# Phtochemical Compounds Test of Lemongrass Extract (Cymbopogon Citratus) Using Maceration Method

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**Abstract :** Lemongrass (Cymbopogon citratus) is a spice plant that contains active compounds that can be used as a cooking spice and medicine. The purpose of this study was to determine the phytochemical compounds in lemongrass a qualitatively and to determine the levels of phenolic compounds quantitatively. The method used to extract the structure of compounds in lemongrass plants is maceration using ethanol as a solvent. Phytochemical test qualitatively by looking at physical and chemical changes. Quantitative phytochemical tests determine the levels of phenolic compounds using the UV-VIS Spectrophotometer instrument and identification of bonds between compounds using the FTIR (Fourier Transform Infra Red) instrument. The results of the phytochemical test qualitatively contained alkaloids, flavonoids, and triterpenoids. The results of the quantitative phytochemical test in the extract contained a total phenolic content of 6.379 mg/g. Based on the identification of phenolic compounds using FTIR, there are wave peaks around 3400-3600 cm-1 indicating the presence of O-H bonds in the phenolic hydroxyl groups. In addition, the presence of a peak wave number around 1600-1700 cm-1 indicates the presence of a C=O bond in the ketone or aldehyde group.

## INTRODUCTION

Indonesia is a tropical country that is rich in a variety of biological natural resources. This diversity provides various benefits for living things such as source of food, medicine, and aromatherapy. One of the plants that can be used as medicine and aromatherapy is lemongrass (Cymbopogon citratus). Lemongrass is usually used as a cooking spice and medicine which is commonly called lemongrass oil because it contains essential oils (Moniharapon, et al., 2014). As a seasoinng, it is still not widely cultivated in Indonesia, because most of it is only used for daily needs as a food ingredient. However, if processed properly, Lemongrass will produce essential oils that have high selling value (Firyanto et al., 2020).

Extraction is a process of separating materials from a mixture using an appropriate solvent (Mukhriani, 2014). The extraction process can be performed by 2 methods, that are hot method and cold method (Febrina et al, 2015). The cold method is percolation and maceration, while the hot method is soxhlet and reflux (Safitri et al, 2018). The maceration method is an extraction process that is carried out by soaking simplicia at room temperature with a suitable solvent. Soaking sample takes 3-5 days with several stirring to speed up the process of dissolving the analyte (Septiani et al, 2021). The solvent used for maceration is ethanol. The character of ethanol is the same as polar solvent and water (Rafsanjani & Putri, 2015). Phytochemical tests were carried out to determine the content of groups of chemical

compounds contained in lemongrass. Phytochemical test can be analyzed qualitatively and quantitatively. Qualitative test is the initial step to determine the class of bioactive compounds by looking at the color change reaction in the sample. Qualitative tests include alkaloid tests, flavonoid tests, tannin tests, saponin tests, steroid and triterpenoid tests (Fauzi & Santoso., 2021). Meanwhile, a quantitative test was carried out to determine the phenolic content of citronella plants. Quantitative tests were carried out using a UV-Vis spectrophotometry instrument to determine the phenolic content in citronella and FTIR (Fourier Transform Infra Red) instruments to analyze the structure between compounds. FTIR is an infrared spectroscopy equipped with a Fourier transform for the detection and analysis of spectrum results (Nurfitriyana, et al., 2022).

## METHOD

### Tools and Materials

The tools used in this study included beakers, stirrers, blenders, funnels, Erlenmeyer, filter paper, rotary evaporators, petri dishes, FTIR tools, 100 and 10 ml volumetric flasks, measuring pipettes, bulbs, vortexes, cuvettes, test tubes, spatulas, vials, and UV-Vis spectrophotometer tools. The materials used in this study were citronella (Cymbopogon citratus), ethyl acetate, gallic acid, 96% ethanol, 0.5 ml Folin-ciocalteu, 7% H2SO4, 12 M concentrated HCl, dragendorf reagent, 5% FeCl3, magnesium, chloroform, and acetic acid.

