

Phytochemical Screening And Antioxidant Activity Testing Of Porang (*Amorphophallus Muelleri Blume*) Leaf Ethanol Extract From Kuta Buluh Region, North Sumatera

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Abstract.

Porang (*Amorphophallus muelleri Blume*) is one type of tuber plant that has the potential to be developed in Indonesia. Porang plants have been reported to contain chemical compounds that have antioxidant activity. This research was conducted on the leaves of the porang plant with the aim of knowing the antioxidant activity, characterization, and phytochemical profile of the compounds contained therein. Compound identification and characterization were performed using standard methods, and antioxidant activity was determined using the DPPH (1,1- Diphenyl-2-picrylhydrazyl) method. The results of the identification of chemical compounds showed that the ethanol extract of porang leaves contained chemical compounds of alkaloids, flavonoids, tannins, saponins, and steroids. The results of the examination of the simplicia characterization of porang leaves included the soluble ethanol content of 33.93%, the water soluble extract content of 17.3%, the ash content of 5.58%, the acid insoluble ash content of 0.235% and the water content of 8%. From the results of the simplicia characterization, it shows that the results meet the specified requirements. The results showed that the ethanol extract of porang leaves had antioxidant activity with an IC_{50} value of 93.04 $\mu\text{g/mL}$, which in this case is included in the category of strong antioxidant activity. Meanwhile, as a comparison, Vitamin C was used, which has an IC_{50} value of $\mu\text{g/mL}$, which is included in the category of very strong antioxidant activity.

Keywords: Porang leaf, ethanol extract and antioxidant activity

I. INTRODUCTION

Indonesia is rich in major carbohydrate sources such as cassava, corn, rice, potatoes, taro, sweet potato, sorghum, sago, as well as minor carbohydrate sources consisting of kimpul, arrowroot, suweg, uwi, canna, and porang, which have another name of iles-iles. The potential for minor carbohydrate sources, namely porang, is very large, but the commercialization of minor carbohydrate sources for alternative food products such as porang is still small (Koswara, 2006). The porang plant (*Amorphophallus muelleri Blume*), or often called iles-iles, belongs to the Araceae family, is a plant that lives in the tropics, and is one of the most abundant tubers in Indonesia. In North Sumatra, the existence of this porang plant is quite abundant and has begun to be widely cultivated due to the high utilization and value of exports abroad. Indonesia is an exporting country for porang tubers, which are sent in the form of cassava and flour to various countries such as the United Kingdom, Japan, New Zealand, Pakistan, Australia, Sri Lanka, and the Republic of Korea (Afifah et al., 2014). Porang (*Amorphophallus Muelleri Blume*), also known as iles-iles, is a tuber plant that grows a lot in the forest. *Amorphophallus sp.* is one type of tuber plant that can grow well in Indonesia and generally grows wild, but now many have started cultivating it. Porang is one type of plant that belongs to the Araceae family and is a shrub (herb) plant that has a single tuber in the soil (Siswanto and Karamina, 2016). *Amorphophallus* is a genus name used for approximately 80 species and is found mostly in the tropics (Saputro et al., 2014).

The main constituent of the porang plant is glucomannan, which is found in the tuber. Glucomannan is a hemicellulose type polysaccharide consisting of several chain bonds, namely galactose, glucose, and mannose (Aryanti and Abidin, 2015). The main chains present in glucomannan are D-glucose and D-mannose. In one chain of glucomannan molecules there is a D-glucose content of 33% and a D-mannose content of 67%. The content of glucomannan in porang tubers varies depending on the type and species, with

