicated by a yield >10%.[10] The higher the yield of the extract is, the higher the content of substances will be attracted to the raw material.[11] It was also presumably due to the maceration time that has been done for three days. The remaceration was done two times so that the chemical compound extraction process could be maximized.

Phytochemicals

Based on the phytochemical identification, lemongrass leaf extract contained alkaloids, flavonoids, saponins, tannins, and steroids. The results of the flavonoid test using TLC showed the spots between the extract and quercetin were nearly by using eluents n-Hexane: ethyl acetate: ethanol: water in a ratio of 2.5:1:0.5:6.5. The Rf value of the extract was 0.51, and the Rf value of quercetin was 0.56. The difference in the Rf value was 0.05. The results would be declared positive containing quercetin if the Rf value had a difference of 0.05.[12]

Results of Total Flavonoid Content Test of Lemongrass Leaf Extract (*Cymbopogon citratus* (DC.) Stapf

Quantitative tests were carried out to determine the total flavonoid content of the lemongrass leaf extract, using quercetin as a standard solution. The maximum wavelength of quercetin was 400 nm. Furthermore, the standard curve of quercetin was made by making several concentrations, namely, 10 ppm, 20 ppm, 25 ppm, 30 ppm, and 35 ppm. Each concentration was measured at a wavelength of 400 nm. Based on these measurements, the linear regression equation for the standard solution of quercetin was y = 0.025x - 0.072, which can be seen in figure 1 below.

