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# Nutritional Value and Inhibitory Activity Alpha-Amylase of Cookies Made from Addition of Mulberry Leaf and the Extract

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**Abstract:** Utilization of mulberry leaves have the potential ability to be developed, especially for diabetics group. From the previous research proved that mulberry leaf extract and the instant powder had inhibitory activity against alpha-glucosidase enzyme. In this study, it had been observed the nutritional value and alpha-amylase inhibitory activity on cookies made from addition of Mulberry leaf and the extract. The formulation of the cookies using "trial and error". The treatment used is the addition of mulberry leaves (10, 20 and 30%) and the addition of the extract (10, 20 and 30%). The collected data were sensory evaluation, nutritional value, tannins and inhibitory activity against alpha-amylase enzyme. The results showed that Mulberry leaf addition up to 20% and the extract addition up to 30% were acceptable by the panelist. The cookies contain: 3.19%±0.11 up to 3.64%±0.03, moisture content, 2.63%±0.04 up to 2.94%±0.01 ash content, 6.75%±0.37 up to 8.41%±0.42 protein content, 9.54%±0.73 up to 15.27%±0.74 fat content, 71.26%±0.86 up to 76.42%±0.98 carbohydrate, 0% up to 2.40%±0.15 tannin. Inhibitory activity alpha-amylase on cookies made from mulberry leaf extract 10, 20 and 30% were 46.56%±0.94; 95.57±0.25% and 75.74%±0.82, respectively. Inhibitory activity alpha-amylase on cookies made from mulberry leaf 10, 20 and 30% were 50.82%±0.47; 76.89±0.57% and 48.96%±0.70, respectively.

**Key word:** Cookies, inhibitory activity, mulberry leaf and the extract, alpha-amylase

## INTRODUCTION

Based on research that has been conducted in Indonesia, the number of people with diabetes mellitus ranged from 1.2-2.3% of the population aged 15 year and above, this is likely to increase in line with economic growth. It is caused by changes in the diet of people from traditional foods to fast food (Dalimartha, 2002). Several attempts have been made to overcome this problem include the use of oral antidiabetic drugs and the use of herbal plants.

Mulberry leaf extract have activity to decrease glucose serum level, it is associated with a content of compounds in the leaves which can suppress the activity of alpha-glucosidase enzyme present in the gut (Miyahara *et al*., 2004; Asano *et al*., 1994). Mulberry leaf extract can reduce sugar levels in mice strongly after being given a carbohydrate, through the inhibition of enzyme activity in the small intestine disakaridase (Miyahara, *et al*., 2004). A study showed that oral administration powder enriched with deoksinojirimisin (DNJ) as much as 0.8 and 1.2 g, significantly suppressed the increase in glucose levels after dinner, this indicates a physiological effect of DNJ contained in mulberry leaves (Kimura *et al*., 2007).

Mulberry leaf extract is also able to reduce fluctuations in blood sugar levels; This may reduce complications in diabetics (Mudra *et al*., 2007). Other studies have also reported that mulberry extract function in vascular disorders recovery in diabetic rats, further explained that this allegedly associated with reduced oxidative stress (Naowaboot, 2009). Containing mulberry DNJ is known strongly to inhibit the activity of alpha-glucosidase enzymes in the intestine (Asano *et al*., 1994) and in mulberry also contain polyphenolic compounds (Arslan *et al*., 2004; Arabshahi *et al*., 2007; Chan *et al*., 2009) and flavonol glycosides (Katsube *et al*., 2006).

Mulberry widely grown in West Sumatra and usually grow around the house and not utilized optimally. Utilization of mulberry leaves have the potential ability to be developed as an adjunct to diet, especially for diabetics group. Sayuti (2010) has been processing mulberry leaves to extract powder and it proved that after it was processed to be instant powder, it still showed inhibitory activity against alpha-glucosidase enzyme. Further demonstrated that DNJ concentration in the extract powder is 0:01±0.82 mg/g. These results indicate that anti-glycosidase DNJ that are contained in mulberry leaves which are water soluble and very high temperature resistance, still shows activity when incorporated in foodstuffs.

