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Influence Analysis of Heating Time Towards Degradation of Anti Oxidant Activity on Water Spinach Leaves (*Ipomoea Aquatica* Forsk)

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Abstracts

The purpose of this research is to analyze the impact of water spinach leaves heating (Ipomoea aquatica Forsk) at 100°C by different time duration on its antioxidant activity. The method was experimental method involved several steps, such as sample preparation, extraction process, and evaluation of antioxidant activity with UV-Visible Spectrophotometer. The result of this research shown that IC_{50} value from K_1, K_2, K_3 to K_4 were 25.25 µg/mL (very high), 96.75 µg/mL (high), 181.47µg/L (low, and 280µg/mL (very low). The IC_{50} value of K_1 to K_2 decreased 71.49 µg/L, The K_2 to K_3 decreased 84.72 µg/L, K_3 to K_4 decreased 98.53 µg/L. By this result, it was predicted that the antioxidant of water spinach will diminish, or even it will be lowered when it is steamed more than 15 minutes in 100 °C.

Key words: Antioxidant Activity; Water Spinach; Heating Process.

1. Introduction

Water spinach (*Ipomoea aquatica* Forsk) is one of vegetables that found in many south east area, india, and china of south east. The plant is growth with spread and float upward in the water usually found in Indonesia as food dish such as *tumis kangkung, cah kangkung, etc.* Indonesian society from each circle, mostly consume this vegetable because it is cheap and easy t get [1].

Water spinach cooking by Indonesian society is usually by a culinary procedure similar to sauteing but at a high temperature, steam, and boiled in basic the cooking activity is with heating. The cooking result is various, such as *tumis* water spinach with soft leaves and stem, *tumis* water spinach with hard leaves and stem, and there is result cooking with fresh green color, yellow green color, and even until black color [2].

Cooking process with heating in long time in high temperature can reduce the nutrition

contents and anti oxidant of vegetables, even though there is a vegetables which cooking with heating process can increase the anti oxidant such as onion leave, pepper, and legumes of various sorts. Anti oxidant contents can be evaporated when heating process activity. The vegetable is enough heating to evaporate the resistant to nutrient essence because if its served in uncooked is not good either [3]. water spinach if its long time cooking will reduce the vitamin C in it and damage the fiber structure [4].

Water spinach contents anti oxidant which is useful for human being [5]. Anti oxidant which contents in water spinach is in great quantities. The rough extract of water spinach is detection contents some bio active component that is alkaloid, steroid, phenol and hydrokinon. Bio active components is assume have many positive physiology activity for body [5].

The benefit of anti oxidant for human is to prohibit the cell damage because the free radical. Anti oxidant neutralized the free radical with receive or donor one electron for vanishing condition "no partner electron". Free radical become stable molecule (no radical) when the molecule neutral process. Anti oxidant molecule will change become radical. Anti oxidant molecule which change become radical usually less reactive than free radical that neutralized. The size of anti oxidant molecule is can be big (to dilute non partner electron), and it can be neutralized by other anti oxidant or have other mechanism to finish the radical condition [6].

Free radical is a molecule, atom or several atom group which have one or more electron that non partner electron in outside orbital [6]. Free radical is made from oxidation reaction in the body. Oxidation reaction is done any time, even though when we are breath, in the human body is done oxidation reaction. This reaction produce an active free radical that can damage structure and cell function. Whereas, re activity of free radical can obstruct by the anti oxidant system which complete the body immune system [7].

The forming of free radical naturally is done on the body, which is side produce from body metabolism process. Free radical that is the body is *hidroksil* (OH•), *anion superoksida* (O_2•), *hidrogen peroksida* (H_2O_2), *asam hipoklorid* (HOCl), *oksigen singlet* ($^1O_2$) and *peroksil* (•OOH) [8]. Free radical is produce in side the cell by *mitokondria,* plasma membrane, *lisosom, peroksisom, endoplasmik retikulum,* and cell nucleus. While out side body, free radical is gain from pollutant, food, and drink, ozone and pesticide residue [6].

