Master's Thesis of Science in Agriculture

Characteristics of Mangiferin Glucoside Synthesized by *Leuconostoc mesenteroides* B-512 F/KM Dextransucrase and Its Bio-functionality

덱스트란수크라이제를 이용하여 합성한
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Characteristics of Mangiferin Glucoside Synthesized by *Leuconostoc mesenteroides* B-512 F/KM Dextransucrase and Its Bio-functionality

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Abstract

Mangiferin as the major constituent in *Mangifera indica* has attracted many interests due to its various physiological activities, such as antioxidant, immunomodulatory, anti-inflammatory, anti-microbial, and antiviral activities. However, its poor solubility in water limits its use and development in food industry and as a medical agent. In this study, enzymatic glycosylation using dextransucrase from *Leuconostoc mesenteroides* B-512F/KM was conducted to increase the solubility of mangiferin. Four novel mangiferin glucosides were synthesized and confirmed by MALDI-TOF, ranging from one to four glucoses attached to mangiferin. The optimized conditions of mangiferin glucoside (Mg-G1) synthesis were obtained through response surface methodology (RSM). The predicted Mg-G1 formation was 10.90 mM at the optimum condition of 19 mM mangiferin, 49 mM sucrose, and 1.2 U/mL dextransucrase. The Mg-G1 was purified by using HPLC and its structure was determined using nuclear magnetic resonance (NMR) (1H, 13C, HMBC, HSQC, and COSY). The structure was confirmed as mangiferin-(1→6)-α-D-glucopyranoside. The water solubility, antioxidant activity (DPPH radical scavenging and SOD-like scavenging activity), and α-glucosidase inhibitory activity were further investigated. The water solubility of Mg-G1 was 824.7 mM, which was 2,322 times higher than that of mangiferin. Mg-G1 also had DPPH

radical scavenging activity than mangiferin, with SC_{50} value of 0.022±0.001 mM. However, the SOD-like scavenging activity of Mg-G1 was lower than mangiferin, with SC_{50} value of 0.035±0.005 mM. Mg-G1 also showed inhibitory activity against α-glucosidase and human intestinal maltase, with the IC_{50} value were 0.663±0.001 mM and 0.224±0.008 mM, respectively. Because of the improvement in its water solubility, mangiferin glucoside could be more preferable than mangiferin as an antioxidant and antidiabetic ingredient in dietary supplement and other biomedical applications.

Keywords: mangiferin (Mg), mangiferin glucoside (Mg-G1), acceptor reaction, solubility, antioxidant

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Literature Review

1. Mangiferin

Mangiferin is the major polyphenol constituent in the leaves, fruits, and stem barks of mango plant (*Mangifera indica*) [1], which also exists in other mangifera genera including *Mangifera persiciformis* [2] and *Mangifera odorata* [3]. Besides that, mangiferin can also be found in other plants such as *Bombax ceiba* [4], *Hypericum perforatum* [5], *Arrabidaea samydoides* [6] and the leaves of various coffee species [7,8,9,10].

Mangiferin structure contains two aromatic rings of 1,3,6,7-tetrahydroxyxanthone with a β-D-glucosyl residue (Fig 1). In the previous study, the *in silico* simulations of mangiferin had been conducted to validate the physiochemical properties of mangiferin (Table 1) [11].

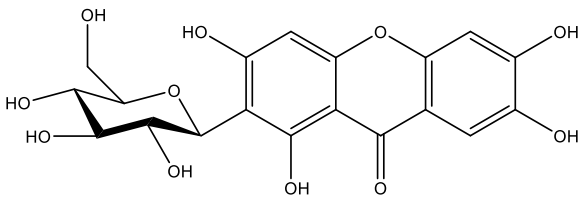

Figure 1. Structure of mangiferin

Mangiferin has attracted a lot of interest due to its various physiological activities, such as antioxidant, immunomodulatory, anti-

inflammatory, and anti-microbial activities [12]. Mangiferin also showed antiviral activity against HIV by inhibiting the HIV-1 replication [13]. The role of mangiferin as antidiabetic showed its potential to prevent the increase of blood sugar level in Type II diabetic model rats [14] and its hypoglycemic effects in diabetic rats [15]. The presence of functional groups contributes to the therapeutic potential of mangiferin [16].

Table 1. *In silico* simulations result of mangiferin [11]

Properties / Parameters	Value
Molecular formula	$C_{19}H_{18}O_{11}$
Molecular weight	422.35 g/mol
Solubility in water	1.44 mg/mL
Solubility fasted-state simulated gastric fluid	0.133 mg/mL
Solubility fasted-state simulated intestinal fluid	1.51 mg/mL
Solubility fed-state simulated Intestinal fluid	4.67 mg/mL
Melting point	274 ºC
log P	-0.59
log D (pH 7.4)	-0.80
P_{eff}	0.27×10^{-4} cm/s
P-gp substrate	Yes

Despite of its great potential, application of mangiferin for orally administered drug faces many challenges, especially due to its low bioavailability [17] and poor solubility [18]. Mangiferin showed a low oral absorption in rats with bioavailability only 1.2% and the plasma maximum were 715.04 ± 600.14 ng/mL [19]. It means that only 715.04 ± 600.14 ng/mL of mangiferin obtained after 0.72 h of its oral intake.

2. Dextransucrase from *Leuconostoc mesenteroides*

Dextransucrase is an extracellular enzyme that is produced by some bacteria species, especially from *Leuconostoc* and *Streptococcus* genera [20]. This enzyme is belong to glucosyltransferases class that hydrolyze sucrose, transfer its glucosyl residue with the release of fructose [21].

There are various strains of *Leuconostoc mesenteroides*, including B-512F, B-1355, and B-1299, which produce different type of dextran. The dextransucrase from *L. mesenteroides* B-512F produces a dextran containing 95% of α-(1,6)- linkages and 5% of α-(1,3)- branch linkages (Fig 2). The dextransucrase from *L. mesenteroides* B-1355 produces a similar dextran structure with B-512F dextran, with alternating sequence of 50% of α-(1,6)- and 50% of α-(1,3)- linkages. The dextransucrase from *L. mesenteroides* B-1299 produces a soluble and a less soluble dextran with the main chain in α-(1,6)- linkages and a single glucosyl residues in α-(1,2)- linkage.

The mutans, *L. mesenteroides* B-512FMC and B-512FMCM had been developed to overcome the low yield and impurity problems of the original commercial strain B-512F. The *L. mesenteroides* B-512FMCM produced more than 100 and 13 times enzyme than B-512F and B-512FMC, respectively [22,20]. Furthermore, by using the optimized purification methods, the purified B-512FMCM dextransucrase showed the highest

specific activity for dextransucrase [23].

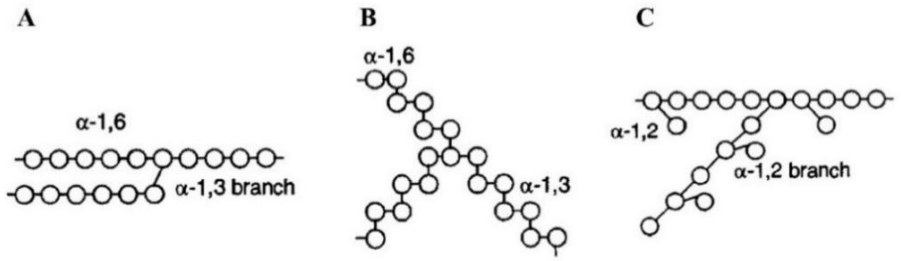

Figure 2. The structure of dextran that synthesized from various *L. mesenteroides* (A: B-512F Dextransucrase, B: B-1355 Dextransucrase, C: B-1299 Dextransucrase) [21]

3. Glycosylation and acceptor reaction

The glycosylation of phenolic compounds has been widely used as a method to improve their water solubility and stability [24, 25, 26, 27]. In general, the glycosylation can be carried out chemically or enzymatically [28]. In the synthesis of phenolic glycosides, these two methods are more economical and efficient compared to the extraction from natural resources. Recently, the enzymatic glycosylation has gained more attention due to its sustainable and environment-friendly methods [28, 29].

Dextransucrase has several mechanism of actions according to its characteristics. The B-512F dextransucrase produces a dextran through the formation of glucosyl intermediate [21]. The glucosyl units are transferred as the dextran chain at the reducing end. This elongation is terminated by acceptor reaction. Generally, in the acceptor reaction, the sugar moieties (glycosyl residue) from donor are broken and transferred to a specific aglycon (acceptor) (Fig 3) [28]. Many compounds including sugar, lipid, protein, flavonoids, and polyphenols that have hydroxyl or carboxyl group can be the acceptor in this reaction.

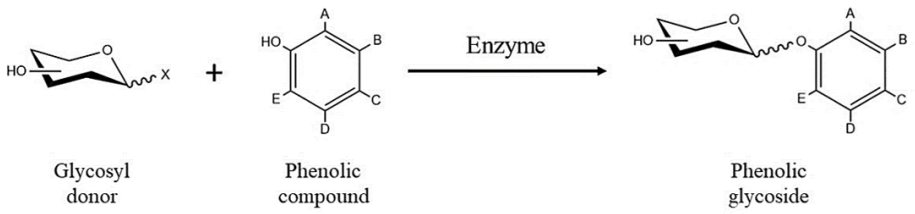

Figure 3. Acceptor reaction with phenolic compound as the aglycon [28]

4. Hypothesis and objectives

Mangiferin has been well known for its great benefits for health. However, the poor water solubility and the oral administration of mangiferin limits its use and development in food industry and as a medical agent. Many several methods have been used to synthesize mangiferin derivatives to overcome the hydrophobicity problem, such as the encapsulation using natural polymers [30], phospholipid complexation [31], β-cyclodextrin complexation [32], and enzymatic glycosylation using β-fructofuranosidase [33]. In this study, the enzymatic glycosylation using dextransucrase was carried out to enhance the water solubility and oral bioavailability of mangiferin, while still maintaining its bio-functionality.