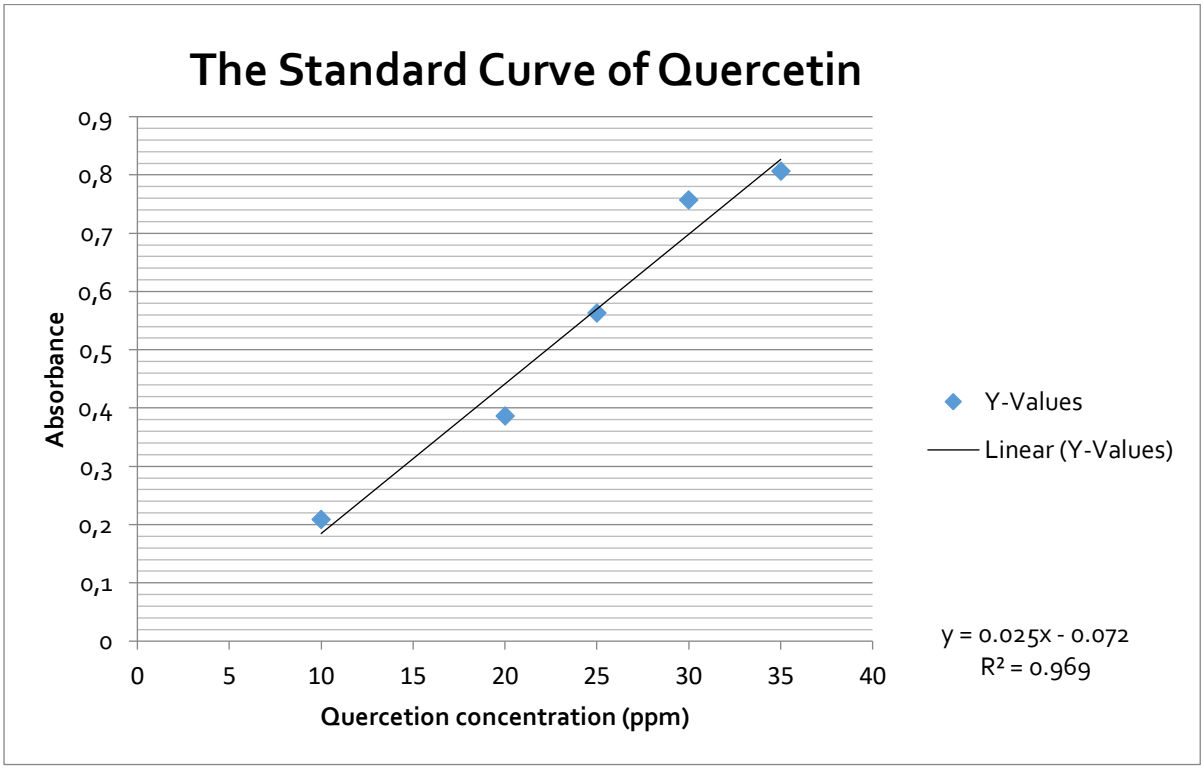


Figure 1. The Standard Curve of Quercetin

Furthermore, the absorbance measurement of the lemongrass leaf extract was made for 3 replications. The sample solution was added with 1 mL of aluminum chloride ($AlCl_3$), which could form a complex, resulting in a shift in wavelength towards the visible indicated by the solution producing a more yellow color. 1 mL of potassium acetate was then added to maintain the wavelength in the visible region [13]. It was measured by using UV-Vis spectrophotometry to identify the extract absorbance (Table 2).

Table 2. Results of Total Flavonoid Content of Lemongrass Leaf Extract (*Cymbopogon citratus* (DC.) Stapf)

Replication	Sample Weight (g)	Absorbance	mg QE/g extract
I	0.01	0.437	20.36
II	0.01	0.427	19.96
III	0.01	0.650	28.88
Average			**23.06**

The results of this study showed that the total flavonoid content of lemongrass leaf extract (*Cymbopogon citratus* (DC.) Stapf was 23.06 mg QE/g extract, which can be seen in table 2. The higher the flavonoid content is, the higher the benefits of flavonoids as antioxidants will be.[14] Sample III has a higher absorbance than the other two samples, probably due to the less precise determination of operating time. The three replications were analyzed in minutes of 10. In previous studies, it was shown that starting in the minutes of 30, the flavonoid compounds had completely reacted with the $AlCl_3$ reagent, and the absorbance reading was stable in the minutes of 30.[15]

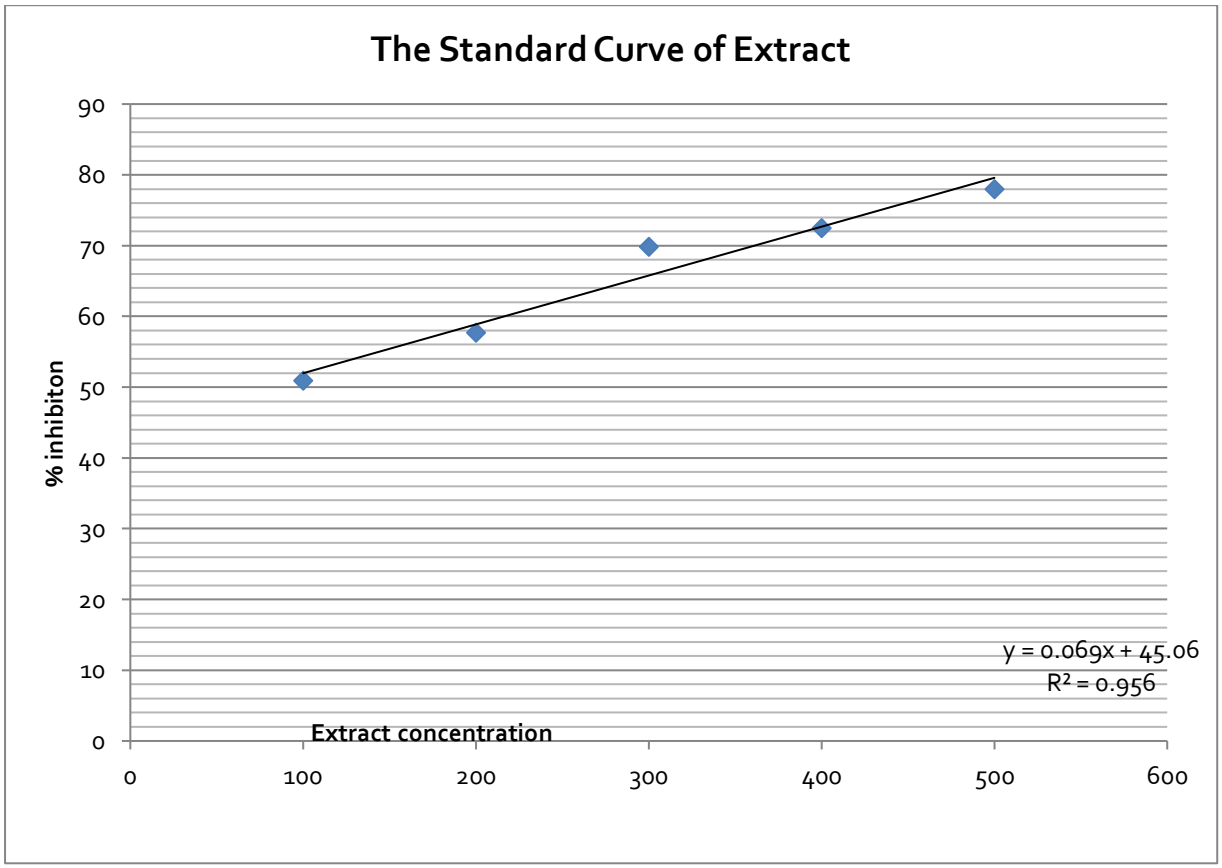
Results of Antioxidant Test of Lemongrass Leaf Extract (*Cymbopogon citratus* (DC.) Stapf)