The increasing free radical on human body is produced continuously and it can be avoid as consequence factor of oxidation stress, UV radiation, air pollution and environment, and also food and beverage that content pesticide residue, saturated fat acid, trans fat acid, dye, and forbidden preservative, so its caused the defense anti oxidant system in the body is not adequate anymore and need additional anti oxidant from out side the body [9].

Anti oxidant from out side the body is gain in the synthetic and natural form. Synthetic anti oxidant such as *buthylatedhydroxytoluene* (BHT), *buthylated hidroksianisol* (BHA) and *ters-butylhydroquinone* (TBHQ) which effectively can obstruct the oxidation. The usage of synthetic anti oxidant in certain time can cause toxic in the body and carcinogenic character so its need save natural anti oxidant. One of the potential source of natural anti oxidant is plant because contents *flavonoid,* clorofil and *tanin* compound [10]. Water spinach can be one of anti oxidant source from out side the body as neutralized of over free radical inside the body.

The disease that faced by human is started from over oxidation reaction inside the body. In the certain condition, existence of oxygen can implicated to various disease and degeneration condition, such as *aging,* arthritis, cancer, etc [7]. Processing water spinach is need reference so that when heating time there is no reduce the anti oxidant activity so the benefit can be taste by human. For that is need research about reduction level of anti oxidant activity of water spinach every 5 minutes if its heating in temperature 100 ^oC. Reduction level of anti oxidant activity of water spinach that gain can produce profile of

reduction level of anti oxidant activity. Data of research result (profile/reduction level of anti oxidant activity) can be used as reference of processing water spinach with heating treatment.

Testing of anti oxidant activity is done used radical submerged method of DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate). Those method is simple and radical DPPH is stable character so it might be done an accurate measurement of anti oxidant activity [10]. This method is considered as easy and useful method for screening or measurement of anti oxidant activity whether pure or complex [11].

2. Experiments Procedure

Sample preparation

The one kg of fresh water spinach leave is divide 4 parts, each part is 250 gram which labeled with K_1, K_2, K_3, and K_4. Sample with label K_1 is let it be without given hating treatment (without steam), sample with label K_2 is steamed 5 minute, sample with label K_3 is steamed 10 minutes, and sample with label K_4 steamed 15 minutes with temperature 100 °C. Table 1 shows the beginning treatment of water spinach sample.

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Table 1. The treatment of sample

No.	Sample code	Steam time
1.	K_1	0 minute
2.	K_2	5 minutes
3.	K_3	10 minutes
4.	K_4	15 minutes

The all samples are kept until dry in temperature room in the place far from direct sun light after the samples become perfect dry after 7 days. Each sample which is dry is blend without solvent. Then, as many as 25 gram dry leaves which delicate and each of the sample is *maserasi* used 250 ml ethyl asetat technique more over 48 hours. As long as the *maserasi*, its done mixing a couple times. Each sample, that have been *maserasi* is filtered and take the filtrate. The filtrate which filtered is thick with *rotary evaporator* with temperature 50 °C.

Test of anti oxidant activity

Test of anti oxidant activity with DPPH method, first time is explain by Blois. Test of anti oxidant activity in this research is used Blois procedure, that is *absorbansi* which is counted from 1 ml sample that mixed with 1 ml DPPH and it is liquidate with 2 ml methanol [17].

Production of DPPH solution

As many as 2 mg of DPPH is solute in 20 ml methanol so it gain concentrate of 100 µg/ml.

Optimum of DPPH long wave

DPPH solution with concentrate of 100 µg/ml is measured the absorbent in long wave of 510-525 nm, it is definitely the optimum long wave so its gain maximum long wave in 515 nm.

