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Chemical Compositions, Extraction and Phytochemical *Cyperus* sp Plant

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Abstract

Research on the chemical composition, extraction, and phytochemicals Plant *Cyperus* sp has been done. *Cyperus* sp is the best plants that who can be used to review biondikator heavy metal contamination. The Objective study present were to determine the chemical composition value, extraction bioactive compound, and phytochemical plant *Cyperus* sp. Research study using AOAC method (proximate), single extraction (maseration) with methanol solvent, and phytochemicals qualitative with harbone method. Results study showed that the chemical composition composed were moisture content (82.8%), ash (1.58%), protein (0.03%), fat 0.09% and 15.4% carbohydrate. rendement extract with metanol (12.30%). The phytochemical test results Cyperus sp conducted to indicate content of alkaloids, steroids, phenols hydroquinone, carbohydrates, and reducing sugars form of monosaccharides. Result phytochemical test indicated to antioxidant activty.

Keywords

Chemical Compotision, *Cyperus* sp, Drugs, Exstraction, Phytochemical

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1. Introduction

Cyperus sp. is one of the aquatic plants were found growing in both environments lentic and lotic (Kaggwa et al. 2001). This plant can grow to a height of 5 m consisting of stems and leaves, for example bracteoles with closed canopy structure (Jones and Muthuri 1997). According to Denny et al. (1995) Cyperus sp. has a sizable ecological functions in aquatic ecosystems that are useful in preventing heavy metal pollution. Currently Cyperus sp. special attention to be directed in the control of water pollution and treatment (Kansiime & Van Bruggen, 2001).

The content of the active chemical compounds found in plants are alkaloids, flavonoids, terpenoida, steroid, tannins and saponins which can be known by phytochemical screening (Ahmad, 2006). water plants that have been widely studied regarding its bioactive components ie watercress (Salama et al., 2011), Genjer (Nurjanah[a] et al. 2014), water spinach (Nurjanah[b] et al. 2014). Cyperus sp has the potential beneficial that the need for information related to the physiological properties and characteristics. Based on the above it is necessary to study on the active ingredient compound with the proximate analysis and phytochemical test of Cyperus sp. as the basis of the development of research on the potential aquatic plants. This purpose study was to determine the chemical composition, Extraction, and phytochemical plant Cyperus sp.

2. Material and Methods

2.1. Materials and Tools

The main material used in this study is a water plant *Cyperus* sp. Other ingredients are ingredients used in the proximate analysis and phytochemical test. The materials used in the proximate analysis is distilled water, H_2SO_4, H_2O_2, selenium catalysts, boric acid (H_3BO_3), indicators bromcherosol green

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and methyl red, NaOH-$Na_2S_2O_3$, HCl, and n-hexane. The materials used in the extraction process and phytochemical test is methanol, reagent Dragendorff reagent Meyer, reagents Wagner, chloroform, anhydride acetic, sulfuric acid, amyl alcohol, alcohol, hot water, HCl 2N, 70% ethanol, a solution of $FeCl_3$ 5%, reagent Molish, Benedict reagent, Biuret reagent, Ninhydrin 0.1% solution.

The tools used in the proximate analysis is a porcelain cup, gege, scales, ovens, electric stoves, furnaces, incineration, desiccator, Kjeldahl flask, distillation equipment, erlenmeyer, burettes, filter paper, cotton fat-free, and the soxhlet. The tools used in the extraction of the sample and test phytochemical that is erlenmeyer, shaker, filter paper, evaporator, glass funnel, beaker, beakers, test tubes, spatula, electric stove, tweezers, pipette, and scales.

2.2. Methods

2.2.1. Chemical Compositions

chemical compotision with proximat methods are an analysis conducted to predict the chemical composition of a substance, including the analysis of moisture, ash, protein and fat

2.2.2. Mositure Content (AOAC 2005)

Analysis of moisture content is based on sample weight difference before and after drying. Empty porcelain cup dried in an oven for 1 hour at 105°C, then cooled in a desiccator for 15 minutes and then weighed. A sample of 5 grams weighed and put into the cup, then put in the oven with a temperature of 105°C for 5 hours or until its weight is constant. Then the cup is inserted into the desiccator to cool and then weighed again. The percentage of moisture content can be calculated by using the formula:

$$\text{mositure content (\%)} = \frac{B-C}{B-A} \text{ x } 100\%$$

Keterangan:

A = Weight empty porcelain cup (gram)