Materials and Methods

1. Synthesis of mangiferin glucosides

The acceptor reaction was conducted according to a procedure reported by Kim *et al.* [34] with slight modification. Briefly, the reaction mixture containing 9.5 mM mangiferin in 20% DMSO, 325 mM sucrose, 2 U/mL B-512F/KM dextransucrase, and 20 mM sodium acetate buffer (pH 5.2) were incubated at 28 ºC for 3 hours. The reaction was terminated by boiling at 60 ºC for 10 min. The reaction mixture was spotted onto a TLC silica gel plate and developed using a solvent mixture of ethyl acetate : acetic acid : formic acid : water (100:11:11:26, v/v/v/v). Mangiferin, fructose, sucrose, and glucose were used as standard materials. Solvents were evaporated and the spot was visualized by developing the TLC plate in the staining solution, containing 0.5% (w/v) N-(1-napthyl) ethylenediamine dihydrochloride in methanol and 5% (w/v) sulfuric acid, followed by heating at 125 ºC.

2. Optimization of acceptor reaction using response surface methodology (RSM)

The acceptor reaction was optimized using Response Surface Methodology (Design Expert 11.0.0, USA), with Central Composite Design (CCD) as the experimental design. There were three independent variables: mangiferin concentration, enzyme concentration, and sucrose concentration. The experimental RSM data were fitted in the second-order polynomial equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2$$

where Y represents the predicted response, β_0 is the constant, β_1, β_2, β_3 are the regression coefficients, and x_1, x_2, x_3 are the independent variables. Statistical analyses of the experimental design were performed to determine the effects of each independent variable. The effects of those variables on the response were presented in three dimensional surface plots. The coefficient of determination (R^2) or adjusted R^2 represented the relationship between the dependent variable and the polynomial model equation.

3. Purification of mangiferin glucosides

3.1. Purification of mangiferin glucosides using Diaion HP-20

In order to purify the product, the reaction mixture was mixed with EtOH (final concentration 60%) and centrifuged at 8000 rpm for 15 min to remove the polymer. The solution was loaded into Diaion HP-20 resin in an open column (300 × 3 mm). Distilled water (pH 7.0) was used at first for elution to remove saccharides, followed by 60% ethanol (v/v) to elute the target compound. The ethanol was evaporated at 45 ºC under vacuum pressure. The mangiferin glucosides mixture was lyophilized using freeze dryer (EYELA, Tokyo) and stored at -20 ºC for further studies.

3.2. Purification of Mangiferin Glucoside using high performance liquid chromatography (HPLC)

The mangiferin glucoside mixture powder was dissolved in 5% acetonitrile and purified using high performance liquid chromatography (HPLC) system 2545 binary gradient module (pump), 2767 sample manager (injector) (Waters, Milford, MA, USA). Compounds were detected at 258 nm with 2998 photodiode array detector (Waters, Milford, MA, USA). The separation was performed in reverse-phase by using Luna® Prep C_{18}, 5 μm, 150×21.2 mm column (Phenomenex, CA, USA). The mobile phase was 100% acetonitrile (A) and 0.2% (v/v) formic acid in water (B) with a gradient as

follows: 6.5% A from 0 to 5 min, 8% A from 5 to 20 min, 10% A from 20 to 32 min, 12% A from 32 to 40 min, and 100% A from 40 to 45 min. The mobile phase flow rate was 17 mL/min. The mangiferin glucosides were lyophilized using freeze dryer (EYELA, Tokyo) and stored at -20 °C for further studies.

4. Structure analysis of mangiferin glucoside

4.1. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

The MALDI-TOF analysis was conducted in the National Center for Inter-University Research Faculties (NCIRF), Seoul National University. Briefly, 1 mg/mL of each mangiferin glucoside sample was diluted with distilled water prior to the analysis. The analysis was carried out by obtaining the mass spectra using a Voyager DESTR MALDI-TOF mass spectrometer (Applied Biosystem, USA). The mass spectra were obtained in the positive reflector mode with delayed extraction (average of 75 laser shots) with a 65 kV acceleration voltage.

4.2. Nuclear Magnetic Resonance (NMR) analysis

The NMR analysis was conducted in the National Center for Inter-University Research Faculties (NCIRF), Seoul National University. The targeted mangiferin glucoside (Mg-G1) was chosen for the structure elucidation. Briefly, 10 mg of sample was dissolved in 600 µl DMSO-d_6 and placed into 5 mm NMR tubes. The NMR spectra was recorded using the AVANCE III 850 system (Bruker, Germany), operating at 850 MHz for ^{1}H and at 213 MHz for ^{13}C, at 25 ºC. The linkage between mangiferin and glucose was determined by ^{1}H, ^{13}C, Homonuclear Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Coherence (HSQC), and Heteronuclear Multiple Bond Correlation (HMBC).

5. Characteristics and bio-functionality of mangiferin glucoside

5.1. Analysis of water solubility

The water solubility of mangiferin and Mg-G1 were determined using a procedure by Kim *et al.* [26] with slight modification. Briefly, the excess mangiferin and Mg-G1 were dissolved in 20 µL of distilled water at 25 ºC for 1h, followed by centrifugation at 12,000 rpm (15 min). The solubilized sample was filtered using a 0.45µm PTFE syringe filter (ANOW, Hangzhou, China). The concentration of solubilized compound was determined by using Waters HPLC-UV in Sunfire^{TM} C_{18}, 5 µm, 4.6 × 100 mm column (Milford, MA, USA) at 258 nm. Acetonitrile (100%) and formic acid in water (0.2%) were used for the mobile phase.

5.2. DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated to analyze the antioxidant activity of mangiferin and Mg-G1. The analysis was carried out by measuring the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging according to a procedure by Kim *et al.* [26] with slight modification. Briefly, each sample in DMSO was mixed with 100 mM DPPH in MeOH. The mixture was incubated at 28 ºC for 30 min, in dark condition. The absorbance of each mixture was recorded at 517 nm using SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). EGCG (Amorepacific), L-ascorbic acid and α-tocopherol (Sigma-Aldrich Chemical Co.) were used as positive controls. DMSO was used as negative control. The relative radical scavenging activity was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = [1-(A_1/A_0)] \times 100$$

where A_0 is the absorbance of negative control and A_1 is the absorbance of sample.

5.3. Superoxide dismutase (SOD)-like scavenging activity

Superoxide dismutase-like scavenging activity was evaluated to analyze the antioxidant activity of mangiferin and Mg-G1. This analysis was carried out by measuring the radical-scavenging activity for autoxidation of pyrogallol according to a procedure by Li [35] with modification. Briefly, sample (12 μL) was mixed with 50 mM Tris-HCL buffer (pH 8.2) containing 10 mM EDTA (180 μL) and 60 mM pyrogallol (2 μL). The mixture was incubated at 37 ºC for 5 min. The absorbance of each mixture was recorded at 325 nm using SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). The pH of reaction was checked before and after analysis. L-ascorbic acid (Sigma-Aldrich Chemical Co.) prepared in DMSO was used as a positive control. DMSO were used as a negative control. The SOD-like activity was calculated as:

$$\text{SOD-like activity (\%)} = [1-(A_1/A_0)] \times 100$$

where A_0 is the absorbance of negative control and A_1 is the absorbance of sample.

5.4. α-Glucosidase inhibitory activity

α-Glucosidase inhibitory activity was evaluated according to a procedure by Telagari and Hullatti [36] with a slight modification. Briefly, α-glucosidase enzyme from baker's yeast (type I) (E.C. 3.2.1.20) solution was prepared by dissolving 1 mg enzyme in 10 mL of 50 mM potassium phosphate buffer (pH 6.8). Acarbose (TCI Chemicals, South Korea) as the positive control, mangiferin, and Mg-G1 were prepared in 5% DMSO. The negative control contained the same volume of 5% DMSO instead of sample. The enzyme solution (0.27 U/mL), samples, and potassium phosphate buffer (1 mM, pH 6.8) were pre-incubated at 37 ºC for 10 min. One mM of p-nitrophenyl α-D-glucopyranoside (PNP-G) was added as a substrate and the mixture was incubated at 37 ºC for 20 min. The absorbance was recorded at 405 nm at initial time (0 min) and final time (20 min). The amount of DMSO did not give any interference with the assay. The inhibition (%) was calculated as:

$$\text{Inhibitory activity (\%)} = [1-(A_{1\text{initial-final}}/A_{0\text{initial-final}})] \times 100$$

where A_0 is the absorbance of negative control and A_1 is the absorbance of sample.

5.5. Human intestinal maltase (HMA) inhibitory activity

The HMA inhibitory activity was evaluated according to a procedure by Nguyen *et al.* [37] with a slight modification. The HMA was expressed in *Pichia pastoris* X-33 as described previously by Nguyen *et al.* [37]. Briefly, 20 µL of enzyme (0.49 U/mL), 5 µL of samples, and 135 µL of 50 mM potassium phosphate buffer (pH 6.0) were pre-incubated at 37 ºC for 10 min. After that, 40 µL of 10 mM maltose was added and the mixture was incubated at 37 ºC for 15 min. The reaction was stopped by adding 0.4 mL of 2 M Tris-HCl (pH 8.0) and 0.6 mL of glucose oxidase-peroxidase assay reagent (Asanpharm, South Korea). Reactions were allowed to develop at 37 ºC for 1 h and absorbance was measured at 500 nm to determine the amount of glucose produced by HMA activity in the reaction. Acarbose (TCI Chemicals, South Korea) as the positive control, mangiferin, and Mg-G1 were prepared in DMSO. The negative control contained the same volume of DMSO instead of sample. The inhibition (%) was calculated as below:

$$\text{Inhibitory activity (\%)} = [1-(A_1/A_0)] \times 100$$

where A_0 is the absorbance of negative control and A_1 is the absorbance of sample

6. Statistical analysis

GraphPad Prism 8 (GraphPad Software, San Diego, USA) was used for statistical analysis. The experimental data were evaluated by means of descriptive statistics with the error values. The ANOVA analysis of the optimization of reaction condition was performed by using Response Surface Methodology (Design Expert 11.0.0, USA). P-values < 0.05 were taken to indicate statistical significances.

