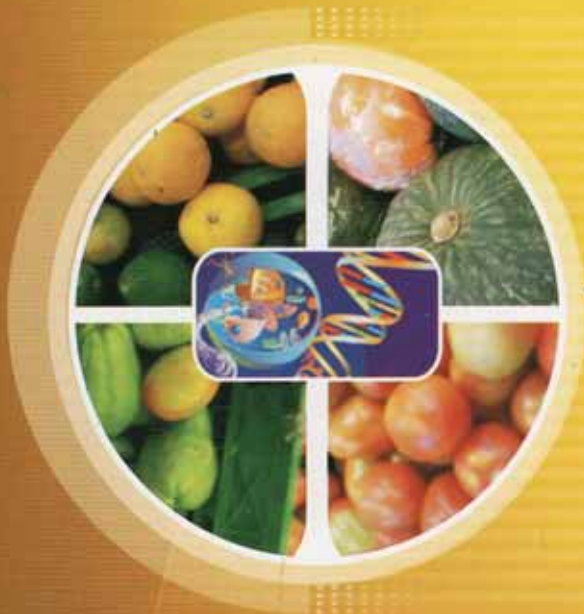




UNIVERSITAS
ATMA JAYA YOGYAKARTA
Fakultas Teknobiologi



PROCEEDING



1st International Seminar on
**“Natural Resources Biotechnology:
From Local to Global”**

September 8th – 9th 2015
Faculty of Biotechnology
Universitas Atma Jaya Yogyakarta

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Welcome Speech Chair of the Seminar Committee

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

It is a great pleasure to welcome all of you to the International Seminar "Natural Resources: From Local to Global". The Faculty of Biotechnology of Universitas Atma Jaya Yogyakarta runs this seminar to commemorate the 50th Anniversary of the Universitas Atma Jaya Anniversary and the 25th Anniversary of the Faculty of Biotechnology. Your presence is your present for the anniversary of our university and faculty as well.

The Anniversary is not the only reason to run this seminar. A greater reason is behind the seminar. Indonesia is rich in biodiversity. It is a challenge for us, as scientist, to maintain the biodiversity and to develop the potential of the biodiversity for the common good. Through this seminar, the scientific research on Indonesian biodiversity can be shared and probably the finding of the new research can inspire us for further exploration. Therefore, the seminars goal is to facilitate the spread of the research on local potential of biodiversity to the global level. Hopefully, it can attract more researchers to explore the wealth of local biodiversity.

The committee invites speakers who are expertise in the research concerning biodiversity. Our invited speakers are Assoc. Prof. Dr. Michael Murkovic from Graz University of Technology Austria (food scientist), Assoc. Prof. Worawidh Wajjwalku from Kasetsart University Bangkok Thailand (Veterinary disease biotechnology), Dr. Kathryn McMahon from Edith Cowan University Australia (Seagrass biotechnology), Prof. Marco Nemesio E. Montano, PhD from University of the Philippines (Seaweed biotechnology), Prof. Jun Kawabata from Hokkaido University Japan (food biochemist), Endang Semiarti, PhD from Universitas Gadjah Mada, Indonesia (Plant biotechnology), Ign. Pramana Yudha, PhD from Universitas Atma Jaya Yogyakarta (Conservation genetics), Dr Machmud Thohari from Technical Team for Environmental Biosafety, Ministry of Environment & Forestry Indonesia (Environmental Biosafety), Dr Harvey Glick from Asia Regulatory Policy & Scientific Affairs Monsanto Company (Regulatory Policy & Scientific Affairs Monsanto). It is a good opportunity to learn from the speakers to enhance and to update our knowledge. I hope this seminar is of benefit to all of us.

In conclusion, I wish you a successful seminar and a pleasant stay in Yogyakarta.

With kind regard
Coordinator of conference program

Dr. rer. nat. Yuliana Reni Swasti, S.TP., MP.

**WELCOME SPEECH
DEAN
FACULTY OF BIOTECHNOLOGY
UNIVERSITAS ATMA JAYA YOGYAKARTA**

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

On behalf of the Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta and the Committee of the International Seminar, I would like to first of all to extend our heart-felt thanks for your presence at this Seminar. This seminar is so significant in a sense that it focuses on natural resources with local content but by utilizing biotechnology they will become global and worldwide products and services as well.