### Research Procedure

#### Extract Sample Preparation

The lemongrass sample weighing 650 grams was dried, then cut into pieces and ground to a powder. The powder was filtered using a 40 mess sieve to get a fine powder. 100 grams of fine citronella powder is soaked in 700 ml of ethanol for 24 hours. The results of the soaking were extracted using a rotary evaporator to obtain a thick lemongrass extract.

#### Phytochemical Qualitative Test

#### Alkaloid Test

A total of 0.5 grams of sample was put in a test tube added to 0.5 ml of 2 M HCl. Added 1-2 drops of dragendorf. Observations are made by looking at the colors that appear. It is said to be positive for alkaloids if it shows an orange or red precipitate (Tiwari, et al., 2011).

#### Flavonoid Test

A total of 200 mg of sample was put into a test tube, added 5 ml of ethanol, then heated for 5 minutes. Added a few drops of concentrated HCl and added 0.2 grams of magnesium. Observations seen from the change in color. It is said to be positive if it shows a dark red (magenta) color in the ethanol layer (Mustikasari & Ariyani, 2010).

#### Saponin Test

A number of samples are put into a test tube then hot water is added and cooled. After chilling, it is shaken for 10 minutes and will form froth or foam if it is positive. If the foam persists and is stable after adding 2M HCl, it indicates a positive saponin (Dewi, 2020).

#### Phenolic Test

A number of samples were put into the Erlenmeyer and added 10 mL of ethanol. Take 1 mL of the solution formed and put it in a test tube. After that, 2 drops of 5% FeCl3 solution were added. If a green or bluish green color is formed, it indicates a positive phenol (Syafitri, et al., 2014).

#### Steroids and Triterpenoids Test

A number of samples were put into a test tube and added 0.5 mL of chloroform and 0.5 mL of acetic acid. After that, 2 mL of concentrated H2SO4 was added through the test tube wall. The sample is said to be positive for triterpenoids if a purple-red color is formed. While steroids are positive if they produce green or blue (Suryanita, et al., 2019).

**Quantitative Test using UV-Vis Spectrophotometry**

**Preparation of Gallic Acid Standard Solution**

The required gallic acid is 1 ppm, so a comparison is used

$$\text{Ppm} = \frac{mg\ gallic\ acid}{1000\ ml\ ethanol}$$

The stock solution that will be used as much as 100 to get the results by using under this formula

$$100\ \text{ppm} = \frac{100\ lg}{1000\ ml} = 0{,}01\ \text{gram}$$

Preparation of standard mother liquor Gallic acid 100 ppm

Gallic acid powder was weighed on an analytical balance as much as 10 mg and then put into a volumetric flask. Dissolved with ethanol up to 100 ml and shaken until homogeneous. Gallic acid standard mother liquor was prepared with various concentrations of 10, 20, 30, 40, 50 ppm. The formula used is below

$$M1.\ V1 = M2.\ V2$$

Information :
M1 = Molarity before dilution
M2 = Molarity before dilution
V1 = Volume before dilution
V2 = Volume after dilution

**Preparation of 7% Na2CO3 Solution**

Na2CO3 powder was weighed as much as 7 grams and then dissolved with distilled water up to 100 ml.

**Maximum Wavelength Determination**

Measurement of the maximum wavelength of gallic acid was carried out by measuring a sample of 10 ppm gallic acid concentration in the wavelength range of 400-800 nm using a UV-Vis spectrophotometer.

**Gallic Acid Standard Solution Curve**

Standard gallic acid solutions were made with various concentrations of 10, 20, 30, 40.50 ppm. Take 1 ml of each concentrate and put it in a test tube and add 0.5 ml of Folin-ciocalteu and then let it stand for 8 minutes while shaking. Into the solution was added 4 ml of 7% Na2CO3 solution and vortexed for 1 minute. If there is a precipitate, centrifuge to separate the precipitate and filtrate. The filtrate was taken and tested with a UV-Vis spectrophotometer. Measurements are made at the maximum wavelength. Create a calibration curve between the concentration of gallic acid (sb x) and the absorbance (sb y). The regression equation is used to find the concentration of the sample. Pay attention to the value of r2 (make sure the value of r2 is close to 1).