Results and Discussion

1. Synthesis and purification of mangiferin glucosides

After acceptor reaction was accomplished in the presence of dextransucrase B-512F/KM, B-1299 and B-1355, the mangiferin glucosides were detected by TLC (Fig 4). The HPLC chromatogram shows the purification profile of mangiferin and mangiferin glucosides from the dextransucrase B-512F/KM containing reaction (Fig 5). The purity of each compound was confirmed by TLC (Fig 6).

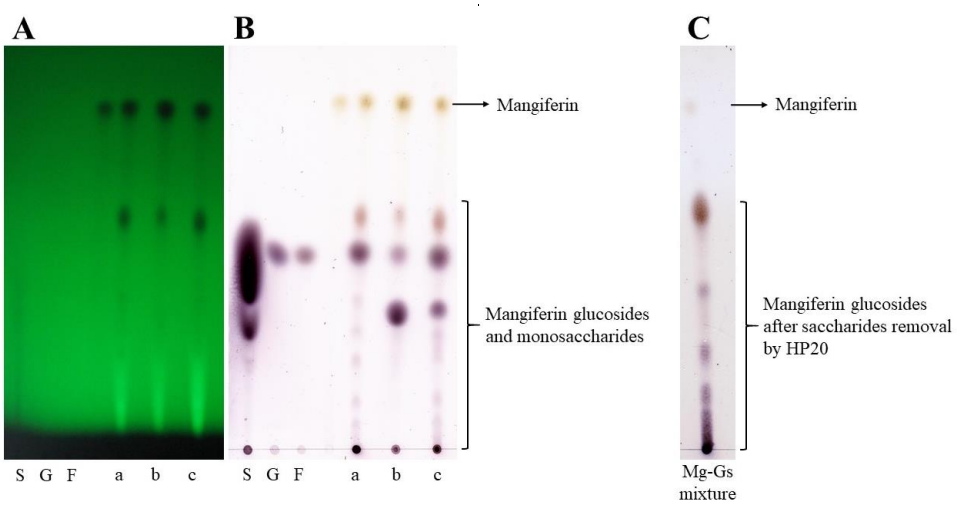

Figure 4. The TLC analysis (A: under UV detection, B: after staining) of mangiferin acceptor reaction by using dextransucrase (a: B-512F/KM, b: B-1299, c: B-1355); C: the synthesis of mangiferin glucosides (by using dextransucrase B-512F/KM) after the saccharides removal

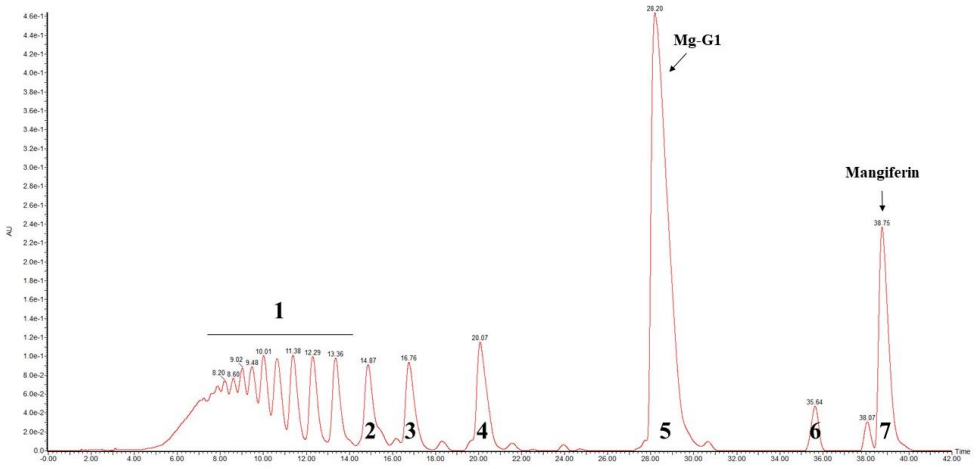

Figure 5. HPLC chromatogram of mangiferin and mangiferin glucoside (Mg-G1) after the saccharides removal using Diaion HP-20 resin column

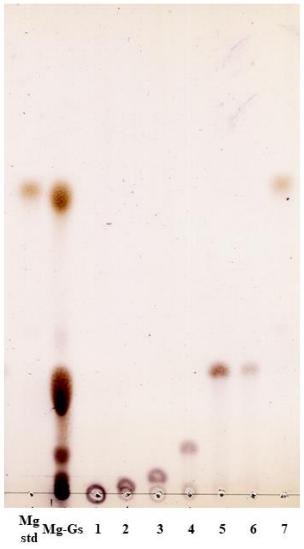

Figure 6. Analysis of glycosylation products by TLC (Mg std: mangiferin standard, Mg-Gs: the mixture of mangiferin glucosides, 1-7: a single compound of mangiferin and its glucosides after purification by using HPLC)

2. Optimum condition for the synthesis of mangiferin glucosides

Response Surface Methodology (RSM) is a statistical method to determine the optimum process condition of multiple variables. In this study, RSM was used to find the optimum condition of mangiferin conversion into the desired mangiferin glucoside (Mg-G1). Three independent factors: mangiferin concentration (8-30 mM), enzyme concentration (0.5-1.9 U/mL) and sucrose concentration (20-78 mM) were determined to investigate the formation of mangiferin glucoside (mM). The experimental design and corresponding RSM responses showed the actual and predicted amount of Mg-G1 (Table 2). The amount of mangiferin converted into Mg-G1 was expressed by the following equation:

$$Y = 0.62303X_1 + 8.91793X_2 + 0.24982X_3 + 0.08684X_1X_2 + 0.00026X_1X_3 + 0.01256X_2X_3 - 0.01495X_1^2 - 4.27447X_2^2 - 0.00234X_3^2 - 9.67709$$

where Y is the amount of Mg-G1 (mM), X_1 is mangiferin concentration (mM), X_2 is enzyme concentration (U/mL), and X_3 is sucrose concentration (mM). According to the equation above, the actual Mg-G1 was 10.9 mM at 19 mM mangiferin, 49 mM sucrose, and 1.2 U/mL dextransucrase. The correlation between independent variables were plotted in 2D contour plots and 3D response surface (Fig 7).

The analysis of variance (ANOVA) showed that the coefficient of determination (R^2) of the experimental data was 0.86 (Table 3). This indicated that the calculated model was able to explain 86.13% of the results. The adjusted coefficient of determination (R^2_{adj}) to measure the goodness-of-fit of the regression equation was 0.74. This indicated that 26% of the total variation was not explained by the model. The model used to fit the independent factors was found to be significant ($p < 0.005$).

Table 2. Experimental design and central composite design response for Mg-G1 sythesis

Run no.	Independent variables			Mg-G1 (mM)	
	X_1	X_2	X_3	Actual	Predicted
1	8.00	1.90	20.00	0.26	7.85
2	19.00	1.20	0.23	10.37	7.85
3	8.00	0.50	20.00	9.45	7.85
4	19.00	1.20	49.00	8.53	11.23
5	30.00	0.50	78.00	7.61	6.44
6	30.00	1.90	78.00	4.86	6.14
7	19.00	1.20	49.00	6.70	7.85
8	8.00	0.50	78.00	9.45	7.15
9	19.00	1.20	49.00	4.86	7.27
10	19.00	0.02	49.00	5.26	5.24
11	19.00	1.20	49.00	5.26	4.41
12	0.50	1.20	49.00	9.88	10.46
13	19.00	1.20	97.77	8.72	7.85
14	19.00	1.20	49.00	11.03	9.26
15	30.00	1.90	20.00	9.88	9.55
16	19.00	1.20	49.00	4.10	4.47
17	37.50	1.20	49.00	13.34	11.29
18	19.00	2.38	49.00	11.03	8.54
19	30.00	0.50	20.00	8.72	8.43
20	8.00	1.90	78.00	7.61	7.85

X_1, mangiferin concentration (mM); X_2, enzyme concentration (U/mL); X_3, sucrose concentration (mM).

