

PHYTOCHEMICAL ANALYSIS OF WATER HYACINTH (*Eichhornia crassipes*) OF AGRICULTURAL WASTE AS BIOSENSITIZER FOR FERRI PHOTOREDUCTION

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ABSTRACT

Analysis of the phenolics, flavonoids and tannins compounds has been done on *water hyacinth leaves* (*Eichhornia crassipes*). The research was carried out in several stages, including: sample preparation, maceration extraction, and evaporation. The total phenolics constituted analysis by Folin Ciocalteu 50%, flavonoids by using aluminium chloride 2% in ethanol, and tannins by using vanillin 4% solution in methanol, continued by using ultra violet visible spectrophotometry (UV-Vis) methods. The analysis showed that the phenolics content of *water hyacinth* with methanol 60% equal to gallic acid 2.72 mg /kg is the highest phenolics content, and the lowest phenolics by distilled water equal to gallic acid 1.07 mg /kg and the flavonoids with methanol 80% equal to 3.29 mg quercetin/kg is the highest flavonoids content, and the lowest by the distilled water of quercetin 1.65 mg /kg and the highest condensed tannins content by methanol 80% of 0.7 mg/kg catechin. The Fe²⁺ content process with methanol 60% solution was 17.67 mg/kg, and with distilled water was 2.94 mg/kg. Solution without extract as sensitizer produced only Fe²⁺ ion at 5.6 mg/kg and the extract solution without light at 0.06 mg/kg.

Keywords: phytochemical, agricultural waste, water hyacinth, biosensitizer, ferric photoreduction

INTRODUCTION

Indonesia is famous with its abundant natural resources of both flora and fauna. North Sulawesi is rich in natural resources with a wealth of biological diversity and has supporting environment for the growth of flora and fauna. In North Sulawesi, the natural products of flora as residue, such as wood and leaves, have not been processed and scattered to decompose naturally.

Water hyacinth (*Eichhornia crassipes*) plants have filled the river surface and Tondano Lake in the district of Minahasa North Sulawesi Province and the government is overwhelmed in eradicating the plants, although plants eradication program has been realized (Figure 1).

These plants are hydrophytes plants, the plants are not edible, even become weeds for other plant life and the lives of other animals. These plants which have the property as a nuisance plant play an important role in reducing the levels of the heavy metals in water, such as iron, zinc, copper, and mercury, and the plants have high cellulose content and are usually used as an alternative fuel.

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Figure 1. Water hyacinth leaves in Tondano Lake (Self Collection)

Water hyacinth is considered as the only water plant that can remove pesticides dissolved in water. Sari (2007) reported that water hyacinth was able to increase DO (dissolved oxygen) in river water that had been polluted by detergents and was able to reduce BOD (biological oxygen dissolved). Water hyacinth is able to increase DO of 8.05 mg/kg and decrease the BOD of 52.119 mg/kg (Moenandir and Hidayat, 1993). According to Pitrawijaya (1992) water hyacinth also has the capacity as bioaccumulator that is able to absorb anion or cation in the waste and able to grow quickly and can survive in poor condition. The leaf stalks are efficacious as medicines for swelling. The leaves contain saponins, flavonoids, tannins and polyphenols.

Agricultural waste is the source of organic matter available in large quantities and is produced continuously, but has not been used optimally. Agricultural waste is generated during the production process in field, during harvest and post harvest. Agricultural wastes contain organic materials that contain primary metabolite and secondary metabolite compounds. Agricultural waste of various types of leaves contain basic or primary sources of abundant phenolics, flavonoids, and condensed tannins

components and can be recycled into biosensitizer materials that are useful for plant growth and improve the soil fertility by the assistance of sunlight.

Chandler and Silva (1974) stated that the nitrogen, phosphor, and potassium concentrations on soybean leaves were reduced by the increasing presence of aluminum concentration. While Foth (1984) stated that aluminum poisoning would reduce the nutrient absorption, one of which was iron (Fe). Iron element plays an important role in the enzymatic system in the chlorophyll synthesis. If iron deficiency occurred, symptoms of chlorosis would be seen on the younger leaves. Area between leaf veins will mostly be affected and the leaf veins will remain dark; such a condition is called chlorosis (Foth, 1984). In general, plants take iron in the form of Fe^{+2} ions from nature, but the availability of iron in nature is in the form of Fe^{+3} ions. Therefore, Fe^{+3} must first be reduced to Ferro (Fe^{+2}), in order to associate with compound factor (Brown, 1969).