B = Weight porcelain with sample (gram) before oven process

C = Weight porcelain with sample (gram) after oven process

2.2.3. Ash Content (AOAC 2005)

Analysis of ash carried by cremate the sample in the furnace. Empty porcelain cup dried in an oven for 1 hour at a temperature of 105°C, then cooled in a desiccator and weighed. A sample of 5 grams weighed and put into the cup, and then burned using an electric stove to not smoke anymore. The plate was then inserted into a furnace to a temperature of 600°C for 1 hour to obtain a white ash-gray. After that, the cup along with a sample cooled in a desiccator to cool and weighed. The percentage of ash content can be calculated using the formula:

$$\text{ash content (\%)} = \frac{C-A}{B-A} \text{ x } 100\%$$

Keterangan:

A = weight empty porcelain (gram)

B = weight porcelain with sample (gram) before tanur process

C = weight porcelain with sample (gram) after tanur process

2.2.4. Protein Content (AOAC 2005)

The principle of the analysis of proteins, namely to determine the crude protein content (crude protein) in a material. The stages are carried out in the analysis of proteins consists of three stages, namely the destruction, distillation, and titration. Measurements of protein made by the Kjeldahl method. The sample is weighed as much as 1 gram, then put into 50 ml Kjeldahl flask, then the catalyst is added selenium (half tablet), 10 ml of concentrated H_2SO_4, and 10 ml of H_2O_2 was added slowly into the flask and allowed to stand 10 minutes under the hood. The tube containing the solution was put in a heating device (didestruksi) at a temperature of 410°C for approximately 2 hours or until the solution became clear green. Pumpkin Kjeldahl washed with distilled water to 75 ml., And then inserted into the distillation equipment.

Distilled accommodated in 125 ml Erlenmeyer containing 25 ml of boric acid (H_3BO_3) 4% containing bromcherosol green indicator methyl red 0.10% and 0.10% with a ratio of 2: 1. Distillation has done by adding 50 ml NaOH-$Na_2S_2O_3$ into distillation equipment to be accommodated 100-150 ml of distillate in distillate erlenmeyer with green outcomes. Then distillate titrated with 0.1 N HCl until the pink color changes occur the first time. The protein content calculated using the formula:

$$\text{Nitrogen (\%)} = \frac{(\text{ml HCl} - \text{ml blanko}) \text{x N HCl x } 14{,}007 \text{ x } 10}{\text{mg sampel}}$$

% protein =% Nitrogen x conversition factor (6, 25)

2.2.5. Fat Content (AOAC 2005)

A sample of 5 grams weighed and put in a filter paper and then both ends covered with fat-free cotton. Once the sample is introduced into a fat pumpkin is dried and weighed, then connected with Soxhlet tube. Fatty sheath inserted into the space soxhlet extractor tube. Fat solvent (n-hexane) was poured into the flask fat taste. The process is done reflux for 6 hours until the solvent drops back into a pumpkin colored clear fat. Solvent present in the fat flask was distilled until all the fat solvent evaporated. Furthermore, fat flask containing the extracted fat was heated in an oven at 105°C, after it was

put in a desiccator until its weight is constant. Furthermore, along with pumpkin weighed fat and fat content calculated using the formula:

$$\text{fat (\%)} = \frac{W3-W2}{W1} \text{ x } 100\%$$

Keterangan:

W1 = weight sample (gram)

W2 = weight pumpkin without fat (gram)

W3 = weight pumpkin with fat (gram)

2.2.6. Extraction Bioactive Compound

his phase consists of several steps, the sample preparation and extraction of active ingredients. At this stage of sample preparation, Cyprus which had been taken from the village Cikarawang, Dramaga, Bogor prepared and then carried steaming. Fresh Cyprus and Cyprus who have experienced steaming, sun-dried. Cyprus which has been dried then crushed with a blender in order to get a smooth texture. The next stage is the extraction of active ingredients. The extraction method used is a single extraction method (Quinn 1988). Solvents used in this study is 96% ethanol.

Samples Cyprus which have weighed as much as 20 grams of crushed and macerated with ethanol 96% as much as 100 mL during 24 jam. Hasil maceration of the solution was then filtered with filter paper Whattman 42 thus obtained and residu. Filtrat filtrate obtained is evaporated until solvent separating the extract using a rotary vacuum evaporator at 50°C.