a range of glucomannan content of between 5-65% (Saputro et al., 2014). Indonesian people use a lot of plants from the Araceae family as medicine or food ingredients. Porang has been studied and reported to have several pharmacological activities, namely antibacterial (Khan et al., 2009) and antioxidant activity (Maimunah, 2015; Firman 2016). Secondary metabolites are metabolites that are not essential for the growth of organisms and are found in unique or different forms from one species to another. The function of secondary metabolites is to defend themselves from unfavorable environmental conditions, for example, to overcome pests and diseases, attract pollinators, and as a signal molecule (Rasyid, 2012). Several secondary metabolites have antioxidant activity. Antioxidants are compounds that can inhibit oxidation reactions by donating electrons so that free radicals will be bound and become more stable (Septiani et al., 2018). Extraction is the process of separating materials from a mixture using a solvent. Extracts are preparations obtained by extracting medicinal plants with a certain particle size and using a certain extraction medium (menstrum) (Agoes, 2007). Extraction or separation of chemical compounds from plant sources is the beginning of the process of isolating bioactive compounds present in plants, including leaves, seeds, roots, and stems. In carrying out this extraction, you will be assisted by a solvent. This solvent must, of course, be selected. What must be considered is the selectivity, toxicity, and ease of evaporation. Extraction is used to obtain the content of chemical compounds that are soluble in solvents.

There are several types of extraction commonly used in the process of separating bioactive compounds from plants in order to determine the yield produced, namely cold extraction and hot extraction (Kiswandono, 2011). Maceration is a simple method of extraction. Maceration is done by soaking the simplicia powder in a liquid filter. The filter fluid will penetrate the cell wall and enter the cell cavity, which contains the active substance. The active substance will dissolve and, because of the difference in concentration between the active substance solution inside the cell and outside the cell, the concentrated solution is pushed out (Voight, 1994). Phytochemical screening is an analytical method to determine the types of secondary metabolites found in plants because of their characteristics that can react specifically with certain reagents (Harbone, 1987). DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a free radical molecule with a purple color that can become a stable compound with a yellow color by reaction with antioxidants, where antioxidants donate one electron to DPPH so that the DPPH free radicals are reduced (Yuhernita, 2011). The content and activity of secondary metabolites obtained will vary. This is because one of them is how the process is extracted and also how the state of the plant is seen (Syahputra, 2021). The content of porang and porang simplicia leaves really needs to be researched to find out what secondary metabolites are present in the two plants, namely extracts from fresh porang leaves and porang leaf simplicia (dried leaves).

II. METHODS

2.1 Materials

The materials used in this study were simplicia and leaf extract of Porang (*Amorphophallus muelleri* Blume), aquadest, 96% ethanol, magnesium powder, concentrated HCl, 10% iron (III) chloride, 2N HCl, Libermann-Bouchard reagent, Bouchard reagent, reagent Mayer, Dragendorff's reagent, amyl alcohol, toluene, DPPH, and ascorbic acid.

2.2 Tools

The tools used in this study were beakers, tube racks, blenders, analytical balances, rotary evaporators, porcelain dishes, maceration vessels, furnaces, water baths, measuring flasks, stirring rods, drip plates, ovens, and UV-Vis spectrophotometer (Shimadzu- UV-1800).

2.3 Sample

The leaves of the porang plant were taken in the Kuta Buluh area of Sumatra using a purposive sampling technique, taking samples intentionally from one area without comparing them with other areas. The plants used are leaves that are still fresh. Then the chopping process is carried out to facilitate the drying process. The leaves were dried in a drying cabinet, then the dried leaves were weighed dry and mashed using a blender.

2.5 Extraction

Extraction of plant leaves from simplicia was carried out using the maceration method. The dried simplicia was weighed and immersed in 96% ethanol solvent in a maceration vessel with a ratio of 1:10 for 3x24 hours at room temperature with several stirrings. Furthermore, the remaining ethanol solvent was evaporated using a rotary evaporator. After that, the liquid ethanol extract was put into a porcelain cup and evaporated again over a water bath at a temperature of 60°C until a thick extract was formed.