Absorbent test of blanko solution

As many as 1 ml of DPPH 100 µg/ml solution is input in reaction test tube then add 2 ml methanol and homogeneous. After that, sample is incubation in water steam bath of 37 °C more over 30 minutes. Finally, the absorbent measurement in optimum long wave.

Extract Test

As many as 25 mg from extract K_1, K_2, K_3, and K_4 (extract of water spinach ethyl asetat), each sample is soluble in 25 ml methanol p.a so its gain concentrate of 1000 µg/ml. Then, doing dilution so its gain solution with concentrate of 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml. Table 2 show the dilution table of main solution with variation concentrtae of 25, 50, 75 and 100 ppm. 1 ml of each sample solution concentrate of K_1, K_2, K_3, and K_4 is go into reaction test tube, add 1 ml DPPH 100 µg/ml and liquid with 2 ml methanol p.a then homogeneous. Each solution in reaction test tube is incubation in water steam bath of 370 C more over 30 minutes and its measured the absorbent in 515nm.

Table 2. Dilution of sample main solution

No	Concentration	Sample main solution+ Metanol(ml)			
		K_1	K_2	K_3	K_4
1.	25 ppm	0.25 + 9.75	0.25 + 9.75	0.25 + 9.75	0.25 + 9.75
2.	50 ppm	0.5 + 9.5	0.5 + 9.5	0.5 + 9.5	0.5 + 9.5
3.	75 ppm	0.75 + 9.25	0.75 + 9.25	0.75 + 9.25	0.75 + 9.25
4.	100 ppm	1 + 9	1 + 9	1 + 9	1 + 9

Data Analysis technique

Data of absorbent result of each sample is used to find % of inhibition.
Calculation used Equation (1):

% inhibition : **Error! Reference source not found.** X 100% (1)

A_{blanko} = absorbent on DPPH without sample
A_{Sampel} = Absorbent on DPPH after add the sample

Result of percentage calculation of inhibition is substitute to the linear Equation (2):

$Y= aX + b$ (2)

Y = % inhibition a = Gradient
X = concentrate (µg/ml) b = constant

Linear equation is produced used to gain value of $IC_{50.}$ value of IC_{50} is concentrate which gain at % inhibition amounted 50 from equation Y=aX+b
On % inhibition = 50, so to calculate the value of $IC_{50,}$ the equation is:

$50 = aX+b$

X = **Error! Reference source not found.**

X is IC_{50} with unit of µg/ml.

3. Result and Discussion

Absorbent measurement in several sample is done in concentrate/diluted 25 ppm, 50 ppm, 75 ppm and 100 ppm with twice decrease. The purpose of dilute is to expand the concentration scope with constant susceptible so the intersection point can be substitute as linear equation by accurate, so later on the IC_{50} can gain that equation [5, 11-13].

A compound can be said that have anti oxidant activity when the compound can donor the hydrogen atom in DPPH radical which signed with changing color of purple become pale yellow. Catching the free radical is caused *diazo* double bond in DPPH is decrease so the its occur the descent absorbent [12].

A great anti oxidant activity is signed with value of IC_{50}, that is sample solution concentrate which is need to obstruct 50% of DPPH free radical. Result data of absorbent value measurement and percentage (%) inhibition of each sample extract can be seen on Table 3, Table 4, Table 5, and Table 6. From data on Table 3, Table 4, Table 5 and Table 6 can be substitute to the linear equation Y=aX+b. The linear equation can be seen in Figure 1, Figure 2, Figue 3 and Table 4, respectively.

Result of extract absorbent measurement of sample K_1 until K_4 from concentrate 25 ppm, 50 ppm, 75 ppm until 100 ppm, its can be seen that the absorbent value is more decrease. It appropriate with theory of absorbent value perusal, that is the influence of concentrate is inversely with absorbent value. The high of concentrate so it will more less the absorbent value, because absorption toward small solution it means the ray that reserved is small [14].