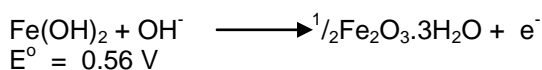
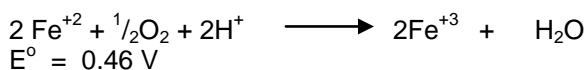
Reduction and oxidation reaction (redox) of metal may occur in almost all soil types. The condition of redox reaction of metal ions in the soil will influence the stability of the compounds

of manganese and iron. According to Aiken *et al.* (1985) the factor compounds in soil can be classified into 3 fractions, namely (1) humin; fraction that is not soluble in acid or alkaline solution, (2) humic acid; fraction that is soluble in alkaline and precipitated by acidification (3) fulvic acid; fraction that is soluble in acid or alkaline solutions. According to Flaig *et al.* (1975), factor compound or humic compound has the ability to reduce some metal ions that are easily oxidized. In the humic compound, there are many clusters that can be used as electron donor, such as OH⁻ cluster at the phenolics compound and cluster that can act as the electron acceptor, the quinone cluster.

In the photosynthesis reaction, plants collect available low level carbon in the atmosphere. When the carbon bonded with the water from the air, it would be converted into organic matter by the aid of sunlight to form chlorophyll. The absorbed elements for plant growth and metabolism called as plant nutrients elements. Mechanism of changes in plant nutrients elements into organic compounds or energy is called metabolism. The iron deficiency symptoms initially emerge on the younger leaves, and develop at the sheet between the bone and the leaves which can eventually cover the entire leaves, that is similar to the magnesium (Mg) deficiency. The color of iron-deficient leaves will become yellowish and the leaf bones will be darker, so the lamina color and the bones become more contrast (Pandey, 1980).

Oxidation rate and the amount of iron species in solution is the function of pH. At acidic pH and neutral pH, ionic species of iron is as Fe⁺² and FeOH⁺ ions, whereas at alkaline pH, Fe⁺² and FeOH⁺ ions will show a reduction in concentration, and at pH above 10, Fe⁺² and FeOH⁺ ions do not present in the solution (Blesa and Matijevic, 1989). According to (Baes dan Mesmer, 1976), Fe⁺² ions in water solution will form a complex ion species hexa aquo ferrat [Fe(H₂O)₆]⁺² in a pale-green color on acidic pH, and are easily oxidized by air to form Fe⁺³ ions [Fe(H₂O)₆]⁺³. The tendency of oxidized Fe⁺² ion species to become Fe⁺³ ions in the open air can be observed based on its electrode potential. Hydrolysis of Fe⁺² ions to form FeOH⁺ ions apt to occur in slightly acidic or neutral solution than in acidic solutions. The green complex formed faster in slightly acidic or neutral solution with

increasing concentration FeOH⁺ ions. Oxidation of Fe⁺² species may occur in acidic or alkaline solution with each E^o worth 0.46 V and 0.56 V. The oxidation reaction can be written as follows:



In alkaline solution, Fe⁺² species oxidation rate is higher than in acidic solutions. In alkaline conditions, solution with low concentration of Fe⁺² will experience hydrolysis to form mononuclear species. Fe⁺² ions will be hydrolyzed into FeOH⁺ ions at pH=8, and at pH=10, Fe⁺² will transform into Fe(OH)₂ which then will dissolve back into the Fe(OH) and Fe⁺² under acidic condition. Aionic species will be formed at pH above 10, such as Fe(OH)₃⁻.

