# Comparison of Pandan Leaf Extract (*Pandanus Amaryllifolius*) Using Ethanol and N-Hexane to The Content of Bioactive Compounds

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**Abstract :** The content of bioactive compounds in pandan leaves is used as traditional medicine. One of the utilization efforts is done by maceration extraction. This extraction involves the presence of a solvent. The choice of solvent is a success factor in the maceration extraction process. This study aims to determine the effect of different types of solvents on the content of bioactive compounds and phenolic content of pandan leaf extract. The research method was carried out through maceration extraction using ethanol and n-hexane solvents. Determination of compound content using qualitative phytochemical tests and quantitative tests of phenolic content using UV-Vis spectrophotometry and identification of compounds using FTIR instruments. The results showed that pandan leaf extract positively contained alkaloids, saponins, steroids, and phenolics. The total phenolic content was 12.207 mg/g GAE in ethanol and 10 mg/g GAE in n-hexane. Also, the FTIR test of ethanol solvent shows the O-H bond spectrum in the 3300 region, C=C in the 1650 region, and C-O in the 1100 region. Meanwhile, in n-hexane solvent a C=O bond spectrum is formed in the 1700 region. Both solvents form a C-H bond in the 2900 region. The spectrum formed indicates the presence of phenolic compounds in pandan leaves.

## INTRODUCTION

Pandan leaves (*Pandanus amaryllifolius*) are plants that can survive in tropical climates so that their presence is almost evenly distributed throughout Southeast Asia, especially Indonesia. This plant is very easy to cultivate and develop and can even grow wild in damp places (Ariana, 2017). Pandan leaves have abundant benefits as traditional medicinal ingredients such as reducing fever, antibacterial, anticancer, antioxidant, and antihyperglycemic (Lomthong *et al*., 2022). In addition, pandan leaves also function as food coloring and natural food aromas that can be used in the long term. This is all inseparable from the presence of bioactive compounds contained in pandan leaves such as phenolic compounds, alkaloids, flavonoids, saponins, and tannins (Hardianti *et al*., 2022). Based on research conducted by Ariana (2017), states that the content of the bioactive compounds above is actually contained in pandan leaves.

Utilization of the content of bioactive compounds from pandan leaves can be done through extraction. Extraction is a mechanism by separating the content of bioactive compounds present in plants using the appropriate solvent (Purwandari *et al*., 2018). One of the extraction methods used is maceration. The maceration method is carried out by immersing plant powders in solvents based on their polarity without heating (Chairunnisa *et al*., 2019). The process of soaking this plant powder requires ideal time so that the bioactive compounds can be extracted

properly. According to research conducted by Kurniawati & Maftuch (2016), explains that the ideal time to soak plant powders through maceration extraction is 24 hours, 48 hours and 72 hours. However, this shows a difference in the study of Dwipayana *et al* (2019) which stated that the best rendement time for maceration extraction of fragrant pandan leaves is 36 hours because it is suspected that the material has been extracted out which causes the soaking to not increase again at 48 hours.

Selection of the appropriate solvent in the maceration method is one of the biggest factors that can determine the level of success in the extraction process. The choice of this solvent is based on the principle of like dissolves like, where polar compounds will dissolve in polar solvents, such as ethanol, methanol, butanol and water. Non-polar compounds will also only dissolve in non-polar solvents, such as ether, chloroform and N-Hexane (Dewatisari, 2020). In this study the solvents used were ethanol and n-hexane. Ethanol is a solvent commonly used in the maceration extraction process because it is polar, has a low boiling point, and is non-toxic. Ethanol solvent has a great influence based on its concentration on the degree of polarity. This can be proven from the research of Riwanti *et al* (2019) that the highest levels of flavonoids occur in 70% ethanol extract. This statement is because 70% ethanol is more polar than 96% ethanol so that the flavonoid compounds tend to dissolve more in 70% ethanol. The results of the study by Suhendra *et al* (2019) also showed that 70% ethanol solvent was able to produce the highest total phenolics in the extraction of *Padina minor* seaweed and *Sargassum polycytum.* This highest increase was due to ethanol having hydroxyl groups which bonded to hydrogen groups causing an increase in phenolic compounds in ethanol. N-hexane is a solvent that is stable, volatile, and has high selectivity (Kumar *et al*., 2017). Based on research conducted by Leksono *et al* (2018) stated that the N-Hexane solvent is used in the extraction process for nonpolar compounds because it produces extracts that are classified as strong in the category of antioxidant activity.

