Antioxidant and Antibacterial Activities of Taro Leaf Herbal Tea (*Colocasia esculenta L.Schoot*) Based on Variations in Drying Time and Steeping Time

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Abstract. Taro leaves are wild plants that are commonly found in Indonesia and are proven to have chemical bioactive compounds that are beneficial to health. Making taro leaf herbal tea has the potential to boost the immune system because it contains antioxidants and is a preventive and curative effort against several diseases caused by bacteria. This research was conducted using activity testing antioxidants and antibacterial taro leaf herbal tea with drying times of 80 minutes, 100 minutes, 120 minutes, and 140 minutes and steeping times of 9 minutes and 11 minutes using the Kirby-Bauer method, namely the diffusion method using disc paper. In antioxidant testing using DPPH, the inhibition zone is measured by taking a horizontal line on the clear zone around the disc using a vernier caliper. The inhibition zones of taro leaf tea against Escherichia coli and Salmonella typhii bacteria were taro leaf herbal tea with 120 minutes of drying (Code C) and 140 minutes of drying (Code D) with 9 minutes of steeping time. Herbal teas code C and D with a steeping time of 11 minutes showed unstable inhibition. Taro leaf herbal tea with 120 minutes of drying and 9 minutes of steeping time has a susceptible inhibition. In Staphylococcus aureus bacteria it is more effective with 140 minutes of drying.

Keywords: Taro Leaf Herbal Tea, Antioxidant, Antibacterial, Drying Time, Steeping Time.

1 INTRODUCTION

Taro (*Colocasia esculenta L. Schoot*) is a tropical plant that grows in Indonesia. In some countries, the taro plant is known as taro or dasheen. In Indonesia, the plant is known as taro or lompong. Taro leaves are widely used as vegetables and herbal medicine. In proving taro leaves as an herbal medicine, taro leaves were converted into an extract dosage form. Several previous studies showed that taro leaf extract was proven to have antimicrobial, antidiabetic, antihepatotoxic, and anti-inflammatory activity. The health benefits of taro leaf extract are due to the phytochemical compounds contained in it. Content Taro leaf extract includes terpenoid, saponin, tannin, and anthocyanin

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compounds such as pelargonidin 3-glucoside, cyanidin 3-rhamnoside, and cyanidin 3-glucoside which have been proven to have antioxidant activity [1-3].

The flavonoid and saponin content plays a role in inhibiting bacterial growth. The best concentration of taro leaf ethanol extract in inhibiting bacteria is 50 mg/ml. The greater the concentration of the extract, the greater the inhibition zone formed [4]. The use of taro leaf extract in treating diabetes mellitus has been widely studied. Tea is a type of drink that Indonesian people much love. In Indonesia, tea is a refreshing drink that has benefits for the body. Tea can be made from other leaves such as Moringa leaves, avocado leaves and others. The drying process is a way of removing or removing some of the water content in a material using heat energy so that the material is not easily damaged when stored. Oven drying is a method of drying tea leaves using an oven [5]. Leaves can be preserved for a long time without losing the nutrients contained in them. Drying or freezing can be done to store the leaves.

Drying is influenced by temperature and drying time. High temperatures can cause the tea leaves to burn, while low temperatures cause the fermentation process. In the process of drying tea leaves, you must pay attention to the drying time. Drying time that is too long will cause the tea to become brittle while drying time that is too fast will cause the water content to remain high. Several previous studies on the temperature and length of drying leaves which have almost the same characteristics as cocoa leaves can produce tea with quite good quality, in the Mulachella study, drying bay leaves at a temperature of 50°C for 50 minutes can produce bay leaf tea with a water content of 7.77%. and the highest antioxidant activity, namely 89.78%, from the organoleptic test, bay leaf tea was acceptable with a slightly favorable score for taste, aroma, and color [6]. In addition, in Anggorawati's research, drying avocado leaves at a temperature of 60°C for 70 minutes produced leaf tea. avocado with an IC50 of 32.255 µg/ml and good texture, color, and aroma.7 This research aims to determine which taro leaf herbal tea with drying time and steeping time has the best antioxidant and antimicrobial activity.

