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Content Analysis Of Vitamin C In Fresh And Processed Moringa Trees By Spectrophotometry And Iodometric Titration Methods

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ABSTRACT

The aim of this research were to examine the vitamin C content found in fresh and processed moringa trees and to know the method/ stage used to analyze the vitamin C content found in fresh and processed moringa trees. This research uses two methods namely spectrophotometric method and iodometry titration method. Initial stage prepare the sample of moringa, which were moringa leaf, moringa stem and moringa seed. The results showed that at the wavelength of 600 nm and after the iodometric titration, all the moringa samples (leaves, stems and seeds) contain vitamin C. Both of this two methods, spectrophotometry and iodometric titration, is able to analysis vitamin C in Morianga trees.

Keyword: *Moringa leaves, moringa stems, moringa seeds, citric acid, iodometric titration, spectrophotometry*

1. INTRODUCTION

Indonesia is an archipelago country that has various natural resources both animals and plants. One of which is the wealth of flora, such as *Moringa oleifera*. From last few decades Moringa oleifera has known a plant multifunction. This is because Moringa trees contains more natural compounds than other plants,which were vitamins, minerals, antioxidants, and essential amino acids.

Moringa oleifera has known as herbal medication. Moringa oleifera belongs to family Moringaceae. A pantropical plant of the Moringaceae family, Moringa oleifera, is one of approximately thirteen species in the monogeneric family. Native to the sub-Himalayas of India, M. oleifera has been naturalized in various tropical and subtropical regions of the world, including the Middle East, Africa, the Americas, Asia, the Philippines, Cambodia, and the Caribbean islands. A wide range of common names for the tree are documented, including benzolive tree, drumstick tree, horse-radish tree, kelor tree, mother's best friend, never die tree, mlonge, moonga,mulangay and numerous others (Rockwood, Anderson, and Casamatta, 2013: 62).

Many people in the word, have used *Moringa oleifera*. They often used to food or some other beneficial properties, sometime they used as a micronutrient powder to treat various ailments, but most of people when they used this trees. They like used only part of leaves. Even though, moringa trees can usefull in all of part such as roots, seeds, branchs, flowers and stem. Many benefits can get it, such as high calcium, high vitamin, and antibacterial.

The usefull of Moringa trees like in seeds. The example, dried seeds are used in ophthalmic preparation, venereal affection anti-inflammatory, purgative and as tonic and then the roots of Moringa trees are bitter, acrid, thermogenic, disgetive, carminative and etc. They are useful in dyspepsia, anorexia, vermitaminosis, diarrhea, colic, flatulence, paralysis. The last, the specific

components of Moringa preparations that have been to have anticancer and antibacteria. That's way, not only in this leaves but roots, seed also usefull and that we know the Moringa trees called by Trees for Life because of it.

Moringa trees are used to overcome malnutrition, especially in infants and nursing mothers. The nongovernmental organizations in particular-Trees for Life, Church World Service and Educational Concerns for Hunger Organization—have advocated Moringa as natural nutrition for the tropics 5. Its leaves as food supplement, recommended for children with moderate malnutrition between the ages of 6 months to 5 years. The leaves have therefore been promoted as a potential low cost high quality food (Devendra, Srinava, Prasad, and Latha, 2011).

Moreover the potencial contain from the Moringa trees is high vitamin. Vitamin is organic compound that's very important affect metabolism process. Vitamin is need to our body but not to much and its always there and it can be maintain our health. The Moringa tress contain many vitamin such as vitamin A, vitamin C. This is nutrional value of *Moringa oleifera* is proteins, calcium (Ca), Iron (Fe) and vitamin C. It has been demonstrated that the dry leaves of *Moringa oleifera* contain seven times more vitamin C than oranes, 10 times vitamin A than carrots, 17 ties calcium milk and 15 times potassium than bananas, 25 times iron than spinach and 9 times proteins than yogurt. Futhermore, they contain various antioxcidants compounds such as ascorbic acid, flavonoid, phenolics, and carotenoids (Koul and Chase, 2015).

Based on the explanation, moringa trees has many benefits in all of part. Therefore, this experiment want to measure the levels of vitamin C in leaves, stems, seeds fresh and dried with spectrophotometry and iodometry titration method and this experiment want to compare both of them which has highly vitamin C in all of part.

