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Comparative Study of Chlorophyll Content in Leaves of Thai *Morus alba* Linn. Species

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Abstract: Chlorophyll is an antioxidant which usually found in nature especially in green leafy plants. The experiment study had an objective to evaluate the content of chlorophyll A in two species of mulberry, Nakhorn Ratchasrima-60 and Burirum-60. The two species of mulberry were extracted by 95% ethanol as a solvent and the chlorophyll A extraction was collected and detected by spectrophotometer by mean of 2 wavelengths, 420 and 680 nm. The experiments were repeated 3 times and chlorophyll A was calculated by using the equation of Lorenzen. The results showed that the amounts of chlorophyll A from Nakhorn Ratchasrima-60 Strain were 15-929, 24.212 and 20.112 mg L^{-1} with the average of 20.084 mg L^{-1} and from Burirum-60 were 19.641, 21.137 and 18.713 mg L^{-1} with the average was 19.668 mg L^{-1}. The amount of chlorophyll A content in Nakhorn Rachasrima-60 species were slightly higher than in Burirum-60 species.

Key words: Mullberry leaf, chlorophyll A, lorenzen, spectrophotometer, solvent, Thailand

INTRODUCTION

Chlorophyll is one of the antioxidant compounds which are stored in the chloroplast of green leaf plants. Normally it has been found in the area of green leaves, stems, flowers and roots. Chlorophyll A is the primary pigment related to the photosynthesis action in plants and produces the energy for plants. The other pigments are known as accessory pigments. Chlorophyll A contents are 2-3 times higher than the level of chlorophyll B.

Chlorophyll is an essential element to the plants and is an important substance that can be used as nutritional approaches in decreasing blood sugar, in detoxification, in digestion, excretion and lowering the allergens. From the above criteria, the researchers are interested in detecting the chlorophyll A content in Thailand *Morus alba* between 2 species, Burirum-60 and Nakhorn Rachasrima-60 and which are easily grown in Nakhon Rachasrima region of Thailand. The experiment was carried out at the Department of Silk Innovation Center, Mahasarakham University. The solvent used for extraction was 95% ethanol (Porra and Scheer, 2000). It was aimed at quantifying chlorophyll A content in 2 different species of Thai *Morus alba* species, Nakhorn Rachasrima-60 species and Burirum-60 species by 95% ethanol solvent extraction method.

This research was an experimental study and aimed at detecting the content of chlorophyll A between 2 species of Thailand *Morus alba* Linn. (Burirum-60 and Nakhon Rachasrima-60) which were extracted by 95% ethanol. Then the extracted solvent from both *Morus alba* species were brought to be detected for the quantity of absorbance (OD) by spectrophotometer (Ronenm and Galun, 1984). The experiment period started from December 2010 to February 2011. The mulberry leaves were measured at medium age which were collected from the leaves no. 9 above the ground. The leaves were homogeneous in shape, equal size and healthy smooth green color. The *Morus alba* (Nakhon Rachasrima-60) leaves appear as heart shape with a very a smooth surface but *Morus alba* (Burirum-60) leaves appear as thicker with soften surface. Some study showed that the rate of photosynthesis is directly proportional to light intensity.

Chlorophyll has the structure similar to human red blood cells and they are useful in the production of tooth paste, bakery products and medicine, etc. Chlorophyll

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helps in protecting gastric mucosa cells, in detoxification in liver and kidney, in lowering blood sugar (Kimura *et al*., 2007) and also helps increasing the immune system.

The active nutrients of mulberry consist of 24.7% protein, 40-68% of carbohydrate, 10-12% of minerals such as calcium, phosphorus, manganese, magnesium, iron, copper and zinc. Some of the research found that there is high content of vitamin C. The dry mulberry 100 g contains 4,320 I.U. of vitamin A, 0.6 mg of green tea and English green tea and mulberry roots also found some content of tyrosinase enzyme.