Biotechnology has been developed very rapidly and it is believed to be "a new wave in the economic world". Biotechnology has contributed in all aspects of humans' life, such as food production, health, industry, environment, etc. The role of biotechnology for the betterment of human beings, however, is still need to be improved. Indonesia, with its huge biodiversity, has a potency to develop and applied biotechnology nationwide.

The role of biotechnology has increased rapidly. Many are believed that biotechnology has become an integral part of modern industries with high economic values. On the other hand, it needs to be closely managed in order to avoid its negative impacts. There are some examples of negative impacts with relate to biotechnology application, such as intellectual property rights, genetically modified organisms (GMOs), environmental degradations, biodiversity issues, indigenous people knowledge, biosafety, etc.

The Seminar covers topics such as: Functional Foods, Food Biotechnology, Biopharmacy, Health/Medical Biotechnology, Environmental Biotechnology, Legal Aspect of Biotechnology, Bioinformatics, and Social-Economic Aspects of Biotechnology. This Seminar will be presented by nine (9) invited speakers with different topics and expertise. There will be some papers and posters to be presented also in this Seminar from some participants from the Philippines and Indonesia.

Henceforth, in commemorating its 50th anniversary Universitas Atma Jaya Yogyakarta (UAJY) and 25th anniversary of Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta (UAJY) on September 2015, it is worthy and appropriate to explore the newest innovations in the field of research and development of biotechnology to be applied in many aspects for the betterment of human beings. The Seminar takes this opportunity to discuss and hopefully find ways to solve problems faced by human beings in the world.

I would like to take this opportunity to express my sincere thanks and gratitude to the Committee and in particular to the honorable speakers. Before closing this remarks, allow me to ask the Rector of Universitas Atma Jaya Yogyakarta to open this Seminar officially.

Finally, this is an opportune time for me to wish you all in the two (2) fruitful days of interesting and beneficial programs and hope you have a pleasant stay in Yogyakarta.

Thank you very much and may God bless us all. Amen.

Yogyakarta, 8 September 2015

Dean

Drs. B. Boy Rahardjo Sidharta, M.Sc

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The Study of Bioactive Compound Lesser Yam (*Dioscorea esculenta*), Wild Yam (*Dioscorea hispida*), and Arrowroot (*Maranta arundinacea*) Tubers as Source of Antioxidants

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Abstract

Indonesia is currently facing the problem of increasing the prevalence of degenerative diseases. It caused a decline in the health status of society. One of the causes of degenerative diseases is a free radical, which is a compound having unpaired electrons that are highly reactive. It snatches electrons from other molecules around it to stabilize them. An increase in free radicals causing oxidative stress and lead to dysregulation of adipose tissue as an early pathophysiology of degenerative diseases such as hypertension, atherosclerosis, coronary heart disease, stroke, diabetes and other vascular diseases. Free radicals can be resisted by antioxidants. Identification of potential bioactive compounds obtained from local tubers such as lesser yam, gadung, and arrowroot that have the physiological effect as an antioxidant, and will be developed as a functional food through nutrigenomics approach. This study was an exploratory study to identify the content of the antioxidant compound on local tubers such as lesser yam, gadung, and arrowroot obtained in the area of Gunungpati, Semarang. The study was conducted in the laboratory of Biochemistry, Department of Biology, Unnes in April-July 2015. The materials needed were gembili, gadung and garut tubers as well as chemicals and equipment necessary for the analysis of total phenol and antioxidant activity. The antioxidants analysis was done descriptively. The results of phenol total on lesser yam was 0,8865%, whereas arrowroot was 1,8959% and wild yam was 2,7132%. While antioxidant activity test by DPPH methods were 21.2422%, 20.2845% and 19.8476%, respectively. Lesser yam, arrowroot and wild yam tubers are potential as a functional food to be applied as an antioxidant

Keywords: tubers, antioxidants, functional food

1. INTRODUCTION

Recently, the rapid development of science impacts the increasing of industrialization, urbanization, development, free markets, and economic and social welfare. It is also impact on the lifestyle changes i.e. low frequency of exercising, a diet that high in calorie, and low-fiber food consumption. This condition is significantly influence the health and nutritional status of people, particularly in developing countries, including Indonesia. Diet is a major cause of obesity. Modern humans tend to be busy with a variety of life activities until could no longer eat healthy and nutritious foods. Instant

and junk food are getting popular for most people who are exposed to modern life. Obesity is happening followed by increasing of fat metabolism would lead to the production of Reactive Oxygen Species (ROS) and free radicals, both in circulation and in adipose cells. Free radicals (ROS) are defined as the atom/ molecule/ compound containing one or more unpaired electrons (Fang *et al.*, 2002). In chemistry, the unpaired molecules of free radicals tend to react with a molecule of body cells. Then cause abnormal compounds (free radicals new, more reactive) and start a chain reaction that cause cell damage (Winarsi, 2007).