2. RESEARCH METHOD

This research is a quantitative research that aims to analyze the levels of vitamin C in fresh and processed maize stems by spectrophotometric method and iodometric titration. The subject of this research is stem of kelor that comes from one tree in Sidikan, Pandeyan, Umbulharjo, Yogyakarta. The object of this study is the level of vitamin C in the stems kelor fresh and processed dried.

a. Determination of Vitamin C using Iodometric Titration

1) Sample Preparation
   In this study, used part of the kelor tree (leaves, stems, and seeds) are washed clean first. Weighed 1.5 grams. To sample the leaves and seeds first crushed / mashed by using mortar while adding 50 mL of distilled water at a time. To sample the rods first enlarged the surface area by using a blender. After a bit smooth, gerus / puree with mortar while adding 50 mL of distilled water at a time. Furthermore, the mixture is filtered by using a buchner filter so that the resulting filtrate can be maximized.
2) Making a 0.1 N $Na_2S_2O_3$ Solution
   Consider carefully 2.48 g of $Na_2S_2O_3.5H_2O$ crystals. Dissolve with 50 mL cold, cold water. Enter the solution in a 100 mL measuring flask and add a few $Hg(CN)_2$ or CCl_4, then add the distilled water to the limit marker.
3) Standardization of $Na_2S_2O_3$ solution with KIO_3
   Consider carefully 0.36 g KIO_3 crystals, dissolve in a measuring flask up to exactly 100 mL. Pipette 12.5 mL of solution into Erlenmeyer, add 0.5 g of KI and 1.5 mL of 3 M sulfuric acid. The solution is diluted with 0.1 N $Na_2S_2O_3$ solution to the straw yolks, then add the starch indicator and continue the titration until the blue color disappears.
4) Determine levels of vitamin C in moringa trees
   Amount 1.5 grams of sample were dissolved with 100 mL distilled water. Pipet as much as 10 mL insert in Erlenmeyer, add 1 g KI, 5 mL acetic acid 2 N, and 10 mL distilled water.

Then add the starch indicator and continue the titration of 0.1 N $Na_2S_2O_3$ solution until the blue color disappears.

$$\% \text{ vit C} = \frac{100}{10} \text{ x } \frac{\text{Vt x N Na2SO3 x BE ascorbat acid}}{\text{sample mass}} \text{ x } 100\%$$

b. Determination of Vitamin C in Spectrophotometric Samples

1) Sample Preparation
In this study, used part of the kelor tree (leaves, stems, and seeds) are washed clean first. Weighed as much as 25.0 grams. To sample the leaves and seeds first crushed / mashed by using mortar while adding 50 mL of oxalic acid 0.4% bit by bit. To sample the rods first enlarged the surface area by using a blender. After a bit smooth, gut / puree with mortar while adding 50 mL of 0.4% oxalic acid bit by bit. Furthermore, the mixture is filtered by using a buchner filter so that the resulting filtrate can be maximized. The filtrate is taken and fed into a 500 ml measuring flask, and append it with 0.4% oxalic acid solution to the marking margin.
2) Preparation of a standard solution of vitamin C
Amount of 100.0 mg of pure ascorbic acid was fed into a 100.0 ml measuring flask and dissolved with 0.4% oxalic acid to 100.0 ml (1000 ppm).
3) Maximum wavelength determination
Dipped to 0.8 mL of vitamin C solution (1000 ppm), then inserted into 10 mL measuring flask (80 ppm concentration), 5% H_2SO_4 added 4 mL, and then added 5% ammonium molybdate to the mark and homogenized. Incubated for 30 minutes, then measured uptake with spectraic 20 in the wavelength range 500 - 630 nm to find the maximum wavelength.
4) Making standard curve
Ascorbic acid solution of 1000 ppm as much as 7 times that is 0.2; 0.3; 0.4; 0.5; 0.6; and 0.7 mL, each inserted into a 10 mL measuring flask, and then added 5 ml of H_2SO_4 by 4 mL and then sufficient volume with 5% ammonium molybdate to the marking, shuffled and homogenized (concentration 20; 30; 40; 50; 60 and 70 ppm), then incubated for 30 minutes. This standard solutian was measured with Spectronic 20 at maximum wavelength.
5) Measurement of vitamin C levels
The sample solution was inserted as much as 1.0 ml into a 10 mL measuring flask. Add a 4 mL of 5% H_2SO_4, then the volume was added to the mark limit with 5% ammonium molybdate, shaken until homogeneous and then incubated 30 minutes later measured uptake at maximum wavelength. (replicated 2 times).
6) Calculation of vitamin C levels
Calculation of vitamin C content is done by extrapolating vitamin C absorption data on linear regression equation from standard curve of vitamin C

3. RESULTS AND ANALYSIS

a. Qualitative Test

The data were obtained based on qualitative test. The experiment was about the level of Vitamin C in the samples tested by iodine solution. The result showed that all the samples contain Vitamin C. It was proved by the loss of color from iodine solution after being dropped on the samples.

b. Quantitative Test

1) Iodometric Titration Method

The data were collected based on the quantitative test on the level of Vitamin C in fresh Kelor leaf and dried Kelor leaf, using iodometric titration method as seen in Table 1.

