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Antioxidant Activity of Sago (Metroxylon sagu Rottb) Pith Waste

Patricia Wally[1], Brechkerts Lieske Angruni Tukayo[1*], Baiq Daraquthni Wandansari[1]
[1]Department of Pharmacy, Poltekkes Kemenkes Jayapura, Jayapura, Indonesia
**email : tukyaolieske@gmail.com*

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A B S T R A C T

Sago pith waste contains flavonoid and tannin compounds. Flavonoids and tannins are well-known phytochemical compounds that act as natural antioxidants that can inhibit free radicals. This study aimed to determine the antioxidant activity of sago (Metroxylon sagu rottb) pith waste extract. Sago pith waste was obtained from Jayapura Regency and extracted by maceration method using 1500 ml of ethanol 70% as a solvent for 5 days. Furthermore, phytochemical screening was carried out on the thick extract. 2,2-Diphenyl-1-picrylhydrazy (DPPH) assay was used to determine antioxidant activity from ethanol extract of sago pith waste with variation in concentration as follows: 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm. The study's results found that the secondary metabolite content of the ethanol extract of sago pith waste were flavonoids, phenolic compounds and tannins. From the antioxidant activity test results, the inhibition percentages of the extracts were 30.98%, 40.66%, 51.15%, 65.90%, and 70%, respectively. The IC50 value of the sago pith waste ethanol extract was found to be 283.11 ppm, which was categorised as weak activity of antioxidants. It can be concluded that sago pulp extract has the potential as a source of natural antioxidants.
Keywords: Antioxidant, DPPH, Sago pith waste

INTRODUCTION

Sago is a carbohydrate-producing agricultural plant widely found in Southeast Asia and has been used as a local food by the community as a substitute for rice (1). In Indonesia, Papua has the largest sago plantations with an area of 4,749,424 Ha. Sago can be processed into various food products, such as sago flour(2). Sago starch has the potential as a raw material for making capsules (edible film) to replace gelatine. Dry sago starch is the best raw material that meets the quality standards of SNI 06-3735-1995 in terms of organoleptic colour, odour, water content, ash content and heavy metal content(3). In addition, research by Suwarda et al. (4) found that edible film from sago acetate starch was relatively more stable when stored at low or high temperatures, had good mechanical properties and was resistant to water vapour so that it could be used as a packaging material in humid environments.

Processing sago into food products usually produces sago pith waste, which is generally just thrown away or burned. So far, sago waste has only been used as animal feed(5). Sago pith waste contains primary metabolites such as carbohydrates, water, protein and cellulose fibre(1). Meanwhile, the secondary metabolites in sago pith waste are phenolics, flavonoids and condensed tannins(6). From the potential content of phytochemical compounds, sago pith waste can be used as a source of antioxidants.

Antioxidants are compounds that can be used to slow down or inhibit the oxidation process (7).
Antioxidants can inhibit the activity of free radicals by donating one electron to free radical compounds(8). The most important benefit is maintaining the integrity and function of lipid membranes, cell proteins, and nucleic acids and controlling signal transduction and gene expression in the immune system. Antioxidants in the body reduce and eliminate free radicals. The antioxidant system that complements the immune system can inhibit the reactivity of free radicals(9). The development of antioxidants from natural ingredients is often done in the form of semisolid preparations such as lotions and creams as one of cosmetic ingredients.

A study by Momuat et al..(10) reported that the total antioxidants, total phenolics and total flavonoids were higher in ethanol extract from flour of dry sago baruk compared to flour from fresh sago baruk extract. On the other hand, flour of fresh sago baruk extract had a slightly higher antioxidant activity compared to flour of dry sago barok extract with the antioxidant activity were 83.08% and 81.49%, respectively.

* Corresponding author: Brechkerts Lieske Angruni Tukayo, Department of Pharmacy, Poltekkes Kemenkes Jayapura, Jayapura, Indonesia. Email: *tukyaolieske@gmail.com*

Another research by Ginting(6) entitled Antioxidant Activity of Water and Ethanol Extracts from Sago Bark (Arenga microcarpha) showed that the water extract and ethanol extract have a total phenolic content of 150.31 and 88.92µg/mL. While the total condensed tannins for water extract and ethanol extract, respectively, are 39.91 and 39.74 µg/mL. Ethanol extract and water extract with concentrations of 56.23 µg/mL and 1949.84 µg/mL have antioxidant activity in the very strong category with IC50 values of 1.75 ppm and 3.29 ppm.