2.2.7. Phytochemical Assay (Harborne 1984)

Phytochemical test is performed to determine whether there is any bioactive components contained in the crude extract cuttlefish which have antioxidant activity. Before the phytochemical test, carried out the extraction process to obtain crude extract samples. Extraction is done is simple extraction (maceration), wherein soaking the samples in solvents with or without stirring. A total of 50 grams of sample immersed in methanol with a comparison between the samples with methanol of 1: 4. Samples were stirred for 48 hours using a shaker and then filtered. The filtrate is then evaporated at a temperature of 50°C using an evaporator. After evaporation process ends obtained crude extract samples followed by phytochemical test. Phytochemical test include tests alkaloids, steroid test / triterpenoids, flavonoids, saponins, phenolic hydroquinone, molisch, benedict, biuret and ninhydrin. This test method is based on Harborne (1984).

I. Alkaloid

ome sample is dissolved in a few drops of sulfuric acid 2N and then each tested with three reagent alkaloid that is, Dragendorff reagents, reagent Meyer, and reagents Wagner. The test results confirmed positive when the precipitation reagent Meyer yellowish white, with a brown sludge and sediment Wagner reagent red to orange with Dragendorff reagent.

II. Steroid/ Triterpenoid

A number of samples were dissolved in 2 ml of chloroform in a dry test tube. Then, to which was added 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid. The formation of a solution of the red for the first time and then turns blue and green indicates a positive reaction.

III. Flavonoid

Some sample added 0.1 mg of magnesium powder and 0.4 ml of amyl alcohol (a mixture of hydrochloric acid 37% and 95% ethanol with the same volume) and 4 ml of alcohol and then the mixture was shaken. The formation of red, yellow or orange in the lining of amyl alcohol showed flavonoids.

IV.4 Saponin

Saponins can be detected by testing the foam in hot water. A total of 1 gram sample was added hot water and then shaken. Stable foam for 30 minutes and did not disappear on the addition of 1 drop of 2 N HCl showed saponin.

V Fenol Hidrokuinon

A total of 1 g sample is extracted with 20 ml of 70% ethanol. The resulting solution is taken as 1 mL was then added 2 drops of 5% $FeCl_3$ solution. The formation of green or blue green color indicates the phenol compound in the ingredients.

VI. Molisch

A total of 1 mL of sample solution were given 2 drops of reagent Molish and 1 mL of concentrated sulfuric acid through the tube wall. Positive test indicates the formation of complex carbohydrates marked purple fluid between the two layers.

VII. Benedict

8 drops of sample solution put into 5 ml reagent Benedict. The mixture is shaken and boiled for 5 minutes. The formation of green, yellow, or brick red precipitate showed a reducing sugar.

VIII. Biuret

A total of 1 mL of sample was added 4 ml Biuret reagent. The mixture is shaken thoroughly. The formation of a purple solution of test results positive for the presence of peptide.

X. Uji Ninhidrin

Sample 2 mL was added with a few drops of Ninhydrin 0.1%. The mixture was heated in a water bath for 10 minutes. The occurrence of a blue colored solution showed a positive reaction for the presence of amino acids.

3. Result and Discussion

3.1. Chemical Composition

The chemical composition of the water plant Cyperus sp. obtained through the proximate analysis that includes moisture, ash, protein, fat, and carbohydrates. Carbohydrate levels obtained through calculation by difference (100% - water - gray - fat - protein). The results of proximate analysis Cyperus sp. can be seen in Table 1. The water content is the amount of water contained in a material. The water content in the aquatic product is estimated 70-80%. The water content in foodstuffs consists of two forms, namely water-free and bound water. Free water is water that is contained in the space between cells and plasma, can dissolve vitamins and mineral salts, and is often used by microbes for growth. Water Bound water molecules bound to other molecules, such as proteins (Winarno, 2008).

Table 1. Chemical compositions *Cyperus* sp.

Chemical composition	*Cyprus* sp (%)	Genjer* (%)	Kale water** (%)
Moisture	82.810%	93.92	90
Ash	1.585%	0.70	0.5
Protein	0.030%	2.38	2.35
Fat	0.099%	0.20	0.5
carbohydrate	15.476%	2.70	6.65

note: * Nurjanah[a] *et al*. 2014 ** Nurjanah[b] *et al* 2014

Analysis of water content aims to determine the amount of water contained in Cyperus sp. The measurement results show that the water content of Cyperus sp. have a high water content, which is 82.8%. The water content measured in this study is the evaporative water and does not bind strongly to tissue material with the aid of heat. Evaporative water is called free water and the water just physically bound in the tissue matrix material that is membrane, capillaries, fibers, and so forth. This water can be utilized for a medium for microbial growth and chemical reactions (Winarno, 2008). Previous research on water spinach (*Ipomoea aquatica*) showed a higher water content, which is 90.00% (Nurjanah[b] et al. 2014).