**Measuring Sample Absorption**

The extract was weighed 0.01 g and dissolved with 10 ml of ethanol and homogenized. The extract solution was taken as much as 1 ml and added 0.5 ml of Folin-ciocalteu, left for 4 minutes while shaking. 4 ml of 7% Na2CO3 solution was added and vortexed for 1 minute. Enter the blank solution (ethanol) into the cuvette. The sample is put in a cuvette and placed in a spectrometer, then the wavelength is in the range of 400-800 nm. The absorbance of the sample is entered into the gallic acid linear regression equation as sb y to obtain the sample concentration (sb x).

**Total Phenolic Content Test (TPC Formula)**

The total phenol content can be calculated using the following formula:

$$\text{TPC} = \frac{C.V.fp}{g}$$

Information:
C = phenolic concentration (x value)
V = volume of extract used (ml)
fp = dilution factor
g = sample weight used

**Qualitative Test Using FTIR Instruments**

Turn on the FTIR tester and the computer connecting the software used for analysis, put the citronella sample into the sample holder,

operate the FTIR tool so that the FTIR spectrum is generated from the sample.

## RESULTS AND DISCUSSION

The lemongrass sample to be used is first subjected to the extraction process. Extraction of lemongrass leaves is carried out using the maceration method so as not to make the chemical content in the lemongrass damaged by heat. This extraction is classified as wet extraction because it does not require heating in the process. This extraction process has the goal that all the compounds contained in the material can be separated, so that it will produce a yield which will later be used to determine the content of existing compounds (Purwandari et al., 2018). The maceration method was carried out by immersing the samples in ethanol for 24 hours. The purpose of immersion is to break down the cell wall caused by pressure on the outside and inside of the cell. The breakdown of this cell wall will result in the compound content in the cytoplasm of the sample soluble in the solvent (Zena, et al., 2019). During the immersion process, the sample must be stirred so that the solvent and sample can be mixed evenly. The solvent used for extraction must fulfill two conditions, namely the solvent must be the best solvent for the material being extracted and the solvent must separate quickly after stirring. The filter liquid or solvent normally used in the extraction method can be water, ethanol, methanol, water-ethanol or other solvents (Febriana & Oktavia, 2019).

One of the suitable solvents for extracting kitchen lemongrass is ethanol. Ethanol is a type of alcohol with two carbon atoms and a polarity value of 0.68 (Febriana & Oktavia, 2019). Ethanol is commonly chosen as a solvent due to its low boiling point, making it easy to evaporate. The volatile nature of ethanol leads to a minimal amount of ethanol remaining in the extract, resulting in a purer extract. Based on this, ethanol is selected as the polar solvent in this study, considering it hinders bacterial and fungal growth and doesn't require excessively high temperatures for heating. Additionally, the principle of "like dissolves like" guides solvent selection, making ethanol suitable for dissolving polar compounds. Ethanol serves as one of the solvents that can bind to active substances like tannins, saponins, flavonoids, alkaloids, and phenols (Nitasari, 2019).

The result of maceration is referred to as the filtrate, which will be evaporated using a rotary evaporator to obtain a concentrated extract. The rotary evaporator serves to separate the solvent from the lemongrass powder. The use of this apparatus is chosen because it can evaporate the solvent below its boiling point, ensuring that the constituents contained within the oil are not damaged by high temperatures (Pangestu & Handayani, 2011). At this stage, ethanol will evaporate, causing the extract to dry and yield a concentrated extract (Rusli, 2016). Drying the extract is performed to prevent enzymatic reactions that could lead to chemical changes in the extract. Additionally, the purity of the concentrated extract will be maintained, and it will be less susceptible to fungal contamination. The obtained concentrated extract will be transferred to a petri dish and covered with plastic wrap (Saifudin, et al., 2011).