The content of pandan leaf bioactive compounds can be detected through phytochemical tests and phenolic tests. Phytochemical test is an effective qualitative test method carried out to determine the presence of bioactive compounds in pandan leaves that are spread in plant tissues (Fithriani *et al.,* 2015). Phenolic test was carried out to determine the phenolic content contained in pandan leaves. This test is classified as a quantitative test using two instruments, FTIR and UV visible spectrophotometry. FTIR spectrophotometry is a method that uses infrared spectroscopy. This spectroscopy is based on the characteristics of the molecular structure where the ability to absorb light of a compound will differ depending on the physicochemical properties, bonds between atoms in the compound and the characteristics of its functional groups (Siregar *et al*., 2015). UV Visible spectrophotometry was performed to identify the levels of phenolic content from maceration extraction of pandan leaves. UV Visible spectrophotometry is an analytical procedure based on the Lambert-Beer law by measuring the absorbance value (Winahyu & Aprillia, 2019).

The choice of solvent type in the extraction process greatly affects the yield of the extract as well as the bioactive components contained in the sample extract. The use of polar or non-polar solvents must be in accordance with the characteristics of the sample, especially pandan leaf extract samples in order to obtain maximum results. Therefore, this research is important to do with the aim of knowing the effect of different types of solvents on the content of bioactive compounds and phenolic content of pandan leaf extract.

## METHOD

### Time and Location of Research

This research is a descriptive study conducted at the Laboratory of Basic Chemistry,

Basic Biology, and Instrumentation Campus II, Sunan Ampel State Islamic University, Surabaya. The research was conducted in June - July 2023.

**Tools and Materials**

The tools used in this study were beaker glass, stirrer, blender, funnel, erlenmeyer, filter paper, rotary evaporator, UV-Vis spectrophotometer, FTIR, 100 mL and 10 mL volumetric flasks, vortex, cuvettes, test tubes, and analytical scales. The materials used in this study were pandan leaves (Pandanus amaryllifolius), ethanol, n-hexane, gallic acid, folin-ciocialteu, Na2CO3, 96% ethanol, 10% aluminum chloride, 1M potassium acetate, distilled water, HCL, dragendorf reagent, 5% FeCl3, magnesium, chloroform, acetic acid, and concentrated H2SO4.

**Research Procedure**

**Sample Preparation**

Samples of fresh pandan leaves with a wet weight of 500 grams were washed thoroughly with water and then cut into small pieces. The plants were dried in the sun for ± 2 days. After drying, the leaves were ground into powder in the hope that the metabolites present in the samples were not damaged and could be extracted as much as possible. Then the sample that has become powder is sieved using a 40mesh sieve to obtain a fine powder sample with a dry weight of 100 grams (Ayuchecaria *et al*., 2020).

**Maceration Extraction**

Pandan leaf dry powder was weighed 100 grams 2 times and put each into a beaker. Then the pandan leaf dry powder was soaked in 700 ml of ethanol and n-hexane at 25ºC-28ºC for 24 hours to obtain filtrate and residue. The filtrate obtained is then evaporated using a rotary evaporator at a predetermined temperature and pressure. The purpose of this step is to remove the solvent so that a thick extract of pandan leaves is obtained (Wijaya, 2014).

**Phytochemical Qualitative Test**

**Alkaloid Test**

A total of 0.5 grams of sample was put in a test tube and 0.5 mL of 2 M HCL was added, after which 1-2 drops of dragendorf were added. A positive test is indicated by the formation of an orange color which indicates a positive alkaloid (Andasari *et al*., 2020).

**Flavonoid Test**

A total of 200 mg of sample was put into a test tube. Then 5 mL of ethanol was added and heated for 5 minutes. After that, a few drops of concentrated HCl were added. Added magnesium as much as 0.2 grams. A positive test is indicated by the formation of a dark red (magenta) color which indicates positive for flavonoids (Andasari *et al*., 2020).