Table 3. ANOVA quadratic model of Mg-G1 synthesis

Source of variation	Sum of square	df	Mean square	F-value	p-value
Model	216.56	9.00	24.06	6.90	0.0029
A-Mangiferin	48.91	1.00	48.91	14.03	0.0038
B-Enzyme	5.72	1.00	5.72	1.64	0.2292
C-Sucrose	19.03	1.00	19.03	5.46	0.0416
AB	3.58	1.00	3.58	1.03	0.3250
AC	0.06	1.00	0.06	0.02	0.9019
BC	0.52	1.00	0.52	0.15	0.7075
A^2	47.14	1.00	47.14	13.52	0.0043
B^2	63.22	1.00	63.22	18.13	0.0017
C^2	55.73	1.00	55.73	15.98	0.0025
Residual	34.87	10.00	3.49		
Lack of Fit	18.61	5.00	3.72	1.14	0.4432
Pure Error	16.27	5.00	3.25		
Cor Total	251.43	19.00			

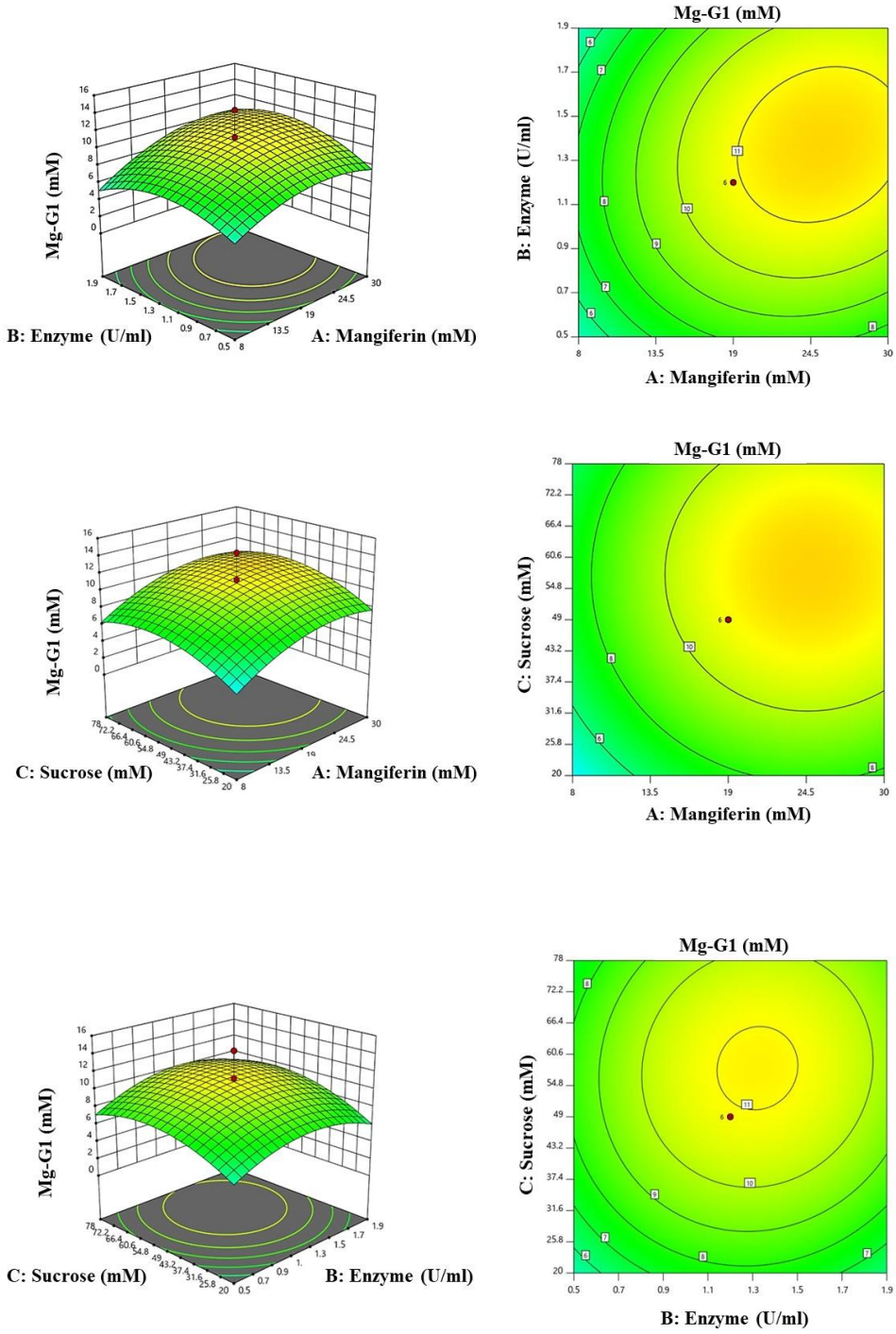

Figure 7. Response surface and contour plots

3. Structure elucidation of mangiferin glucoside (Mg-G1)

The molecular weight of mangiferin and each purified mangiferin glucosides was analyzed by using MALDI-TOF MS (Fig 8 and 9). Based on MALDI-TOF MS analysis, the mangiferin glucoside (Fig 5, peak 2) was observed at m/z 1093.77 ($M + Na^+$), indicating it has four glucosyl residues attached. The mangiferin glucoside (Fig 5, peak 3) was observed at m/z 931.66 ($M + Na^+$), indicating it has three glucosyl residues attached. The mangiferin glucoside (Fig 5, peak 4) was observed at m/z 769.56 ($M + Na^+$), indicating it has two glucosyl residues attached. The mangiferin glucoside (Fig 5, peak 5) was observed at m/z 607.40 ($M + Na^+$), indicating it has one glucosyl residue attached. Compound in the last peak (Fig 5) was observed at m/z 445.33 ($M + Na^+$), indicating it was mangiferin.

The mangiferin glucoside with one glucosyl residue attach was chosen for the structure analysis by nuclear magnetic resonance (^1H, ^{13}C, COSY, HSQC, HMBC) (Fig 10). The double signal at 98.61 pp (J=3.68 Hz) was assigned as the anomeric proton. This showed that the glucosyl residue was connected to mangiferin by α-linkage (Table 4). The carbon signals of Mg-G1 were almost identical to mangiferin, except the signals at C-6′. These indicated the transferred glucosyl residue had attached to C-6′ in the mangiferin. According to HMBC data, the C-6′ of the mangiferin was observed at 66.96 ppm, and the coupling occurred between proton H-1″ of

the glucosyl residue and C-6′ of the mangiferin. The structure of Mg-G1 was designated as mangiferin-(1→6)-α-D-glucopyranoside (Fig 11).

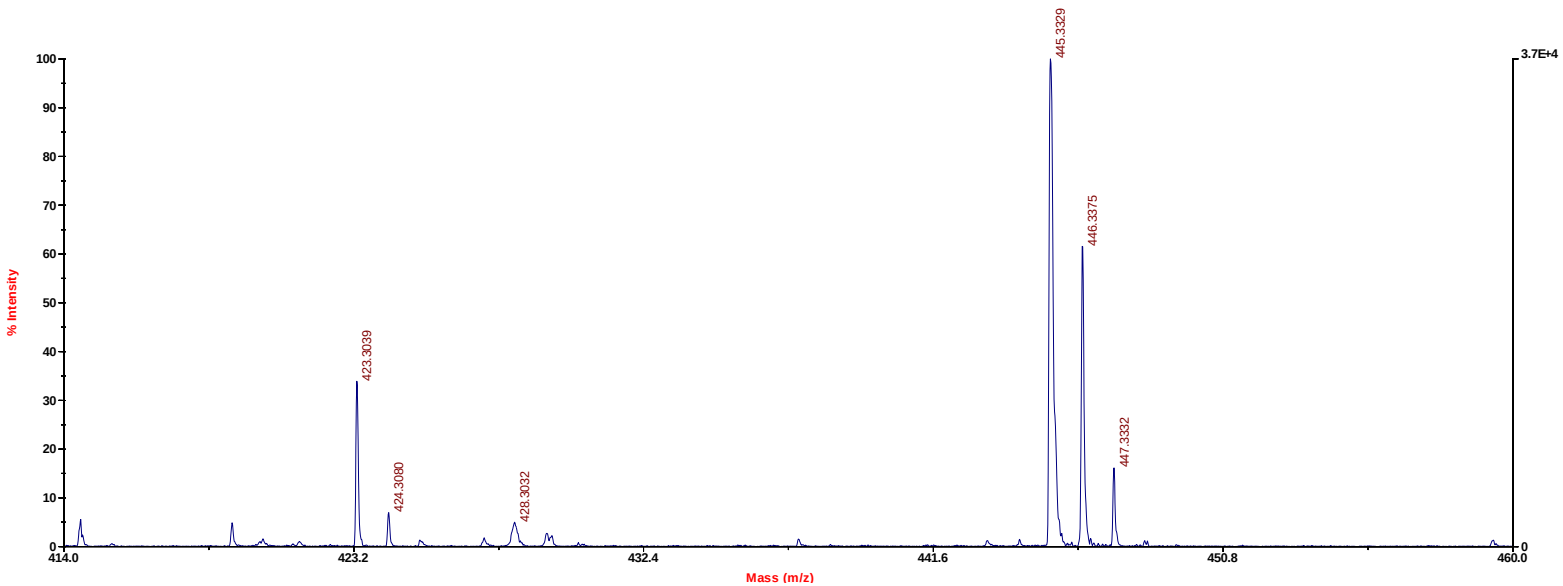

Figure 8. Analysis of mangiferin molecular weight using MALDI-TOF MS observed at m/z 445.33 (M + Na+)