### Phytochemical Screening Qualitative Test

Phytochemical screening test is the initial step to determine the groups of chemical compounds present by observing the physical color changes. Qualitative phytochemical screening is conducted using reagents that will induce color changes in the prepared extract sample (Vifta & Advistasari, 2018). The results of phytochemical screening are influenced by the solvent used and the extraction method applied. In this test, the solvent used is ethanol because the chemical content in lemongrass is more easily extracted using ethanol. Based on the phytochemical test conducted on the kitchen lemongrass leaf extract sample, the following results were obtained.

Table 1: Phytochemical Qualitative Test Results of Lemongrass Leaf Extract

| Test | Etanol | Reaction |
|---|---|---|
| Alkaloid | + | Jingga |
| Flavonoid | + | Dark Red |
| Saponin | - | Tidak berbuih |
| Fenolik | + | Green |
| Steroid | - | - |
| Triterpenoid | + | Purple |

Lemongrass dissolved in ethanol shows a reaction when dissolving alkaloids because it is polar in nature and can bind together. Alkaloids are organic compounds containing negatively oxidized nitrogen and possess active physiological properties, making them widely utilized in medicine (Titis, et al., 2013). The alkaloid reaction is characterized by the presence of a red or orange precipitate due to the formation of covalent bonds from potassium ion K, forming potassium alkaloid (Anindita, et al., 2023). In this experiment, the kitchen lemongrass sample shows a positive alkaloid reaction as it turns orange after being treated with Dragendorff's reagent. In a study by Tuasalamony et al. (2020), it was stated that kitchen lemongrass indeed contains alkaloids, thus having potential to inhibit bacterial growth with specific inhibition zones. Even in Fahdi et al.'s study (2022), an oral antiseptic formulation was made from kitchen lemongrass extract with the addition of 96% ethanol solvent. This mouthwash formulation has been proven to inhibit the growth of S. mutans. Badriyah & Farihah (2022) mentioned that lemongrass treated with 96% ethanol contains high alkaloid content that can restrain the growth of pathogenic organisms by preventing nucleic acid synthesis and inhibiting enzyme activity.

The flavonoid test on kitchen lemongrass yielded positive results, indicated by a color change to orange. Flavonoids are polyphenolic compounds containing an aromatic system. Flavonoids assist in photosynthesis, exhibit antiviral and antimicrobial properties in plants (Akasia et al., 2021). Flavonoids also function as antioxidants, neutralize cytotoxicity, and can alter genes (Iling, et al., 2017). The color change to orange or red is due to the presence of leukonidin, wherein the orange color indicates the presence of flavonols and the darker red color indicates the presence of flavonones (Mosse, et al., 2021). Leukonidin is one of the flavonoid compounds resulting from the formation of flavillium salts when treated with HCl and Mg. Similar to the conducted experiment, Adiguna & Santoso (2017) stated that lemongrass contains antibacterial and fungicidal flavonoid compounds. This is also supported by Hairi et al.'s study (2016), which claimed that the flavonoid content in lemongrass stimulates the development of fibrin threads, leading to faster wound healing and drying of the wound tissue.

The result of the saponin test in this experiment is negative. This is indicated by the absence of froth or foam when the extract is treated with HCl. Saponins are a group of glycoside chemical compounds known for their strong odor. They are easily soluble in water and possess soap-like properties. Saponins are potent compounds that generate froth or foam at high concentrations (Iling, et al., 2017). The formation of foam occurs due to saponin hydrolysis, resulting in the creation of both polar and nonpolar compounds. When shaken, this difference causes saponins to form micellar structures, leading to stable foam (Muthmainnah, 2017). In the study by Sinaga et al. (2020), it was stated that kitchen lemongrass extract indeed does not contain saponin compounds as it does not produce foam when shaken. However, the research by Pujawati et al. (2019) indicated that kitchen lemongrass contains saponins, showing foam formation after being treated with 2N HCl and distilled water. Diana et al. (2017) mentioned that kitchen lemongrass extract contains saponins capable of breaking down bacterial cell walls and forming collagen, thus accelerating wound healing.