2 METHODS

2.1 Making Taro Leaf Herbal Tea

Prepare ± 500g of taro leaves, then wash them until clean and separate them from the twigs. After washing thoroughly, the leaves are then drained and separated from the yellow leaves. The leaves that have been separated are then dried in the sun until dry, avoid drying them in direct sunlight so that the nutrients are not lost. The dried leaves are then blended into small pieces. The drying time will be differentiated from 80 minutes, 100 minutes, 120 minutes, and 140 minutes with steeping times of 5 minutes, 7 minutes, 9 minutes, and 11 minutes.

2.2 Analysis of Antioxidant Levels

A stock solution was taken from each sample and then a stock solution was made with a concentration of 1 mg/mL. Next, the solution is diluted in ethanol to several concentrations. A total of 100 µL was added to a 96-well plate, then 100 µL of 125 µM

DPPH solution in ethanol was added, and the incubation process continued for 30 minutes at room temperature. Absorbance was measured at a wavelength of 517 nm using a microplate reader [8,9].

2.3 Antibacterial Activity Test

Antibacterial activity testing was carried out using 10 ml of EMB media, then poured into a petri dish and left until it solidified. Next, add a suspension of Escherichia coli, Salmonella typhi, and Staphylococcus aureus bacteria using a sterile cotton swab so that the suspension is absorbed into the media. Then, in the petri dish, a disk was placed that had previously been soaked in the test sample (according to the concentration. The treatment was carried out in duplicate to ensure the results were obtained. Next, all the media were incubated in an incubator. Incubation was carried out at a temperature of 370C for 24 hours. Then the zone diameter was measured. Clear form is formed using a millimeter ruler. Antibacterial activity is obtained by measuring the clear zone on solid media and being an indication of whether or not bacteria are growing in each treatment. The parameter observed was the diameter of the area inhibiting bacterial growth. The tools used in the test are analytical balance, oven, blender, Erlemeyer, water bath, autoclave, Petri dish, test tube, test tube rack, tube needle, volume pipette, measuring cup, Bunsen, container, caliper, stir bar, rotary evaporator, and paper disc. Meanwhile, the materials used in this research were taro leaf extract, Escherichia coli bacteria, 96% ethanol, nutrient agar, parchment paper, sterile distilled water, nutrient broth, and ciprofloxacin 500mg. The chemicals used are $FeCl_3$, HCl 2M, magnesium powder, Wagner's reagent. Interpretation of Inhibitory Power [10].

≤ 15 mm = Resistant
16-18 mm = Intermediate
≥ 19 mm = Susceptible

3 RESULT AND DISCUSSION

Herbal tea is one of the functional beverage products from herbal plants that can help treat disease and as a refreshing drink for the body [11]. Herbal tea can be made from industrial tree flowers, seeds, leaves, and roots. In making herbal tea, it must go through a drying process. Drying is one of the processes used to extend shelf life. Drying is one of the common preservation methods for food ingredients. Drying is a method to remove or eliminate most of the water from a material by applying heat energy. Drying can reduce the water content of the material so as to inhibit the growth of bacteria and fungi, and reduce the activity of enzymes that can damage the material, so as to extend the shelf life and preservation. If water is removed, it can affect the physical condition of the material and cause changes in the color, texture, and aroma of food ingredients [12].

The temperature used for drying is 50°C. Based on previous research, it was shown that a drying temperature of 50°C is the drying temperature with the best antioxidant results. drying at a temperature of ±50°C can increase antioxidant activity in materials

from moderate to very strong, but antioxidant activity will decrease if the drying time is too long because the antioxidant compounds have been damaged by heating. The drying time for taro leaf herbal tea was modified to 80 minutes, 100 minutes, 120 minutes, and 140 minutes. Based on the antioxidant test results, it shows that the optimal drying time is at a temperature of 50° for 120 minutes. Drying for more than 120 minutes shows a decrease in antioxidant levels. Drying aims to reduce the water content of food so that it can inhibit the growth of unwanted microbes.

