

**ANTIOXIDANT AND ANTIHYPERGLYCEMIC
ACTIVITIES OF *Lygodium microphyllum*
AGAINST ALLOXAN-INDUCED DIABETIC RATS**

DG SYAHIDAH NADIAH BINTI ABDULL MAJID

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2019**



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DG SYAHIDAH NADIAH BINTI ABDULL MAJID

**THESIS SUBMITTED IN THE FULLFILMENT FOR
THE DEGREE OF MASTER OF SCIENCE**

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**BIOTECHNOLOGY RESEARCH INSTITUTE
UNIVERSITI MALAYSIA SABAH
2019**



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JUDUL: **ANTIOXIDANT AND ANTIHYPERGLYCEMIC ACTIVITIES OF *Lygodium microphyllum* AGAINST ALLOXAN-INDUCED DIABETIC RATS**

IJAZAH: **SARJANA SAINS (BIOTEKNOLOGI)**

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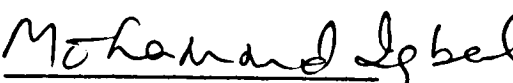
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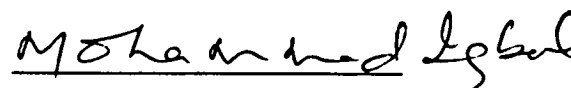
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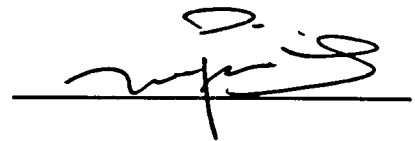
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07th May 2018



ABSTRACT

Diabetes mellitus is the most common endocrine disorder that affects more than 415 million people worldwide. *Lygodium microphyllum* or better known as Old World Climbing Fern from the family Lygodiaceae, is an invasive climbing fern species that is distributed around Sabah Borneo and possesses many medicinal values in treating several diseases and for health care maintenance. The present study was designed to evaluate the antihyperglycemic potential of *L. microphyllum* leaves extract against alloxan induced diabetes in rats. *In vitro* studies have shown that the extract possessed strong antioxidant activity and has ability to scavenge DPPH free radicals effectively. Phytochemical studies revealed the presence of pharmaceutically active compounds of flavonoids, alkaloids, tannins, and steroids. Total phenolic content of leaves was found to be 966.7 ± 0.03 mg/100g dried sample. The total flavonoid content of leaves was found to be 42.9 ± 0.01 mg/100g dried sample. Besides that, experiments designed for antihyperglycemic activity of *L. microphyllum* against alloxan induced diabetes in rats. Body weight and blood glucose level were measured on interval of 14 days. Thirty-six Sprague Dawley rats were taken and randomly divided into six groups (n=6). Injection of alloxan (i.v.) at a dose of 100 mg/kg body weight in rats resulted in a significant increase in serum glucose as compared to saline treated control. Also, oxidative stress was noticed in pancreatic tissue as evidenced by a significant decrease in glutathione level, glutathione reductase, glutathione-s-transferase, catalase activities, also a significant increase in malondialdehyde when compared to normal saline treated control group. Pancreases were also examined histopathologically by haematoxylin and eosin staining. Additionally, serum biochemistry and oxidative stress markers were consistent with the pancreatic histopathological studies. Treatment of diabetic rats with *L. microphyllum* at a dose level of 100, 200 and 400 mg/kg body weight leaves extract for 14 days significantly prevented these alterations and attenuated alloxan-induced oxidative stress. Therefore, the aqueous extract of *L. microphyllum* may be a good source of natural antioxidant. The results of the present study indicates that the antihyperglycemic potentials of *L. microphyllum* might be ascribable to its antioxidant and free radical scavenging properties. Thus, it concluded that *L. microphyllum* may be helpful in the prevention of diabetic complications associated with oxidative stress.