Table 1. The level of Vitamin C in Samples

No.	Samples	Volume $Na_2S_2O_3$ average (mL)	Vitamin C levels (%)	Vitamin C levels (ppm)
1.	Fresh leaf	0,3	1,736	$1,736 \times 10^{-4}$
2.	Boiled leaf (15 minutes)	0,25	1,477	$1,477 \times 10^{-4}$
3.	Boiled leaf (30 minutes)	0,125	0,724	$7,24 \times 10^{-5}$
4.	Dried leaf	0,1	0,579	$5,79 \times 10^{-5}$
5.	Fresh stem	0,075	0,434	$4,34 \times 10^{-5}$
6.	Boiled stem (15 minutes)	0,05	0,289	$2,89 \times 10^{-5}$
7.	Boiled stem (30 minutes)	0,05	0,289	$2,89 \times 10^{-5}$
8.	Dried stem	0,05	0,289	$2,89 \times 10^{-5}$
9.	Boiled seeds (15 minutes)	0,175	1,013	$1,013 \times 10^{-4}$
10.	Boiled seeds (30 minutes)	0,05	0,289	$2,89 \times 10^{-5}$

2) Spectrophotometric Method

The data were collected from the quantitative test about the level of Vitamin C in fresh Kelor leaf and dried Kelor leaf as the samples, using spectrophotometric method. The level of Vitamin C were presented in the Table 2.

Table 2. The level of Vitamin C in the samples

Samples	Average Absorbance	Levels of Vitamin C (ppm)
Fresh leaf	0,172	37,92
Boiled leaf (15 minutes)	0,1555	33,90
Boiled leaf (30 minutes)	0,0665	12,19
Dried leaf	0,05	8,17
Boiled seeds (15 minutes)	0,056	9,63
Boiled seeds (30 minutes)	0,047	7,44
Fresh stem	0,0665	12,19
Boiled stem (15 minutes)	0,05	8,17
Boiled stem (30 minutes)	0,0465	7,32
Dried stem	0,012	-1,097

Based on the study, the result of this test showed that both samples which are fresh kelor leaf and dried kelor leaf contained Vitamin C. The result was proven by the loss of color from iodine solution after being dropped on the samples. Betadine contains 10% povidone iodine active compound equivalent to 1% iodine. Vitamin C reacts with iodine with the following reaction equation.

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I^- + 2H^+$$

To produce I_2 compound which will be used in redox titration, the addition of excess I^- ion is needed in one sample which also contains some ion iodate (IO_3).

$$IO_3^-(aq) + 5I^-(aq) + 6H^+(aq) \rightarrow 3I_2(aq) + 3H_2O(aq)$$

The excess of Iod (I_2) are titrated using standard thiosulfate solution ($S_2O_3^{2-}$) with starch indicator (kanji). The starch indicator will give a blue color when the end of the titration is reached with the following reaction:

$$I_2(aq) + S_2O_3^{2-}(aq) \rightarrow 2I^-(aq) + S_4O_6^{2-}(aq)$$

Based on the data in table 1, the whole titrated samples contain Vitamin C with various levels on each samples. The different levels of Vitamin C are caused by the different treatment toward the samples. Vitamin C is easily dissolve in water solvent, besides Vitamin C is also easily degraded by the heat. In this experiment the treatment such as washing, boiling, and drying the samples could decrease the levels of vitamin C.

The principle of iodine titration is that iodine meets the double bonds of vitamin C on the C 2nd and 3rd carbon atoms, the double bonds iodine adds will break into a single bond. If all of the vitamin C has been overdone by iodine then the iodine that drips further during the titration will react with the indicator solution of the amylum to form blue iodamilum. The formation of blue color indicates that the titration process has been completed, because all of the vitamin C has been added by iodine so that the required iodine volume when titration is equal to the amount of vitamin C.

The second method conducted is spectrophotometric. Spectrophotometric is an analytic method that based on the measurement of absorption from monochromatic light by a colored solution lane at specific wavelengths by using a prism monochromator or diffraction grating and vacuum phototube. In this experiment, the instrument was spectronic-20 that work to measure both the absorption and transmittance in the colored solution samples.

The samples absorption measurement was done on the maximum wavelength which is 600 nm. The level of vitamin C on the fresh kelor leaf and dried kelor leaf were collected by converting absorbance data in Table 2 into concentration (ppm) through straight line equation with the result of the equation is $y = 0.0041\ x + 0.0165$. It proves that the level of Vitamin C in absorbance concentration have positive corelation and the standards curve have decent accuracy on determining the concentration.

The result on dried stem samples showed that dried stem did not contain any Vitamin C. The cause of this thing is probably because the amount of Vitamin C in dried stem is very low and the dried treatment make the Vitamin C in it is degraded. The level of accuracy of the instruments used can also affect the results obtained in this experiment.

Based on two methods conducted for quantitative test on knowing the level of Vitamin C in the samples, the spectrophotometric method gave more accurate result rather than iodometric titration method. The level of accuracy using spectronic-20 instrument in spectrophotometric method is higher than iodometric titration method.

4. CONCLUSION

Both of two methods was able in determining the level of Vitamin C in Moriangan trees. But the study showed that the spectrorotometric methods is prefer in analyzing vitamin C level ratherthan the iodometric titration method.

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