For the solution of Fe⁺² with a high concentration, the solution will be saturated by precipitated Fe(OH)₂, at pH <10 Fe⁺² species that exist in the form of cationic such as FeOH⁺, and on pH>10 Fe⁺² anionic species will be transformed into Fe(OH)₃⁻ (Blesa and Matijevic, 1989). In the neutral and slightly acid solution, certain anions such as Fe⁺² species can be oxidized in air to form green complex. Green complex will be precipitated as green rush. Types of green complexes are formed depending on the type of anion and the mole ratio between species Fe⁺² and Fe⁺³. In neutral or slightly acidic solution containing SO₄²⁻, FeOH⁺ ions will be oxidized by air to form a green complex I with a ratio Fe⁺² and Fe⁺³ = 2:1. Green complex [II] which has ratio of Fe⁺² and Fe⁺³ = 1:1 is formed from air oxidation process to FeOH⁺ ions in neutral or slightly acid media containing Cl⁻ ions. Next, green complexes I and II will be a green complex precipitated as green precipitate I and green precipitate II (Baes and Mesmer, 1976).

Interaction of either divalent metal ion or trivalent with functional cluster in the phenolics compounds in water medium at pH close to 7 can take place through one or more of the reaction mechanism between metal ion and phenolics compounds (Stevenson, 1994).

According to Suryanto (2008) eugenol compound with chemical formula of C₃H₅C₆H₃(OH)OCH₃ is phenolics compounds that have

several functional clusters such as allyl, hydroxide and methoxy. Due to the presence of these functional clusters, eugenol compounds can be transformed into derivative compound that can be used directly or as the basic materials for making other compound. Phenolics compounds have been proven as the protector toward free radical and are able to reduce the risk of cancer, coronary heart disease, stroke, atherosclerosis, osteoporosis, inflammation, and other neurodegenerative diseases associated with stress. Besides, phenolics compounds also have multifunctional properties such as to contribute as reductant (as antidote to free radical), metal chelators and singlet oxygen quencher. Binding between transitional metal with phenolics compound begins chelate structure. Stevenson (1994) also stated that with the increasing metal ion concentration, the ionic character of the formed bonds between metal ions with phenolics compound would increase. Goodman and Cheshire (1982) have studied the reduction of molybdenum ion (Mo) by phenolics compound. The result obtained that some ions of MoO_4^{-2} (Mo^{+6}) in the solution will be reduced by phenolics compound to Mo^{+5} . Either ion MoO_4^{-2} or Mo^{+5} is absorbed by phenolics compound through ion exchange mechanism.

Phenolics compound analysis is conducted to determine the biosensitizer potential in certain extract. The phenolics compounds in the plant extract or in the agricultural waste are determined based on phenolics compound ability in the extract which can react with phosphomolibdat-phosphotungstat in the reagent of Folin-Ciocalteu that is yellow in color and later turns to blue (Suryanto, 2008). According to Markham (1988) it is estimated about 2% of the carbon production as the results of photosynthesis in plants is converted into flavonoids or its derivative compound. Most of the tannins compounds are also derived from flavonoids. Flavonoids is one of the largest classes of natural phenols because the flavonoids compound presents in all green plants. Stated in Suryanto (2010), flavonoids compounds have effect on the health to prevent skin bleeding and also function as natural antibiotic, as in *Lantana camara* L.

The flavonoids compound can be extracted by using hot water or ethanol that produced red color indicating the presence of

flavonoids as the consequences of reduction by hydrochloric acid and magnesium. Condensed tannin compounds are divided into two compound groups and each compound group may provide different color reaction of ferrychloride compound (FeCl_3) 1%. The hydrolyzed tannin compound group will produce blackish blue, and condensed tannins will produce blackish green color. At the time of the addition, it was estimated that FeCl_3 would react with one of the hydroxyl clusters in the tannin compound, and the output reaction led to producing color. Widespread use of the compound FeCl_3 was to identify the phenolics compound including tannins (Figure 2).