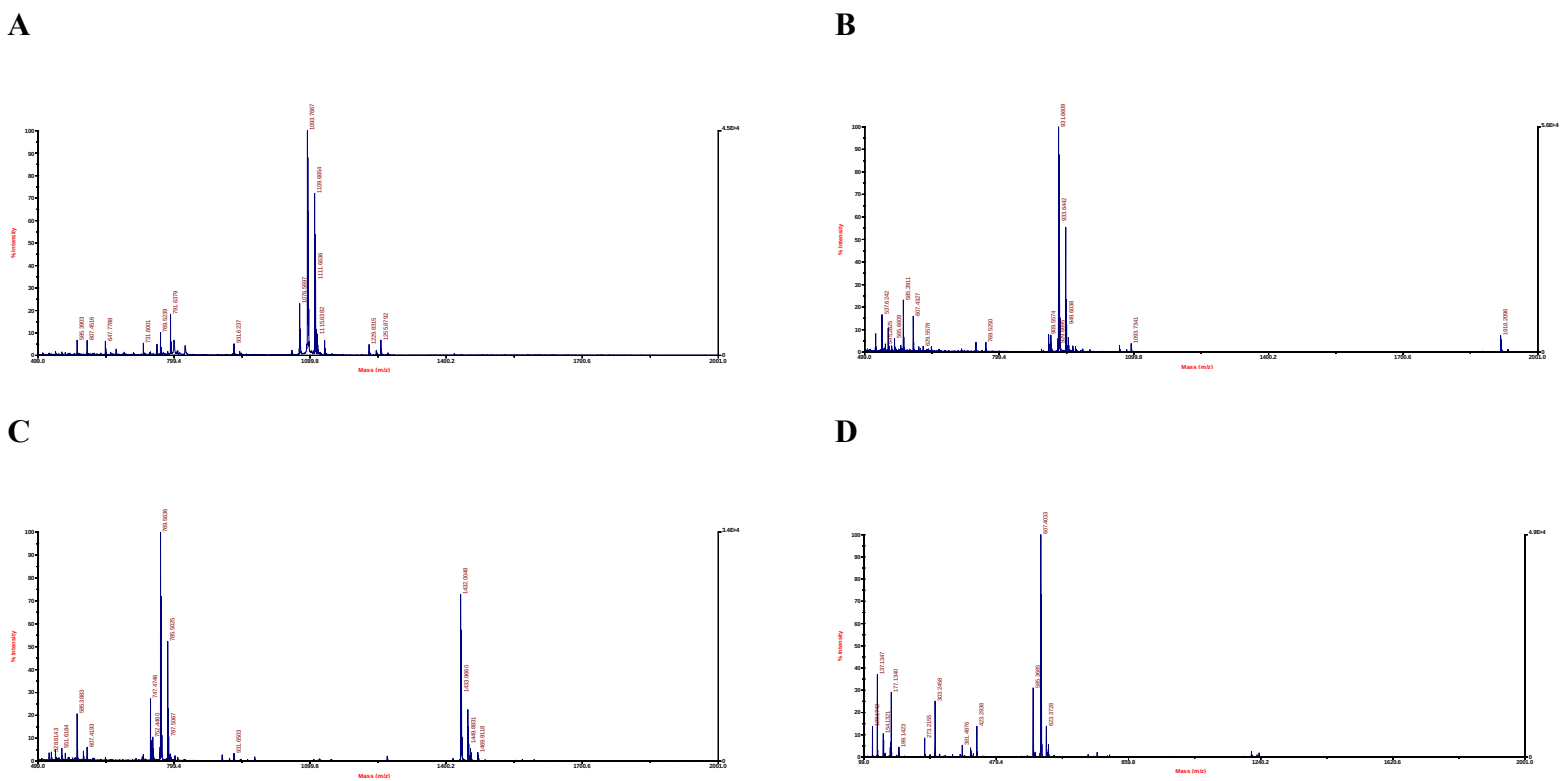

Figure 9. Analysis of mangiferin glucosides using MALDI-TOF MS (A: Mg-G with four glucosyl residues at m/z 1093.77 ($M + Na^+$), B: Mg-G with three glucosyl residues at m/z 931.66 ($M + Na^+$), C: Mg-G with two glucosyl residues at m/z 769.56 ($M + Na^+$), D: Mg-G with one glucosyl residue at m/z 607.40 ($M + Na^+$))

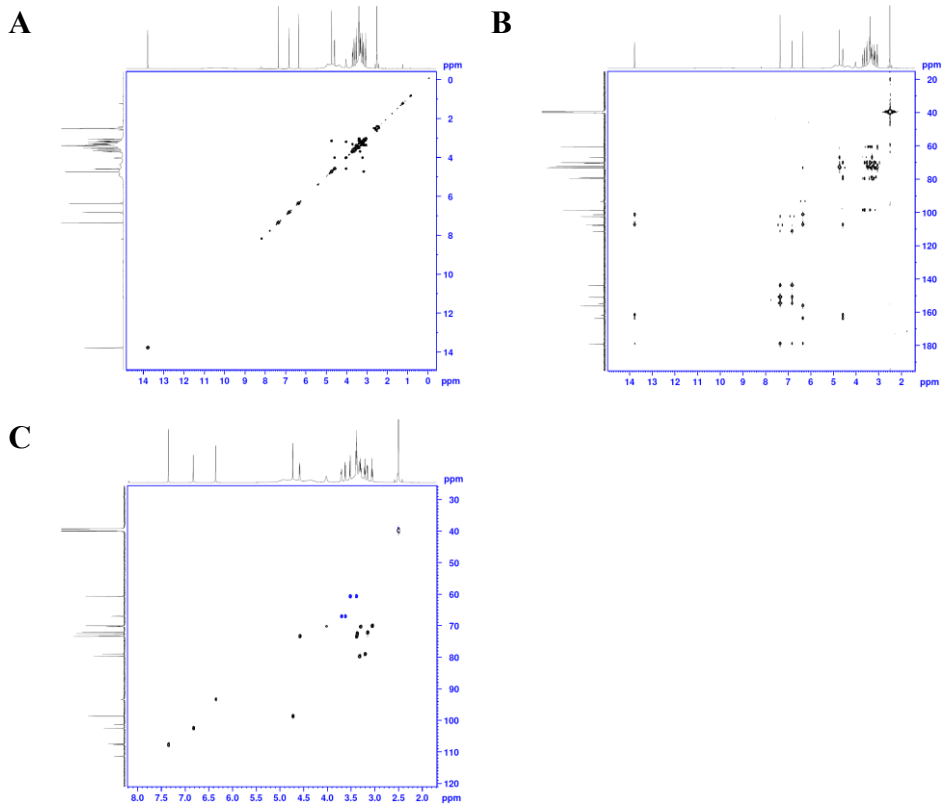

Figure 10. NMR spectra of Mg-G1; A. COSY, B. HMBC, C. HSQC

Table 4. ^{13}C- and ^{1}H-NMR spectral data for Mg-G1

Carbon position	**^{13}C-NMR (δ)**	**^{1}H-NMR [δ, J(Hz)]**
1	161.73	13.78, s
2	107.43	
3	163.73	
4	93.31	6.34, s
4a	156.2	
5	102.46	6.83, s
6	154.66	
7	143.91	
8	107.73	7.35, s
8a	111.36	
9	178.99	
9a	101.28	
10a	150.95	
1′	73.24	4.58, d, *J=9.76*
2′	70.16	4.02, s
3′	78.95	3.21, t, *J=6.83*
4′	70.26	3.35
5′	79.68	3.33, m
6′	66.96	3.7, dd, *J=4.88, 11.62*
1″	98.61	4.73, d, *J=3.68*
2″	72.77	3.05, m
3″	72.07	3.15, dd, *J=3.71, 9.60*
4″	69.99	3.05, m
5″	72.86	3.38, m
6″	60.63	3.52, d, *J=10.88*

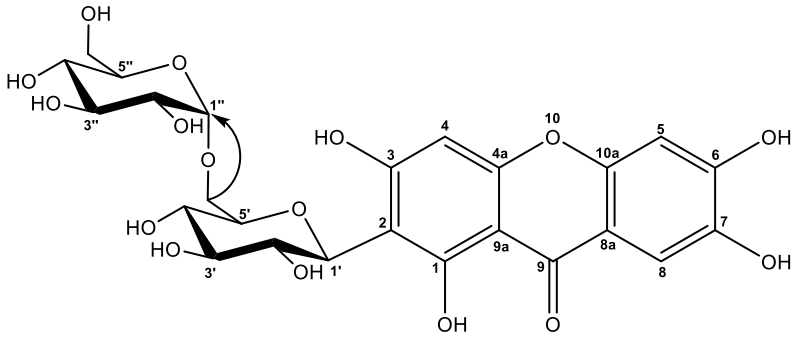

Figure 11. Structure of Mg-G1

4. Characteristic and bio-functionality of mangiferin glucoside (Mg-G1)

4.1 Analysis of water solubility

The water solubility of mangiferin and Mg-G1 was 0.4 mM and 824.7 mM, respectively. It indicated that the water solubility of mangiferin was improved 2,322-fold due to the attachment of one glucosyl residue. This result was consistent with the previous report that showed the increase in mangiferin solubility after glycosylation as compared to mangiferin itself [33]. In addition, our previous report also showed higher solubility of quercetin [25], EGCG [26], and caffeic acid [27] after glycosylation. The increased solubility was because the glycans have a higher affinity for the aqueous solvent that can increase the hydration potential of less soluble compounds. In this study, the type of glycosyl moiety also contributed to the increase of solubility in glycosylated mangiferin. The attachment of one glucosyl residue in α-linkage showed the improvement in mangiferin water solubility 14% higher than the β-fructosylated mangiferin [33]. It indicated that glucose has an important role to increase the water solubility of mangiferin. Moreover, the α-linkage which has lower dissociation energy than β-linkage also contributed to the higher water solubility.

4.2 Analysis of antioxidant capacity

Reactive oxygen species and free radicals have an important role in various types of disorders including diabetes [38, 39]. The antioxidant capacity of a phenolic compound can be measured in vivo and in vitro. There are many kinds of in vitro methods to analyze the antioxidant capacity, which generally can be categorized as electron transfer (ET)-based assay and hydrogen atom transfer (HAT)-based assay [40]. The example of the assay based on HAT is ORAC assay, whereas the DPPH, SOD, ABTS, and FRAP assays are classified as ET-based assay.

4.2.1 DPPH radical scavenging activity

DPPH radical scavenging assay is the most widely used method to analyzed the antioxidant activity of phenolic compounds based on their hydrogen donor ability. The principle of this assay is the reduction of DPPH free radical by accepting hydrogen from the antioxidant compound (carrying –OH, –SH, and –NH groups), indicated by the change of DPPH color from purple to yellow [41].

In this study, ascorbic acid, α-tocopherol and EGCG were used as the positive controls. Ascorbic acid is a well-known vitamin C, α-tocopherol is a well-known vitamin E, and EGCG has been recognized for its great antioxidant activity. The SC_{50} value of ascorbic acid, α-tocopherol and EGCG were 0.261±0.006 mM, 0.105±0.028 mM, and 0.022 ±0.007 mM, respectively (Fig 12). The SC_{50} value of mangiferin and Mg-G1 were 0.054±0.004 mM and 0.022±0.001 mM, respectively (Fig 12).