The ash is inorganic substances remainder a result of burning organic material. Foodstuffs consists of 96% organic material and water. The remainder consists of mineral elements, also known as inorganic substances or ash content. The process of burning, burning organic components, but inorganic components are not, therefore called ash (Winarno, 2008). The results of the analysis show that the total ash content Cyperus sp. containing ash content is 1.58%. High and low ash content can be caused by differences in habitat and the environment. Each marine environment can provide mineral intake is different for aquatic organisms that live in it. In addition, each individual organism also has a different ability to regulate and absorb minerals into the body, so it will be an impact on the value of the ash content in each material (Susanto, 2010).

Protein is the main component in living cells, both plants and animals. Protein is the largest component after the water in most tissues of the body. However, the results of the analysis of protein content Cyperus sp. low, amounting to 0,030%. This is because the protein levels in plants in general have a lower quality than animal protein levels (Winarno, 2008). Previous research on water spinach (Ipomoea aquatica) also have low levels of the protein, which is 2.35% (Nurjanah[b] et al. 2014).

Fat is a component that is soluble in organic solvents, for example hexane, ether and chloroform. Animal fats are generally solid form at room temperature, whereas fats are derived from plants in the form of a liquid. The results of the analysis of fat content Cyperus sp. ie 0,09%. The water content is generally inversely related to levels of fat (Yunizal et al., 1998). Cyperus sp. have a high water content, so that the fat content may be lower. Besides water spinach (Ipomoea aquatica) also has a fat content is quite low, ie 0.5% (Nurjanahb et al. 2014). Plant is not a source of protein and fat, therefore the levels of protein and plant fats also lower than the animals.

Carbohydrates are the main source of calories consumed more frequently than protein and fat. Carbohydrate calories as much as 4.2 calories per 1 gram (Ketaren, 2008). The result of the calculation of carbohydrate content by the method by difference showed that Cyperus sp. contains carbohydrates large enough, ie 15.47%. The results of carbohydrate counting method by difference is a method of determining the carbohydrate content in food roughly, the raw fiber are also counted as carbohydrates (Winarno, 2008).

3.2. Extraction Component Bioactive

Extraction is the process of withdrawal of the active substance component of a material by using certain solvents. The purpose of this process is to obtain certain parts of the material containing the active components (Harborne 1984).

According to Ansel (1989) and Winarno et al. 1997, the extraction can be done in two ways, namely phase cairdan organic phase. How the liquid phase is done by using water, while the way the organic phase is done by using an organic solvent. Based on the principle, the extraction process can take place if there are similarities in the nature of polarity between the compound extracted with a solvent compounds. A substance has dissolved different abilities in different solvents. This suggests an interaction between substances soluble in the solvent. Polar compounds will be soluble in polar solvents as well, and vice versa.

Important properties that must be considered in the selection of the solvent is a polar compound seen from the polar group (such as OH groups, COOH, 7 and so forth). It is to be considered in the selection of the solvent is selectivity, the ability to extract, toxicity, ease of evaporated, and the price (Harborne 1984). Harborne (1984) grouped into two extraction methods, extraction is simple and specific extraction. Simple extraction include maceration, percolation, reperkolasi, and allocated while the specific extraction consists of soxhletation, backflow and ultrasonic.

This study extraction is carried out by way of a single maceration. According Hanani et al. 2005 extraction with a single maceration can produce ekstraka higher yield than the storied maceration.

Solvents used in this study is ethanol. The use of this solvent given the nature of ethanol which is a polar solvent. According Filho (2006) in Yusro (2011) extraction by using ethanol are very effective in isolating bioactive compounds. The process of evaporating filtrate from the maceration solvent genjer produce extracts in the form of pasta and green translucent.

Extract products have many advantages, among others all plant bioactive compounds contained in concentrate form, and is still in the form of a natural matrix (Hanani et al., 2005). yield calculation results obtained that Cyprus bernilai 12,30% yield.