A study about the iron oxide colloid photo reductive solution was done in natural water with and without dissolved phenolics compound. The results show that the presence of dissolved phenolics was able to accelerate the photo reductive solution of iron oxide. According to Harborne (1987) the organic component can serve as a metal *chelating* agent because of the presence of one carboxyl cluster and two hydroxyl clusters close to each other and can react with metal ions to form a stable complex. The potential is indicated by the position of its hydroxyl cluster that is able to capture free radicals by simultaneously chelating Fe and stabilizing Fe. The research about the photoreduction of iron metal by using humic compound as the electron donor, not many studies revealing about this photoreduction of iron metal were conducted by using phenolics, flavonoids, and tannin compounds from waste of various plants, such as *water hyacinth* plants as an electron donor. The purposes of this study are: to find the concentration of the phenolics, flavonoids and tannin compounds in the agricultural waste such as *water hyacinth* by using UV-Vis spectrophotometer instrument, to find out the effectiveness of phenolics, flavonoids, and tannin extracts in the leaves of *water hyacinth* to absorb ultra violet ray (UV) that can act as electron donor in biosensitizer formation at the iron photoreduction of soil.

MATERIALS AND METHODS

MATERIALS

The samples used in the research is the leaves of *water hyacinth* that were taken from Tondano Lake in the district of Minahasa, North

Sulawesi Province. The samples were washed, dried and extracted by maceration. Chemical materials such as distilled water, CH₃OH, Folin Ciocalteu reagent 50%, Na₂CO₃ solution 3%, chloride aluminium solution 2%, HCl solution, C₂H₅OH, vanillin solution 4% were used as needed. The implementation was conducted for eight (8) months from February to September 2011. The research was conducted in the laboratory of Advance Chemistry of FMIPA Sam Ratulangi University and the Laboratory of Research and Industry Standardization of North Sulawesi Province.

METHODS

The samples were extracted according to extraction-maceration method and then evaporation rotary was applied and dried in the oven, then followed by phytochemical analysis. The phytochemical analysis included phenolics,

flavonoids and tannins compounds (Rorong and Suryanto, 2010). The phenolics compound analysis was according to Folin Ciocalteu 50% methods by Conde *et al.* 1997. The flavonoids compound analysis was according to aluminum chloride 2% in ethanol by Meda *et al.* 2005. The tannin condensed compound analysis was according to vanillin 4% in methanol by Julkuren-Titto, (1985) methods. The phenolics, flavonoids and tannin condensed compounds produced from the above analysis were continued by advanced analysis using ultra violet spectrophotometry methods. The phenolics compound analysis uses 750 nm wave length and gallic acid as standard solution, while the flavonoids compound analysis uses 415 nm wave length and cuercetin as standard solution.

Finally, the tannins condensed compound analysis uses 520 nm wave length and standard solution catechin as standard solution.