After glycosylation, the Mg-G1 showed 2.4-fold higher DPPH radical scavenging activity compared to mangiferin. Moreover, the antioxidant activity of Mg-G1 was almost as high as EGCG, which possessed the highest antioxidant activity among all compounds tested in the present study. This result was consistent with the previous report that showed the higher antioxidant activity of fructosylated mangiferin [33]. Moreover, our previous study showed that α-glucosidic link could increase

the antioxidant activity of hydroquinone glucosides than the β-glucosidic linkages [42].

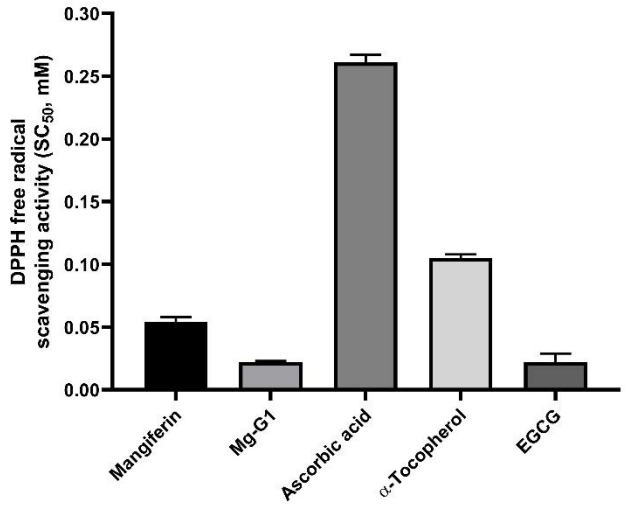

Figure 12. DPPH radical scavenging activity of ascorbic acid, α-tocopherol, EGCG, mangiferin and Mg-G1

4.2.2 Superoxide dismutase (SOD)-like scavenging activity

Superoxide radical is a by-product of oxygen metabolism that can damage other cells. Basically, superoxide dismutase (SOD) is an enzyme that triggers a biochemical reaction. SOD does not work like most antioxidants which give a spare electron to an unstable radical molecule. It breaks down the highly damaging superoxide radical ($O_2^{\bullet -}$) into oxygen (O_2) and hydrogen peroxide (H_2O_2). However, the administration of SOD is difficult because it cannot be absorbed into the gastrointestinal tract. Furthermore, the injected SOD is generally unstable and rapidly cleared by the kidneys. Therefore, it becomes necessary to find alternative safe compounds that possess similar activity to SOD.

Different from DPPH, the basic principle of SOD assay is based on the competition between autoxidation of pyrogallol and dismutation of radical by SOD [43]. In the alkaline solution, pyrogallol can be autoxidized to produce anion radicals ($O_2^{\bullet -}$) [35]. Some antioxidants with acidic groups can contribute to the inhibition mechanism.

In this study, the 325 nm was selected to detect the radicals. This wavelength was more sensitive to detect the generation of $O_2^{\bullet -}$ [35]. The ascorbic acid was used as the positive control, with the SC_{50} value was 0.010±0.001 mM. The lactone group of ascorbic acid can be hydrolyzed to generate –COOH in the alkaline solution [35]. In this study, the SC_{50} value

of mangiferin and Mg-G1 were 0.131±0.005 mM and 0.035±0.005 mM, respectively (Fig 13). Mangiferin contains eight –OH groups, which four of them attach to the norathyriol skeleton and contribute to antioxidant activity [44]. In Mg-G1, the attachment of glucosyl moiety to the glucopyranosyl system of mangiferin contributed to increase the SOD-like scavenging activity of mangiferin. There was no pH difference in all samples, before and after reaction.

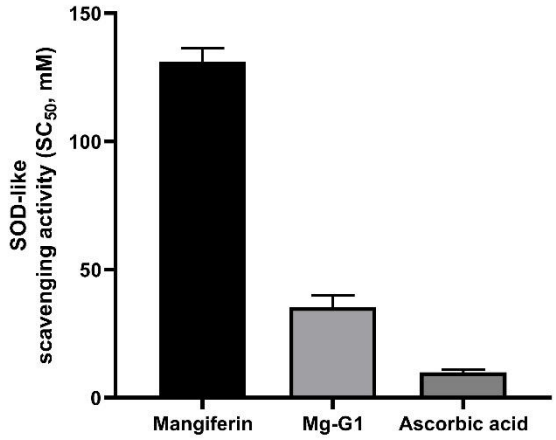

Figure 13. SOD-like scavenging activity of ascorbic acid, mangiferin, and Mg-G1

4.3 Analysis of antidiabetic properties

Diabetes is a chronic carbohydrate metabolic disorder caused by an abnormal insulin regulation. Insulin is a hormone that is produced by pancreas, which regulates the blood glucose level in the body. There are three main types of diabetes [45]. Type I diabetes (insulin-dependent) occurs when the body cannot produce insulin due to autoimmune system. Type II diabetes is the most common case (80-90% of all diabetes cases), which occurs when the body cannot use insulin properly. Gestational diabetes occurs during the pregnancy.

Generally, there are six enzymes involve in the carbohydrate digestion. Two α-amylases (salivary and pancreatic) and four α-glucosidases including maltase-glucoamylase (N-terminal subunit: NtMGAM, C-terminal subunit: CtMGAM) and sucrase-isomaltase (N-terminal subunit: NtSI, C-terminal subunit: CtSI) [45]. The inhibition of both α-amylase and α-glucosidase can delay glucose absorption into the blood and suppressing postprandial hyperglycemia [46]. Therefore, inhibition of these enzymes has been being the target for the treatment of Type II diabetes.

Currently, there are some widely used therapeutic agents to treat diabetes, such as acarbose, voglibose, and miglitol. Among these, acarbose is the most prescribed drug and showed the best result in the study of

inhibitors. However, there are some side effects of consuming these drugs, such as diarrhea and abdominal pain. Therefore, it becomes necessary to discover a safe second generation agents from nature [47]. In general, the inhibitors are classified as sugar-mimicking and non-sugar type inhibitors [48]. Recently, non-sugar type inhibitors gained many interest due to the limitation of sugar-mimicking inhibitors.

4.3.1 α-Glucosidase inhibitory activity

α-Glucosidase is a carbohydrate hydrolyzing enzymes that catalyze the breakdown of complex carbohydrates. In general, this enzyme is categorized as Type I (yeast) and Type II (mammals). Acarbose is the most widely used α-glucosidase inhibitor and classified as sugar-mimicking type because of its oligosaccharide structure. It showed a competitive inhibition mode against α-glucosidases [49, 48]. The N-glycosdic bond, the first glucose and the second glucose of acarbose, had the same binding site as α-maltose [49]. This action inhibited the cleavage of glyosidic bond in carbohydrates into small sugars. Different from acarbose, polyphenolic compounds are classified as non-sugar type inhibitor.

In this study, acarbose was used as the positive control with the IC_{50} value was 0.881±0.066 mM. Meanwhile, the IC_{50} value of mangiferin and Mg-G1 were 0.497±0.039 mM and 0.663±0.001 mM, respectively (Fig 14). Both mangiferin and Mg-G1 showed more potent inhibitory activity than the positive control. This result was agreed with the previous study that mangiferin had higher inhibitory activity than acarbose, with a non-competitive inhibition mode [48, 50]. Polyhydroxyl groups and aromatic rings of mangiferin are the two key in the inhibitory action. A docking simulation study has reported that the binding mode of 1,3,7-trihydroxyxanthone exist in the non-competitive domain of α-glucosidase,

in which H-bonding is the most important binding interaction [51].

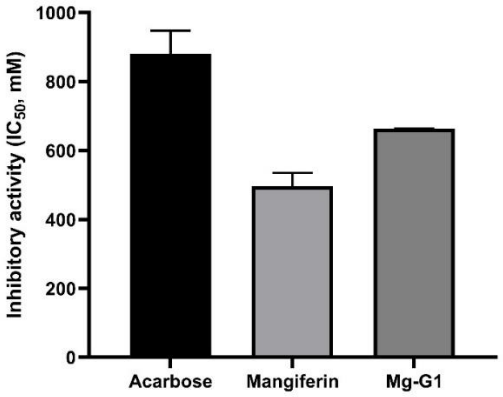

Figure 14. Inhibitory activity of mangiferin and Mg-G1 against α-glucosidase

In this study, Mg-G1 showed lower inhibitory activity than mangiferin. The attachment of one glucose into Mg-G1, weakens the inhibitory potent of mangiferin. Previous study has reported that mangiferin (the glucosylated form of norathyriol) showed lower inhibitory activity than the norathyriol itself [48]. It could be concluded that the more attachment of another group (i.e. glucosyl group) reduced the inhibitory activity of phenolic compound. In addition, this case was also similar to the previous study that showed an addition of a rutinose group in rutin at the 3-position of C-ring, reduced its inhibitory activity [49, 52]. However, even though Mg-G1 showed slightly weaker inhibitory activity, it could be a good alternative for the development of antidiabetic drugs due to its high water solubility.

4.3.2 Human intestinal maltase inhibitory activity

Human intestinal maltase (HMA) is an N-terminal catalytic domain of human intestinal maltase-glucoamylase (MGA) that is responsible to hydrolyze the α-1,4-linkages of maltose [53]. The inhibition of HMA becomes important to treat Type II diabetes due to its role in carbohydrate digestion.

In this study, acarbose was used as the positive controls with IC_{50} value was 0.046±0.013 mM. Acarbose has been reported to inhibit HMA in competitive mode [54, 55]. The analysis of structure showed that acarbose binds to the HMA active site through numerous hydrogen bonds with the acarvosine unit [56]. The inhibitory activity of mangiferin and Mg-G1 were weaker than that of acarbose, with IC_{50} value of 0.210±0.006 mM and 0.224±0.008 mM, respectively (Fig 15). This result was agreed with the previous study that mangiferin showed a less potent inhibitory activity than acarbose [57]. Mg-G1 had an inhibitory activity similar to that of mangiferin, which indicates that the attachment of one glucosyl moiety did not affect the HMA inhibitory activity of mangiferin.