In 1930 Hans Fisher studied the structure in chlorophyll. The chlorophyll is consumed by human oral intakes; it will increase level of Heme production. Yoshita Hakiwara also found that the chlorophyll can absorp in the small intestine totally. Andallu *et al*. (2001) on plasma and erythrocyte lipids membrane in Diabetes type-2 patients.The study found that the efficacy in lowering blood sugar. Mulberry is higher than the efficacy of glibenclamide with statistically significant. There was the effect in lowering of fast blood glucose with statistically significant but no effect in the level of HbA1c.

MATERIALS AND METHODS

This research was experimental study which aimed at detecting the quantity of chlorophyll A content in Mulberry leaves of 2 species, Nakhorn Rachasrima-60 and Burirum-60 from the Division of Silk Innovation Center, Mahasarakham University, Thailand. The study was conducted 3 times in order to determine the content of chlorophyll A by using Light Absorbance values (OD) from spectrophotometer. Results were calculated by using Lorenzen equation. The process began as follows:

- Preparation of fresh and blended mulberry leaves as samples (Hiscox and Israleistam, 1979)
- The extraction of chlorophyll A with 20 mg ethanol 95% as a solvent (15 min) and fllter the solution
- Detecting the chlorophyll A by measuring the light absorbance (OD) by spectrophotometer with 2 wavelengths at 420 and 680 nm
- Calculation of the amount of chlorophyll A in each species of mulberry by Lorenzen equation:

Chlorophyll A content = 11.4 K [(A420-A680)-B420-B680)] VS/L (mg L^{-1})

- When A 420 for the absorbance of light of sample solution (wavelength at 420 nm)
- When A 680 for the absorbance of light of sample solution at (wavelength at 680 nm)
- When B 420 for the absorbance of standard solution (at wavelength 420 nm)
- When B 680 for the absorbance of standard solution (at wavelength 680 nm)

V = Volume of the extraction (mL)
L = The thickness of cuvette (cm)
S = Volume of filtered solution (mL)
K = Constant = 2.43

RESULTS AND DISCUSSION

The analytical study of chlorophyll A content was derived from Lamberts law which illustrated that the concentration of extracted chlorophyll was directly proportional to the absorption of monochromatic light and was not affected by the reflection of light at the surface of the extracted solution Beer's law also stated that the absorption of monochromatic light was directly proportional to the quantity of medium which absorbed the light. The absorbance of light was direct proportional to the concentration of medium solution and the length of the light which transmitted through the solution.

The analysis of chlorophyll A content in Nakhorn Ratchasrima-60 mulberry species was started with the extraction of mulberry 4 g for 20 mL, 95% ethanol extraction solvent by macerating them for 15 min. Then, the solvent was blended and was filtered to obtain the solution which was measured the content of chlorophyll A by visible light spectrophotometer at 420 and 680 nm with the calculating of Lorenzen equation (Ritchie, 2006).

The result of the absorbance at 420 nm was found at 2.332, 2.554, respectively and the average at 2.360. The result of absorbance at 680 nm wavelength light was also found at 1.745, 1.468 and 1.658 with the average of 1.623 by using the Lorenzen equation (Ronen and Galun, 1984) (Table 1). We can calculate the content of chlorophyll A as follows:

Formular: Chlorophyll A content
= 11.4k [(A420-A680)-(B420 - B680)] VS/L

First analysis: Chlorophyll A content
= 11.4×2.43×[(2.332 - 1.745)-(0.074-0.062)]×20/20×1
= 15.929 mg L^{-1}

Second analysis: Chlorophyll A content
= 11.4×2.43×[(2.354-1.468)-(0.074-0.062)]×20/20×1
= 24.212 mg L^{-1}

Table 1: Absorbance of light (nm) and content of chlorophyll A in Nakhorn Rachasrima-60 mulberry species

	The absorbance of light			
Wave length (nm)	First	Second	Third	Mean
420	2.332	2.354	2.390	2.360
680	1.740	1.468	1.658	1.623
Chlorophyll A content (mg L^{-1})	15.929	24.212	20.112	20.084

Table 2: The absorbance of light and the content of chlorophyll A in Burirum-60 mulberry species

	The absorbance of light			
Wave length (nm)	First	Second	Third	Average
420	2.369	2.339	2.357	2.355
680	1.648	1.564	1.689	1.633
Chlorophyll A content (mg L^{-1})	19.641	21.137	18.173	19.668

