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Phytochemical analysis of secondary metabolite compounds of Pandanwangi leaf extract (*Pandanus amaryllifolius*)

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ABSTRACT
The majority of people use pandan as a dye, food fragrance, and natural medicine because it contains secondary metabolites such as alkaloids, saponins, and phenolics. Therefore, this research aims to explore the secondary metabolites of *Pandanus amaryllifolius* using qualitative and quantitative methods. The research begins by taking samples of pandan leaves, which are then extracted and tested qualitatively and quantitatively. The qualitative test results showed that pandan leaf extract contained flavonoids, polyphenols, and tannins. In the quantitative test of pandan leaf phenolic compounds using a UV-Vis spectrometer with gallic acid concentrations of 80 ppm, 100 ppm, 120 ppm, and 140 ppm, Based on the quantitative test, the total phenolic content of pandan extract is 114 mg/L.

Keywords:
Phenolic, Pandanus, Secondary Metabolites

Introduction

Pandan (*Pandanus amaryllifolius*) is a type of monocot plant from the Pandanaceae family, which is found in many tropical areas. In Indonesia, this plant is known as pandanwangi leaves (Nguyen et al., 2021). Pandan is an upright shrub with a height of 3-7 m, branched, thorny in several types, and has a taproot around the base of the stem. Pandan Wangi has parallel leaves with a length of 1-3 m and a width of 8-12 cm; the tip of the leaf is sharp, there are thorns on the edges of the leaves and the mother of the leaf bones, the leaves have a waxy texture (Antonius et al., 2021).

Pandanwangi is usually used as a natural dye and aroma enhancer for food or drinks. It is used as a natural fragnant because this plant has a distinctive fragrant aroma that can provide a calming effect (Mataliana et al., 2015). Pandan can be used as a green dye because it contains chlorophyll in pandan leaves. Meanwhile, the distinctive aroma of pandan is produced from the volatile compound 2-acetyl-1-pyrroline. Pandan is also widely used for medicines because it contains secondary metabolites including alkaloids, saponins, and phenolic compounds such as flavonoids and tannins (Bhuyan & Sonowal, 2021).

The use of fragrant pandan as a traditional medicine includes preventing hair loss, blackening hair, eliminating dandruff, healing weak nerves (neurasthenia), increasing appetite, rheumatic pain, and pain accompanied by anxiety (Marina & Astuti, 2012). In addition, pandanwangi leaves also have pharmacological activity, namely antidiabetic activity in aqueous extracts, antioxidants in aqueous and methanol extracts, anticancer in ethanol and methanol extracts, and antibacterial in ethanol and ethyl acetate extracts (Mardiyaningsih & Aini, 2014).

Pandan Wangi leaves contain several secondary metabolites, such as alkaloids, steroids, triterpenoids, flavonoids, saponins, and phenol hydroquinones. Phenolic compounds are a source of natural antioxidants that have hydroxyl groups as an antidote against free radicals. Antioxidant activity occurs when phenolic compounds interact with oxidants to stop the chain of free radical reactions. Antioxidants can be obtained naturally in plants such as pandan (Thatsanasuwan & Srichamnong, 2015).

Phytochemicals are components that are owned by plants that are not in the nutritional substance class but have very important health functions. Phytochemical testing is carried out to determine the characteristics of active compounds that have toxic or beneficial effects (Antonius et al., 2021). Based on that, the purpose of this study was to determine the presence or absence of secondary metabolites and the total phenolic content in Pandan Wangi leaf extract. The tests carried out were qualitative tests by means of phytochemical screening and quantitative tests of phenolic compounds using a spectrophotometer.

Methods

This research was conducted in November 2022 in Chemical Laboratory UIN Sunan Ampel Surabaya. This research used filter paper, stirrer, spatula, dropper pipette, measuring cup, erlenmeyer, petri dish, t est tube, test tube rack, hot plate, rotary evaporator, UV-Vis spectrophotometer, micropipette, yellow tip, blue tip, vials 10 mL, and there are materials that be used: *P. amaryllifolius* leaf extract, Ethyl acetate, n-hexane, methanol, 70% ethanol, ethanol, magnesium powder, concentrated HCl, 2N HCl, 10% NaCl, 1% FeCl3, gallic acid, methanol, 1% folic ciocalteu reagent, 1M sodium carbonate, distilled water 2L.