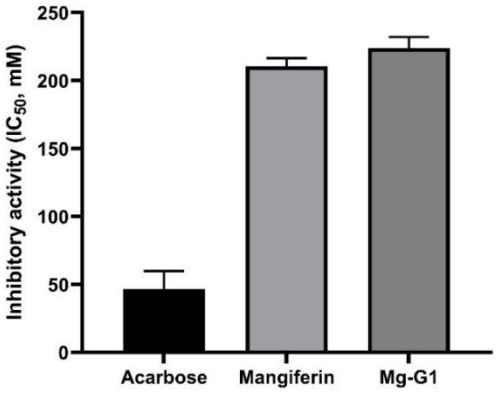

Figure 15. Inhibitory activity of mangiferin and Mg-G1 against human intestinal maltase

4.4 Relation between antioxidant and antidiabetic activity

Many previous studies have shown that the generation of reactive oxygen species and oxidative stress are associated with diabetes. In general, oxidative stress is a condition of excess highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS include free radicals such as superoxide ($^{\bullet}O_2^-$), hydroxyl ($^{\bullet}OH$), peroxyl ($^{\bullet}RO_2$), hydroperoxyl ($^{\bullet}HRO_2^-$), meanwhile RNS include free radicals such as nitric oxide ($^{\bullet}NO$) and nitrogen dioxide ($^{\bullet}NO_2^-$). Among these radicals, $^{\bullet}O_2^-$ and $^{\bullet}NO$ play important roles in the diabetic complications [58]. In diabetes, the oxidative stress originates from the oxidative biochemistry of glucose, which can undergo autoxidation and generate •OH radicals [59]. In addition, the metabolism of glucose is enhanced through the polyol pathway, which also results in the production of •O2- [58].

As discussed previously, the presence of SOD is important to fight the exceed •O2- in the body, due to its ability to converts •O2- to H_2O_2 immediately. The previous study showed that in the heart of experimental models with diabetes, SOD and glutathione peroxidase activity are decreased, meanwhile catalase is increased [60]. The heart is an important target in diabetes and dispose to diabetic cardiomyopathy leading to chronic heart failure. Therefore, the enough availability of SOD or SOD-like agents are important.

Conclusion

Mangiferin is the major polyphenol in *Mangifera indica*, which also exist in the leaves of various coffee species. Mangiferin has attracted a lot of interest due to its various physiological activities like antioxidant, immunomodulatory, anti-inflammatory, and anti-microbial activities. However, application of mangiferin for orally administered drug undergo many challenges, especially due to its low bioavailability and poor solubility in water. Many previous studies have been carried out to synthesize mangiferin derivatives to overcome the hydrophobicity problem.

In this study, the enzymatic glycosylation using dextransucrase from *Leuconostoc mesenteroides* B-512F/KM was chosen to enhance the water solubility and oral bioavailability of mangiferin. The optimum condition to synthesize mangiferin glucoside (Mg-G1) was 19 mM mangiferin, 49 mM sucrose, and 1.2 U/mL dextransucrase, with the yield 10.899 mM. The structure of Mg-G1 was confirmed as mangiferin-(1→6)-α-D-glucopyranoside. The water solubility of mangiferin was improved 2,322-fold after glycosylation, with the solubility value of Mg-G1 was 824.7 mM. Moreover, Mg-G1 showed 2.4-fold higher DPPH radical scavenging activity compared to mangiferin, with the SC_{50} value were 0.022±0.001 mM. Mg-G1 also showed higher SOD-like scavenging activity compared to mangiferin and ascorbic acid, with SC_{50} value was 0.035±0.005 mM. It

indicates that Mg-G1 could be considered as a potent antioxidant. However, further studies are needed to prove the antioxidant activity of Mg-G1 in molecular level. Besides the antioxidant activities, Mg-G1 also showed the inhibitory activity against α-glucosidase and human intestinal maltase, with the IC_{50} value were 0.663±0.001 mM and 0.224±0.008 mM, respectively. According to these results, because of the improvement in its water solubility, mangiferin glucoside could be more preferable than mangiferin as an antioxidant and antidiabetic ingredient in dietary supplement and other biomedical applications.

References

1. Viswanadh EK, Rao BN, and Rao BSS. *Antigenotoxic effect of mangiferin and changes in antioxidant enzyme levels of swiss albino mice treated with cadmium chloride*. Hum Exp Toxicol. 2010. **29**(5): 409-418.
2. Wu JF*, et al. Xanthone glycosides from herbs of polygala hongkongensis hemsl and their antioxidant activities*. J Asian Nat Prod Res. 2008. **10**(7): 665-670.
3. Li XF*, et al. Mangiferin identified in a screening study guided by neuraminidase inhibitory activity*. Planta Med. 2007. **73**(11): 1195-1196.
4. Faizi S*, et al. Temperature and solvent dependent nmr studies on mangiferin and complete nmr spectral assignments of its acyl and methyl derivatives*. Magn Reson Chem. 2006. **44**(9): 838-844.
5. Jurgenliemk G, and Nahrstedt A. *Phenolic compounds from hypericum perforatum*. Planta Med. 2002. **68**(1): 88-91.
6. Pauletti PM*, et al. New antioxidant c-glucosylxanthones from the stems of arrabidaea samydoides*. J Nat Prod. 2003. **66**(10): 1384-1387.
7. Campa C*, et al. A survey of mangiferin and hydroxycinnamic acid ester accumulation in coffee (coffea) leaves: Biological implications*

and uses. Ann Bot-London. 2012. **110**(3): 595-613.

8. Trevisan MTS*, et al. Quantitation by hplc-uv of mangiferin and isomangiferin in coffee (coffea arabica) leaves from brazil and costa rica after solvent extraction and infusion*. Food Anal Method. 2016. **9**(9): 2649-2655.

9. Chen XM, Ma ZL, and Kitts DD. *Effects of processing method and age of leaves on phytochemical profiles and bioactivity of coffee leaves*. Food Chem. 2018. **249**(143-153.

10. Talamond P*, et al. First report on mangiferin (c-glucosyl-xanthone) isolated from leaves of a wild coffee plant, coffea pseudozanguebariae (rubiaceae)*. Acta Bot Gallica. 2008. **155**(4): 513-519.

11. Khurana RK*, et al. Exploring and validating physicochemical properties of mangiferin through gastroplus (r) software*. Futur Sci Oa. 2017. **3**(1):

12. Matkowski A*, et al. Mangiferin - a bioactive xanthonoid, not only from mango and not just antioxidant*. Mini-Rev Med Chem. 2013. **13**(3): 439-455.

13. Wang RR*, et al. Mangiferin, an anti-hiv-1 agent targeting protease and effective against resistant strains*. Molecules. 2011. **16**(5): 4264-4277.

14. B D, Mitra A, and Manjunatha M. *Studies on the anti-diabetic and hypolipidemic potentials of mangiferin (xanthone glucoside) in streptozotocin-induced type 1 and type 2 diabetic model rats*. International Journal of Advances in Pharmaceutical Sciences. 2010. **1**(1): 75-85.

15. Masibo M, and He Q. *Mango bioactive compounds and related nutraceutical properties—a review*. Food Reviews International. 2009. **25**(4): 346-370.

16. Jaiswal S*, et al. Mangiferin as chain transfer agent: Effect on the molecular weight of poly(methyl methacrylate) and polystyrene*. Polymer Bulletin. 2015. **72**(6): 1407-1416.

17. Tian XT*, et al. Pharmacokinetics of mangiferin and its metabolite-norathyriol, part 1: Systemic evaluation of hepatic first-pass effect in vitro and in vivo*. Biofactors. 2016. **42**(5): 533-544.

18. Ma HQ*, et al. Improving permeability and oral absorption of mangiferin by phospholipid complexation*. Fitoterapia. 2014. **93**(54-61.

19. Liu HH*, et al. Metabolism and pharmacokinetics of mangiferin in conventional rats, pseudo-germ-free rats, and streptozotocin-induced diabetic rats*. Drug Metab Dispos. 2012. **40**(11): 2109-2118.

20. Kim D*, et al. Dextran molecular size and degree of branching as a*

function of sucrose concentration, ph, and temperature of reaction of leuconostoc mesenteroides b-512fmcm dextransucrase. Carbohyd Res. 2003. **338**(11): 1183-1189.

21. Naessens M*, et al. Leuconostoc dextransucrase and dextran: Production, properties and applications*. J Chem Technol Biot. 2005. **80**(8): 845-860.

22. Kim D*, et al. Development of constitutive dextransucrase hyper-producing mutants of leuconostoc mesenteroides using the synchrotron radiation in the 70-1000 ev region*. Biotechnol Tech. 1997. **11**(5): 319-321.

23. Kim DM, and Kim DW. *Facile purification and characterization of dextransucrase from leuconostoc mesenteroides b-512fmcm*. J Microbiol Biotechn. 1999. **9**(2): 219-222.

24. Jiang JR*, et al. Conversion of puerarin into its 7-o-glycoside derivatives by microbacterium oxydans (cgmcc 1788) to improve its water solubility and pharmacokinetic properties*. Appl Microbiol Biot. 2008. **81**(4): 647-657.

25. Moon YH*, et al. Synthesis and characterization of novel quercetin-alpha-d-glucopyranosides using glucansucrase from leuconostoc mesenteroides*. Enzyme Microb Tech. 2007. **40**(5): 1124-1129.

