

**HYPOGLYCEMIC AND ANTIOXIDANT  
ACTIVITY OF POLYPHENOL GLYCOSIDE  
CATALYZED BY TRANSGLYCOSYLATION  
REACTION OF CYCLODEXTRIN  
GLUCANOTRANSFERASE DERIVED FROM  
*TRICHODERMA VIRIDE***



PERPUSTAKAAN  
UNIVERSITI MALAYSIA SABAH  
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UNIVERSITI MALAYSIA SABAH

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THE DEGREE OF DOCTOR OF PHILOSOPHY**

**FACULTY OF FOOD SCIENCE AND  
NUTRITION  
UNIVERSITI MALAYSIA SABAH  
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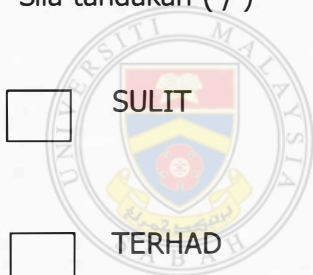
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IJAZAH: **DOCTOR OF PHILOSOPHY (FOOD SCIENCE)**

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## DECLARATION

I hereby declare that the thesis entitled, "Hypoglycemic and Antioxidant Activity of Polyphenol Glycoside Catalyzed By Transglycosylation Reaction of Cyclodextrin Glucanotransferase Derived From *Trichoderma Viride*" submitted for the degree of Doctor of Philosophy in Food Science to the Universiti Malaysia Sabah is the result of the work carried out by me under the guidance of Dr. Joko Sulistyono in the Faculty of Food Science and Nutrition, during the period 2014-2018.

I further declare that the results of this work have not been submitted for the award of any other degree or fellowship.

29 March 2018



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# CERTIFICATION

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VIVA DATE : **28 FEBRUARY 2018**



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## ACKNOWLEDGEMENT

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## ABSTRACT

Present study was conducted to evaluate the ability of *Trichoderma viridae* as a source of cyclodextrin glucanotransferase (CGTase) that has shown transglycosylation activity in the presence of polyphenolic constituents extracted from *Moringa oleifera* leaves as its acceptor to catalyze synthesis of polyphenolic glycosides as transglycosylation products. The study had indicated that some commercial substrates such as corn flour, wheat flour, rice flour and cassava flour could be utilized as potential substrates to synthesis transfer products in the presence of polyphenolic acceptors extracted from seaweed, clove, ginger, torch ginger and star anise. The enzymatically synthesized polyphenolic glycosides were then purified using octa-dodecyl-functionalized silica gel column chromatography prior to analysis using high performance liquid chromatography (HPLC) and identified using nuclear magnetic resonance (NMR) spectroscopy. The result of HPLC analysis presented that the isolated transfer products had retention times at 1.446, 1.431, and 1.474, respectively, compared to the retention time of arbutin (1.474) that was applied as an external authentic standard for polyphenol glycoside. Moreover, observation using  $^1\text{H}$  NMR as well as  $^{13}\text{C}$  NMR showed that structures of the transglycosylation products were identified as gallic acid-4-O- $\beta$ -D-glucopyranoside, ellagic acid-4-O- $\beta$ -D-glucopyranoside, and catechin-4'-O- $\beta$ -D-glucopyranoside, respectively. Ellagic acid-4-O- $\beta$ -D-glucopyranoside (EAGP) was evaluated for its antioxidant properties as it had the same RF value as compared to arbutin and was subjected to assess its antioxidant potential using various *in vitro* analyses.  $\text{IC}_{50}$  value of EAGP for DPPH free radical scavenging test was calculated as 46.12 $\mu\text{g/ml}$  as compared to the standards ascorbic acid (35.96 $\mu\text{g/ml}$ ), BHT (39.73 $\mu\text{g/ml}$ ) and  $\alpha$ -tocopherol (42.62 $\mu\text{g/ml}$ ), respectively. In  $\text{ABTS}^+$  radical scavenging assay,  $\text{IC}_{50}$  value for EAGP was recorded as 64.01 $\mu\text{g/ml}$  as compared to ascorbic acid (30.13 $\mu\text{g/ml}$ ), BHT (38.09 $\mu\text{g/ml}$ ) and  $\alpha$ -tocopherol (54.84 $\mu\text{g/ml}$ ) respectively. The  $\text{IC}_{50}$  value of EAGP as percentage of metal chelating ability was determined as 45.42 $\mu\text{g/ml}$  where EDTA as standard showed 28.24 $\mu\text{g/ml}$ . In  $\text{H}_2\text{O}_2$  radical scavenging assay,  $\text{IC}_{50}$  value of EAGP was found to be 51.90 $\mu\text{g/ml}$  while BHT and  $\alpha$ -tocopherol were 44.18 $\mu\text{g/ml}$  and 47.18 $\mu\text{g/ml}$ , respectively. The assays demonstrated that the presence of moderate antioxidant properties of enzymatically synthesized EAGP as compared to the ascorbic acid, BHT and  $\alpha$ -tocopherol. Furthermore, hypolipidemic and antidiabetic effect of enzymatically synthesized EAGP in normal and alloxan- induced diabetic rats were investigated. Diabetes was induced in rats by a single dose administration of alloxan (150 mg/kg, i.p.) for 28 days. Blood glucose levels were determined at day 0 until day 28 of treatment and blood glucose levels on four day after treatment with alloxan were significant ( $p < 0.05$ ) increasing in all diabetic rats as compared to normal control rats. At day 12, three different dosages of EAGP at concentration 100 mg/kg, 200