An antioxidant activity test was carried out to determine the IC_{50} value of the lemongrass leaf extract. The maximum wavelength of the FRAP solution was 595 nm. Qualitative analysis was carried out by taking a sample solution of lemongrass leaf extract. The FRAP reagent was added, and then color changes were observed. As a result, the sample turned purplish blue, indicating that the sample had antioxidant activity.[3] Furthermore, vitamin C was used to compare as it functions as a secondary antioxidant. It can reduce free radicals and prevent chain reactions.[16] The IC_{50} value of vitamin C was 23.60 ppm. Quantitative tests were conducted by making leaf extract of lemongrass (*Cymbopogon citratus* (DC.) Stapf) in five concentration series, namely, 100, 200, 300, 400, and 500 ppm. It was added with FRAP reagent at each concentration series and read the absorbance at the maximum wavelength of 595 nm.

Table 3. Results of Antioxidant Activity Test of Lemongrass Leaf Extract (*Cymbopogon citratus* (DC.) Stapf)

Extract Concentration (ppm)	Absorbance Average	% inhibition
100	0.355	50.89
200	0.306	57.67
300	0.218	69.84
400	0.199	72.47
500	0.159	78.00

The results of the absorbance measurement were used to obtain the % inhibition value. The value of % inhibition was used to find the IC_{50} value to determine the strength of antioxidant activity in the sample. Data in the form of concentration and % inhibition of lemongrass leaf extract showed a linear regression equation y = 0.069x + 45.06 (Figure 2) to calculate the IC_{50} value.



The calculation of the IC_{50} value of lemongrass leaf extract was as follows:

y = 0.069 x +45.06

50 = 0.069 x +45.06

50 – 45.06 = 0.069x

$$x = \frac{4.94}{0.069}$$

x= 71.59 ppm

The IC_{50} value of lemongrass leaf extract (*Cymbopogon citratus* (DC.) Stapf) was 71.59 ppm. Specifically, phytochemicals are categorized as highly strong antioxidants when the IC_{50} value is <50 ppm, strong when the IC_{50} value is 50-100ppm, moderate when the IC_{50} value is

100-150ppm, and weak when the IC_{50} value is 151-200ppm.[17]

CONCLUSION

Based on the result of this research, it can be concluded that the total flavonoid content of the lemongrass leaf extract (*Cymbogon citratus* (DC.) Stapf) was 23.06 mg QE/g. There was antioxidant activity in the lemongrass leaf extract indicated by the formation of a purplish blue color on the test with FRAP solution. Furthermore, the IC_{50} value of lemongrass leaf extract was 71.59 ppm, classified as a strong antioxidant.

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