Increased of ROS in adipose cells could cause the balance of oxidation-reduction reactions (redox) is disturbed, resulting in decreased of the antioxidant enzyme in the circulation. This situation called oxidative stress (Fang *et al.*, 2002). The increasing of oxidative stress is causing the dis-regulation of adipose tissue as well as an early pathophysiology of degenerative diseases such as hypertension and atherosclerosis, diabetes mellitus, cancer, hyper-lipid with sickness "derivatives" such as coronary heart disease (CHD), stroke, failed kidneys, arthritis, Alzheimer and Parkinson (Furukawa *et al.*, 2004; Hernani, 2005; Yunanto *et al.*, 2009).

Indonesia is currently facing more nutritional problems such as obesity, vascular disease (coronary heart disease and atherosclerosis), diabetes mellitus and cancer, or commonly known as degenerative diseases. Degenerative diseases triggered by unhealthy eating patterns, causing obesity and the increase of free radicals (ROS) and oxidative stress (Caves and Munos, 2003).

In fact, human need to get antioxidants from diet. It produces by the body to experience a decline in circulation as a result of competition with free radicals. Antioxidants from the outside can be obtained from fruits and vegetables, or other foods that contain antioxidants, known as exogenous antioxidants. Various studies and studies of antioxidants have been widely performed as the content of the antioxidant compounds in seaweed (Olsen *et al.*, 2013; Namvar *et al.*, 2013; Fiedor and Burda 2014), honey (Bohdanov *et al.*, 2008), antioxidants red fruit (Tjahjani and Khiong, 2010).

The study in antioxidants is still needed to be done in the view of great benefits to health. The study on the local sources of antioxidants such as local tubers i.e. lesser yam, wild yam, arrowroot is potential. Those tubers are containing with bioactive compounds such as antioxidant, anthocyanin, dioscorin, diosgenin, and phenol (Mar'atirosyidah and Teti, 2015). As one of the efforts to optimize the use of natural sources of Indonesia as well as improving public health, it is necessary to study to explore bioactive compounds that have physiological effects as antioxidants in the local tubers.

Tubers group can be regarded as a functional food because it contains one or more compounds that have a specific physiological function and health benefits. When the bioactive compounds in the tubers are either directly or indirectly affect the human genome, which in its action can change the expression of the gene structure which called asnutria-genomics (Muller and Kersten, 2003).

2. MATERIALS AND METHODS

2.1 Materials

The raw materials used in this study included lesser yam, wild yam, and arrowroot

obtained from the Gunungpati, Semarang, Central Java with a shelf life of no more than seven days after harvesting. The chemicals were water, NaCl, sodium metabisulfite, methanol, alcohol, acetone, distilled, concentrated HCl, solution Buffer KCl, Na-acetate buffer solution ($\text{CH}_3\text{CO}_2\text{Na}\cdot 3\text{H}_2\text{O}$), petroleum ether, ethanol 98% of Bratacho chemical, Folin Ciocalteu phenol from Merck, gallic acid from Sigma, sodium carbonate from Merck, aluminum foil (Klin pack), radical DPPH (2,2-diphenyl-1-picryldihydrazil radical) from Sigma.

2.2. Methods

2.2.1 Preparation and Treatment of Materials

Lesser yam, wild yam and arrowroot flour was prepared in the following manner: the tubers were sorted, peeled, washed, sliced with a thickness of ± 0.2 cm and soaked in water with the addition of sodium metabisulphite for ± 5 minutes. Slices of each tuber then drained and dried in the sun for ± 2 days to dry, milled and sieved using a sieve of 80 meshes. The next flour packed in plastic and stored at room temperature.

2.2.2 Estimation of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method (Lachman *et al.*, 2000). Samples (100 μL) were mixed thoroughly with 2 ml of 2% Na_2CO_3 . After 2 min. 100 mL of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 750 nm against a blank. Total phenolic content was expressed as grams of gallic equivalents per 100 grams of dry weight (100g g-1DW) of the plant samples (Ruba and Mohan, 2013; Therasin and Baker, 2009).

