Phytochemical and Antioxidant Analysis of Fermented ‘Dayak’ Wild Yam (*Dioscorea hispida* Dennst), Purple Yam (*Dioscorea alata*) and Air Potato (*Dioscorea bulbifera* L.) Tuber Flour

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Abstract. Tubers of Dioscorea spp. contain many compounds that have physiological activity that can act as antioxidants and are proven to have various health uses. Antioxidant compounds have a very important role in the health of the human body. Various scientific evidence show that antioxidant compounds reduce the risk of various chronic diseases such as cancer and coronary heart disease. The study aimed to determine the phytochemical content and antioxidant activity of fermented Dayak wild yam (*Dioscorea hispida* Dennst), Purple Yam (*Dioscorea alata* var. Purpurea) and Air Potato (*Dioscorea bulbifera*) tubers flour was carried out. Phytochemical testing was carried out by the Harborne method (1987) and antioxidant activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The data obtained were analyzed descriptively from the average value of 3 replications for each parameter. Phytochemical analysis results showed the presence of alkaloids, tannins, triterpenoids and carbohydrates. While the results of the antioxidant activity testing showed that the IC50 value of fermented Dayak wild yam flour (WYF) was 227.43 ppm, air potato tuber flour (APF) was 15.77 ppm and purple yam tuber flour (PYF) was 7.70 ppm. Thus it can be concluded that all three tubers belonging to Dioscorea spp. all of them contain antioxidants with the order of the strength of antioxidant activity sequentially from the strongest to the weakest are PYF, APF and WYF.

Key words: antioxidant, Dioscorea spp., fermented flour, phytochemical.
1. Introduction

Tubers of Dioscorea spp. contain many compounds that have physiological activity that can act as antioxidants and are proven to have various health uses. Antioxidant compounds have a very important role in the health of the human body. Various scientific evidence show that antioxidant compounds reduce the risk of various chronic diseases such as cancer and coronary heart disease. The main character possessed by antioxidant compounds is their ability to capture free radicals [1].

Wild yam plants are tubers that fall into the category of food sources that contain carbohydrates and are not widely known by the public. People are more familiar with wild yam after being processed in the form of chips, whereas wild yam as one of the commodities has quite good prospects. This is because wild yam cultivation techniques do not require complex maintenance and can grow anywhere. Dioscorea spp group contain high carbohydrates. Therefore, wild yam is often used to be processed into flour as a basic ingredient in making crackers. As a source of carbohydrates, processed wild yam products have the potential to be developed and consumed, even though the carbohydrate content is lower than rice.

Exploration of new food sources based on local potential by preparing appropriate technology tools that can be applied by the community so far is still not optimal. The potential of local raw materials that are feasible to be developed at this time are tubers belonging to the dioscorea species. The use of tubers such as Kalimantan Dayak wild yam (Dioscorea hispida), purple yam (Dioscorea alata) and air potato (Dioscorea bulbifera) as food ingredients by the community is still very limited. Tubers included in the dioscorea species are local plants that contain high carbohydrates, reaching an average of 23.3% which is very potential to be used as a staple food for rice. The development of the use of dioscorea tubers is not yet developed due to the uncontrolled information related to its potential and excellence, and the technology of exploration and diversification has not been mastered into a quality product by the community.

One type of tuber included in the Dioscoreaceae family is the Dayak wild yam or more popularly known as wild yam which is also called bitter yam. Although poisonous, this tuber has the potential as a raw material for savory and delicious wild yam chips. Wild yam plants usually grow wild or as intercropping plants that are less treated. Some farmers plant wild yam as a side crop. In fact, if cultivated, this plant can produce large tubers and can be used as a variety of foods. Wild yam plants can be harvested after 6-12 months. Harvest time should be done when the dry season when the plants begin to die. At this time wild yam tubers have high starch content. Each plant can produce 6-12 kg of wild yam with a weight of one tuber can reach 5 kg. Meanwhile, wild yam is less cultivated, whereas from the nutrient content, wild yam has a high content of chromydrate and protein.

Then purple uwi (Dioscorea alata) is one of the potential tuber varieties as a source of non-rice carbohydrate food ingredients. Aside from being a non-rice food source, Diosorea alata is beneficial for health. Local purple varieties contain substances that are beneficial for health and other benefits that are not widely known by the public. Dioscorea alata has yellowish white tubers to dark blue. Yellow Uwi is also one of the uwi varieties included in the Dioscorea alata variety. The umbrellas are commonly called Uwi Menjangan, branching branches with a length of 35-60 cm, 7-10 cm thick. Purple Uwi is an alternative food substitute for carbohydrates that is healthy for consumption because there are contents that are useful for the body and also suitable for people with diabetes mellitus. Purple Uwi has the potential as a functional food ingredient because it has a low IG value. Besides that, purple uwi has functional components such as mucin, dioscin, allontin, cholin and essential amino acids [2].