Table 3. Absorbent and Percentage (%) Inhibition on sample K_1

No	Concentration (ppm)	K_1 repeat 1			K_1 repeat 2			Mean % Inh
		Abs. DPPH	Abs. Sample	% Inh	Abs. DPPH	Abs. Sample	% Inh	
1	25	0.832	0.432	49.16	0.833	0.425	48.98	49.07
2	50	0.832	0.385	53.73	0.833	0.388	53.42	53.58
3	75	0.832	0.361	56.61	0.833	0.367	55.94	56.28
4	100	0.832	0.354	57.45	0.833	0.359	56.90	57.18

Table 4. Absorbent and Percentage (%) Inhibition on sample K_2

No	Concentration (ppm)	K_2 repeat 1			K_2 repeat 2			Mean % Inh
		Abs. DPPH	Abs. Sample	% Inh	Abs. DPPH	Abs Sampel	% Inh	
1	25	0.834	0.447	46.40	0.835	0.448	46.35	46.37
2	50	0.834	0.428	48.68	0.835	0.431	48.38	48.53
3	75	0.834	0.423	49.28	0.835	0.427	48.86	49.07
4	100	0.834	0.419	49.76	0.835	0.417	50.06	49.91

Table 5. Absorbent and Percentage (%) Inhibition on sample K_3

No	Concentration (ppm)	K_3 repeat 1			K_3 repeat 2			Mean % Inh
		Abs. DPPH	Abs. Sample	% Inh	Abs. DPPH	Abs Sampel	% Inh	
1	25	0.837	0.479	42.77	0.839	0.483	42.43	42.60
2	50	0.837	0.475	43.25	0.839	0.480	42.79	43.02
3	75	0.837	0.460	45.04	0.839	0.461	45.05	45.04
4	100	0.837	0.451	46.12	0.839	0.454	45.89	46.005

Table 6. Absorbent and Percentage (%) Inhibition on sample K_4.

No	Concentration (ppm)	K_4 repeat 1			K_4 repeat 2			Mean % Inh
		Abs. DPPH	Abs. Sample	% Inh	Abs. DPPH	Abs Sampel	% Inh	
1	25	0.841	0.526	37.46	0.843	0.528	37.37	37.415
2	50	0.841	0.518	38.41	0.843	0.517	38.67	38.54
3	75	0.841	0.506	39.83	0.843	0.504	40.21	40.02
4	100	0.841	0.495	41.14	0.843	0.498	40.93	41.035