mg/kg, 400 mg/kg of body weight showed significant decrease (5-7%) ( $p < 0.05$ ) in blood glucose levels in alloxan induced diabetic rats as compared to untreated diabetic group. All the hyperglycemic groups which were treated with various dosages of EAGP recorded significantly ( $p < 0.05$ ) lower biochemical parameters (Cholesterol, triglycerides, HDL, LDL, creatinine, urea, alkaline phosphatase) of blood serum as compared to untreated diabetic group. Pancreatic tissue analysis of alloxan induced diabetic groups exhibited significant ( $p < 0.05$ ) reduction in catalase activity, GSH levels and lipid peroxidation (MDA) levels as compared to normal control group, while treated diabetic groups showed significant ( $p < 0.05$ ) elevation as compared to untreated diabetic group and activities were quite near to normal rats. In brief, the results of the recent research discovered that the hypoglycemic and hypolipidemic potentials of enzymatically synthesized polyphenol glycoside (EAGP) might be attributed to its free radical scavenging and antioxidant properties. Hence, it is resolved that EAGP may be suitable in the prevention of hyperglycemic complications linked with oxidative stress.



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## ABSTRAK

### **AKTIVITI HYPOGLYCEMIC DAN ANTIOXIDANT POLYPHENOL GLYCOSIDE CATALYZED OLEH REAKSI TRANSGLYCOSYLATION OF CYCLODEXTRIN GLUCANOTRANSFERASE DIBERIKAN DARIPADA TRICHODERMA VIRIDE**

Kajian ini telah dijalankan untuk menilai keupayaan *Trichoderma viride* sebagai sumber siklodekstrin glukano transferase (CGTase) yang telah menunjukkan aktiviti transglukosilasi dalam kehadiran polifenol yang diekstrak daripada daun *Moringa oleifera* sebagai penerima untuk memangkin sintesis polifenol glikosida sebagai produk transglukosilasi. Kajian ini telah menunjukkan bahawa beberapa substrat komersial seperti tepung jagung, tepung gandum, tepung beras dan tepung ubi kayu boleh digunakan sebagai substrat berpotensi untuk sintesis produk pemindahan dalam kehadiran sebatian polifenol yang diekstrak daripada rumpai laut, cengkik, halia, bunga kantan dan bunga lawang. Polifenol glikosida hasil sintesis enzim seterusnya dimurnikan menggunakan kromatografi turus silika gel octa-dodecyl berfungsi sebelum analisis menggunakan kromatografi cecair berprestasi tinggi (HPLC) dan dikenalpasti menggunakan spektroskopi resonans magnetik nuklear (NMR). Hasil analisis HPLC menunjukkan bahawa produk pemindahan yang diasingkan mempunyai masa tahanan masing-masing adalah 1.446, 1.431 dan 1.474, berbanding masa tahanan daripada arbutin (1.474) yang digunakan sebagai piawaian luar yang sah untuk polifenol glikosida. Selain itu, pemerhatian menggunakan  $^1\text{H}$  NMR serta  $^{13}\text{C}$  NMR menunjukkan bahawa struktur produk pemindahan transglukosilasi telah dikenalpasti masing-masing sebagai gallic acid-4-O- $\beta$ -D-glucopyranoside, ellagic acid-4-O- $\beta$ -D-glucopyranoside, dan catechin-4'-O- $\beta$ -D-glucopyranoside. Ellagic acid-4-O- $\beta$ -D-glucopyranoside (EAGP) telah dinilai untuk ciri-ciri antioksidannya kerana ianya mempunyai nilai RF yang sama berbanding dengan piawaian arbutin dan digunakan untuk pengujian potensi antioksidan menggunakan pelbagai analisis *in vitro*. Pengiraan nilai  $\text{IC}_{50}$  daripada EAGP untuk ujian memerangkap radikal bebas DPPH masing-masing adalah  $46.12\mu\text{g/ml}$  berbanding piawaian asid askorbik ( $35.96\mu\text{g/ml}$ ), BHT ( $39.73\mu\text{g/ml}$ ) dan  $\alpha$ -tokoferol ( $42.62\mu\text{g/ml}$ ). Pengiraan nilai  $\text{IC}_{50}$  daripada EAGP untuk ujian dalam memerangkap radikal  $\text{ABTS}^+$  masing-masing adalah  $64.01\mu\text{g/ml}$  berbanding asid askorbik ( $30.13\mu\text{g/ml}$ ), BHT ( $38.09\mu\text{g/ml}$ ) dan  $\alpha$ -tokoferol ( $54.84\mu\text{g/ml}$ ). Penentuan nilai  $\text{IC}_{50}$  daripada EAGP untuk nilai peratusan dalam keupayaan mengelat logam adalah  $45.42\mu\text{g/ml}$  yang mana EDTA sebagai piawaian adalah menunjukkan  $28.24\mu\text{g/ml}$ . Dalam pengujian memerangkap radikal  $\text{H}_2\text{O}_2$ , nilai  $\text{IC}_{50}$  daripada EAGP yang didapati adalah  $51.90\mu\text{g/ml}$ , manakala BHT dan  $\alpha$ -tokoferol masing-masing adalah  $44.18\mu\text{g/ml}$  dan  $47.18\mu\text{g/ml}$ . Pengujian menunjukkan bahawa kehadiran ciri-ciri antioksidan yang sederhana daripada EAGP hasil sintesis enzim berbanding dengan asid askorbik, BHT dan  $\alpha$ -tocopherol. Tambahan pula kesan EAGP hasil sintesis enzim dalam merendahkan lipid dan antidiabetik dalam tikus-tikus normal