Preparation sample

Pandan leaf (*Pandanus amryllifolius*) samples were collected and then washed clean on flowing water. Pandan leaf was chopped into small pieces with ± 1 cm; in the next step, pandan leaf samples were dried in an oven at 65°C for 8 hours. Mash the dried pandan leaf samples using a blender.

Extraction

The extraction process used the maceration method; the pandan leaf sample was added to 96% and then left for three days while stirring each day. After the third day, the samples were filtered, and the filtration of the samples was carried out using a rotary evaporator to separate the ethanol and pandan leaf extract and obtain samples in the form of a paste.

Qualitative test

Flavonoids

About 0.10 g of pandan leaf extract into the Erlenmeyer. Add 5 ml of shaken ethanol, then heat the sample; after heating, the sample is shaken again. The heated sample was filtered, and the filtrate was taken. Added 0.20 gram Mg into the filtrate. Plus, three drops of HCl. Observations were made for changes in color, if there was a change in color to reddish, indicating that the sample contained flavonoid compounds.

Polyphenols

About 0.10 grams of pandan leaf extract into the Erlenmeyer. Add 5 ml of distilled water. Heat until boiling for about 5 minutes. Filtered and took the filtrate. Add five drops of 1% $FeCl_3$

to the filtrate. Observations were made for changes in color; if there was a change in color from bluish-green to blackish, it indicated that the sample contained polyphenolic compounds.

Tannins

I took about 0.50 grams of pandan leaf extract and put it into the Erlenmeyer. Plus 5 ml of distilled water. Heat until boiling for about 5 minutes. Filtered and took the filtrate. Add five drops of 1% FeCl3 to the filtrate. Observations were made for changes in color, and if there was a change in color to dark blue or blackish green, it indicated that the sample contained tannin compounds.

Phenolic Quantitative Test

Gallic Acid Standard Solution

About 50 mg of gallic acid powder. Dissolved with 100 ml of methanol to prepare a stock solution with a concentration of 500 ppm. Then, a series of concentrations of gallic acid standard solutions of 80, 100, 120, and 140 ppm were made. Take 0.5 ml of each concentration of gallic acid standard solution. Added 5 ml of 1% Folin Ciocalteu reagent and 4 ml of 1M sodium carbonate. Incubated for 15 minutes and measured the absorbance value using a spectrophotometer with maximum wavelength.

Determination of Total Phenol Content

Weigh as much as 30 mg of each sample extract transfer it into a test tube. 10 ml of methanol was added to each sample. Take 0.5 ml of the extract solution and then add 5 ml of 1% Folin Ciocalteu reagent and 4 ml of 1M sodium carbonate. Incubation was carried out for 15 minutes. The absorbance value was measured using a spectrophotometer with the maximum wavelength. Total phenol was calculated using the linear regression equation of the gallic acid calibration curve with a wavelength.

Results and Discussions

Extraction

The sample used in this study was pandan leaves from Jalan Ngelom Megare No. 649-A, Taman, Sidoarjo Regency. Pandan leaves are sorted and dried using an oven for 8 hours and then mashed. Pandan Wangi leaf powder was extracted using the maceration method with 96% ethanol solvent. Extraction is a procedure performed to obtain plant metabolites such as alkaloids, phenolics, flavonoids, glycosides, and others using selective solvents. The selective solvent will dissolve materials contained in cells and can cause protoplasm to swell (Prayoga et al., 2019).

The extraction process can be influenced by several factors, including plant parts, harvest time, temperature, concentration, type of solvent, and extraction method. Extraction of fragrant pandan leaf powder was carried out using the maceration method, which is a simple and widely used extraction process. The advantage of using the maceration method is that there is minimal damage to chemical components in plants. The extract material used is mashed first to form a powder, then it is dissolved with a solvent according to the required extract and metabolite (Damanik et al., 2014).