The result of the phenolic compound test in this study showed a positive outcome, indicated by the color change of the sample from orange to green. Phenols are compounds known for their high antioxidant activity. In the research by Yanti et al. (2022), the phenolic content in

lemongrass and various other plants is used as a raw material for producing herbal teas that combat free radicals in the body. According to Mirghani et al. (2013), a high phenolic content in a sample can be beneficial as a preventive measure against hypertension and diabetes. Meanwhile, in Yeni et al.'s study (2022), it is explained that low concentrations of phenols can cause cell leakage. This is because at low concentrations, phenols can damage the cell wall and cytoplasm. At high concentrations, phenols can easily lyse cell membranes due to protein coagulation (Hairi et al., 2016). Other benefits are highlighted in Sujianti et al.'s study (2020), which stated that phenolic content in lemongrass can prevent lipid oxidation in fish ball products, thereby maintaining the fat content that affects the quality of the fish balls.

The steroid test yielded a negative result in the lemongrass sample. This is evident from the lack of color change to green or blue in the lemongrass sample. In the study conducted by Balfa & Rahmawati (2022), no steroids were found in lemongrass either. Steroids are derivative/lipid compounds that are not hydrolyzed (Ratnasari et al., 2022). The presence of steroids in a sample would result in acetylation reactions of the -OH group when treated with acetic acid (Agustina et al., 2016). The acetylation reaction caused by adding acetic acid would yield acetyl steroid complexes, resulting in a color change to green or blue (Ilyas, et al., 2015). The absence of a color change to green or blue in the lemongrass sample indicates the absence of steroid compounds (Alviani et al., 2022). Steroids have various benefits, including being antimicrobial, antioxidant, anti-asthma, and antiviral agents (Mohan, et al., 2012). In the research by Wardhani & Supartono (2015), it is stated that steroids have the property of being insoluble in lipids, which enables them to easily penetrate bacterial cell walls. This suggests that sufficiently potent steroid content has significant potential as an antimicrobial agent.

The result of the triterpenoid test on kitchen lemongrass showed a positive outcome, indicated by the color change to green. Triterpenoids are secondary metabolite compounds derived from terpenoids, which can be cyclic or acyclic. Triterpenoids contain alcohol, aldehyde, or carboxylic acid groups that have numerous benefits for both plants and humans (Andayani & Gunawan, 2013). In human life, triterpenoids are useful as anti-cancer, antibacterial, anti-inflammatory agents, and can help manage cholesterol levels. In plants, they can prevent pest, fungal, bacterial, and viral disturbances that might affect growth (Nassar, et al., 2020). In Djahi et al.'s study (2021), it was stated that triterpenoid content has been proven to control blood sugar levels in the body. This is because triterpenoids can stimulate insulin release and help reduce glucose levels in the body.

Ethanol is a compound that possesses polar properties, similar to water and methanol (Ergina et al., 2014). Additionally, ethanol is also considered universal or nearly capable of dissolving all substances, whether polar, nonpolar, or semi-polar (Erviana et al., 2016). Ethanol can dissolve compounds present in the lemongrass sample. This is because the compounds contained in lemongrass can be easily extracted when the solvent used is polar in nature (Ariyani et al., 2017). Furthermore, ethanol has more hydroxyl groups compared to other compounds like methanol, which makes ethanol's polarity suitable for the lemongrass sample. Moreover, in the research by Miftahurrahmah et al. (2023), it is explained that the yield obtained from the lemongrass sample extraction process using ethanol was greater compared to using other solvents.