In this study, it had been tried to add the mulberry leaves and the extracts into snack food. One of the snack food is cookies. Cookies are snacks eaten between main meals made from flour. This research studied the effect of cookies made from addition mulberry leaves and extracts on the nutritional value and enzyme inhibition activity of alpha-amylase.

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## MATERIALS AND METHODS

**Materials:** The main ingredient is added in making cookies are mulberry leaves and the extracts. It is also necessary ingredients are flour, milk powder, margarine and salt. The tools used in this study were the vortex (Velp), spectrophotometer (Shimadzu 1800 UV-Vis), oven (Buchi), furnace (Buchi), Kjedhal pumpkin, pumpkin fat, desiccator, micropipette (Brand) and glass tools.

**Making cookies and mulberry leaf extract:** The formulation of cookies conducted by using "trial and error". The treatment used in this study is the addition of mulberry leaves (10, 20 and 30%) and the addition of the extract (10, 20 and 30%) and without the addition of mulberry leaves and extracts as a controls. Mulberry leaf extract obtained by boiling the leaves according to treatment (10, 20 and 30% of the weight of the total) in 1 L of water until the volume becomes 30 mL. Then the extract is mixed with wheat flour, milk, margarine and salt and then baked in the oven, so it becomes a cookies. This is used as a sample.

**Observation:** The observations were sensory evaluation, nutritional value include: moisture content, ash content, protein content, fat content, carbohydrate content, determination of tannins and inhibitory activity against alpha-amylase enzyme.

**Sensory evaluation:** Sensory evaluation is done by looking at the level of preference of 25 panelists for texture, color, flavor and taste. A level of preference from the panelists was assessed from Strongly disliked, disliked, neutral, liked and really liked.

**Determination of nutritional value and tannin:** Nutritional value include; moisture content using the oven method by drying the sample in an oven (Buchi) at a temperature of 105°C and moisture content is determined based on the dry weight percentage the initial sample weight. Ash content determination is done by burning the samples in a furnace (Buchi) at a temperature of 550°C and ash content expressed as a percentage of ash weight to dry weight of the sample. The determination of protein content using the Micro Kjedhal (stages in this process include the destruction, distillation and titration, total nitrogen obtained is multiplied by a conversion factor to obtain the percentage of total protein samples). The determination of fat content using Soxhlet extraction method. Determination of carbohydrates by different methods. (Sudarmadji *et al*.,1997).

**Determination of tannins (Sudarmadji *et al*.,1997):** Five grams of finely ground material was added 400 mL of distilled water and then boil for 30 min. After cooled put into 500 mL volumetric flask and add distilled water to mark boundaries, then filtered (filtrate 1). Taken as much as 10 mL of the filtrate was added 25 mL of solution 1 indigocarmin and 750 mL distilled water. Next titrated with 0,1 N $KMnO_4$ solution until the yellow color, eg: required A mL. 100 mL of the filtrate was taken in a row 1+50 mL gelatin, 100 mL of acid salt, 10 g kaolin powder, then shaken vigorously a few minutes and filtered (filtrate 2). 2 taken 25 ml filtrate, mixed with a solution of 25 mL indigokarmin and 750 mL distilled water. then titrated with 0.1 N $KMnO_4$ solution until the yellow color of gold, for example, it takes B mL. standardize $KMnO_4$ solution with Na-oxalate:

$$\text{Tannin} = \frac{(50A - 50B) \times \frac{N}{0.1} \times 0.00416}{5} \times 100\%$$