The solvent used in the extraction is 96% ethanol. According to Alasa et al. (Alasa et al., 2017), an ethanol solvent can maximally attract secondary metabolites. In addition, ethanol solvents have lower toxicity potential compared to other solutions. Ethanol is a solvent that is safe for consumption when used as a solvent for natural products for food or medicinal purposes. Ethanol has been widely used to extract phenolic-derived antioxidant compounds from natural products

with good results (Hikmawanti et al., 2021). Extraction of fragrant pandan leaves using 96% ethanol solvent in the form of a thick green-black paste.

Qualitative Test of Pandan Wangi Leaf Extract

The results of the tests that were carried out are shown in table 1. The test results showed that the pandan leaf sample (*Pandanus amryllifolius*) contained flavonoids, polyphenols, and tannins.

Table 1. Secondary Metabolic Compound Test Results

Compound	Existence
Flavonoids	+
Polyphenols	+
tannins	+

Flavonoid Test

Flavonoids are compounds derived from the phenol group consisting of C6-C3-C6 and are often found in various plants in the form of glycosides. They can be found in all types of plants. Flavonoid compounds are a source of purple, red, blue and some yellow dyes in plants. Flavonoid compounds are derived from the main compound flavones(Harborne, 1987).

Flavonoid compounds are characterized by a change in color to reddish. In the tests carried out, the sample of flavonoids that had been mixed with Mg and then added with HCl resulted in a reddish color change. The purpose of adding Mg and HCl metal is to reduce the benzopirone nucleus contained in the flavonoid structure to form red or orange flavilium salts(Ergina et al., 2014).

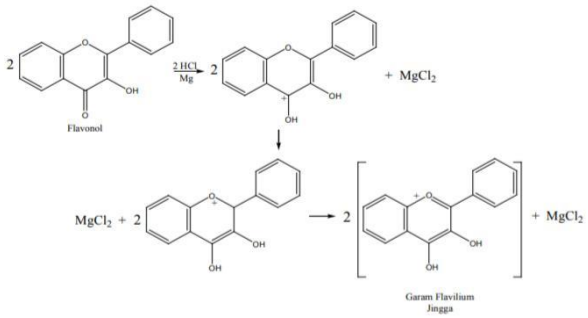

Figure 1. Reaction of flavonoids with Mg and Cl metals (Septyaningsih, 2010)

Polyphenol Test

Tests were also carried out on polyphenolic compounds. In the test conducted, the sample that had been dripped with 1% $FeCl_3$ showed a change in color to bluish-green which indicated the presence of polyphenolic compounds in the sample. The color change that occurs is formed due to the reaction of $FeCl_3$ with the sample, making the formation of color in this test. The role is the Fe^{3+} ion, which undergoes hybridization(Manongko et al., 2020), as shown in the following figure 2.

Tannin Test

Testing of tannin compounds in samples was also carried out. In the test that was carried out, the sample that was dripped with $FeCl_3$ had a reaction that changed color to black-green. A color

proverb reaction occurs due to the formation of complex compounds between tannins and $FeCl_3$. Phytochemical tests using $FeCl_3$ can show the presence of phenol groups; if phenolic compounds are present, then it is likely that they also contain tannins, because tannins are polyphenolic compounds (Syahputra et al., 2021). The color change in the qualitative test of tannins was due to the reaction of tannins with $FeCl_3$, which produced aromatic compounds. The reaction of tannins with $FeCl_3$ forms complex compounds because there are Fe^{3+} ions that bind two donor atoms, namely at the 4' and 5' dihydroxy positions in tannins to produce complex compounds(Ergina et al., 2014).

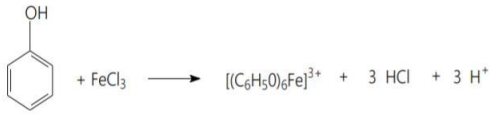

Figure 2. The reaction of polyphenols with $FeCl_3$ (Manongko et al., 2020)

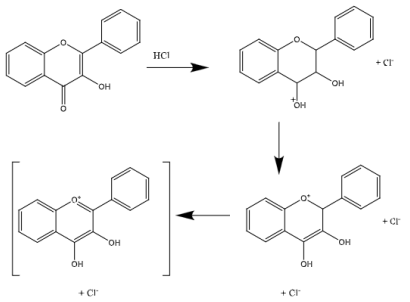

Figure 3. Reaction of Tannins and FeCl3 (Fitriyah et al., 2021)

Quantitative Test of Secondary Metabolites

The gallic acid calibration curve is a UV-Vis spectrophotometer calibration analysis using gallic acid as a standard. The gallic acid concentrations used were 80, 100, 120, and 140 mg/L (Table 2).