26. Kim J*, et al. Functional properties of novel epigallocatechin gallate*

glucosides synthesized by using dextransucrase from leuconostoc mesenteroides b-1299cb4. J Agr Food Chem. 2016. **64**(48): 9203-9213.

27. Nam SH*, et al. Synthesis and functional characterization of caffeic acid glucoside using leuconostoc mesenteroides dextransucrase*. J Agr Food Chem. 2017. **65**(13): 2743-2750.

28. Xu LJ*, et al. Recent progress in the enzymatic glycosylation of phenolic compounds*. J Carbohyd Chem. 2016. **35**(1): 1-23.

29. Miguez N*, et al. Fructosylation of hydroxytyrosol by the beta-fructofuranosidase from xanthophyllomyces dendrorhous: Insights into the molecular basis of the enzyme specificity*. Chemcatchem. 2018. **10**(21): 4892-4901.

30. de Souza JRR*, et al. Spray-drying encapsulation of mangiferin using natural polymers*. Food Hydrocolloids. 2013. **33**(1): 10-18.

31. Ma H*, et al. Improving permeability and oral absorption of mangiferin by phospholipid complexation*. Fitoterapia. 2014. **93**(54-61.

32. Yang X*, et al. Host-guest inclusion system of mangiferin with beta-cyclodextrin and its derivatives*. Mater Sci Eng C Mater Biol Appl. 2013. **33**(4): 2386-2391.

33. Wu XM*, et al. Efficient enzymatic synthesis of mangiferin*

glycosides in hydrophilic organic solvents. Rsc Adv. 2013. **3**(41): 19027-19032.

34. Kim GE*, et al. Glucosylation of the flavonoid, astragalin by leuconostoc mesenteroides b-512fmcm dextransucrase acceptor reactions and characterization of the products*. Enzyme Microb Tech. 2012. **50**(1): 50-56.

35. Li XC. *Improved pyrogallol autoxidation method: A reliable and cheap superoxide-scavenging assay suitable for all antioxidants*. J Agr Food Chem. 2012. **60**(25): 6418-6424.

36. Telagari M, and Hullatti K. *In-vitro alpha-amylase and alpha-glucosidase inhibitory activity of adiantum caudatum linn. And celosia argentea linn. Extracts and fractions*. Indian J Pharmacol. 2015. **47**(4): 425-429.

37. Thi THN*, et al. Inhibitory effects of epigallocatechin gallate and its glucoside on the human intestinal maltase inhibition*. Biotechnol Bioproc E. 2012. **17**(5): 966-971.

38. Yang H*, et al. Oxidative stress and diabetes mellitus*. Clin Chem Lab Med. 2011. **49**(11): 1773-1782.

39. Halliwell B. *Free radicals and antioxidants: Updating a personal view*. Nutr Rev. 2012. **70**(5): 257-265.

40. Dudonne S*, et al. Comparative study of antioxidant properties and*

total phenolic content of 30 plant extracts of industrial interest using dpph, abts, frap, sod, and orac assays. J Agr Food Chem. 2009. **57**(5): 1768-1774.

41. Nenadis N, and Tsimidou M. *Observations on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (dpph center dot) tests*. J Am Oil Chem Soc. 2002. **79**(12): 1191-1195.

42. Seo ES*, et al. Synthesis and characterization of hydroquinone glucoside using leuconostoc mesenteroides dextransucrase*. Enzyme Microb Tech. 2009. **45**(5): 355-360.

43. Magnani L, Gaydou EM, and Hubaud JC. *Spectrophotometric measurement of antioxidant properties of flavones and flavonols against superoxide anion*. Anal Chim Acta. 2000. **411**(1-2): 209-216.

44. Gomez-Zaleta B*, et al. Uv/vis, h-1, and c-13 nmr spectroscopic studies to determine mangiferin pk(a) values*. Spectrochim Acta A. 2006. **64**(4): 1002-1009.

45. Santos CMM, Freitas M, and Fernandes E. *A comprehensive review on xanthone derivatives as alpha-glucosidase inhibitors*. Eur J Med Chem. 2018. **157**(1460-1479.

46. Du X, and Myracle AD. *Fermentation alters the bioaccessible phenolic compounds and increases the alpha-glucosidase inhibitory*

effects of aronia juice in a dairy matrix following in vitro digestion. Food Funct. 2018. **9**(5): 2998-3007.

47. Li YH, Huang THW, and Yamahara J. *Salacia root, a unique ayurvedic medicine, meets multiple targets in diabetes and obesity*. Life Sci. 2008. **82**(21-22): 1045-1049.

48. Shi ZL*, et al. In vitro and in vivo effects of norathyriol and mangiferin on alpha-glucosidase*. Biochem Res Int. 2017.

49. Proenca C*, et al. Alpha-glucosidase inhibition by flavonoids: An in vitro and in silico structure-activity relationship study*. J Enzym Inhib Med Ch. 2017. **32**(1): 1216-1228.

50. Wu CM*, et al. The alpha-glucosidase inhibiting isoflavones isolated from belamcanda chinensis leaf extract*. Rec Nat Prod. 2012. **6**(2): 110-120.

51. Liu Y*, et al. Binding mechanism and synergetic effects of xanthone derivatives as noncompetitive alpha-glucosidase inhibitors: A theoretical and experimental study*. J Phys Chem B. 2013. **117**(43): 13464-13471.

52. Li YQ*, et al. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase*. J Agr Food Chem. 2009. **57**(24): 11463-11468.

53. Rossi EJ*, et al. Inhibition of recombinant human maltase*

glucoamylase by salacinol and derivatives. Febs J. 2006. **273**(12): 2673-2683.

54. Ryu HJ*, et al. Expression, purification, and characterization of human intestinal maltase secreted from pichia pastoris*. Food Sci Biotechnol. 2011. **20**(2): 561-565.

55. Breitmeier D, Gunther S, and Heymann H. *Acarbose and 1-deoxynojirimycin inhibit maltose and maltooligosaccharide hydrolysis of human small intestinal glucoamylase-maltase in two different substrate-induced modes*. Arch Biochem Biophys. 1997. **346**(1): 7-14.

56. Sim L*, et al. Human intestinal maltase-glucoamylase: Crystal structure of the n-terminal catalytic subunit and basis of inhibition and substrate specificity*. J Mol Biol. 2008. **375**(3): 782-792.

57. Mukherjee A, and Sengupta S. *Characterization of nimbidiol as a potent intestinal disaccharidase and glucoamylase inhibitor present in azadirachta indica (neem) useful for the treatment of diabetes*. J Enzym Inhib Med Ch. 2013. **28**(5): 900-910.

58. Johansen JS*, et al. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice*. Cardiovasc Diabetol. 2005. **4**(5.

59. Turko IV, Marcondes S, and Murad F. *Diabetes-associated nitration*

of tyrosine and inactivation of succinyl-coa : 3-oxoacid coa-transferase. Am J Physiol-Heart C. 2001. **281**(6): H2289-H2294.

60. Maritim AC, Sanders RA, and Watkins JB. *Diabetes, oxidative stress, and antioxidants: A review*. J Biochem Mol Toxic. 2003. **17**(1): 24-38.

Abstract in Korean

*Mangifera indica L*의 주요 성분인 망기페린(Mangiferin)은 항산화, 면역조절, 항염증, 항균성, 항바이러스 등 다양한 건강 기능성으로 많은 관심을 끌었다. 그러나 수용성이 낮기 때문에 식품산업과 의료품 개발의 사용과 발전이 제한된다는 단점이 있다. 이 연구에서는, 망기페린의 용해도를 높이기 위해 *Leuconostoc mesenteroides* 512-F/KM에서 얻은 덱스트란수크레이즈를 이용하여 당전이 반응을 실시했다. 이 반응에서 총 4개의 망기페린 배당체가 합성된 것을 MALDI-TOF에 의해 확인하였으며, 망기페린에 합성된 글루코스는 1개에서 4개까지 다양했다. 또한, Mg-G1의 최적화를 위하여 response surface methodology (RSM)을 실시하였고 이에 따라 최적조건인 덱스트란수크레이즈 1.2 U/mL, 망기페린 19 mM, 수크로스 49 mM 에서 10.90 Mm의 최대Mg-G1의 수율값을 도출하였다. Mg-G1은 HPLC를 이용하여 정제하였으며, nuclear magnetic resonance (NMR) (1H, 13C, HMBC, HSQC, COSY)를 이용하여 구조를 결정하였다. 이 구조물은 mangiferin-(1→6)-α-D-glucopyranoside로 확인되었다. 기능성 확인으로는 수용화능, 항산화능(DPPH 자유 래디컬 소거능

활성 및 SOD 유사 소거능 활성) 및 항당뇨능(α-글루코시다아제 억제 활성)을 분석하였다. Mg-G1의 수용성은 824.7 mM으로 기존의 망기페린보다 2,322배 높았다. 또한 Mg-G1은 망기페린보다 DPPH 자유 래디컬 소거능 활성을 보였으며 SC_{50} 값은 0.054±0.004 mM이었다. 그러나 Mg-G1의 SOD 유사 소거능 활성은 망기페린보다 낮았고, SC_{50} 값은 0.035±0.005 mM이었다. 또한 Mg-G1에서 α-glucosidase에 대한 억제 능력이 있는 것을 확인하였고, IC_{50} 값은 0.663±0.001 mM이었다. Mg-G1에서 human intestinal maltase (HMA)에 대한 억제 능력이 있는 것을 확인하였고, IC_{50} 값은0.224±0.008 mM이었다. 수용성이 증가됨에 따라, 망기페린 배당체(Mg-G1)가 망기페린보다 식이 보충제 및 기타 건강기능식품에서 항산화제 및 항당뇨 성분으로서 더 선호될 수 있음을 기대할 수 있다.

키워드: 망기페린(Mg), 망기페린 배당체(Mg-G1), 수용체 반응, 수용성, 항산화제