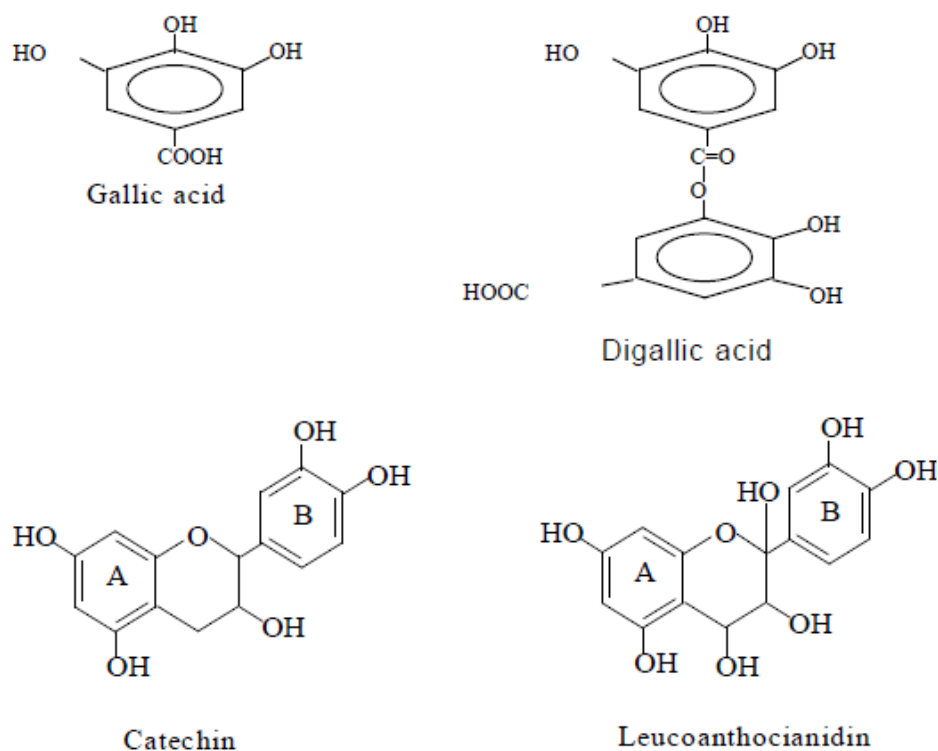


Figure 2. Phenolics coumpounds of derivate structure

RESULTS AND DISCUSSION

The phenolics content in water hyacinth leaves was determined by the ability of phenolics compounds in the leaves extract which reacted with acid phosphomolibdophosphotungstate in Folin-Ciocalteu reagent (yellow) which produced complex compound of blue molybdenum-tungstate. The yellow color in Folin-Ciocalteu reagent would change the color to blue due to the reaction with the extract. The darker color intensity of the solution showed the larger the phenolics compound in the extract (Julkunen-Tiito, 1985). The absorbance results obtained then converted into concentration (mg/Kg) by using standard solution of gallic acid. The standard solution concentration of gallic acid used was 0, 5, 10, 15, and 20 mg/Kg, obtained with linear line equation $y = 0.1461x - 0.0611$ with correlation coefficient (R^2) 0.9870. From the equation, the slope value of 0.046 was obtained and used to determine the phenolics in the extract.(Figure 3).

The content of phenolics in the sample extract was related directly with its activity as the electron donor in Fe^{+3} to Fe^{+2} . Aiken *et al* (1985) indicated that the phenolics compounds had the ability to reduce some oxidized metal

ion. There were many clusters of phenolics compound that could be used as electron donor, such as OH^- cluster on the phenolics compounds. The formation of complex compound was the reaction of metal ion with ligand (an organic compound that is able to clamp metal cations) through electron pair sharing. The reaction results were called metal coordination compound. In this case the metal acted as the receiver electron pair while the ligand was the electron pair donor. Metal ions acted as central atom, and the organic ions were negatively charged as a ligand that was coordinated and moved around the central atom. Some organic ligand could bind metal ions with more than one functional donor cluster. This type of binding formed heterocyclic ring and was called chelation. The binding between transition metal with phenolics compound began to occur at site that produced strong binding through formation of chelating structure. The weaker binding occurred after strong site saturated. Metal ion concentration, the ionic character at the formed binding between metal ion with phenolics compound, would increase. The flavonoids compound content in water hyacinth leaves is revealed in Figure 4.

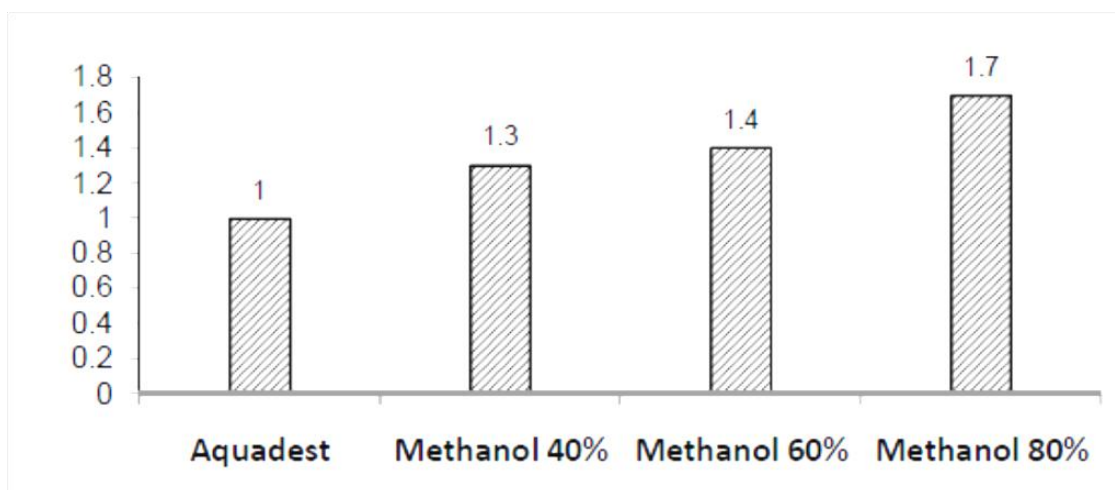


Figure 3. Phenolics compounds content of water hyacinth leaves, that is extracted with solvents of distilled water, methanol 40, 60 and 80%