N = Normal $KMnO_4$
One milliliter $KMnO_4$ 0.1 N = 0,00416 g tannin

**Determination of inhibitory activity against alpha-amylase enzyme**

**Preparation of reagents:** One unit/mL alpha-amylase enzyme dissolved in cold distilled water. Activity of the enzyme alpha-amylase inhibition was detected using the substrate starch solution (1%) in 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. Besides DNS reagent (dinitrosalisilat acid) is made by mixing a solution of 20 mL of Na-K-tartaric, 50 mL distilled water control and to obtain a final volume of 100 mL. Solution of Na-K-tartaric obtained by dissolving 30 g of Na-Ktartarat in 20 mL of 2 M NaOH over heating plate. DNS solution obtained by dissolving 3.5 dinitrosalisilat 1094.88 mg of acid into 50 mL of distilled water at a temperature of 45-50°C. Sample solution is made by dissolving cookies with a concentration of 1%.

**Analysis procedures:** Reaction mixture consisting of the blank (200 mL and 100 mL buffer solution of 1% starch), control A (100 mL buffer, 100 and 100 mL enzyme solution 1% starch), control B (100 mL sample, 100 and 100 mL buffer starch solution 1%) and samples (100 mL sample, 100 and 100 mL enzyme solution of 1% starch). Each reaction mixture was incubated in a water bath at a temperature of 37°C for 30 min. Then added to each DNS solution of 200 mL and incubated for 5 min in boiling water. Then 4,000 mL of distilled water was added and the absorbance was measured with a spectrophotometer (Shimadzu UV-Vis 1800) at λ 540 nm.

Inhibitory activity of the sample is calculated using the formula:

$$\text{Inhibition}(\%) = \frac{A1 - A2}{A1} \times 100\%$$

Explanation:

- A1 = Absorbance control A-Absorbance blanco
- A2 = Absorbance sample-Absorbansi control B

**Statistical analysis:** The design used in this study is completely randomized design with three replications. If the results show a difference due to the treatment, then followed by Duncan's test New Multiple Range Test (DNMRT) at the 5% significance level.

## RESULTS AND DISCUSSION

**Sensory analysis:** The results showed the addition of extract (10, 20 and 30%) and the addition of mulberry leaves (10%) in the raw materials produced cookies slightly better than the control. While the addition of mulberry leaves 20% on raw materials produced cookies with slightly lower acceptance rate than controls, but still acceptable. The addition of leaves 30%, resulting in a less than acceptable cookies (Fig. 1).

Table 1, shows the effect of mulberry leaves and the extracts on preference test. Highest acceptance average is the addition 20% extract, with a preference level for the texture, color, flavor and the taste were 3.92±0.57, 4:04±0.61,3.84±0.69 3.92±0.76, respectively.

The addition of the extract up to 30%, did not show any changes in texture, color, flavor and the taste of cookies. While the addition of the leaf 10, 20 and 30%, giving effect to color, texture, flavor and the taste. The addition of 20% leaves, produce cookies with a hard texture and taste of the leaves deeply felt. The addition of mulberry leaves, which have the highest value is the addition of mulberry leaves 10%, with an average value of the panelist on texture, color, flavor and the taste were 3.92±0.64, 4.08±0.91, 3.84±0.80, 3.68±0.80, respectively.

**Nutritional value of the cookies:** Analysis of nutritional value conducted on nutritional content on the cookies that are acceptable by the panelists that was the addition of leaf extract 10, 20 and 30% and the addition 10 and 20% of leaves.

Table 2 shows that moisture content ranging from 3.19%±0.11 up to 3.64%±0.03. By using statistically analysis showed that addition of the extract have significantly different on the moisture content, ($p<0.05$), that are between addition 10% extract and addition 20%, 30% extract, but there are not significant difference between addition 20 and 30% extract. Similarly, the addition of 10 and 20% of leaves, there is significant differences, which increase the number of leaves causes increasing water content. Although if viewed from the numbers, do not show a big difference, which is about 3%.