Third analysis: Chlorophyll A content

$= 11.4\times2.43\times[(2.396-1.658)-(0.074-0.062)]\times20/20\times1$

$= 20.112$ mg L^{-1}

Average chlorophyll A content

$= 11.4\times2.43\times[(2.360-1.623)-(0.074-0.062)]\times20/20\times1$

$= 20.084$ mg L^{-1}

The analysis of chlorophyll A content in Burirum-60 mulberry species: The process of analysis was carried out the same as in Nakhorn Rachasrima mulberry species. The results revealed that the aborbance of the light with 420 nm were 2.369, 2.339, 2.357 within the average at 2.355 and the result of the absorbance of light with 680 nm. Were 1.648, 1.564, 1.689 within the average at 1.633 and the calculation of chlorophyll A from Lorenzen equation as follows: the content of chlorophyll A (Table 2).

$= 11.4\times k\times[(A420-A680)-(B420-B680)]\times(V/L\times S)$

$= 19.641$ mg L^{-1}

$$\text{The content of chlorophyll A} = 11.4\times2.43\times\begin{bmatrix}(2.369-1.648)-\\(0.074-0.062)\end{bmatrix}\times20/20\times1 = 19.641 \text{ mg } L^{-1}$$

Secondly:

$$\text{The content of chlorophyll A} = 11.4\times2.43\times\begin{bmatrix}(2.339-1.648)-\\(0.074-0.062)\end{bmatrix}\times20/20\times1 = 21.137 \text{ mg } L^{-1}$$

Thirdly:

$$\text{The content of chlorophyll A} = 11.4\times2.43\begin{bmatrix}(2.357-1.689)-\\(0.074-0.064)\end{bmatrix}\times20/20\times1 = 18.1725 \text{ mg } L^{-1}$$

$$\text{The average content of chlorophyll A} = 11.4\times2.434\begin{bmatrix}(2.355-1.633)-\\(0.074-0.062)\end{bmatrix}\times20/20\times1 = 19.668 \text{ mg } L^{-1}$$

CONCLUSION

The result revealed that there was slightly higher in chlorophyll A content in Nakhorn Rajchasrima-60 mulberry species than in Burirum-60 mulberry species. The average content of chlorophyll A in Nakhorn Rachasrima was 20.084 mg L^{-1} and the average content of chlorophyll A in Burirum-60 species was 19.668 mg L^{-1}. The method of extraction in this research was called destructive because we have to macerate and blend mulberry leaves in 95% ethanol. Some experimental studies were used in the detection method by using chlorophyll meter to measure the chlorophyll content with non-destructive method by Marquard and Tipton.

This study will be benefit to chlorophyll content research in various species of plants which can classify the content of chlorophyll. The study can also apply to the different wavelengths of light in order to detect the chlorophyll content. The chlorophyll seems to have an important compound for health which can bring the essential nutrients to human haemoglobins and human cells. The comparative study in clinical efficiency of chlorophyll from *Morus* species of other plants in many indications of therapeutic choices should be very interesting in order to produce the natural health products as more convenient dosage forms in the future.

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REFERENCES

Andallu, B., A.V. Suryakantham, B.L. Srikanthi and G.K. Reddy, 2001. Effect of mulberry (*Morus indica* L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. Clin. Chim. Acta, 314: 47-53.

Hiscox, J.D. and G.F. Israelstam, 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot., 57: 1332-1334.

Kimura, T., K. Nakagawa, H. Kubota, Y. Kojima and Y. Goto, 2007. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. J. Agric. Food Chem., 55: 5869-5874.

Porra, R.J. and H. Scheer, 2000. ^{18}O and mass spectrometry in chlorophyll research: Derivation and loss of oxygen atoms at the periphery of chlorophyll macrocycle during biosynthesis, degradation and adaptation. Photosyn. Res., 66: 159-175.

Ritchie, R.J., 2006. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynth. Res., 89: 27-41.

Ronenm, R. and M. Galun, 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. J. Environ. Exp. Bot., 24: 239-245.