The flavonoids content is stated as quercetin equivalent in mg/Kg extract. From the standard solution concentration of quercetin 0, 5, 10, 15 and 20 mg/Kg, linear equation $y = 0.0278x - 0.0022$ with correlation coefficient (R^2) of 0.9910 was obtained. From the equation, the slope value of 0.0278 was obtained and used to determine the total content of flavonoids in the extract.

The polarity levels would determine the extraction results and anti oxidant activity contained in the extracts. The condensed tannins content in water hyacinth leaves is shown in Figure 5. The condensed tannins content was expressed as catechin equivalents in mg/Kg extract. From the concentration solution of standard catechin 0, 5, 10, 15 and 20 mg/kg, linear equation $y = 0.009x - 0.023$ with a correlation coefficient (R^2) 0.9310 was obtained. The equation slope 0.009 was obtained and used to determine the condensed tannins content in the extract.

The content of Fe^{2+} formed through process of water hyacinth extract. The sample was extracted with solvent of distilled water, methanol 40, 60, and 80%. The extract that did not contain erythrosine as sensitizer and extract solution with light was used as control. Extract

with methanol solvent of 60% shows the Fe^{2+} ion production in methanol 60% reached the highest content (17.67 mg\Kg) followed by that of methanol 40% (12.44 mg/kg), distilled water (2.94 mg/kg), and methanol 80% (1.11 mg/kg). Solution without extract as sensitizer produced only Fe^{2+} ion at 5.67 mg\Kg and extract solution without light was at 0.06 mg/kg.

CONCLUSIONS

The phenolics content of water hyacinth extracted by methanol 80% which was equal to 1.71 mg gallic acid/Kg constituted the highest result. The flavonoids content extracted by methanol 80% which was equal to 3.29 mg quercetin/Kg constituted the highest result, and the lowest extract with distilled water solvent was equal to 1.65 mg quercetin/kg. The tannin condensed content only detected in an extract of methanol solvent 80% was equal to 0.74 mg catechin/Kg which was the highest condensed tannins extract. Extract using methanol solvent 60% produced the Fe^{+2} ion at 17.67 mg\Kg which was the highest. Fe^{+2} ion at 5.67 mg/kg was produced without extract as sensitizer, and extract solution without light produced Fe^{+2} at 0.06 mg/kg.

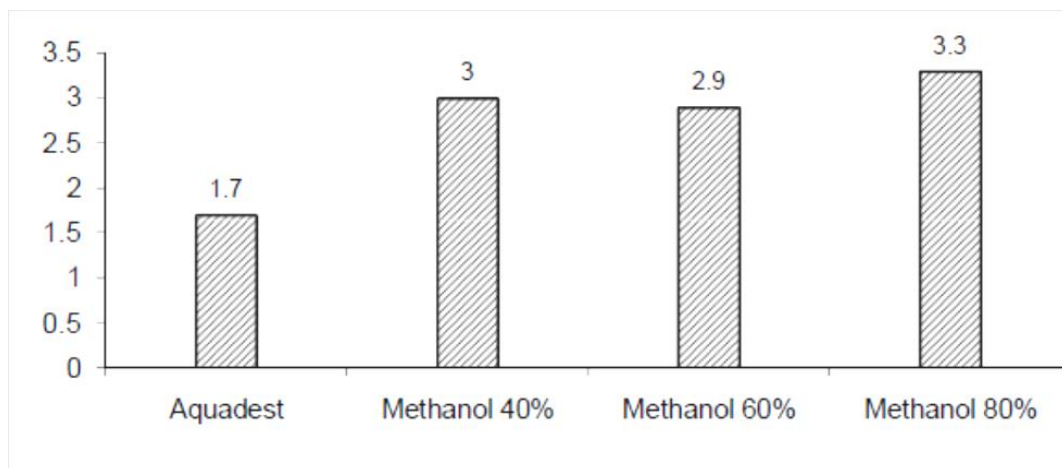


Figure 4. Flavonoids compounds content in water hyacinth leaves sample that extracted with solvents of distilled water, methanol 40, 60 and 80%