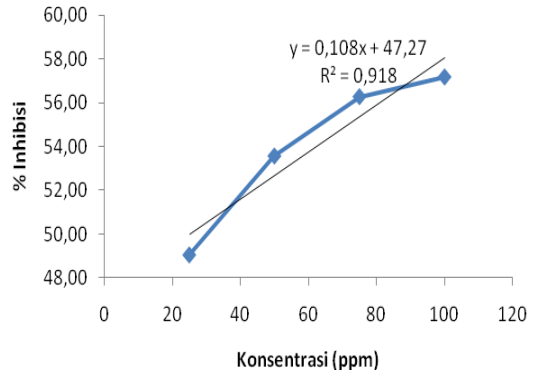

Figure 1. Percentage graph (%%) inhibition extract of ethyl asetat on sample K_1

Linear equation is y = 0.1081 x – 47.27

IC_{50} = **Error! Reference source not found.** = 25.254 µg/mL

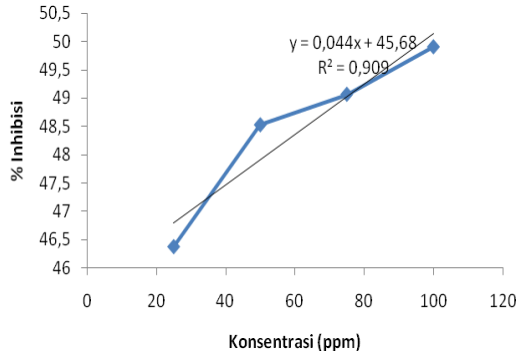

Figure 2. Graph % inhibition extract of ethyl asetat sample K_2

Linear equation is, y = 0.0446x + 45.685

IC_{50} = **Error! Reference source not found.** = 96.748 µg/mL

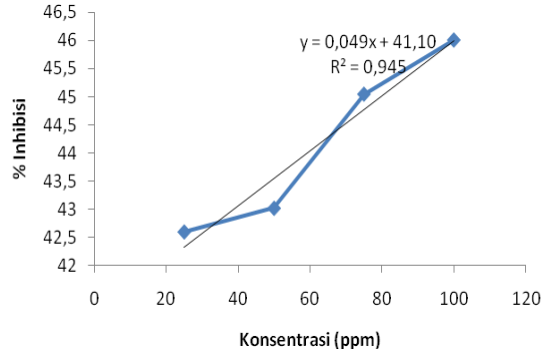

Figure 3. Graph % inhibition extract of ethyl asetat on sample K_3.

Linear equation is, y = 0.049x + 41.108

IC_{50} = **Error! Reference source not found.** = 181.469 µg/mL

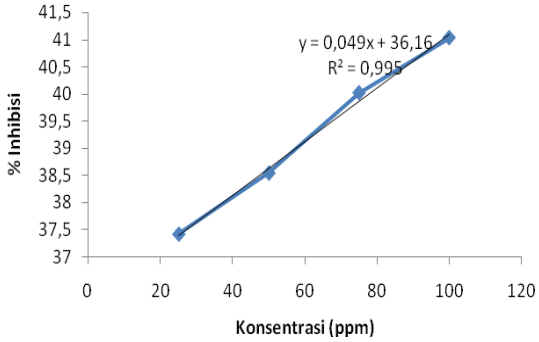

Figure 4. Graph percentage (%) inhibition extract of ethyl asetat on sample K_4

Linear equation is, y = 0.0494x + 36.168

IC_{50} = **Error! Reference source not found.** = 280 µg/mL

Anti oxidant activity is declare in IC_{50}. Based on measurement result of absorbent and percentage (%) value of inhibition is gained value of IC_{50} from each of ethyl asetat extract of water spinach leave. More and more small of IC_{50} value means that more high anti oxidant activity. The compound is called as very strong anti oxidant if the value of IC_{50} is less from 50 µg/ml, strong anti oxidant if the value of IC_{50} is between 50-100 µg/m, average anti oxidant if the value of IC_{50} is between 100-150 µg/ml, and weak anti oxidant if the value of IC_{50} is between 150-200 µg/ml [15]. value of IC_{50} from each ethyl asetat extract of water spinach leave can be seen at Table 7.

Table 7. IC_{50} value of each ethyl asetat extract of water spinach leave.

No	Sample Code	IC_{50} value (µg/mL)	Category
1.	K_1	25.25	Very strong
2.	K_2	96.75	strong
3.	K_3	181.47	weak
4.	K_4	280	Very weak

From Table 7 can be gain pole diagram of IC_{50} value of each ethyl asetat extract of water spinach leave that can be seen on Figure 5. Y coordinate explain IC_{50} value, X coordinate explain heating time. Picture 5 shows that IC_{50} value in succession from the small is K.1=25.25 µg/mL, K.2=96,75 µg/mL, K.3=181,47µg/L, and K.4=280µg/mL. From IC_{50} value that is gain is in the form of pattern graph of anti oxidant activity of water spinach leave. Pattern of anti oxidant activity can be seen in Figure 6. In the Figure 6 can e read that the more long of heating time, the graph IC_{50} value is increase, it means that anti oxidant activity is more weak. IC_{50} value from K_1 to K_2 has increased amounted 71,49 µg/mL, from K_2 to K_3 has increased amounted 84,72 µg/mL, from K_3 to K_4 has increased amounted 98,53µg/mL.