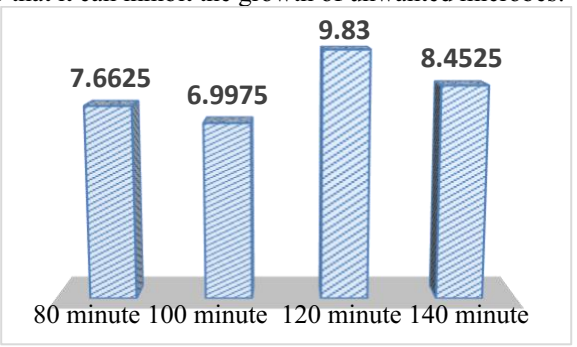

Fig. 1. Antioxidant levels are based on the drying time

There are two factors influencing drying, namely factors related to the drying air and factors related to the nature of the material being dried. Factors included in the first group are temperature, drying airflow volume speed, and air humidity. The second group of factors is the size of the material and the initial water content in the material. Time can affect the drying process, the longer the drying time will cause a decrease in the water content because the heat energy provided will be greater so that the water content in the material will evaporate into the air.

Drying time affects antioxidant activity, the longer the drying time, the antioxidant activity will also decrease. the results of this study showed a decrease in antioxidant activity in taro leaf herbal tea when dried for 140 minutes. The drying process that is too long results in a decrease in the active substances contained in a food ingredient, the decrease in antioxidant activity is influenced by the enzymatic oxidation process which causes polyphenols to oxidize and decrease.12 Taro leaf herbal tea has optimal antioxidant activity when dried at a temperature of 50°C with a drying time of 120 minutes.

The duration of steeping herbal tea leaves affects the quality of herbal tea. Based on antioxidant tests for making herbal tea, it show that the steeping time of 11 minutes has higher antioxidant activity than 5 minutes, 7 minutes, and 9 minutes. Brewing with water will separate the components contained in it. The tea brewing process is influenced by the temperature used and the brewing time. In this research, the temperature used was boiling water temperature of 100°C. The brewing time that has the highest antioxidant activity is 11 minutes. The brewing time will affect the content of dissolved bioactive compounds, tea aroma, and color intensity. Taro leaf herbal tea has a greenish appearance. The longer the brewing time will increase the total flavonoids and total phenolics which contribute to increasing antioxidant activity. The

antioxidant activity of taro leaf tea is influenced by the brewing time. The longer the brewing time will increase the antioxidant activity, however, after reaching the optimum point, the antioxidant activity will decrease. This is because if the brewing time is too long, thermal degradation will occur, causing a decrease in antioxidants. In this study, the duration of brewing taro leaf tea did not experience thermal degradation because there was no decrease in antioxidant activity compared to brewing for 9 minutes. Overall, the highest antioxidant levels of taro leaf herbal tea modified from drying and steeping time can be seen in Figure 3.

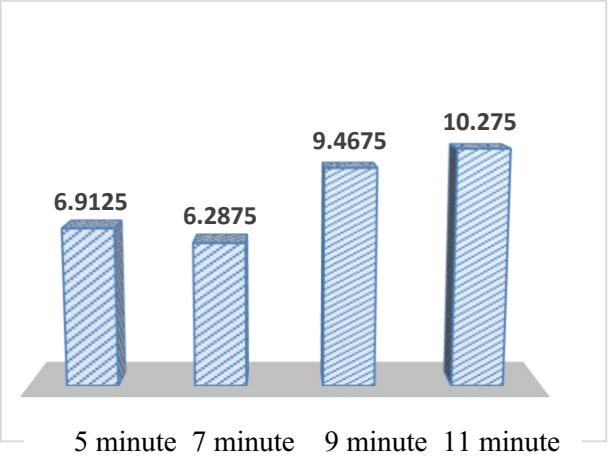

Fig. 2. Antioxidant levels based on steeping time

Antioxidant tests on all groups showed that C11 had the highest antioxidant levels. This shows that taro leaf herbal tea will have optimal antioxidant properties if the taro leaf herbal tea leaves are dried for 120 minutes and brewed for 11 minutes. The tea-making process generally includes washing, sorting, withering, rolling, drying and packaging. Tea is made through a drying process to obtain the final result in the form of dry tea which will last a long time when stored. Drying is influenced by several factors such as temperature and drying time. Drying at high temperatures can cause the tea to burn, while low temperatures cause the fermentation process to continue. Apart from that, a drying time that is too long will cause the tea to become brittle, while a drying time that is too fast will cause the water content to remain high [13].