2.2.3 Determination of antioxidant activity with DPPH (1,1-Diphenyl-2 Picrylhidrazyl)

Sample extract of tubers with a concentration of 20,000 ppm was taken as 2 ml, and then it poured into a test tube. DPPH solution was made by 7.5765×10^{-5} mol/l in ethanol, and then it was taken for 1 ml and was added by 3 ml of distilled water. The absorbance was measured at 516 nm wavelength and must obtained absorbance at 0.8. In order to measure the sample absorbance, as much as 1 ml sample of the antioxidant was added by 3 ml of DPPH solution. The mixture was measured at a wavelength of 516 nm for 20 minutes. Determination of DPPH radical capturing capability was measured against standard curve of Gallic acid (0, 50, 100, 250, 300 ppm).

3. RESULTS AND DISCUSSION

The results of the percentage of total phenol content was highest in the wild yam (*Dioscorea hispida*) (2.7132%), followed by arrowroot (*Maranta arundinacea*) (1.8959%), and lesser yam (*Dioscorea esculenta*) (0.8865%). In determining the levels of phenolic compounds, total gallic acid used as a standard solution. Gallic acid obtained maximum absorption at a wavelength of 750 nm. In the beginning, the standard curve of Gallic acid was made as a standard level to determine the levels of total phenolic compound. Making the standard curve was useful to help determine the levels of phenol in the sample through a regression equation of the standard

curve. Based on the standard curve, the regression equation $Y = 0,0021x + 0.733$ was obtained. The correlation coefficient (R^2) = 0.9926. The value of R^2 value proved that the regression equation was linear. Tables and Gallic acid standard curve can be seen in Table 1 and Figure 1.

The concentration of the sample solution can be determined by using a calibration curve by measuring the absorbance of samples, and then the total phenolic content in the tubers was calculated using linear regression equation. Total phenolic content of the ethanol extract of the tuber lesser yam (*Dioscorea esculenta*), arrowroot (*Maranta arundinacea*) and Wild Yam (*Dioscorea hispida*) are presented in Table 2.

Table 1. Results of measuring the absorbance of standard solution of Gallic acid at a wavelength of 750 nm using a spectrophotometer

No	Concentration (mg/L)	Absorbance
1	0	0,7328
2	100	0,8953
3	150	1,1021
4	250	1,2961
5	300	1,3819

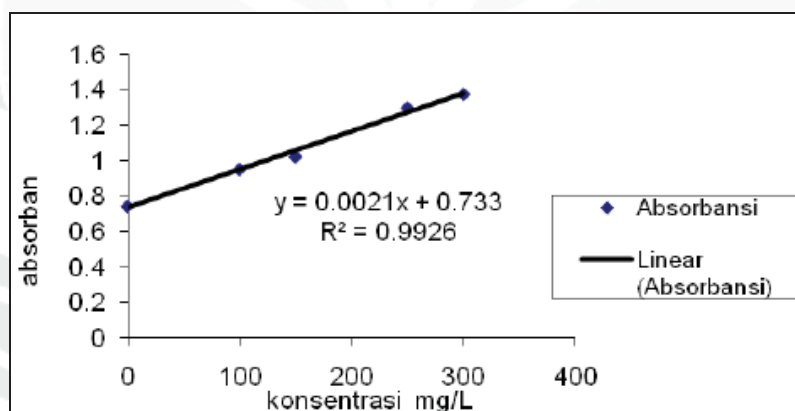


Figure 1. Calibration curve of Gallic acid in a solution of phenol at a wavelength of 750 nm

Table 2. Percentage Content of phenols several types of tubers

No	Tubers	Mean of Absorbance	Fenol concentration (%)
1	Lesser yam (<i>Dioscorea esculenta</i>)	0,8952	0,8865
2	Arrowroot (<i>Maranta arundinacea</i>)	0,9123	1,8959
3	Wild yam (<i>Dioscorea hispida</i>)	1,2865	2,7132

3.1 Radical capturing activity with DPPH (1,1-Diphenyl-2-Picrylhidrazyl)

The ability to capture free radicals is another term for the activity of antiradical compound. This activity is measured by the value of DPPH. The ability to capture the highest percentage of radicals contained in lesser yam (*Dioscorea esculenta*) was 21.2422%, arrowroot (*Maranta arundinacea*) was 20.2845% and the lowest was wild yam (*Dioscorea hispida*) 19.8476%. This condition indicates that the activity was not affected by the antiradical polyphenol compounds contained. However, high amount of polyphenol compounds did not affect the activity.