Ash content ranged between 2.63%±0.04 up to 2.94%±0.01. By using statistically analysis shows, addition of leaf and the extract have significancy difference on ash content of the cookies ($p<0.05$), that are addition 10% extract with addition 20% extract, 30% extract, 10% leaf and 20% leaf and between addition 20% extract, 30% extract and 10% leaf with 20% leaf have significantly difference. But the addition 20% extract, 30% extract and 10% leaf have no significantly difference. This data show that increase the number of Mulberry leaf and the extract cause can increase the ash content on the cookies. It was assume that is related with the ash content of the Mulberry leaf. Syahrir *et al.* (2009) showed that the ash content of mulberry leaves aged 30 days was 10.92% and the age of 60 days was 13.23 and 16.60% in the leaf extract.

Ash contained in the materials associated with the mineral. Minerals is in mulberry leaves: calcium and

Table 1: Average of value of preference test on cookies made from addition of Mulberry leaf and the extract

| | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | Extract 10% | Extract 20% | Extract 30% | Leaf 10% | Leaf 20% | Leaf 30% |
| Texture | 3.80±0.58 | 3.88±0.83 | 3.92±0.57 | 3.96±0.73 | 3.92±0.64 | 3.30±0.90 | 3.32±0.18 |
| Color | 3.80±0.65 | 4.04±0.61 | 4.04±0.61 | 3.92±0.76 | 4.08±0.91 | 3.60±0.91 | 2.96±1.17 |
| Flavor | 3.60±0.65 | 3.68±0.63 | 3.84±0.69 | 3.76±0.66 | 3.84±0.80 | 3.50±0.92 | 3.48±1.05 |
| Taste | 3.48±0.82 | 3.64±0.86 | 3.92±0.76 | 3.72±0.74 | 3.68±0.80 | 3.40±1.08 | 3.20±1.15 |

5 = Really liked, 4 = liked, 3 = Neutral, 2 = Disliked, 1 = strongly disliked

Table 2: Average of nutritional value of the cookies made from addition of Mulberry leaf and the extract

| | Variable (%) | | | | |
|---|---|---|---|---|---|
| Treatment | Moisture content | Ash content | Protein content | Fat content | Carbohydrate |
| Extract 10% | 3.19±0.11$^{a}$ | 2.63±0.04$^{a}$ | 7.25±0.13$^{ab}$ | 13.05±0.28$^{b}$ | 75.89±0.57$^{b}$ |
| Extract 20% | 3.64±0.03$^{c}$ | 2.75±0.05$^{b}$ | 6.75±0.37$^{a}$ | 12.95±0.35$^{b}$ | 73.92±0.79$^{b}$ |
| Extract 30% | 3.54±0.02$^{bc}$ | 2.76±0.02$^{b}$ | 8.41±0.42$^{c}$ | 13.96±0.47$^{bc}$ | 71.35±0.09$^{a}$ |
| Leaf 10% | 3.28±0.03$^{a}$ | 2.75±0.01$^{b}$ | 7.44±0.09$^{ab}$ | 15.27±0.74$^{c}$ | 71.26±0.86$^{a}$ |
| Leaf 20% | 3.46±0.04$^{b}$ | 2.94±0.01$^{c}$ | 7.65±0.21$^{b}$ | 9.54±0.73$^{a}$ | 76.42±0.98$^{c}$ |
| p | 0.002 | 0.001 | 0.014 | 0.001 | 0.004 |

The number was followed by the same alphabet, is not significantly different at $\alpha$ = 5% by using DNMRT

Control

10% extract

20% extract

30% extract

10% mulberry leaf

20% mulberry leaf

Fig. 1: Continued

30% mulberry leaf

Fig. 1: Cookies control, cookies of mulberry leaf extract (10%, 20%, 30%), cookies of mulberry leaf (10%, 20%, 30%)

Table 3: Tannins content of cookie

| Treatment | Tannin (%) |
|---|---|
| Extract 10% | 0.00±0.00[a] |
| Extract 20% | 0.21±0.00[ab] |
| Extract 30% | 0.42±0.00[bc] |
| Leaf 10% | 0.52±0.14[c] |
| Leaf 20% | 2.40±0.15[d] |

The number was followed by the same alphabet, is not significantly different at alpha = 5% by using DNMRT

phosphorus, where the mineral content of young leaves is lower than the old leaves. This shows that the addition of leaf extract and can increase the mineral content in the cookies.