The antioxidant activity of taro leaf herbal tea will increase with the length of drying time. This is because the drying process results in an increase in the active substances contained in tea leaves. The results of the research on taro leaves showed that the highest antioxidant activity was at a drying time of 120 minutes at a temperature of 50°C. This is the same as research before, the highest antioxidant activity in Moringa and roselle leaf tea was at a drying time of 120 minutes at a temperature of 60°C. Drying time can increase the amount of active substances in tea leaves such as phenolic acids, selenium, flavonoids, and anthocyanins [14].

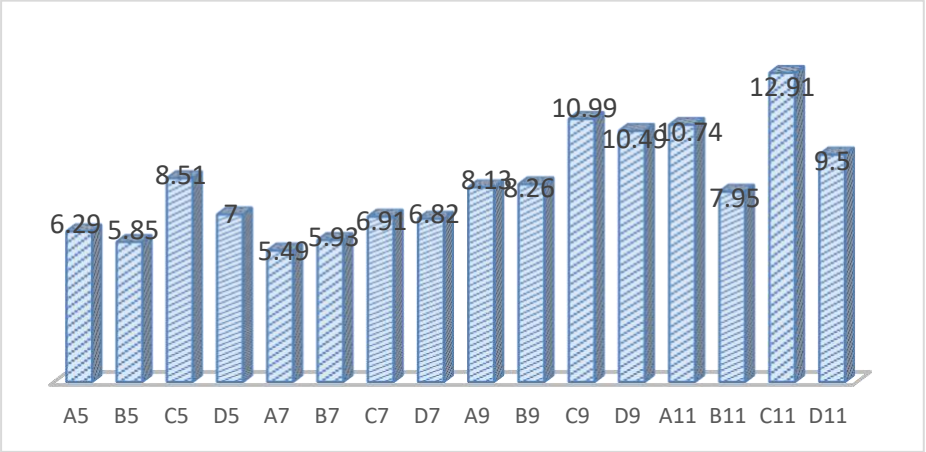

Fig. 3. Antioxidant level

According to research by Ana (2011), the temperature used in drying Moringa leaves is 50, 60, and 70°C with a time of 100, 160, and 180 minutes, but the best temperature for the formation of flavonoids which can form optimal antioxidants is with a temperature treatment of 60° C and drying time 160 minutes. This can be influenced by several factors, including variations in drying time and the content of antioxidant compounds in the material itself [14]. An improper drying process can cause a decrease in antioxidant activity.

Table 1. The bacterial inhibitory effect of taro leaf herbal tea on Escherichia coli bacteria

Steeping time (minute)	Tea code	Weight (gram)	Zone (mm)		Inhibitory Power	Resistant
			I	II		
9	C	5	24	19	21.5	Susceptible*
		10	20	19	19.5	Susceptible*
		15	22	19	20.5	Susceptible*
	D	5	18.5	24	21.25	Susceptible
		10	18.5	15	16.75	Intermediate
		15	18.5	14	16.25	Intermediate
11	C	5	-	-	-	-
		10	-	-	-	-
		15	23	20	21.5	Susceptible
	D	5	15	-	15	Resistant
		10	18.5	20	19.25	Susceptible
		15	-	-	-	

The results of the research as seen in Table 1 show that the stable one has inhibitory power against Escherichia coli bacteria, namely taro leaf herbal tea with drying of 120 minutes (Code C) and drying of 140 minutes (Code D) with a steeping time of 9

minutes. Herbal teas coded C and D with a steeping time of 11 minutes tested unstable. Taro leaf herbal tea with drying for 120 minutes with a steeping time of 9 minutes has a susceptible inhibitory effect.

Table 2. The bacterial inhibitory effect of taro leaf herbal tea on Salmonella typhi bacteria

Steeping time (minute)	Tea code	Weight (gram)	Zone(mm)		Inhibitory Power	Resistant
			I	II		
9	C	5	29.5	28	28.75	Susceptible*
		10	25	17	21	Susceptible*
		15	28.5	34	31.25	Susceptible*
	D	5	33.5	33.5	33.5	Susceptible
		10	27	25.5	26.25	Susceptible
		15	20	17.5	18.75	Intermediate
11	C	5	-	-	-	-
		10	21.5	25	23.25	Susceptible
		15	23.5	31.5	27.5	Susceptible
	D	5	18	12	15	Intermediate
		10	-	-	-	-
		15	15	13	14	Resistant

The results in Table 2 show that the stable one has inhibitory power against Salmonella typhii bacteria, namely taro leaf herbal tea with drying of 120 minutes (Code C) and drying of 140 minutes (Code D) with a steeping time of 9 minutes. Herbal teas coded C and D with a steeping time of 11 minutes tested unstable. Taro leaf herbal tea with drying for 120 minutes with a steeping time of 9 minutes has a susceptible (strong) inhibitory power.