The method used in part of testing of the antioxidant activity was DPPH radical absorbance method. This method is simple, easy, and requires only samples in small amounts, a short time (Hanani *et al.*, 2005). In the determination of the ability to capture radicals, Gallic acid was used as a standard solution. Gallic acid obtained maximum absorption at a wavelength of 516 nm. Before the determination of ability to capture radicals, the standard curve of Gallic acid was made. The standard curve was useful to help to determine the ability to capture radicals in the sample through linear regression equation of the calibration curve. From the determination of the standard solution of Gallic acid calibration curve obtained by regression equation $Y = 0,0014x + 2.4078$ and correlation coefficient (R^2) = 0.9819. Table and figure of Gallic acid standard curve can be seen in Table 3 and Figure 2. The concentration of the sample solution can be determined by using a calibration curve by measuring the absorbance of samples, and then the percentage of radical capture capabilities was calculated using linear regression equation. Percentage ability to capture radicals in the ethanol extract of tubers lesser yam (*Dioscorea esculenta*), arrowroot (*Maranta arundinacea*) and wild yam (*Dioscorea hispida*) are presented in Table 4.

Table 3. The results of the absorbance measurement of a standard solution of DPPH at 516nm using a spectrophotometer

No	Concentration (mg/L)	Absorbance
1	50	2,1642
2	100	2,2604
3	250	2,1437
4	300	2,0271

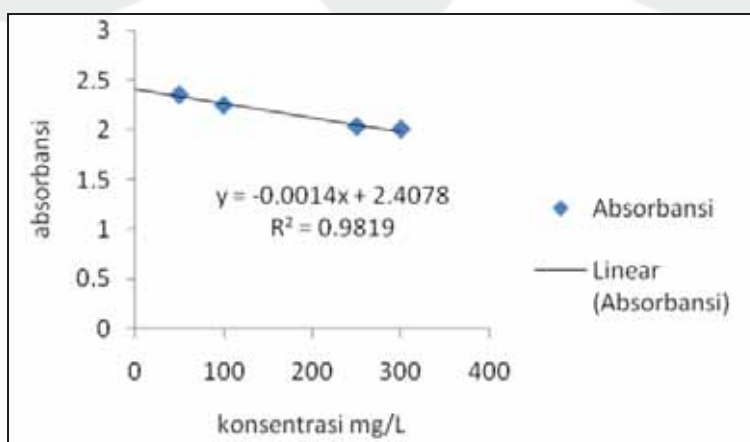


Figure 2. Calibration curves DPPH gallic acid in solution at a wavelength of 516 nm

Table 4. Percentage of antioxidant activity of several types of tubers

No	Tubers	Mean of absorbance	Anti free radicals activity (%)
1	<i>Lesser yam (Dioscorea esculenta)</i>	0,8952	21,2422
2	<i>Arrowroot (Maranta arundinacea)</i>	0,9123	20,2845
3	<i>Wild yam (Dioscorea hispida)</i>	1,2865	19,8476

On a sample of tubers after DPPH solution added, it was resulting in a color change in the solution of DPPH in ethanol, which was originally colored dark violet to yellow. This was in accordance with Andayani *et al.*, (2008), the measurement of the antioxidant activity of the sample performed at a wavelength of 516 nm which is the wavelength of maximum DPPH. The presence of the antioxidant activity of the sample was proved by a color change. DPPH is a free radical that is stable at room temperature and is often used to evaluate the antioxidant activity of several compounds or extracts of natural ingredients. DPPH accept electrons or hydrogen radicals will form a stable diamagnetic molecule. DPPH antioxidant interaction with either the transfer of electrons or hydrogen radicals on DPPH will neutralize free radicals of DPPH character. If all the electrons in the free radical DPPH are into pairs, then the color of the solution changed from dark purple to yellow light and the absorbance at 517 nm wavelength will be lost. These changes can be measured in accordance with the stoichiometric amount of electrons or hydrogen atoms captured by DPPH molecules due to antioxidants (Gurav *et al.*, 2007).

4. CONCLUSIONS

Phenol content and antioxidant activity of tubers in this study can be concluded as follows:

1. The percentage of the highest content of phenolic compounds found in wild yam (2.7132%), arrowroot (1.8959%), and lowest was found in the lesser yam (0.8865%).
2. Antiradical activity was highest in lesser yam (21.2422%), followed by arrowroot (20.2845%), and was lowest in wild yam (19.8476%).

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