**Saponin Test**

A total of 0.5 grams of sample was put into a test tube. Then added hot water and cooled. After chilling, shake for 10 minutes. A positive test is indicated by the formation of foam and adding 2 M HCl, the foam is still there, indicating positive saponins (Suleman *et al*., 2022).

**Phenolic Test**

A total of 0.5 grams of sample was put into the erlenmeyer and 10 mL of ethanol was added. Take 1 mL of the solution formed and put it in a test tube. After that, 2 drops of 5% FeCl3 solution were added. A positive test is indicated by the formation of green or bluish green color which indicates positive phenol (Maharani *et al*., 2014).

**Test Steroids and Triterpenoids**

A total of 0.5 gram of sample was 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 $H_2SO_4$ was added through the test tube wall. A positive test is indicated by the formation of a purple-red color which indicates a positive triterpenoid. While positive steroids are indicated by the formation of green or blue color (Wijaya, 2014).

**Quantitative Test Using UV-Vis Spectrophotometric Instruments**

**Preparation of Gallic Acid Standard Mother Solution**

A 100 ppm gallic acid mother liquor is prepared by weighing 10 mg of gallic acid powder and then placing it in a volumetric flask. Then add ethanol up to 100 mL in a volumetric flask, after which it is homogenized by shaking (Ayuchecaria *et al.*, 2020).

**Preparation of 7% $Na_2CO_3$ Solution**

$Na_2CO_3$ powder was weighed as much as 7 grams and then dissolved with distilled water up to 100 ml (Fawwaz *et al*., 2017).

**Maximum Wavelength Determination**

The maximum wavelength of gallic acid is by measuring a 10ppm concentration of gallic acid solution in the wavelength range of 400-800 nm using a UV-Vis spectrophotometer. The maximum wavelength is the highest absorbance of each measurement (Gustriani *et al*., 2016).

**Gallic Acid Standard Solution Curve**

The standard curve of gallic acid standard solution was made with various concentrations of 10, 20, 30, 40, 50 ppm. Each standard solution of gallic acid with a certain concentration is taken 1 mL and put into a test tube. Then, 0.5 mL of Folin-ciocalteu was added to the solution and allowed to stand for 8 minutes while shaking. After that, 4 mL of 7% Na2CO3 solution was added to the solution and stirred for 1 minute. The filtrate was taken and tested with a UV-Vis spectrophotometer. Measurements are made at the maximum wavelength. Next, make a calibration curve between the concentration of gallic acid (x-axis) and its absorbance (y-axis). The regression equation is used to find sample concentration (Fawwaz *et al*., 2017).

**Sample Absorption Measurement**

Measurement of sample absorption by means of 10 mg of extract dissolved in 96% ethanol to a solution volume of 10 mL and homogenized. Take 1 mL of the extract solution and add 0.5 mL of folin-ciocalteu, leave for 4 minutes while shaking. 4 mL of 7% $Na_2CO_3$ solution was added and vortexed for 1 minute. Then, centrifugation was carried out and continued with the UV-Vis spectrophotometry test. The absorbance is calculated by the maximum wavelength. The absorbance of the sample is entered into the gallic acid linear regression equation as sb y to obtain the sample concentration (sb x). The total phenol content can be calculated using the following formula (Toripah, 2014):

$$TPC = \frac{c \,.\, v \,.\, fp}{g} \qquad (1)$$

Information:

TPC : Total phenolic content (mg/g GAE)
c : Concentration (x value) (ppm)
v : Extract volume (mL)
fp : Dilution factor
g : Sample weight (gram)

**Quantitative Test Using FTIR Instruments**

Identification of active compounds in pandan leaf extract was carried out using FTIR instruments. The FTIR tester and the software interface computer are turned on for analysis. Then, 96% ethanol was placed first on the sample holder as a background. The extract sample is placed into the sample holder, then the FTIR tool is operated so that the FTIR spectrum is produced from the sample. At this stage, the FTIR tool will send infrared light to the sample and record the resulting spectrum response. This process generates the FTIR spectrum of the sample being analyzed. Finally, read the results of the FTIR spectra (Purwakusumah *et al*., 2014).