3.3. Phytochemical Test

Plants have small molecular chemical compounds that it is limited and is often referred to as secondary metabolites (Sirait, 2007). These secondary metabolites are bioactive compounds that can give you health in the human body (Hasler, 1998). Phytochemicals have an important role in the research of drugs produced from plants. Phytochemical content on Cyperus sp. can be seen in Table 2. Figure 1 and 2 below.

Figure 1. Pyhtochemical test flavonoid, saponin, molisch, biuret and fenol hidroquinon.

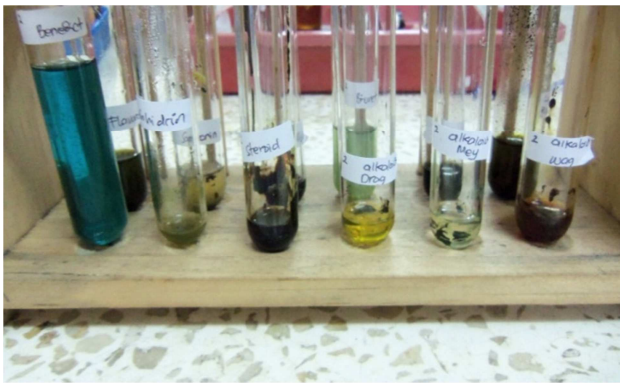

Figure 2. Phytochemical test benedict, ninhidrin, flavonoid dan alkaloid.

Table 2. Phytochemical test *Cyperus* sp.

Phytochemical	Result	Indicator of color
a. Alkaloid:		
b. Dragendorf	-	yellow
c. Meyer	+	brown
Wergner	-	no precipitate green
Steroid	+	Turquoise color
Flavonoid	-	green
Saponin	-	Foaming at the beginning, but not frothy after addition HCl 2 N
Fenol Hidroquinon	+	Green
Molisch	+	Purple
Benedict	+	Turquoise color
Biuret	-	Reseda
Ninhidrin	-	Yellow

Phytochemical test results can be seen that *Cyperus* sp. have indicated phytochemical content of the test alkaloids, steroids, phenols hydroquinone, molisch and benedict. Alkaloids were detected in the plant *Cyperus* sp. It is characterized from meyer reagent solution which gives a positive result although other reagents are dragendorf and wagner only give negative results. Alkaloids generally include alkaline compounds containing one or more nitrogen atoms. In general, no alkaloid found in plants gymnosperms, ferns, mosses, and low-level plants (Harbone, 1984). In plants containing alkaloids usually has astringent and bitter taste. At certain concentrations can be as toxic alkaloids (Lenny, 2006). In steroid test showed positive (+) that is on steroids test produced turquoise color. The content of this steroid interesting and important in the pharmaceutical field. Steroids is a chemical compound that is widely used in the medical field. Steroids can be used as an anti-bacterial, anti-inflammatory, and painkillers (Kumar et al., 2009).

Phenols include various compounds derived from plants and have the same characteristics, namely the aromatic ring containing one or two hydroxyl groups. Quinone compound is colorless and has a basic chromophore, such as a chromophore in benzokuinon, which consists of two carbonyl groups conjugated with two carbon-carbon double bonds. Quinone for identification purposes can be divided into four groups, namely benzokuinon, naftokuinon, anthraquinone,

and isoprenoid quinones. The results showed *Cyperus* sp. test positive for phenol hydroquinone. From the test results showed that phytochemicals are also positive (+) on carbohydrate testing. The positive results marked the onset of a purple ring on molisch test indicates their carbohydrate content on *Cyperus* sp. The purple color that appears is a condensation reaction that occurs between purfurat in carbohydrates with naphthol in reagent benedict. A positive result indicates that the test benedict in *Cyperus* sp. there is a reducing sugar content. Reducing sugar is a monosaccharide reducing compound.

4. Conclusion

Cyperus sp chemical cmpostions contains mostly composed of water and carbohydrates. Cyperus sp has a high water content and low in fat. *Cyperus sp* based qualitative phytochemical contains alkaloids, steroids, phenols hydroquinone, carbohydrates, and reducing sugars such as monosaccharides. single extraction with methanol to produce a large yield.

References

[1] *Association of Official Analytical of Chemist*. Arlington: The Association of Official Analytical Chemist, Inc.

[2] Denny, P., Bailey, R., Tukahirwa, E. and Mafabi, P. (1995): Heavy metal contamination of Lake George (Uganda) and its wetlands. *Hydrobiologia* 297: 229-239.