dan diabetik yang teraruh aloksan telah disiasat. Diabetik pada tikus telah teraruh dengan pengambilan dos tunggal aloksan (150 mg/kg, ip) selama 28 hari. Tahap gula darah telah ditentukan pada hari 0 sehingga hari 28 rawatan dan tahap glukosa darah pada hari 4 selepas rawatan dengan aloksan adalah semakin meningkat ketara ( $p < 0.05$ ) dalam semua tikus diabetik berbanding dengan tikus kawalan normal. Pada hari 12, tiga dos yang berbeza daripada EAGP pada kepekatan 100 mg/kg, 200 mg/kg, 400 mg/kg daripada berat badan menunjukkan penurunan yang ketara (5-7%) ( $p < 0.05$ ) keatas tahap glukosa dalam darah tikus diabetik yang teraruh aloksan berbanding kumpulan tikus diabetik yang tidak dirawat. Semua kumpulan hiperglisemia yang telah dirawat dengan pelbagai dos EAGP menunjukkan parameter biokimia (Kolesterol, trigliserida, HDL, LDL, kreatinin, urea, alkali fosfatase) secara ketara ( $p < 0.05$ ) lebih rendah dalam serum darah berbanding kumpulan diabetik yang tidak dirawat. Analisis tisu pankreas daripada kumpulan diabetik teraruh aloksan mempamerkan pengurangan yang ketara ( $p < 0.05$ ) dalam aktiviti katalase, tahap GSH dan peroksidasi lemak (MDA) berbanding dengan kumpulan kawalan normal, manakala kumpulan diabetik yang dirawat menunjukkan peningkatan secara ketara ( $p < 0.05$ ) berbanding kumpulan diabetik yang tidak dirawat dan aktiviti nya agak berhampiran dengan kumpulan tikus normal. Secara ringkas, disimpulkan bahawa hasil daripada penyelidikan ini mendapati bahawa potensi hipoglisemik dan hipolipidemik daripada polifenol glikosida (EAGP) hasil sintesis enzim adalah disebabkan oleh pemerangkapan radikal bebas dan sifat-sifat antioksidan. Oleh itu, mungkin dapat disimpulkan bahawa EAGP adalah sesuai untuk pencegahan komplikasi hiperglisemik yang berkaitan dengan tekanan oksidatif.

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## LIST OF ABBREVIATIONS

<b>WHO</b>	- World Health Organization
<b>NCEs</b>	- Novel Chemical Entities
<b>USA</b>	- United States of America
<b>CGTase</b>	- Cyclodextrin glucanotransferase
<b>NMR</b>	- Nuclear Magnetic Resonance spectroscopy
<b><i>M. oleifera</i></b>	- <i>Moringa oleifera</i>
<b><i>T. viride</i></b>	- <i>Trichoderma viride</i>
<b>HPLC</b>	- High Performance Liquid Chromatography
<b>-OH</b>	- Hydroxyl
<b>TLC</b>	- Thin Layer Chromatography
<b>ODS</b>	- Octa-Dodecyl Functionalized Silica Gel
<b>mM</b>	- Millimolar
<b>MIC</b>	- Minimum Inhibitory Concentration
<b>ppm</b>	- Parts Per Million
<b>BHT</b>	- Butylated Hydroxytoluene
<b>ml</b>	- Milliliter
<b>CD</b>	- Cyclodextrin
<b>w/v</b>	- Weight/Volume
<b>TE</b>	- Transfer efficiency
<b>IgE</b>	- Immunoglobulin E
<b>EC</b>	- Epicatechin
<b>MS</b>	- Mass Spectrophotometry
<b>UV</b>	- Ultra Violet