In the area around Lake Sentani, Jayapura district, where sago pith waste was taken in this study, had high productivity of Sago. Some sago plants around lake Sentani have a fairly high starch content, such as phara, yebha, osukul, and folo(11). Based on the researcher's initial survey, around Lake Sentani, especially in the Nendali village area, sago pith waste has few benefits and is simply thrown away. So, researchers are interested in studying the antioxidant activity of sago pith waste

MATERIALS AND METHODS

This research was laboratory experimental research to test and observe the antioxidant activity of sago (Metroxylon sagu rottb) pith waste. The research was conducted at the Pharmacognosy Laboratory of the Jayapura Ministry of Health Polytechnic, the Chemistry Laboratory of the Chemistry Department of Cendrawasih University and the Pharmacy Department of Pharmacy, Cendrawasih University. The research was conducted in May – June 2021.

The object of this study was sago (Metroxylon sagu rottb) pith waste as a sample taken from Nendali village, Jayapura Regency, Papua Province, was 3 kg.

Sample Preparation

Sago (Metroxylon sagu rotted) pith waste from sago processing was taken and then air-dried for 1 x 24 hours at room temperature, then dry sorting was carried out, after which the sago (Metroxylon sagu rotb) pith waste was ground using a blender grinder (12). The grinding was carried out until the sago pith waste became smooth at the lowest speed, and then the grinding results were sieved to obtain sago pulp that was the same size.

Extract Making

Sago (Metroxylon sago rottb) pith waste extraction in this study was carried out using the maceration method. The sago (Metroxylon sagu rottb) pith waste was weighed for 300 grams and then macerated using 1500 ml of 70% ethanol 70% as the solvent (1:5b/v). Soaking was done for 24 hours, at room temperature, until the colour of the solvent changed from initially clear to brown, which matched the colour of sago (Metroxylon sagu rottb) pith waste. Stirring was done 2 times a day, and after the filtering process, the residue was soaked again with the solvent, and the filtrate was collected. This method was repeated for the 2nd and 3rd days. The filtrate results were evaporated using a vacuum rotary evaporator at a temperature of 70 C to separate the solvent from the filtrate; then, a water bath was used until a thick extract with less water content was obtained.

Flavonoid Examination

A total of 3 ml of extract solution was added with Magnesium powder, 2 ml of HCl and 2 ml of amyl alcohol, then shaken vigorously until 2 layers were formed, either it was red, orange or yellow, which was shown the presence of amyl alcohol layer(13).

Condensed tannin examination

1 mL of extract was reacted with iron (III) chloride (FeCl3) solution. If a greenish-black or bluish-black colour is formed, it indicates the presence of condensed tannins(13).

Examination of phenolic compounds

1 mL of solution was put into a test tube, then 2 drops of 1% iron (III) chloride solution was added. If the results are positive, the solution changes colour to green or blue-green(13)

Antioxidant activity testing

Determination of antioxidant activity using the DPPH method: the initial step was to make a 0.1 mm DPPH solution, with 2 mg of DPPH powder dissolved using methanol pa to a volume of 50 mL in a 50 mL measuring flask then placed in a bottle covered with aluminium foil(14).

Before conducting the antioxidant test, the liquid extract was centrifuged at 3000 rpm for 1 hour. The antioxidant test was carried out after obtaining the sago pith waste extract sediment from the centrifugation results. A total of 0.025 grams of sago pith waste powder was dissolved with ethanol in a 50 ml measuring flask to make a stock solution of 500 (mg/l). This stock solution was used to prepare solutions with variance of concentration as follows: 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm.

The procedure for making a positive control solution was 0.002 grams of vitamin C powder dissolved with ethanol in a 50 ml measuring flask to make a stock solution of 20 mg/L. This solution was used as a stock solution to make solutions of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. The test was carried out by pipetting 1 ml of sample solution from various concentrations (100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm) and then adding 3 ml of DPPH to each. Furthermore, the mixed solutions were incubated at a temperature of 37 C in a dark room. The absorbance was measured at 517 nm wavelength.