Protein content ranged from 6.75%±0.37 up to 8.41%±0.42. Based on the results of analysis of variance showed that the addition of mulberry leaves and extracts have a statistically significant effect on levels of protein cookies ($p<0.05$).

There is a significant difference between the addition of the extract 10%, 20% with 30% extract, getting more extract can increase the protein level on the cookie. This protein come from protein in the leave. According to Saddul *et al.* (2004) the protein content in dry leaves of Mulberry is 26.9-35.8 %.

Based on the analysis of variance, the addition of mulberry leaf and extract have significant different to fat content ($p<0.05$). An interesting data showed that the fat content of the addition 20% of leaves is the most lowest among other treatment (9.54%). It can cause by the lipid bounded with coarse fibers contained in the leaves, or lost due to the roasting cakes that contain lots of leaves. Mulberry leaves are low in fat (6.89%) which mean the source of fat in the cookie comes from the addition of margarine as a raw material.

Carbohydrate content analyzed by using by different method, derived from calculations performed the carbohydrate content of cookies are 71.26%±0.86, 76.42%±0.98. The carbohydrate content of cookies from mulberry extract and leaf showed a significant difference based on the statistical analysis of variance ($p<0.05$).

**Tannins content of cookie:** Table 3 showed that the tannins content of cookie range from 0%±0.00, 2.40%±0.15. High concentration of leaf and extract can cause the high presence of tannin.

Based on the results of analysis of variance followed by DNMRT test the addition of mulberry extract and leaves showed significant effect on levels of tannins in cookies ($p<0.05$). Tannins are polyphenols that are part of the antioxidant. The addition of the leaves have a better effect to increase the tannins content of cookies. On the addition of the extract 10%, tannins undetected by using such method, however, by increasing the number of leaves and extracts will increase the content of tannins in cookies.

The addition of leaves result the highest content of tannins. It can caused by the mulberry leaves contain tannins that are not soluble in. Levels of tannins contained in cookies with the addition of mulberry leaves ranged from 0.52%± 0.14 - 2.40%±0.15. While the levels of tannins in the cookies with the addition of mulberry leaf extract ranged between 0.00%±0.00 - 0.42%±0.00. According to Hagerman (2002), tannins are classified into two groups, namely condensed tannins and hydrolyzable tannins. Hydrolyzable tannin compound usually amorphous, hygroscopic, yellow and brown soluble in water (especially hot water) to form a colloidal solution, whereas the more pure condensed tannins, the less solubility in water and become more available in the form of crystals. The low content of tannins contained in cookies added with mulberry leaf and extract allegedly caused by the use of solvent extraction process leaves the water so that is extracted in the form of hydrolyzed tannins.

**Inhibitory activity of the alpha-amylase enzyme:** The inhibitory Activity of the alpha-amylase enzyme of the cookie from the mulberry extract range from 46.56%±0.94, 95.57%±0.25 and 75.74%±0.82, respectively. The inhibitory activity of the alpha-amylase enzyme of the cookie from the mulberry leave range from 50.82%±0.47, 76.89%±0.57 and 48.96%±0.70.

Figure 2 showed that the inhibitory of alpha-amylase enzyme increase followed by the addition of leave and extract up to 20%. Same result showed by Syahrir *et al.* (2009) that the addition of mulberry leaf extract can inhibit hydrolysis of disaccharides and polysaccharides into monosaccharides, resulting in decreased body weight of mice.