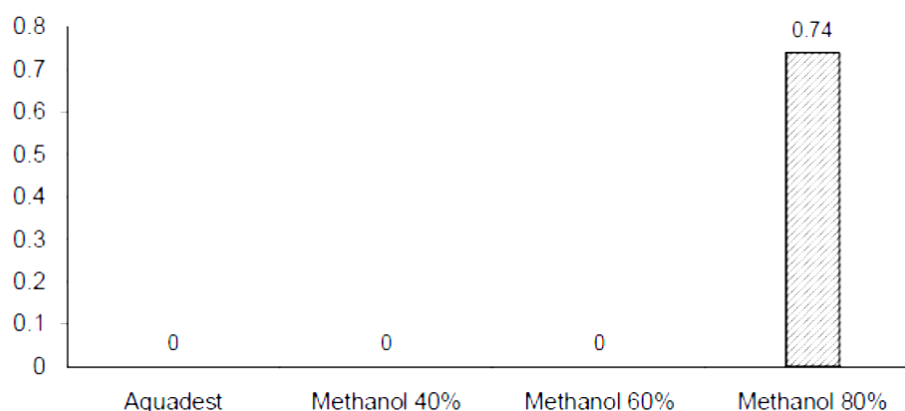


Figure 5. The condensed tannins content in water hyacinth leaves sample extracted with solvents of distilled water, methanol 40, 60 and 80%

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152 AGRIVITA VOLUME 34 No. 2 JUNE-2012 ISSN : 0126-0537 [http://dx.doi.org/ 10.17503/Agrivita-2012-34-2-p152-160](http://dx.doi.org/10.17503/Agrivita-2012-34-2-p152-160) PHYTOCHEMICAL ANALYSIS OF WATER HYACINTH (*Eichhornia crassipes*) OF AGRICULTURAL WASTE AS BIOSENSITIZER FOR FERRI PHOTOREDUCTION Johnly Alfreds Rorong 1) , Sudiarso 2) , Budi Prasetya 2) , Jeany Polii-Mandang 3) and Edi Suryanto 3) 1*) Chemistry Department, Faculty of Natural Science, Sam Ratulangi University Jl. Campus Kleak Manado 95115 North Sulawesi Indonesia 2) Postgraduate Program Faculty of Agriculture University of Brawijaya Jl. Veteran Malang 65145 East Java Indonesia 3) Postgraduate Program Faculty of Agriculture and Faculty of Natural Science University of Sam Ratulangi Jl. Campus Kleak Manado 95115 North Sulawesi Indonesia *) Corresponding author Phone: 0431-864386 Email: rorongjohnly@yahoo.co.id Received: January 23, 2012/ Accepted: April 24,2012 ABSTRACT Analysis of the phenolics, flavonoids and tannins compounds has been done on water hyacinth leaves (*Eichornia crassipes*). **The research was carried out in several stages, including:** sample preparation, maceration extraction, and evaporation. The total phenolics constituted analysis by Folin Ciocalteu 50%, flavonoids by using aluminium chloride 2% in ethanol, and tannins by using vanillin 4% solution in methanol, continued by using ultra violet visible spectrophotometry (UV-Vis) methods. The analysis showed that the phenolics content of water hyacinth with methanol 60% equal to gallic acid 2.72 mg /kg is the highest phenolics content, and the lowest phenolics by distilled water equal to gallic acid 1.07 mg /kg and the flavonoids with methanol 80% equal to 3.29 mg quercetin/kg is the highest flavonoids content, and the lowest by the distilled water of quercetin 1.65 mg /kg and the highest condensed tannins content by methanol 80% of 0.7 mg/kg catechin . The Fe 2+ content process with methanol 60% solution was 17.67 mg/kg, and with distilled water was 2.94 mg/kg. Solution without extract a

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