[3] Hanani E, Mun'im A, Sekarini R. 2005. Identifikasi Senyawa Antioksidan Dalam Spons *Callyspongia* sp. dari Kepulauan Seribu. *Majalah Ilmu Kefarmasian* 2(3): 127-133.

[4] Harborne JB. 1987. *Metode Fitokimia.* Edisi kedua. Padmawinata K, Soediro I, penerjemah. Bandung: ITB. Terjemahan dari: Phytochemical methods 2nd edition.

[5] Hasler CM. 1998. Functional foods: Their role in disease prevention and health promotion. *Journal Food Technology* 52(11): 63-70.

[6] Jones M, Muthuri F (1997) Standing biomass and carbon distribution in a papyrus (*Cyperus papyrus* L.) swamp on Lake Naivasha, Kenya. *J Tropic Ecol* 13: 347–356.

[7] Kaggwa, R. C., Mulalelo, C. I., Denny, P. and Okurut, T. O. (2001): The impact of alum discharges on a natural tropical wetland in Uganda. *Water Research*, 35 (3): 795-807.

[8] Kansiime, F. and van Gruggen, J. J. (2001): Distribution and retention of faecal coliforms in Nakivubo wetland in Kampala, Uganda. Water Science Tecnology, 44: 199-206.

[9] Ketaren S. 1986. *Pengantar Teknologi Minyak dan Lemak Pangan*. Jakarta: UI Press.

[10] Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Arivindhan P, Padmanabhan N, Krishnan MRV. 2009. Phytochemical investigation on a tropical plant, *Syzgium cumini* from Kattuppalayam, Erode Distric, Tamil Nadu, South India. *Journal of Nutrition Pakistan* 8(1): 83-85.

[11] Lenny S. 2006. Senyawa flavonoida, fenilpropanoida dan alkaloida. Laporan penelitian. Medan: Departemen Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Sumatera Utara.

[12] Nurjanah, Hardjito L, Monintja D, Bintang M, Agungpriyono DR. 2009. Aktivitas antioksidan lintah laut (*Discodoris* sp.) dari perairan Pulau Buton Sulawesi Tenggara. *Prosiding Seminar Nasional Pengolahan Produk dan Bioteknologi Kelautan danPerikanan*. Hal: 49-58.

[13] Nurjanah, Abdullah A, Sudriman S. 2014. Aktivitas antioksidan dan komponen bioaktif kangkung air *(Ipomea aquatic*). *Jurnal Inovasi dan Kewirausahaan*. 3(1): 68-75.

[14] Nurjanah, Jacoeb AM, Nugraha R, Permatasari M, Sejati TKA. 2014. Perubahan komposisi kimia, aktivitas antioksidan, vitamin C, dan mineral tanaman genjer (*Limnocharis flava*) akibat pengukusan. *Jurnal Inovasi dan kewirausahaan*. 3(3): 185-195.

[15] Salamah E, Purwaningsih S, Permatasari E. 2011. Aktivitas antioksidan dan komponen bioaktif pada selada air. Jurnal Pengolahan Hasil Perikanan Indonesia. 14(2): 85-91.

[16] Sirait. 2007. *Penuntun Fitokimia dalam Farmasi.* Bandung: Penerbit ITB.

[17] Susanto IS. 2010. Aktivitas Antioksidan dan Komponen Bioaktif pada Keong Mas (*Pomacea canliculata Lamarck*) [skripsi]. Bogor: Fakultas Perikanan dan Ilmu Kelautan. Institut Pertanian Bogor.

[18] Winarno FG. 2008. *Kimia Pangan dan Gizi*. Jakarta: PT. Gramedia Pustaka Utama.

[19] Winarno FG. 1997. *Kimia Pangan dan Gizi*. Jakarta: PT. Gramedia.

[20] Yunizal, Murtini JT, Dolaria N, Purdiwoto B, Abdulrokhim, Carkipan. 1998. *Prosedur Analisis Kimiawi Ikan dan Produk Olahan Hasil-Hasil Perikanan*. Jakarta: Pusat Penelitian dan Pengembangan Perikanan.

[21] Yusro F. 2011. Rendemen Ekstrak Etanol Dan Uji Fitokimia Tiga Jenis Tumbuhan Obat Kalimantan Barat (Rendement of Ethanol Extracts and Phytochemical Tests In Three of Species Medicinal Plants of West Borneo) [makalah]. Kalimantan Barat: Universitas Tanjungpura.