**Quantitative Test Using UV-Vis Spectrophotometry**

UV-Vis spectrophotometry is an analytical method that reviews the molecular interactions that occur between chemicals and ultraviolet electromagnetic radiation. This reaction occurs from near electromagnetic radiation (190 to 380 nm) and visible light (380 to 780 nm) using a spectrophotometer. The principle of this instrument is that there is an electronic transition of a molecule caused by the

absorption of energy in the form of electromagnetic radiation at a certain frequency by the molecule itself. Measurement of the absorption of radiation on the sample will be detected by the detector at various wavelengths. The results of radiation absorption by this detector will be continued to the recorder to produce a spectrum which will provide information for identifying the presence or absence of chromophore groups (Shahmoradi et al., 2022).

The first step before carrying out the test is weighing the sample to be used and adding some chemicals. The added materials are Na2CO3 and folin. Sodium carbonate (Na2CO3) is a salt compound that can dissolve in water about 30% by weight. Na2CO3 solution can be used as an activator because it dissolves easily in water and does not cause metal oxides so that it can reduce the metal content in activated carbon. The Na2CO3 compound will decompose in carbon from the sodium metal compound into the following reaction Na2CO3+ 2C -> 2Na + 3CO. The purpose of giving Na2CO3 to the sample is to form complex compounds so that a shift in visible wavelengths occurs (Meilianti., 2020). While folin is a reagent that has a function as an oxidizing agent in UV-Vis spectrophotometry tests. Folin has a principle by forming a blue complex compound. This complex compound can be measured at 675 nm. The purpose of giving folin reagent is to maintain the wavelength in the visible region. The steps that must be taken after adding Na2CO3 and folin are followed by centrifuging to obtain a clear supernatant which will be tested on UV-Vis spectrophotometry (Listiana, et al. 2022).

Preparation of a calibration curve for gallic acid standard solution was carried out before determining the level of total phenolic compounds. This aims to facilitate the determination of phenol levels and is obtained through the regression equation of the calibration curve. Making a regression curve shows that there is a relationship between concentration and absorption. This relationship is indicated by the value of r (correlation coefficient), and R2 (coefficient of determination). The curve is made from a comparison between concentration and absorption, where the greater the concentration, the greater the absorption value (Marjoni et al., 2015). A standard solution will be made from gallic acid and will be used to determine the total phenolic content, so it is necessary to make a calibration curve for gallic acid standard solutions with concentrations of 10, 20, 30, 40, and 50 µg/mL (Marjoni, et al., 2015). The results of the calibration curve for gallic acid standard solutions with concentrations of 10, 20, 30, 40, and 50 µg/mL can be seen in Table 2 as follows.

Table 2: Gallic Acid Standard Solution Calibration Curve

| Number | Concentration (µl/mL) | Serapan |
|---|---|---|
| 1 | 10 | 0,525 |
| 2 | 20 | 0,863 |
| 3 | 30 | 1,531 |
| 4 | 40 | 2,007 |
| 5 | 50 | 2,392 |

Measurement of the maximum wavelength needs to be done to find out the largest concentration unit at the maximum wavelength, so that the maximum analytical sensitivity will be obtained. The determination of the peak wavelength is carried out because the wavelength of a compound can vary when measured under different conditions and devices. The goal is that the change in absorbance per unit concentration reaches the highest value at the peak wavelength, so that the maximum analytical sensitivity is achieved (Rosalina, 2018). The peak wavelength (λ max) is the wavelength at which the absorbance peak occurs due to electronic excitation. The measurement of the peak wavelength is carried out because the change in absorbance per unit concentration has the highest value at the peak wavelength, so that the maximum analytical sensitivity is achieved. The process of determining the peak wavelength of gallic acid was carried out by measuring a solution of gallic acid at a concentration of 10 ppm in the wavelength range of 400-800 nm. In this study

the maximum absorption of gallic acid was obtained at a wavelength of 753 nm (Leo & Daulay 2022).