Air potatoes (Dioscorea bulbifera L.) include the wild yam or Dioscorea tribes. Air potato tubers are similar to gembili bulbs but larger in size. Air potatoes contain lots of calories, minerals, such as iron, calcium and phosphorus [3]. Air potatoes growth in the armpit of a leaf (hanging) which grows on a tree trunk. Air potato tuber plants are still quite extensive in rural areas but their sustainability has begun to be threatened. Air potato tubers are usually boiled and have a chewy texture. One of the content of air potato tubers is diosgenin. Diosgenin belongs to
the group of saponins that have bitter taste [4]. Thin-skinned air potatoes with dark brown to young colors, greenish yellow flesh and low HCN [5].

2. Material and Method

Material and Equipment

The research main material used include ‘Dayak’ wild yam tubers (*Discorea hispida* Dennst), Purple Yam (*Dioscorea alata*) derived from Lempake, Samarinda, East Kalimantan and air potato (*Discorea bulbifera* L.) derived from Berau regency in East Kalimantan. While the supporting materials used include: ethanol, DPPH (1,1-diphenyl-2-picrylhydrazyl), ascorbic acid, bismut (III) nitrate, asetic acid, kalium iodida, HCl and deionized water. Whereas the equipment used consisted of: pan, oven cabinet, sieve of mesh 60, weighing bottle, analytical balance (Ohaus BSA 2245), desiccator, glass beaker 50 mL and 250 mL (Pyrex), reaction tube with its shelf, erlenmeyer 250 ml (Pyrex), vortex, spectrophotometer (Shimadzu), centrifuge (Macrocentriguge-sartorius sigma-2-16 Model KL-Germany), bottle centrifuge, autoclave (Hiratubera HL 36, Japan), shaker waterbath (Memmert D-91126, Germany) and other relevant tools.

Experiment Method

The study was carried out experimentally by means of descriptive data processing. The data obtained is calculated the average value and plotted in the form of tables and histograms to facilitate interpretation.

Making Ethanol Extract

Modified Dayak wild yam, purple yam and air potato tuber flour were extracted by ethanol with maceration method for 2 x 24 hours. The maceration results were filtered and concentrated using a rotary vacuum evaporator at 38ºC until all ethanol was evaporated and a thick extract was obtained.

Phytochemical Analysis

Phytochemical analysis was carried out qualitatively on color changes referring to Harborne [6] and Kokate [7] to examine the presence of active compounds which included:

a. Alkaloids (Kokate, 2001)

   Identification is carried out using Dragendorff's solution. The stages of making Dragendorff's solution are as follows:
   - Solution I: 0.5 g of bismuth (III) nitrate + 6 mL of acetic acid and 24 mL of distilled water.
   - Solution II: 12 g of potassium iodide + 30 ml of distilled water
   - Solution I + solution II (1 mL: 1 mL)
   - 1 mL of mixed solution plus 2 mL of acetic acid and 10 mL of distilled water, then the solution is ready for use.

   A total of 5 mL of extract was added with 2 mL of HCl, then 1 mL of Dragendorff's solution was added. The color change of the solution to orange or red indicates that the extract contains alkaloids.

b. Flavonoid [7]

   As much as 1 mL of plant extracts are given a few drops of dilute sodium hydroxide (1% NaOH). The appearance of a clear yellow color in the extract solution and becomes colorless after the addition of dilute acid (1% HCl) which indicates the presence of flavonoids.

c. Saponin [6]

   The test is carried out by inserting as much as 10 mL of hot water into a test tube containing 1 mL of test sample which has been dissolved in acetone. Then the solution is cooled and
shaken for 10 seconds. The formation of solid foam for about 10 minutes with a height of 1-10 cm and not lost when added 1 drop of HCl 2N indicates that the extract tested contains saponins.

d. Tannin [7]
The test was carried out by inserting 10 mL of the extract solution into a test tube and adding a solution of lead acetate (CH3COO)2Pb 1%. Tannins are positive if the yellow precipitate is formed.

e. Triterpenoid dan Steroid [6]
Identification was carried out using a mixture of acetic acid anhydride and concentrated sulfuric acid commonly known as Liebermann-Burehard reagent. In this test 10 drops of acetic acid anhydride and 2 drops of concentrated sulfuric acid were added sequentially into 1 mL of the test sample which had been dissolved in acetone. Then the test sample was shaken and left for a few minutes. The reaction is followed by a change in color, if it was seen red and purple in the colour, the test is positive for triterpenoids and if you look green and blue, the test is positive for steroids.