2.6 Simplicity Characterization

Examination of simplicia characterization includes determination of water content; determination of water-soluble extract content; determination of ethanol-soluble extract content; determination of total ash content; and determination of acid-insoluble ash content (Directorate General of POM, 1995).

2.7 Phytochemical Screening Test

The viscous ethanol extract obtained in the maceration process was added with reagents: 10% NaOH, FeCl₃, Meyer, Dragendroff, Leiberman Burchard, and n-hexane. Record the color change of the solution before and after adding the color reagent.

2.8 Antioxidant Activity

2.8.1 Maximal wavelength measurement

A total of 1.0 mL of 0.254 mM DPPH solution was put into a measuring flask, and 3.0 mL of ethanol p.a. was added. The solution was then incubated in the dark for 30 minutes, and then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 400-650 nm.

2.8.2 Preparation of sample solution

In this study, the mother liquor sample was made with a concentration of 1000 µg/mL by weighing 10 mg of the thick extract that had been obtained and then put into a 10 mL volumetric flask and adding ethanol p.a to the mark.

2.8.3 DPPH Test

The mother liquor sample of 1000 µg/mL was then made into several concentrations for analysis, namely 12.5; 25; 50; 100; and 150 µg/mL. Each test solution received 1.0 mL of 0.254 mM DPPH and 2.0 mL of ethanol p.a. and was shaken until homogeneous. Then the solution was incubated in the dark for 30 minutes, and the absorbance was measured at the maximum wavelength. Preparation of a blank solution using 3.0 ml of methanol p.a and adding 1.0 ml of 0.254 mM DPPH, then incubated in a dark room for 30 minutes before measuring the absorption. Ascorbic acid was used as a comparison with a concentration variation of 0.25; 0.5; 0.75; 1; 1.25 µg/mL.

III. RESULT

3.1 Phytochemical Characterization and Screening of Porang Leaf Ethanol Extract

In this study, the extraction method used was the maceration method. This method was chosen because the process is easy, the equipment used is simple, and it does not damage the compounds contained in the test sample. A simplicia is said to be qualified if it meets the quality requirements stated in the simplicia monograph contained in the Indonesian Herbal Pharmacopoeia. The results obtained by the phytochemical test can be seen in (Table 1).

Based on the results of the simplicia characterization of porang leaves, the juice content was carried out to see the amount of soluble compounds in polar and non-polar solvents. The ethanol soluble extract content was 33.93% and the water soluble extract content was 17.3%. Examination of ash content is useful to see the mineral content of simplicia. The ash content was 5.58% and the acid insoluble ash content was 0.235%. The water content was carried out to see the amount of water contained in the simplicia and 8% of the water content was obtained. The results of the determination of simplicia characterization show that the results meet the requirements and are guaranteed to be of high quality based on *Materia Medika Indonesia* (MMI).

Table 1. Porang Leaf Phytochemical Screening

No.	group of chemical compounds	Porang Leaf Powder	Porang Leaf Ethanol Extract
1.	Alkaloid	+	+
2.	Flavonoid	+	+
3.	Tanin	+	+
4.	Saponin	+	+
5.	Steroida	+	+

Description:

(+) = contains the substance examined

(-) = no contains substances examined.

Based on the results obtained, porang leaf extract contains metabolites of alkaloids, flavonoids, tannins, saponins, and steroids. Phytochemical screening was carried out to obtain information on the class of secondary metabolites contained in the simplicia ethanol extract of porang leaves. These secondary metabolites are thought to have activity as anti-free radicals because of the functional groups present in these compounds, such as OH groups, which in their heterolytic breakdown will produce O radicals and H radicals. These radicals will react radically with DPPH so that it can reduce the wavelength of the DPPH.

3.2 Antioxidant Activity

Determination of antioxidant activity using the DPPH method or 1,1-diphenyl-2-picrylhydrazyl as a free radical. Antioxidants are electron-donating compounds that can inactivate oxidation reactions by complementing the electron deficiency of free radicals so that the chain reaction will be inhibited and free radicals will become stable.