The research results seen in Table 3 showed that the stable one had inhibitory power against Staphylococcus aureus bacteria, namely taro leaf herbal tea with 120 minutes of drying (Code C) and 140 minutes of drying (Code D) with a steeping time of 9 minutes. Herbal teas coded C and D with a steeping time of 11 minutes tested unstable. Taro leaf herbal tea with drying for 140 minutes with a steeping time of 9 minutes has a susceptible inhibitory effect.

Table 3. The bacterial inhibitory effect of taro leaf herbal tea on Staphylococcus aerus bacteria

Steeping time (minute)	Tea code	Weight (gram)	Zone (mm)		Inhibitory Power	Resistant
			I	II		

9	C	5	20	21	20.5	Susceptible
		10	21.5	21	21.25	Susceptible
		15	17.5	20	18.75	Intermediate
	D	5	24	25	24.5	Susceptible*
		10	24	23.5	23.75	Susceptible*
		15	24	22.5	23.25	Susceptible*
11	C	5	25	25	25	Susceptible
		10	20	20	20	Susceptible
		15	-	-	-	-
	D	5	15	20	17.5	Intermediate
		10	14	13	13.5	Resistant
		15	-	-	-	-

Taro leaves have secondary metabolite compounds in the form of flavonoids, saponins, and alkaloids which have antibacterial activity. Flavonoids as polyphenol compounds in taro leaves have antibacterial activity by forming complex compounds against extracellular proteins that will disrupt the integrity of bacterial cell membranes so that they have the potential as antibiotics [15]. Flavonoids can inhibit bacterial growth in the form of quorum sensing or through enzymatic processes [16]. Treatment using medicinal plants can reduce the side effects caused by chemical drugs that are commonly consumed. This can be used as an alternative in the treatment system. In gram-negative and positive bacteria, the mechanism of action of flavonoids is by disrupting the function of the bacterial cell membrane. Membrane permeability will be disrupted due to the presence of antibacterial compounds [17]. Saponins have a high level of toxicity so they can disrupt membrane stability and can act as antibacterials [18]. Alkaloids in taro leaves have antibacterial properties. The mechanism is by disrupting the peptidoglycan components in bacterial cells, so that the bacterial cell wall layer is not formed completely and causes cell death [19].

Taro leaf herbal tea has antibacterial activity on Salmonella typhi and Staphylococcus aureus bacteria. Salmonella typhi and Escherichia coli are gram-negative bacteria while Staphylococcus aureus is gram-positive bacteria. The bacterial cell wall will experience protein denaturation due to flavonoids contained in taro leaf herbal tea, so the protein is hard and stiff causing damage to the bacterial cell wall [20].

Taro leaf herbal tea can ward off Escherichia coli and Salmonella typhi bacteria if made with a drying time of 120 minutes with a steeping time of 9 minutes. Meanwhile, to ward off Staphylococcus aureus bacteria, dry it for 140 minutes. To ward off gram-positive bacteria, a longer drying time is needed. This is because gram-positive bacteria have cell walls composed of a thicker peptidoglycan layer, while gram-negative bacteria have a thinner peptidoglycan layer.

It is known that flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA. The results of this study indicate that taro leaf herbal tea has antibacterial compounds, both gram-positive and negative bacteria.

4 CONCLUSION

Based on research results drying taro leaf herbal tea leaves have the highest antioxidant content with 120 minutes of drying. The steeping time for taro leaf herbal tea which have the highest antioxidant activity is 9 minutes and 11 minutes. The bacterial inhibitory power of taro leaf herbal tea against Escherichia coli and Salmonella typhi bacteria is effective in herbal leaf tea with drying of 120 minutes with a steeping time of 9 minutes. In Staphylococcus aureus bacteria it is more effective with 140 minutes of drying.

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