A decrease in inhibitory activity for the addition of leaves and extracts of 30%. As usually, the inhibitory activity should followed by the amount of leaves added into the cookies. Which means more DNJ on cookies will increase the inhibition activity with the high concentration of extract and leaves. At addition of 10% showed the

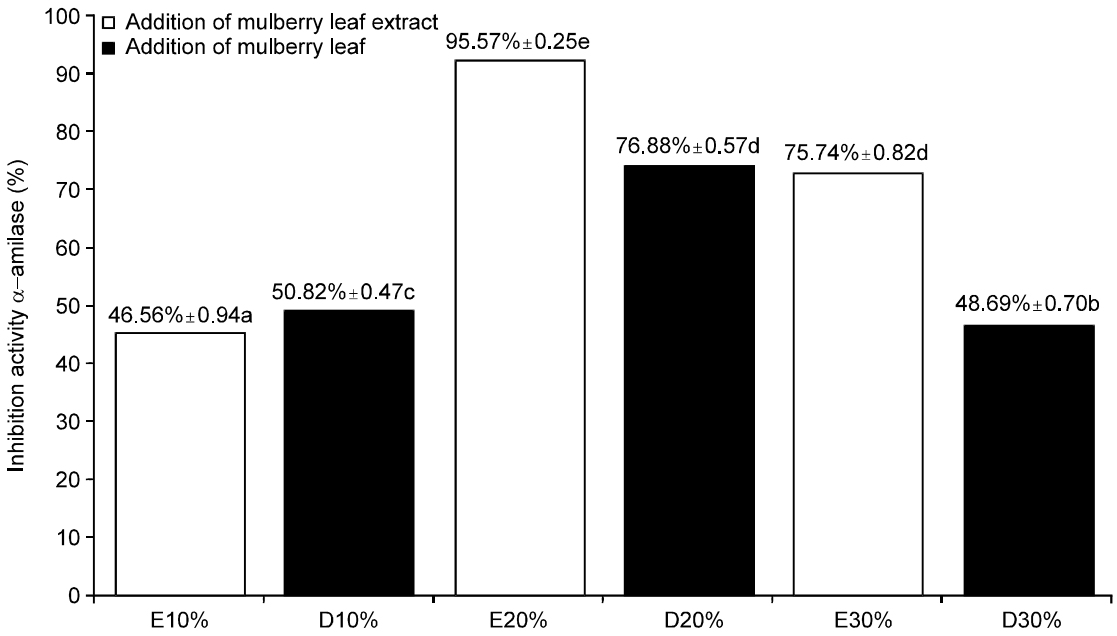


Fig. 2: Inhibitory Activity of the alpha-amylase enzyme in cookie the number was followed by the same alphabet, is not significantly different at $\alpha$ = 5% by using DNMRT

lowest activity to inhibit the alpha-amilase enzyme. The result showed that tannins have no effect to inhibit the alpha-amilase enzyme, but DNJ and crude fiber showed the activity in inhibit the alpha-amilase enzyme. The mulberry leaves contain more crude fiber than the extract.

**Conclusion:** The addition of 30% of extract and 20% of leave resulted acceptable by the sensory test. The moisture content of cookie were 3.19%±0.11, 3.64%±0.03, ash content were 2.63%±0.04, 2.94%±0.01, the protein content were 6.75%±0.37, 8.41%±0.42, the fat content were 9.54%±0.73, 15.27%±0.74, carbohydrate content were 71.26%±0.86, 76.42%±0.98, tannin content were 0%±0.00, 2.40%±0.15. The Inhibition Activity of the alpha-amylase enzyme added with the Mulberry extract 10%, 20% and 30% were 46.56%±0.94, 95.57%±0.25 and 75.74%±0.82, respectively, while he Inhibition Activity of the alpha-amylase enzyme added with the mulberry leave 10, 20 and 30% were 50.82%±0.47, 76.89%±0.57 and 48.96%±0.70, respectively. Diabetic people can also eat these cookies.