<b>µg</b>	- Microgram
<b>mg</b>	- Milligram
<b>DMSO</b>	- Dimethyl sulfoxide
<b>v/v</b>	- Volume/Volume
<b>g</b>	- Gram
<b>LDL</b>	- Low density lipoprotein
<b>IDDM</b>	- Insulin dependent diabetes
<b>T1D</b>	- Type 1 diabetes
<b>NIDDM</b>	- Non-insulin dependent diabetes mellitus
<b>T2D</b>	- Type 2 diabetes
<b>HLA</b>	- Humanoid leukocyte antigen
<b>IGT</b>	- Impaired glucose tolerance
<b>NEFA</b>	- Non-esterified fatty acids
<b>TNF</b>	- Tumor necrosis factor
<b>GLUT4</b>	- Glucose transport system
<b>H<sub>2</sub>O<sub>2</sub></b>	- Hydrogen peroxide
<b>NADPH</b>	- Nicotinamide adenine dinucleotide phosphate
<b>ANG II</b>	- Angiotensin II
<b>ROS</b>	- Reactive oxygen species
<b>ATP</b>	- Adenosine triphosphate
<b>AGE</b>	- Advanced glycation end products
<b>AR</b>	- Aldose reductase
<b>PKC</b>	- Protein kinase C
<b>GSH</b>	- Reduced glutathione
<b>VEGF</b>	- Vascular endothelial growth factor

<b>IGF-1</b>	- insulin-like growth factor-1
<b>CVD</b>	- Cardiovascular disease
<b>NFκB</b>	- Nuclear factor kappa b
<b>PDA</b>	- Potato dextrose agar
<b>LPO</b>	- Lipid peroxidation
<b>CAT</b>	- Catalase activity
<b>MDA</b>	- Malondialdehyde
<b>HDL</b>	- High density lipoprotein
<b>S.E.M</b>	- Standard error of mean
<b>ANOVA</b>	- Analysis of variance
<b>TP</b>	- Transfer product
<b>EAGP</b>	- Ellagic acid 4-O-β-D-glucopyranoside
<b>C</b>	- Carbon
<b>H</b>	- Hydrogen
<b>DPPH</b>	- 2, 2-Diphenyl-2-picrylhydrazyl
<b>ABTS</b>	- 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
<b>dl</b>	- Deciliter
<b>ALP</b>	- Alkaline phosphatase
<b>mmol</b>	- Millimole
<b>nm</b>	- Nanometer
<b>TG</b>	- Triglycerides
<b>TC</b>	- Total cholesterol
<b>rpm</b>	- Revolutions per minute

## LIST OF SYMBOLS

<b>%</b>	- Percentage
<b><math>\beta</math></b>	- Beta
<b><math>\alpha</math></b>	- Alpha
<b><math>^{\circ}\text{C}</math></b>	- Degree celsius
<b><math>\gamma</math></b>	- Gamma
<b><math>\mu\text{g}</math></b>	- Microgram
<b><math>\mu\text{M}</math></b>	- Micromolar



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# CHAPTER 1

## INTRODUCTION

### 1.1 General introduction

Malaysia is situated in Southeast Asia and consists of 13 states and 3 federal territories with a total landmass of 329,847 square kilometers (127,350 square miles). Malaysia is separated by the South China Sea into Peninsular Malaysia and East Malaysia. Malaysia is within the equatorial region, where a tropical rainforest climate is apparent year round. The capital city is Kuala Lumpur, and Putrajaya is the seat of the federal government. By 2015, with a population of more than 30 million, Malaysia became the 43rd most populous country in the world, and it is a multicultural society in which 67.4% of the population are ethnic Malays, 27.3% Chinese, and 7.3% Indians, according to the 2010 census.

Malaysia's gross domestic product is US\$326.9 billion, according to 2014 figures from the World Bank, and the country has an open, upper middle income economy, with a growth rate of more than 7% per year for at least the last 25 years. Today, Malaysia has a diversified economy and has become a leading exporter of electrical appliances, electronic parts and components, palm oil, and natural gas. The national language of Malaysia is Bahasa Melayu (literally "Malay language"), though English is widely spoken as second language (Hussein *et al.*, 2015).

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). The level of hyperglycaemia associated diabetes increases the risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to the related microvascular complications, increased risk of macrovascular complications (ischaemic heart