## RESULT AND DISCUSSION

Pandan leaf extract was obtained through the maceration extraction method by soaking pandan leaf powder in ethanol and n-hexane solvents. Pandan leaves need to be converted into powder form first so that the results obtained

from the extraction process are more optimal. Soaking was carried out for 24 hours at room temperature to prevent damage to the compounds in the extract. During the immersion process, the sample solution is stirred using a stir bar so that the contact between the solvent and the sample is even. The success of the extraction process is determined by the type and quality of the solvent that matches the characteristics of the compounds in the sample. The solvent used must meet several properties, including being able to dissolve a substance properly, having a low boiling point, not being toxic, and not flammable (Tetti, 2014). In this study we used ethanol and n-hexane solvents.

The difference in polarity in the solvent can affect the total content of bioactive compounds in the extract (Megha *et al*., 2014). Polar solvents will also dissolve polar compounds, according to the principle of "like dissolves like" (Kayadoe *et al*., 2015). Ethanol is commonly used because it is an extraction solvent that can extract a greater number of phytochemical compounds and for almost all low molecular weight compounds (Ningsih *et al*., 2020). N-hexane is also one of the solvents that is often used because it has various advantages, including stability, selectiveness, and volatility so that it is a suitable solvent for extracting non-polar compounds (Constanty & Tukiran, 2021). The filtrate that has been produced is filtered, then evaporated using a rotary evaporator with the aim of evaporating the solvent, so that the extract is not easily damaged at high temperatures (Damayanti & Fitriana, 2012).

**Phytochemical Qualitative Test**

Phytochemical test is a test to identify important compounds contained in a plant. The simple identification of chemical compounds with the aim of knowing and providing an overview of the presence of secondary metabolites in plant extracts is known as the process of phytochemical screening. (Alviani *et al*., 2022). Based on the phytochemical tests that have been carried out on samples of ethanol extract and n-hexane of pandan leaves (*Pandanus amaryllifolius*) the following results are obtained.

Table 1: Qualitative Phytochemical Test Results of Pandan Leaf Extract

| No | Phytochemical Test | Sample | Result |
|---|---|---|---|
| 1 | Alkaloids | Pandan Leaf Ethanol Extract | + |
| | | Pandan Leaf N-Hexane Extract | + |
| 2 | Flavonoids | Pandan Leaf Ethanol Extract | - |
| | | Pandan Leaf N-Hexane Extract | - |
| 3 | Saponins | Pandan Leaf Ethanol Extract | + |
| | | Pandan Leaf N-Hexane Extract | - |
| 4 | Phenolic | Pandan Leaf Ethanol Extract | + |
| | | Pandan Leaf N-Hexane Extract | + |
| 5 | Steroids | Pandan Leaf Ethanol Extract | + |
| | | Pandan Leaf N-Hexane Extract | + |
| 6 | Triterpenoids | Pandan Leaf Ethanol Extract | - |
| | | Pandan Leaf N-Hexane Extract | - |

Information:
+ : contain bioactive compounds
- : Doesn't contain bioactive compounds

The results of the phytochemical tests of the ethanol extract and n-hexane extract of pandan leaves both positively contained alkaloid compounds as evidenced by the presence of an

orange precipitate (Assauqi *et al*., 2023). Alkaloids are one of the important secondary metabolites and have potential antibacterial activity (Bufo & Karaman, 2019; Othman *et al*., 2019). One of the characteristics possessed by compounds belonging to the alkaloid group is the presence of N atoms in their structure. The orange precipitate is a potassium alkaloid, in which the N atom will form a coordinate covalent bond with potassium tetraiodobismutat from Dragendorff's reagent (Ergina *et al*., 2014).