The data obtained from the antioxidant activity test of sago pith waste was first calculated for the percentage of antioxidant activity using the formula below:

Antioxidant Activity (%) = (Ab-As) / Abx 100%, where Ab is the Blank absorbance and As is the sample absorbance

After getting the inhibitory percentages from each sample concentration, a linear regression calculation (X, Y) was carried out to obtain the IC50 (Inhibition Concentration 50) value. X was the concentration, and Y was the percentage of antioxidant activity (%). To obtain the IC50 value, the Microsoft Excel program was used to determine the linear regression and the IC50 data was obtained:

$$y = a + bx$$

Where y is the response variable, b is the slope coefficient, x is the predictor variable, and a is the y-intercept of the line. The lowest IC50 value indicates the highest antioxidant activity.(14)

RESULTS AND DISCUSSION

This study's samples were taken from sago processing facilities in the Nendali village area, East Sentani District, Jayapura Regency. In this village, sago processing uses a grater machine, and the grated results are filtered and soaked in a storage tank to separate the starch from the pith waste. The sago starch produced is usually sold or consumed by the residents of Nendali village themselves as a source of carbohydrates; the remaining or unused sago pith waste is used as an object in this study. Jayapura has two types of sago: thorny sago or Metroxylon rumphii Mart and non-thorny sago or Metroxylon sagu Rottb(15). This study used a type of sago without spines.

Results of Ethanol Extract from Sago Pith Waste

In this study, sago pith waste was made into powder form to expand the surface areas so in the extraction process, it can accelerate the extraction of metabolite compounds by the solvent. The extraction method was a maceration process with the aim of extracting efficacious substances that are heat resistant or not heat resistant(16). The extraction process was carried out by soaking the sago pith waste with 70% ethanol to draw all the chemical components in the sago pith waste. The principle of this method is diffusion on the cell wall by the solvent that will enter the cell containing the active substances so that the substances will dissolve in the solvent and come out due to the difference in saturation or the concentration equilibrium inside and outside the cell wall. The filtrate obtained was brownish because the sago pith waste used was brownish in colour due to enzymatic activity during storage, where the polyphenol compound in the sago was oxidised into quinone and became a polymer that formed a brown colour (6).

The filtrate obtained was then concentrated using a rotary evaporator to evaporate the solvent below its boiling point to obtain a thick or liquid extract. The liquid extract obtained was then centrifuged to separate the filtrate from the sediment. Centrifugation is the separation of particles based on their particle weight; particles with a higher density than the solvent will sink to the bottom (6,10).

The results of sago pith waste extraction with 70% ethanol using the maceration method can be seen in Table 1:

Table 1. Sago pith waste extract yield

Powder weight (g)	Extract weight (g)	% Yield
300	0.036	0.012

Based on Table 1, the brown sago pith waste liquid extract was 0.036 g, yielding 0.012%. The yield compares the extract obtained from the initial simplicias (17). The higher the yield value, the more extract is produced. The quality of the extract produced is usually inversely proportional to the amount of yield produced(16,18).

Phytochemical Compound Identification Results

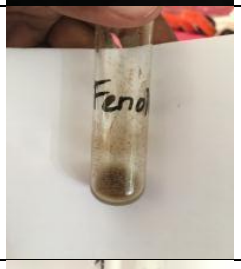
The sago pulp extract produced was then phytochemically screened to determine secondary metabolite compounds. This study conducted tests to determine the presence of flavonoids, phenolics, and tannins, which are natural antioxidants from plants. Identification of flavonoids was carried out by adding magnesium powder, hydrochloric acid, and amyl alcohol, which showed positive results in the form of a yellow colour that was attracted to amyl alcohol. There were condensed flavonoids, phenolics and tannins as a result of phytochemical compounds identifying process in the ethanol extract of sago pith waste, as can be seen in Table 2.

…A colour change occurs in flavonoid identification because the flavonoid compound is reduced by adding magnesium powder and hydrochloric acid. Phenolic identification was made by adding Iron Chloride (III) / FeCl3 to the liquid extract, and a blackish-blue colour was formed. The reaction between the phenol group and FeCl3 causes the formation of the blackish-blue colour. Furthermore, the test for the presence of tannins was done by adding FeCl3, and positive results were obtained, with a greenish-black colour change caused by the reaction between FeCl3 and one of the hydroxyl groups in the tannin compound(13).

Antioxidant Test Results

…The antioxidant activity of sago pith waste extract was measured to determine the ability of secondary metabolite compounds from sago pith waste to reduce free radicals from DPPH. Ethanol extract of sago pith waste that has been added with DPPH can be seen in Figure 1, and the test results are in Table 3.

Table 2. Results of Phytochemical Compounds in Sago Pith Waste

Class of compounds	Colour parameters	Results	Picture	Conclusions
Flavonoid	Shown in red, orange and yellow	Formation of yellow colour		+
Phenolic	Shown in green–blue	Blackish blue color		+
Condensed tannins	Shown as greenish-black or bluish-black	Greenish black colour		+

A.