The measurement results showed that the greater the concentration of the standard gallic acid solution measured, the greater the absorbance obtained. This is because the higher the concentration of the tested compound, the higher the concentration of the compound. In addition, the Lambert-Beer law shows that changes in the concentration of a particular sample will change the absorbance at each wavelength with a constant factor (Pratama & Zulkarnain, 2015). Making a standard gallic acid calibration curve can be done by plotting a standard gallic acid solution (x-axis) and absorbance (y-axis), after which the two points can be connected with a straight line.

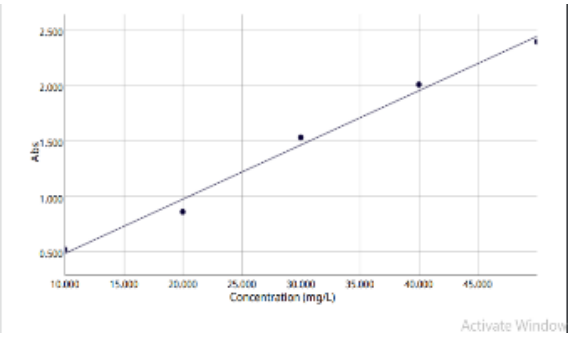


Figure 1: Absorption Measurement Results of Gallic Acid Standard Solution of Lemongrass Leaf Ethanol Extract

Based on the results of measuring the absorption of gallic acid standard solution at a wavelength of 753 nm with a UV-VIS Spectrophotometer, the linear regression curve shows that there is a close relationship between concentration and absorption. The greater the concentration, the greater the absorption value. From the calibration curve, the regression equation y=0.049 x-2.561 is obtained and the coefficient of determination $R^2$ = 0.990 which means that 99.9% of the absorption is affected by concentration.

Total Phenolic Content (TPC) is a total phenolic content test carried out using the Folin-Ciocalteu reagent. The principle of this reagent is the formation of a blue solution which comes from the oxidation of phenolic compounds by Folin-Ciocalteu (Monica, 2017). The choice of Folin Ciocalteu reagent in the total phenolic content test is because phenolic compounds will react with Folin Ciocalteu and form a solution whose absorbance can be measured. The blue colored complex produced by this reaction can be measured at a maximum wavelength of 753 nm. The greater the concentration of phenolic compounds will produce more phenolic ions resulting in the reduction of heteropoly acids (phosphomolybdate-phosphotungstate) into molybdenum-tungsten complexes. The reduction that occurs can produce a deeper blue color (Putri et al., 2022). The formula and results for calculating the TPC content of a sample of lemongrass are as follows:

$$\text{TPC} = \frac{\frac{c.v.Fp}{g}}{\frac{6{,}739.1.10}{0{,}01}}$$

$$\frac{\frac{67.39}{0{,}01}}{}$$

$$= 6{,}739 \text{ ppm}$$

Information :

| | |
|---|---|
| TPC | : total phenolic content (mg/g GAE) |
| c | : concentration (x value) (ppm) |
| v | : extract volume (ml) |
| fp | : dilution factor |
| g | : sample weight (gram) |

Research conducted by Marjon et al., (2015) states that the limit value of quantization = 6.61 µg/mL. The total phenolic content obtained in the extract was 6.379 mg/g GAE, thus exceeding the quantization limit value. The quantization limit value is the smallest quantity of analyte present in a sample that still meets the standard value criteria. If the phenolic content in the extract exceeds the quantization limit value, then the measurement result can be considered as a reliable result and indicates that the extract has a high phenolic content.