Apart from alkaloids, there is another test, namely flavonoids. The flavonoid test on pandan leaf extract gave negative results because no orange precipitate was formed in the pandan leaf extract sample when mixed with Mg and HCl. This reinforces the statement of Wahyuni *et al* (2018), that the principle of the flavonoid test gives a positive result when an orange precipitate forms. Flavonoids are organic compounds with a structure in the form of two aromatic rings with more than one hydroxyl group. The more hydroxyl groups present in flavonoids, the polarity of the compound increases, so it will easily dissolve in polar solvents, including ethanol (Panche *et al*., 2016). Flavonoids have antibacterial activity due to their ability to integrate with bacterial cell membranes and extracellular proteins (Aini & Mardyaningsih, 2016). The addition of magnesium powder and HCl has the aim of reducing benzopyran in the flavonoid structure to an orange flavylium salt (Yuniati *et al.*, 2020).

The saponin test on pandan leaf extract samples between ethanol and n-hexane solvents showed different results. The ethanol solvent showed positive results as evidenced by the presence of foam after shaking. This is in accordance with the opinion of Wahyuni (2018), that positive results from the saponin test are shown by the presence of foam which does not disappear after adding HCl and shaking for 30 seconds. Meanwhile, the n-hexane solvent showed negative saponin results because the foam disappeared after being shaken. The difference in results is due to the reaction between the polarity of the solvent and HCl. A positive result was obtained because HCl is soluble in ethanol which is equally polar. N-hexane cannot dissolve HCl because it is non-polar so the results show negative saponins. The content of saponins in pandan leaves makes this plant have antibacterial properties. This is based on its cytotoxic properties and its ability to affect the permeability of the microbial cytoplasmic membrane, causing lysis of microbial cells (Aini & Mardyaningsih, 2016).

The steroid and triterpenoid tests showed different results between the two solvents. Triterpenoid compounds were not identified in ethanol and n-hexane solvents. Meanwhile, steroids were identified in the two solvents (Suryani *et al*., 2017). Steroids are complex molecules of the lipid group derived from saturated compounds that have a core with 4 rings (Mursyida *et al*, 2021). Steroids can dissolve in n-hexane because it is an alkane hydrocarbon with the chemical formula $C_6H_1$ resulting from refining crude oil. All hexane isomers are often used as organic solvents which are inert because of their non-polarity (Utomo, 2016). Triterpenoids did not show positive results during the test because there was no color change to red or purple. This is not in accordance with the results of the literature from Kiyato *et al* (2022), which states that a triterpenoid is positive if it turns red or purple. The results obtained were negative because of the reaction between the reagents namely acetic acid and chloroform with each solvent. In accordance with the principle of like dissolves like, polar acetic acid cannot dissolve in non-polar n-hexane, so the result is negative (Wiradnyani *et al*., 2014). Meanwhile, chloroform is nonpolar and cannot react with ethanol which is polar.

Tests on pandan leaf extract using n-hexane and ethanol solvents were found to be positive for phenolic compounds. Phenolic compounds were indicated by the occurrence of a black color change in the test sample but not too concentrated after being added with 1% $FeCl_3$. This is in accordance with the literature which states that phenolic compounds are tested positive if there is a bluish-black to dark black color change when 1% $FeCl_3$ is added (Habibi *et al*., 2018) and $FeCl_3$ can react with aromatic –OH

groups (Haryati *et al*., 2015). Compounds that are polar are phenolic compounds (phenolics, flavonoids, and saponins), while alkaloids and steroids are compounds that are non-polar (Romadanu *et al*., 2014).

**Total Phenolic Content Test**

Pandan leaves that have been extracted using the maceration method are then subjected to a phenolic test using a UV-Vis spectrophotometry instrument to determine the levels of phenolic compounds present in the sample. Phenol compounds are secondary metabolites derived from pentose phosphate, shikimate, and phenylpropanoids found in plants. Phenolic compounds have only one hydroxyl group. Phenolic compounds have benefits as antioxidants that can counteract free radicals. (Badriyah *et al*., 2017). UV-Vis spectrophotometry is an instrument to test the levels of secondary metabolites based on light absorption. This instrument uses ultraviolet and visible light to produce curves. Phenolic compounds in pandan leaves were tested using UV-Vis spectrophotometry because they are colored and have strong absorption in the UV region (Satria *et al*., 2022). Testing for total phenolic levels was carried out using the Folin-Ciocalteu method. The principle of the Folin-Ciocalteu method is that phenolic compounds can be oxidized by the Folin-Ciocalteu reagent so that the test solution is blue which can be measured with a visible spectrophotometer at certain wavelengths (Monica, 2017).