The principle of the DPPH method is that the color changes from purple to weak purple or yellow. This decrease in color intensity occurs due to the reduction of conjugated double bonds in the DPPH structure after receiving hydrogen atoms from antioxidant compounds. In this research, measurement of DPPH solution without the addition of test solution with a concentration of 50 µg/mL that has been incubated in a dark place for 30 minutes obtained a maximum wavelength of 517 nm. From the measurement of the DPPH solution, the absorbance was 0.637. The result was declared good because it was still in the range of 0.2-0.8 nm. This absorbance is used to calculate the % attenuation (%) inhibition. This can be seen in Figure 1.

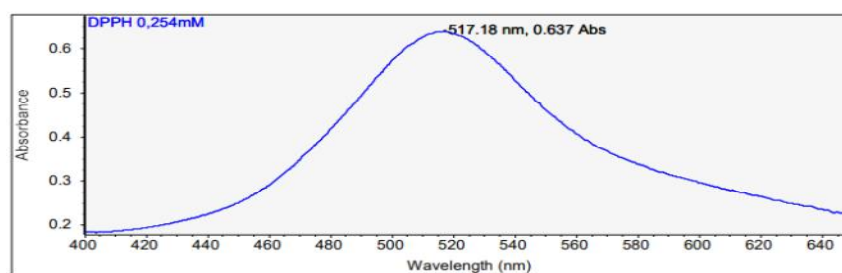


Fig 1. Determination of the maximum wavelength of DPPH solution in ethanol by Visible Spectrophotometry

Measurement of DPPH absorbance with the addition of Vitamin C Control as a comparison with a concentration of 0.25; 0.5; 0.75; 1; 1.25 µg/mL was measured at a maximum wavelength of 517 nm. after being incubated for 30 minutes in a dark place. The results of the measurement of DPPH and Vitamin C with various concentrations can be seen in (Table 2).

Table 2. Measurement of DPPH absorbance after addition of Vitamin C

No	Standard	Concentration (µg/mL)	Absorbance (nm)
1	Vitamin C	0.25	0.387
2		0.5	0.355
3		0.75	0.348
4		1	0.338
5		1.25	0.320

The table above shows that the higher the concentration, the lower the absorbance produced and the more DPPH is attenuated. Then the measurement of DPPH absorbance with an ethanol extract of porang leaves with a concentration of 1.25; 25; 50; 100; 150 $\mu\text{g/mL}$ was measured at a maximum wavelength of 517 nm. after being incubated for 30 minutes in a dark place. The results of the measurement of DPPH and ethanol extract of porang leaves with various concentrations can be seen in (Table 3).

Table 3. Measurement of DPPH absorbance after the addition of Porang Leaf Ethanol Extract

No	Sample	Concentration ($\mu\text{g/mL}$)	Absorbance (nm)
1	Porang Leaf Ethanol Extract	12.5	0.517
2		25	0.459
3		50	0.394
4		100	0.262
5		150	0.238

The DPPH solution after adding porang leaf ethanol extract and Vitamin C standard will change color, from purple to light purple and light yellow. This color change occurs because the electron acceptor radical from the secondary metabolite compound in the sample will oppose the DPPH compound so that it becomes a non-radical compound. The results of the determination of % attenuation (% Inhibition) can be seen in (Table 4)

Table 4. Inhibition percentage Porang leaf ethanol extract and Vitamin C.