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## REFERENCES

Arabshahi-Delouee, S. and A. Urooj, 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. Food Chem., 102: 1233-1240.

Arslan, O., M. Erzengin, S. Sinan and O. Ozensoy, 2004. Purifikasi of mulberry (Morus alba L) polyphenol oxidase by affinity chromatography and investigation of its kinetic and elektrophoretic properties. Food Chem., 88: 479-484.

Asano, N., E. Tomiyoko, H. Kizu and K. Matsui, 1994. Sugars with nitrogen in ring isolated from leaves of mulberry bombycis. Carbohydr Res., 253: 235-245.

Dalimartha, S., 2002. *Ramuan Tradisional Untuk Pengobatan Diabetes Melitus*. Jakarta: Penebar Swadaya.

Hagerman, A.E., 2002. Tannin Chemistry. USA: Miami University Oxford.

Katsube, T., N. Imawaka, Y. Kawano, Y. Yamazaki, K. Shiwaku and Y. Yamane, 2006. Antioxidant flavonol glycosides in mulberry (*Morus alba* L.) leaves isolated based on LDL antioxidant activity. Food Chem., 97: 25-31.

Kimura, T., K. Nakagawa, H. Kubota, Y. Kojima, Y. Goto, K. Yamagishi, S. Oita, S. Oikawa and T. Miyazawa, 2007. Food-Grade Mulberry Powder Enriched with 1-Deoxynojirimyn Suppresses the Elevation of Postprandial Blood Glucose in Humans. J. Agric. Food Chem., 55: 5869-5874.

Kuei-huan, Chan., Ho Hsieh-Hsun, Chen-Ning Huang, Ming-Cheng Lin, Hsiang-Mei Chen and Chau-Jong Wang, 2009. Mulberry leaf extract inhibits vascular smooth muscle cellmigration involving a block of small GTPase and akt/NF-kB signal. J. Agric. Food Chem., 57: 9147-9153.

Miyahara, C., M. Miyazawa, S. Satoh, A. Sakai and S. Mizusaki, 2004. Inhibitory effects of mulberry leaf extract on postprandial hyperglycemia in normal rats. J. Nutr. Sci. Vitaminol, 50: 161-164.

Mudra, M., N. Ercan-Fang, L. Zhong, J. Furne and M. Levitt, 2007. Influence of mulberry leaf extract on the blood glucose and breath hydrogen response to ingestion of 75 g Sucrose by Type 2 Diabetic and Control Subjects. Diabetes Care. Volume 30, number 5.

Naowaboot, J., P. Pannangpetcha, V. Kukongviriyapana, S. Nakmareong and A. Itharat, 2009. Mulberry leaf extract restores arterial pressure in streptozotocin-induced chronic diabetic rats. Nutr. Res., 29: 602-608.

Saddul, D., Z.A. Jelan, J.B. Liang and R.A. Nalim, 2004. The potrential of Mulberry (Morus alba) as a fodder crop: the effect of plant maturity in yield, persistence and nutrient component on plant fraction. J. Anim. Sci., 17: 1657-1662.

Sayuti, K. and N. Muto, 2010. Kadar 1-deoksijirimisin dan aktivitas penghambatan terhadap enzim a-Glukosidase dalam ekstrak, tepung ekstrak dan tepung instant Murbai (*Morus alba* L). J. Teknologi and Industri Pangan Vol XXI nomor, 2: 160-164.

Syahrir, S., K.G. Wiryawan, A. Parakkasi and W. Winugroho Ramdania, 2009. Daya hambat hidrolisis karbohidrat oleh ekstrak daun murbai. Agripet Volume 9 Nomor, 2: 1-9.

Sudarmaji, S., B. Haryono and Suhardi, 1997. Prosedur Analisis Untuk Bahan Makanan dan Pertanian. Liberty. Yogyakarta.