Antioxidant Activity Analysis
The method used in the study is DPPH method [10] with the following procedure:

a. Preparation of DPPH Solution: DPPH solution is made in such a way that it has a final concentration of 0.12 M. The method is 23.5 mg DPPH is weighed and put into 100 ml of flask. 50 mL of methanol is put into a measuring flask, then dissolved. 100 mL volumetric flask is measured by using methanol. DPPH solution has a concentration of 0.6 M. To get DPPH solution with a concentration of 0.12 M, the solution is diluted using methanol (1: 5 v/v).

b. Ascorbic Acid Stock Solution or Trolox Solution is made by 5 mg Trolox weighed and put into a 2 mL tube. 1 mL of methanol is added to the tube and Trolox is dissolved.

c. Following are the steps in carrying out the DPPH method:
1. Making a Standard Curve. The standard compound used is usually ascorbic acid or Trolox solution. For use of Trolox stock solutions, 50 microliters of Trolox stock solution is diluted to 950 microliters of methanol. From the solution (1 mM), a number of concentrations are prepared by diluting it in methanol. The Trolox solution concentration series consisted of 0, 24, 28, 32, 36 and 40 microMolar.
2. The sample solution was prepared with the concentration of DPPH inhibition percentage reaching a range of 20-80%.
3. A total of 100 microliters of samples or Trolox solution were mixed with 100 microliters of DPPH solution in a microplate then measured using a microplate reader (spectrophotometer) at a wavelength of 515 nm.
4. Reaction between DPPH and samples or Trolox can be carried out in a test tube with each volume that is 1: 1.
5. A mixture of sample / Trolox solution with DPPH solution is stored in a dark place at room temperature for 30 minutes.
6. After 30 minutes, the solution is measured using a spectrophotometer at a wavelength of 515 nm.
7. The antioxidant capacity of the sample was calculated as a DPPH radical percentage (% I) at a wavelength of 515 nm using the following formula:

\[
\% \text{ IC} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Information:
Ao = absorbance of DPPH in the control solution / methanol (blank) after 30 minutes.
A1 = absorbance of the sample / Trolox solution after incubation for 30 minutes.
3. Result and Discussion

Phytochemical Analysis Results

The results of phytochemical testing of modified flour of Dayak wild yam (*Dioscorea hispida* Dennts), purple yam (*Dioscorea alata* var. Purpurea) and Air Potatoes (*Dioscorea bulbifera*) can be seen in Table 1.

| Fermented Sample | Saponin | Alkoloid | Flavonoid | Tannin | Steroid/Triterpenoid | Karbohidrat |
|------------------|---------|----------|-----------|--------|----------------------|-------------|
| Wild yam flour    | -       | +        | -         | -      |                      | +           |
| Purple yam flour  | -       | +        | -         | +      | Tristerpenoid        | +           |
| Air potato flour  | -       | +        | -         | +      |                      | +           |

Information:

+: Samples contain phytochemical compounds

-: The sample does not contain phytochemical compounds

Based on Table 1 it can be seen that fermented purple yam tuber flour (PYF) contains more phytochemical compounds than modified air potato flour (APF) and modified Dayak wild yam flour (WYF) modified flour. Phytochemical analysis results in modified flour of *Dioscorea* spp. tubers showed the presence of alkaloids, tannins, triterpenoids and carbohydrates. This result is also in line with research from Rao and Pandey [8] who reported that the results of phytochemical screening of Tubers and Leaf extracts of *Sagittaria sagittifolia* L. were also positively containing glycosides, steroids, tannins, saponins, terpenoids, flavonoids, carbohydrates, alkaloids, and phenols.

Antioxidant Analysis Result

Modified flour of Dayak wild yam (*Dioscorea hispida* Dennts), purple yam (*Dioscorea alata* var. Purpurea) and Air Potato (*Dioscorea bulbifera*) studied all had antioxidant activity with different activity levels. The results of antioxidant activity testing (Figure 1) showed that the IC50 value of modified Dayak wild yam tuber flour (WYF) was 227.43 ppm, air potato tuber flour (APF) was 15.77 ppm and purple yam tuber flour (PYF) was 7.70 ppm. Whereas IC50 ascorbic acid (Vitamin C) was used as positive control in this study was 3.06 ppm. Thus it can be concluded that all three tubers belonging to *Dioscorea* spp. all of them contain antioxidants with the order of strength of antioxidant activity sequentially from the strongest to the weakest are PYF, APF and WYF.

![Fig 1. Antioxidant Activity of Modified Dioscorea Flour](image-url)
Based on IC$_{50}$ [9] that the levels of antioxidant activity obtained from the measurements of the three tuber samples can be classified into the following categories: concentrations <50 ppm have strong antioxidant activity, concentrations of 50-100 ppm have active antioxidant activity, concentrations of 100-250 have moderate antioxidant activity, concentrations of 250-500 ppm have weak antioxidant activity, and concentrations> 500 ppm do not have antioxidant activity. Therefore, based on the categorization, PYF and APF are included in the category of having strong antioxidant activity, while WYF is included in the category of moderate levels of antioxidants.

4. Conclusion
Fermented flour of Dioscorea spp. tubers showed the phytochemical component presence of alkaloids, tannins, triterpenoids and carbohydrates. While the antioxidant strength showed that the IC50 value of the fermented Dayak tuber flour (WYF) was classified as medium level (227.43 ppm), while the air potato air tuber flour (APF) and purple yam flour (PYF) were included in strong category which were 15.77 ppm and was 7.70 ppm respectively.

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