Testing for phenolic content was carried out using the Folin-Ciocalteu reagent, 7% $Na_2CO_3$ solution, and gallic acid as a standard solution (Wibisono *et al*., 2020). Gallic acid standard solution is a stable and simple phenolic compound derived from hydroxybenzoic acid (Indra *et al*., 2019). Based on the results of the UV-Vis spectrophotometer, the maximum wavelength was 753 nm for ethanol solvent and 749 nm for n-hexane solvent.

Standard solutions of various concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm were prepared by dilution. The dilution will go through a centrifugation process. The purpose of centrifugation is to separate substances with different molecular weights, then the liquid or supernatant is transferred to a new test tube to be tested using a UV-Vis spectrophotometer. Measurements were made using the maximum wavelength of each solvent. The results of measuring the absorbance value of gallic acid standard solution at various concentration variations obtained a linear regression curve between the concentration of gallic acid standard solution and its absorbance to obtain the regression equation for ethanol solvent, is

$$y = 0{,}046^{*}x + 0{,}097 \qquad (2)$$

And the regression equation for the n-hexane solvent, is

$$y = 0{,}037^{*}x - 0{,}012 \qquad (3)$$

The regression equation can be used to determine the concentration of the sample, the y value is the absorbance and the x value is the gallic acid concentration (ppm) which can be seen in Figures 1 and 2. The correlation coefficient ($r^2$) in ethanol solvent is 0.975 and in n-hexane solvent is 0.999. The value of the correlation coefficient is a number to determine the strength and weakness of the correlation index between the variables studied. The results for the $r^2$ values for the two solvents show a value close to 1, meaning that the correlation between the concentration variable (x) and absorbance (y) is very strong so that a linear curve is formed (Primadiamanti *et al*., 2020).

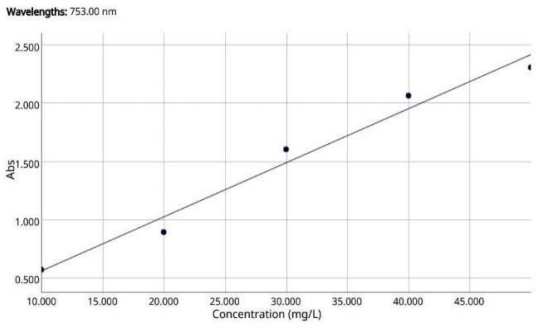


Figure 1: Graph of the Measurement Result Curve of the Absorbance Value of a Standard Solution Gallic Acid of Ethanol Extract.

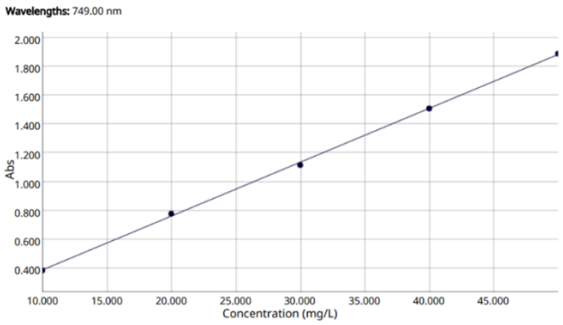


Figure 2: Graph of the Measurement Result Curve of the Absorbance Value of a Standard Solution Gallic Acid of N-Hexane Extract.