B.

Figure 1. Antioxidant activity test
A. Extract before the addition of DPPH; B. Extract after the addition of DPPH

Table 3. Antioxidant Activity of Sago Pith Waste Ethanol Extract and Vitamin C

Sample	Concentration (ppm)	% Inhibition	IC50
Sago Pith Waste Extract	100	30.98%	283.11 ppm
	200	40.66%	
	300	51.15%	
	400	65.90%	
	500	70.00%	
Vitamin C	2	14.26%	9.55 ppm
	4	18.52%	
	6	25.57%	
	8	40.98%	
	10	56.23%	

The table above showed that the IC50 for sago pulp extract was 283.11 ppm and had weak activity for antioxidants. Vitamin C was used as the positive control. Vitamin C had an IC50 of 9.55 ppm, and therefore, it can be concluded that Vitamin C had strong antioxidant activities. An antioxidant activity test was done by reacting DPPH free radicals with sago pith waste extract. This method's working principle of antioxidants is the capture of free radicals by donating H atoms to DPPH radicals, as shown in Figure 2 (19). DPPH solution is easily oxidised, indicated by a change in colour from purple to pale yellow. This indicates that DPPH is in a paired state (stable condition)(6,7,10,19).

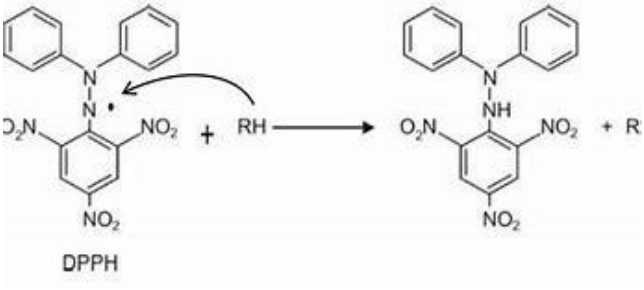


Figure 2. Mechanism of DPPH free radical scavenging

It is known that the higher the concentration, the higher the percentage of inhibition of sago pith waste, which means that the inhibitory power against DPPH radicals increases with increasing concentration. The highest concentration, 500 ppm, had a percentage inhibition value of 70%, so sago pith waste ethanol extract has great potential to counteract DPPH free radicals. Sago pith waste ethanol extract can release hydrogen atoms on the violet-coloured DPPH radical and convert it into diphenylpicrylhydrazine, a yellow-coloured non-radical substance (10.19).

From the percentage of inhibition obtained, a comparison curve was made to compare the percentage of inhibition of sago pith waste ethanol extract against the concentration of sago pith waste extract. From this curve, a linear regression equation was calculated to determine the IC50 value(6). The IC50 value of sago pulp extract was 283.11 ppm, classified as a weak antioxidant, and the IC50 value for the positive control of vitamin C was 9.55 ppm, classified as a strong antioxidant. The ability of a compound to ward off free radicals is inversely proportional to the IC50 value, where the smaller the IC50 value of a compound, the stronger its antioxidant activity(9).

The activity of an extract in counteracting DPPH free radicals depends on the ratio of flavonoid, phenolic and condensed tannin compounds(6,10). The DPPH free radical scavenging effect increases with the increase in the amount of extract. Flavonoid compounds work as antioxidants by donating their hydrogen atoms to free radicals to form stable radicals with low energy levels, which come from compounds that have lost their hydrogen atoms. In the aromatic ring structure, a resonance process will occur to form a more stable antioxidant radical. As a result, it will be difficult to experience other radical reactions. While tannin compounds function as secondary antioxidants because tannins can chelate iron ions and slow down the oxidation process(9,10).

Vitamin C is used as a positive control because it is a natural antioxidant that is easy to obtain, cheap, and easy to consume from nature. Vitamin C has the property of being easily soluble in water; however, the oxidation process, heat and alkali can easily damage vitamin C. Vitamin C is mainly found in vegetables and fruits. As an antioxidant, Vitamin C works by binding to Oxygen so that it does not support oxidation reactions (oxygen scavenger)(20).

CONCLUSION

Sago pith waste ethanol extract has weak antioxidant activity, with secondary metabolites found in the extract flavonoids, phenolics and condensed tannins.

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CONFLICT OF INTEREST

There are no conflicts of interest in research or publication that could influence the research results.

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