Vitamin C	% Inhibition with Variation of Concentration				
	0.25 $\mu\text{g/mL}$	0.5 $\mu\text{g/mL}$	0.75 $\mu\text{g/mL}$	1 $\mu\text{g/mL}$	1.25 $\mu\text{g/mL}$
	39.48 %	44.66 %	46.31 %	46.62 %	49.95 %
Porang Leaf Ethanol Extract	% Inhibition with Variation of Concentration				
	12.5 $\mu\text{g/mL}$	25 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	150 $\mu\text{g/mL}$
	19.67 %	27.79 %	38.70 %	59.76 %	64.73 %

In determining antioxidant activity, the parameter used to determine the ability of antioxidant compounds is IC_{50} . The IC_{50} value is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%. The IC_{50} value is obtained based on the linear regression equation, which states the relationship between the concentration of the extract as the x-axis and % reduction as the y-axis, this can be seen in Figures 2 and 3. The smaller the IC_{50} value, the more active the extract will be as an antioxidant compound. The measurement results of the antioxidant activity of the ethanol extract of porang leaves and vitamin C showed that vitamin C had very strong antioxidant activity. This can be seen from the IC_{50} value of Vitamin C obtained, which is $\mu\text{g/mL}$, while the ethanol extract of porang leaves has antioxidant activity in the strong category because the IC_{50} value obtained is 93.04 $\mu\text{g/mL}$. Compounds that are classified as natural antioxidants are also extracted in phenol or polyphenol test compounds, which can be in the form of flavonoids, cinnamic acid derivatives, and tocopherols. The flavonoid group that has antioxidant activity includes flavonols, isoflavones, flavones, catechins, flavanones, and chalcones (Kumalaningsih, 2006). Flavonoid compounds have a role as free radical scavengers because the hydroxyl groups they contain donate hydrogen to radicals, so they can neutralize atoms with unpaired electrons so that they get electron pairs and are no longer radicals (Silalahi, 2006).

Fig 2. The results of measuring the absorbance of Vitamin C with various concentrations

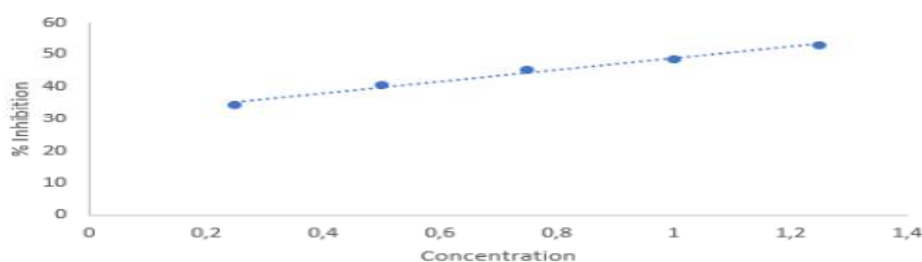
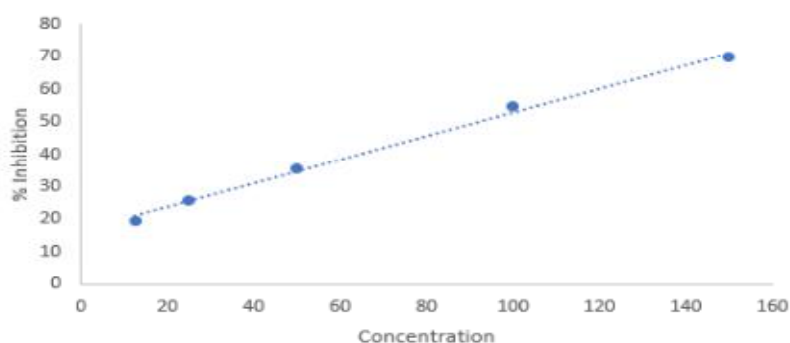


Fig 3. Absorbance measurement results of Porang leaf ethanol extract with various concentrations

IV. CONCLUSION

Based on the results of the research that has been conducted, it can be concluded that the ethanol extract of porang leaves contains alkaloids, flavonoids, saponins, tannins, and steroids. Porang leaf ethanol extract has antioxidant activity in the strong category with an IC_{50} value of 93.04 $\mu\text{g/mL}$ and vitamin C has antioxidant activity in the very strong category with an IC_{50} value of 1.01 $\mu\text{g/mL}$.

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