The Folin-Ciocalteu method uses colorimetric oxidation and reduction reactions on samples suspected of containing phenolic compounds. The Folin-Ciocalteu reagent is a solution of a polymeric ion complex derived from phosphomolybdic acid and heteropolyphosphotungstic acid. The Folin-Ciocalteu reagent will oxidize phenolics and reduce heteropoly acids to produce a blue molybdenum-tungsten (Mo-W) complex (Asmara, 2017). The blue sample shows that it contains phenolic compounds which react with the Folin-Ciocalteu reagent to form complex compounds. The intensity of the blue color density in the sample depends on the amount of phenolic content. The amount of phenolic content in the sample can be influenced by the type of solvent used in the extraction process (Indra *et al.*, 2019). The reaction between phenolic compounds and the Folin-Ciocalteu reagent proceeds slowly under acidic conditions, so it is necessary to add an alkaline solution in the form of sodium carbonate or $Na_2CO_3$ so that the reaction goes faster (Karim, 2017). The reaction under alkaline conditions aims to dissociate the protons contained in phenolic compounds into phenolic ions. The Folin-Ciocalteu reagent is used because it can react with phenolic compounds and produce blue samples whose absorbance can be measured using UV-Vis spectrophotometry (Primadiamanti *et al.*, 2020).

The sample solution is then centrifuged and then the liquid or supernatant is transferred to be tested using a UV-Vis spectrophotometer with maximum wavelength and 2 repetitions are carried out. The absorbance value of the sample is used as a quantitative analysis based on Lambert-Beer's Law. The absorbance value of the pandan leaf extract sample in the eatnol solvent was 0.664 while that of the n-hexane solvent was 0.256, both assorbancy values included good absorbance. A good absorbance value is between 0.2-0.8 in the absorption area of ultraviolet or visible light. A high sample absorbance value correlates with a high concentration of phenolic contained in the sample. UV-Vis spectrophotometry will produce a curve derived from the interaction between UV radiation and the molecules contained in the test sample. Electromagnetic radiation will be absorbed when it hits certain molecules or atoms with the presence or absence of a chromophore group structure (Satria *et al*., 2022). Phenolic compounds have a bond structure in the benzene core so that when exposed to ultraviolet light a resonance occurs with electron transfer (Lisnawati & Nurlitasari, 2019).

The absorbance of the sample measurements was then analyzed by linear regression to determine the concentration of the sample by inserting the absorbance value of the sample into the regression equation so that the value of total phenolic content was obtained. The total phenolic content in pandan leaf extract samples is described as mg of Gallic Acid Equivalent (GAE) per gram of dry extract (Wibisono *et al.*, 2020). The total phenolic content was calculated using the TPC (Total Phenolic Content) formula.

Table 2: Results of Measuring Total Phenolic Levels of Padan Leaf Extract

| Solvent | Absorbance Value | Total Phenolic Content (mg g/GAE) |
|---|---|---|
| Ethanol | 0,664 | 12,207 |
| N-Hexane | 0,256 | 6,521 |

Based on the results of the analysis, the total phenolic content in the ethanol solvent pandan leaf extract was 12.207 mg GAE/g,

meaning that in every gram of pandan extract the ethanol solvent was equivalent to 12.207 mg of gallic acid. While the total phenolic content of n-hexane solvent pandan leaf extract was 6.521 mg g/GAE, meaning that in every gram of n-hexane solvent pandan extract is equivalent to 6.521 mg of gallic acid. These results indicate that the content of phenolic compounds in pandan leaf ethanol extract is greater than pandan leaf extract n-hexane. Research by Mangkolsilp *et al.*, (2004) regarding five Thai medicinal plants stated that the higher the total phenolic content in the sample, the higher the free radical scavenging activity.

**Identification of Compound Structures Through FTIR Instruments**

The working principle of FTIR is to measure changes that occur in the total reflection of infrared light when there is contact between the sample and infrared light (Septiani & Roswien, 2018). Quantitative analysis with FTIR spectroscopy is generally used to identify functional groups present in a compound being analyzed (Sari *et al*., 2018). FTIR has several advantages and disadvantages. The frequency measurement is faster than using other methods through the scanning process and has high sensitivity and accuracy compared to the manual dispersion method, due to the large amount of radiation emitted without having to enter the gap first. Despite these advantages, FTIR has several drawbacks. During the process of generating data, the two IR ports must be facing each other. This causes the process to be long because it takes time to send data. In addition, IR radiation is very dangerous for the eyes because it can cause irritation and even blindness (Lubis, 2015). The FTIR test results of the ethanol and n-hexane extracts can be seen in the following graph:

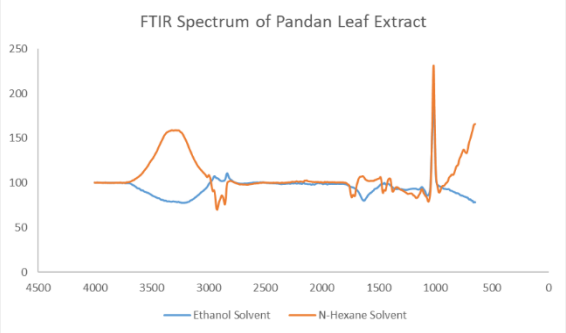


Figure 3: FTIR Spectrum Results of Pandan Leaf Extract with Ethanol & N-Hexane Solvents.

The FTIR results of pandan leaf n-hexane extract identified O-H compound groups found at the peak at wave 3300, the C-H compound group was found at wavenumber 2900 $cm^{-1}$, the C=C compound group was found at the peak wavenumber 1650 $cm^{-1}$, and the C-O compounds were found at the peak at wavenumber 1100 $cm^{-1}$. This is in accordance with research conducted by Wibisono *et al* (2020), which stated that at wave numbers 3000-3500 $cm^{-1}$ there are O-H groups, at wavelengths 2800-3000 $cm^{-1}$ there are C-H groups, at wave numbers 1600-1700 $cm^{-1}$ there is a C=C compound group, and at wave numbers 1100-1200 $cm^{-1}$ there is a C-O compound group. According to Asmara (2017), the bond between the C-C groups is a bond belonging to the vinyl group, the double bonded C atom most likely belongs to the aromatic group. The unsaturated characteristic of C=C aromatic is 1.5 times that of aliphatic C=C due to the influence of the conjugation of three double bonds in six cyclic carbon atoms. This results in the strength of the double bond being weaker than the double in aliphatic so that the strain frequency is also lower. The presence of phenolic compounds is further emphasized by the spectra in the fingerprint region, where there are groups of C-O compounds. The typical characteristic of the presence of C–O bonds in phenolic compounds is indicated by a peak with a strong intensity at wavenumber 1100 $cm^{-1}$ which indicates bond strain between C–O.

In the results of the FTIR spectrum with ethanol solvent, it can be seen that pandan leaves contain alkanes and aldehydes. The alkane

functional group identified at ± 2900 wavelength and at ± 1700 wave vibration is the aldehyde functional group. Aldehydes are carbon compounds that have a carbonyl group (C=O) where the carbonyl group is at the end of the main carbon chain and ends with a hydrogen atom. Aldehydes are polar compounds that can boil at high temperatures. Aldehydes are compounds in which the carboxyl functional group is bound by an alkyl group, composed of the elements carbon, hydrogen and oxygen obtained from the oxidation of primary alcohols, chlorides and also glycol acids (Toar *et al*., 2021). Compounds containing aldehyde groups are easier to oxidize with weak oxidizing agents. Oxidation of these aldehydes can produce carboxylic acids. Aldehydes are usually used for antiseptics, corpse preservatives, raw materials for the melamine plastic industry and also bakelite (Martin, 2012). According to a statement from Adiyasa, (2014) compounds in pandan leaves belonging to the alkanes, alkenes, and aldehydes are volatile. In addition, pandan leaves contain flavonoids, alkaloids, saponins, tannins, and polyphenols (Dewi *et al*., 2019). These compounds belong to the phenolic group. The existence of this phenolic content is in accordance with research by Agustianingsih *et al*., (2010) which has been qualitatively proven, that phenolic compounds found in plants have an aromatic ring containing one hydroxyl group as a special feature.

## CONCLUSION

Based on the research that has been done, it can be concluded that:

1. The different types of ethanol and n-hexane solvents have an influence on the phytochemical screening (qualitative test) through the saponin test. While the alkaloid, flavonoid, phenolic, steroid, and triterpenoid tests had no effect.
2. Total phenolic content of pandan leaf extract in ethanol solvent of 12.207 mg GAE/g and n-hexane of 6.521 mg g/GAE gave a significantly greater effect on ethanol extract than n-hexane extract.

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