Anti oxidant activity of water spinach from K1 until K4 is more decreased its guess because of the damage of seconder metabolite compound structure. Seconder metabolite compound structure that exist on water spinach such as *tanin, flavonoid, alkaloid* and *fenol hidrokuinon,* its predictable can broken because of heating in high temperature, so the compound of seconder metabolite that should be function as anti oxidant can not obstruct free radical of DPPH along addition of heating time. It is because of between the factor that influence seconder metabolite compound structure on water spinach is temperature [16].

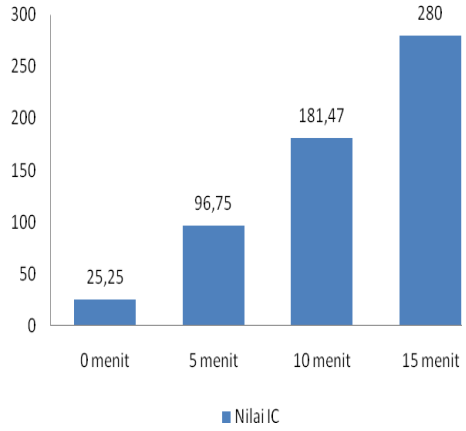

Figure 5. Pole diagram of IC_{50} value of each ethyl asetat extract of water spinach leave.

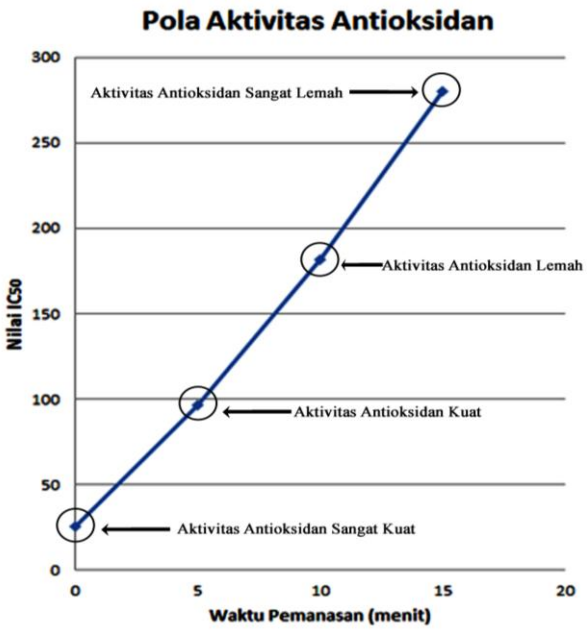

Figure 6. Pattern of anti oxidant activity of water spinach leave

Based on research result, it can said that water spinach if it steam more than 10 minutes in temperature 100 °C, the anti oxidant activity is more weak, even if its steam more longer the anti oxidant activity is used up. Processing of water spinach with steam is better done in time between 5-8 minutes in temperature 100 °C in order that the nutrient resistant essence can lost and anti oxidant activity is relatively strong.

4. Conclusion

Based on research result, anto oxidant activity of water spinach leave (*Ipomoea aquatica* Forsk) in different heating time of 5 minutes in temperature 100°C in totally from sample K_1, K_2, K_3 until K_4 experience descent/weak. IC_{50} value of sample K_1, K_2, K_3 until K_4 in succession is 25.25 µg/mL (very strong), 96.75µg/mL (strong), 181,47 µg/mL (weak), 280 µg/mL (very weak). The descent of anti oxidant activity can be seen from the increasing of IC_{50} value. More big of IC_{50} value so the anti oxidant activity is more weak. IC_{50} value from K_1 to K_2 faced increasing IC_{50} value amounted: 71,49 µg/mL, from K_2 to K_3 faced increasing IC_{50} value amounted: 84,72 µg/mL, from K_3 to K_4 faced increasing IC_{50} value

amounted: 98.53µg/mL. From research result, it can be predicted that water spinach if heating more than 10 minutes in temperature 100°C, the anti oxidant activity is more weak, even if its steam more longer the anti oxidant activity is used up.

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