Table 2 Gallic Acid Absorbance Results

Concentration (mg/L)	absorbance
80	0.250
100	0.269
120	0.283
140	0.500

Based on the gallic acid calibration curve (Figure 4), we get the regression equation y = 0.004x + 0.096, and the value of the correlation coefficient (R2) is 0.711. The linear regression equation states the mathematical relationship between the concentration of gallic acid and its absorbance in measurements using a UV-Vis spectrophotometer. The value of the correlation coefficient (R) represents the correlation between concentration (x-axis) and absorbance (y-axis). The gallic acid regression equation is used in determining the total phenol content by entering the absorbance obtained in each sample to y, and x will be obtained as mg/L gallic acid content. The gallic acid level (mg/L) is used to calculate the total phenol content, namely the amount of gallic acid content (mg/L) per sample content (g/L)(Pamungkas et al., 2016). Based on the calculation results, the total phenolic content of pandan extract is 114 mg/L.

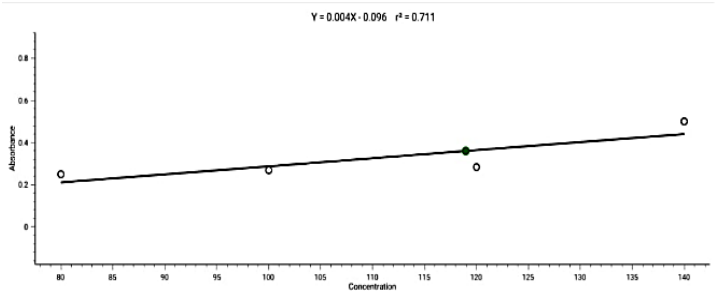

Figure 4. Gallic Acid Calibration Curve

The total phenol content test uses the Folin-Ciocalteu reagent, so it is also called the Folin-Ciocalteu method. Folin-Ciocalteu reagent is a polymeric ion complex solution that is strengthened from phosphomolybdic acid and heteropolyphosphotungstic acid. The basic principle of the Folin-Ciocalteu method is a colorimetric oxidation and reduction reaction to measure all phenolic compounds in the test sample. The phenolic compounds react with the oxidizing agent phosphomolybdate under alkaline conditions to produce phenolic compounds and a blue molybdenum-tungsten complex. Gallic acid is used as a standard solution because it has hydroxyl groups and conjugated double bonds in each benzene ring so that it easily reacts to form complexes with the Folin-Ciocalteu reagent and is a building block for phenolic compounds (Figure 5).

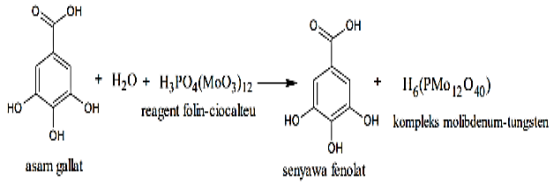

Figure 5. Gallic Acid Reaction With Folin-Ciocalteu Reagent (Adawiah et al., 2015).

Phenol compounds are known to have an important role in antioxidant activity. Phenol will stabilize free radicals by donating hydrogen electrons. The higher the phenol content, the higher the antioxidant activity. The process of biosynthesis of many phenolic compounds occurs in the cytoplasm of the leaves, so the extraction of leaves can produce high levels of phenol (Ristiana, 2017).

Conclusion

*Pandanus amaryllifolius*or fragrant pandan has the potential to be an antioxidant agent because it contains several secondary metabolite compounds. The results of qualitative and quantitative tests showed that Pandan Wangi leaf extract contains phenolic compounds that have potential as antioxidants.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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