**Qualitative Test Using FTIR**

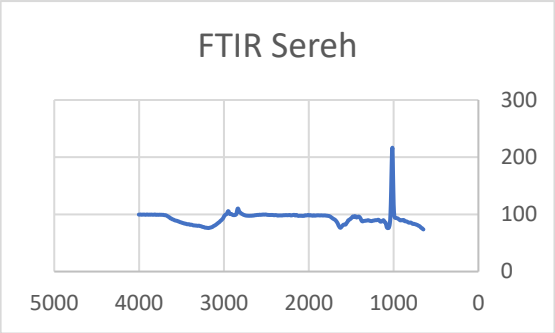


Figure 2: Lemongrass FTIR results

The phenolic test of citronella extract using Fourier Transform Independent Red (FTIR) is an analytical method used to determine the presence of phenolic compounds in citronella extract quantitatively and qualitatively (Mulyani, et al., 2021). Extraction is carried out using a suitable solvent, namely ethanol to obtain citronella extract which will be tested using FTIR spectroscopy. In the FTIR analysis, the lemongrass extract sample is placed in a small cup or transparent container according to the FTIR tool so that the infrared light will hit the sample and record the resulting infrared spectrum (Abd Hamid, 2023). In the infrared spectrum there are a series of peaks and curves that represent the molecular vibrations of the compounds present in the lemongrass extract. The will of phenolic compounds can be revealed through the characteristic peaks seen in the infrared spectrum (William, et al., 2023). Based on the research results, the FTIR spectrum of citronella extract showed a distinctive peak that could be associated with phenolic bonds. Peaks at wave numbers around 3400-3600 cm-1 indicate the presence of OH bonds in the phenolic hydroxyl groups (Muhiddin, 2019). In addition, the presence of peaks at wave numbers around 1600-1700 cm-1 indicates the presence of a C=O bond in the ketone or aldehyde group (Sapitri, 2021).

Interpretation of the FTIR spectrum, the presence of peaks at wave numbers indicating the presence of phenolic bonds indicates that the citronella extract contains phenolic compounds. The phenolic content in lemongrass extract has important implications for the potential antioxidant activity and other biological properties of the extract (Fajriah, et al., 2022). Phenolic compounds are known to have strong antioxidant activity due to their ability to fight free radicals and prevent oxidative damage in the body. Therefore, the phenolic content contained in sereh extract can provide potential health benefits such as protecting the body from oxidative stress and several other diseases (Marpaung, et al., 2020).

The structural composition of the compounds found in the spectrum results of the lemongrass extract also needs to be identified. Based on the results of the FTIR chromatogram analysis in Figure 2, the component N,N'-Hexamethylenebis (methacrylamide) is obtained which is a synthetic amine compound mixed with water. It is proven that there is an absorption band of 3289.26cm with a transmittance percent of 50,209 which means that the intensity is in the –OH group. Whereas in the absorption band of 1376.45cm with a transmittance percent of 86,402 which means that the weak intensity is in the –CH3- or C-H alkyl groups. This is in accordance with the opinion of Nurhasanah (2012) that the higher the absorption band on the FTIR chromatogram will affect the strength and weakness of the energy intensity.

## CONCLUSION

The lemongrass sample was first extracted using ethanol as the solvent and the maceration method to preserve the compound content in the sample. Phytochemical screening yielded positive results for alkaloids, flavonoids, saponins, phenolics, and triterpenoids, while steroids were not detected. The calibration curve results showed an absorbance of 0.52 at a concentration of 10 µl/ml, 0.863 at 20 µl/ml concentration, 1.531 at 30 µl/ml concentration, 2.007 at 40 µl/ml concentration, and 2.392 at 50 µl/ml concentration. The linear regression curve demonstrated a direct relationship between concentration and absorbance, with the

regression equation y=0.049x-2.561 and a coefficient of determination R2=0.990, indicating that 99.9% of the absorbance is influenced by concentration. The total phenolic content, calculated using the TPC formula, was 6.739 ppm. FTIR analysis revealed the presence of N-N - Hexamethylenebis (methacrylamide) component. The absorption band at 3229.26 cm with a transmittance percentage of 50.209 (medium) corresponds to the -OH group, while the absorption band at 1376.45 cm with a transmittance percentage of 86.402 (weak) corresponds to the -CH3- or C-H alkyl functional group.

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