ABSTRAK

AKTIVITI ANTIOKSIDATIF DAN ANTIHIPERGLIKEMIA TUMBUHAN *Lygodium microphyllum* TERHADAP TIKUS-TIKUS DIABETIS YANG DISEBABKAN OLEH ALLOXAN

Diabetes mellitus merupakan gangguan endokrin paling biasa yang memberi kesan kepada lebih daripada 415 juta orang diseluruh dunia. Lygodium microphyllum yang lebih dikenali sebagai Paku Memanjat Dunia Lama daripada keluarga Lygodiaceae, ianya adalah spesies paku-pakis memanjat yang invasif. Ia banyak ditemui diseluruh Sabah Borneo disamping mempunyai pelbagai nilai perubatan dalam merawat beberapa penyakit dan juga untuk penjagaan kesihatan. Kajian ini dibuat untuk menilai potensi antihyperglykemia ekstrak daun L. microphyllum terhadap tikus-tikus diabetes yang disebabkan oleh alloxan. Kajian in vitro telah menunjukkan bahawa ekstrak daun mempunyai aktiviti antioksidan yang kuat dan mempunyai keupayaan untuk memusnahkan radikal bebas DPPH secara berkesan. Kajian fitokimia mendedahkan kehadiran sebatian farmasi aktif iaitu flavonoid, alkaloid, tannin, dan steroid. Jumlah kandungan fenolik daripada daun adalah 966.7 ± 0.03 mg/100g sampel kering. Jumlah kandungan flavonoid daripada daun adalah 42.9 ± 0.01 mg/100g sampel kering. Selain itu, eksperimen telah direka untuk aktiviti antihyperglykemia L. microphyllum terhadap tikus-tikus diabetes yang disebabkan oleh alloxan. Berat badan dan tahap kandungan gula dalam darah telah diambil dalam masa 14 hari. Tiga puluh enam tikus Sprague Dawley telah digunakan dan dibahagikan secara rambang kepada enam kumpulan (n=6). Suntikan alloxan (i. v.) pada dos 100 mg/kg daripada berat badan tikus memberi kesan peningkatan yang signifikan dalam serum glukosa berbanding tikus yang dirawat dengan air garam (kumpulan kawalan). Tekanan oksidatif telah diperhatikan dalam tisu pankreas, sebagai bukti dengan pengurangan yang signifikan dalam tahap glutathion, glutathion reductase, glutathione-s-transferase, aktiviti catalase, juga peningkatan yang signifikan dalam malondialdehyde apabila dibandingkan dengan kumpulan kawalan yang dirawat dengan air garam. Pankreas juga diperiksa secara histopatologi dengan pewarnaan haematoxylin dan eosin. Sebagai tambahan, penanda serum biokimia dan tekanan oksidatif adalah konsisten dengan kajian terhadap histopatologi pankreas.



Oleh itu, ekstrak akueus L. microphyllum boleh menjadi sumber antioksidan semulajadi yang baik. Hasil kajian ini menunjukkan bahawa, potensi antihiperglikemia L. microphyllum mungkin boleh dianggap sebagai memiliki sifat antioksida dan radikal bebas. Dengan itu, disimpulkan bahawa L. microphyllum mungkin boleh membantu dalam pencegahan daripada komplikasi diabetes yang terkait dengan tekanan oksidatif.



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Magnification 80X.



LIST OF ABBREVIATIONS

%	: Percentage
°C	: Degree Celsius
α	: Alpha
β	: Beta
$\mu\text{g/mL}$: Microgram per milliliter
μL	: Microliter
μm	: Micrometer
μmol	: Micromol
c	: Concentration
df	: Dilution Factor
dH ₂ O	: Distilled Water
g	: Gram
h	: Hour
i.v	: Intravenous
m	: Mass
mg/dL	: Microgram per deciliter
mg/g	: Milligram per gram
mg/kg	: Miligram per kilogram
mL	: Milliliter
mmol/L	: Milimol per liter
mm	: Millimeter
n	: Number
nm	: Nanometer
pH	: Potential of Hydrogen
Abs	: Absorbance
ABSL3	: Animal Biosafety Laboratory Level 3
ADA	: American Diabetes Association
AEC	: Animal Ethics Committee
ALX	: Alloxan
ANOVA	: Analysis of Variance



ATP	: Adenosine Triphosphate
BRI	: Biotechnology Research Institute
BSA	: Bovine Serum Albumin
CAT	: Catalase
CDNB	: 1-chloro-2,4-dinitrobenzene
CE	: Catechin Equivalent
CVD	: Cardiovascular Disease
DM	: Diabetes mellitus
DNA	: Deoxyribonucleic Acid
DPPH	: 1,1-diphenyl-2-picrylhydrazyl
DPPIV	: Dipetyl Peptidase IV
DPX	: Distyrene, a plasticizer, and xylene
DTNB	: 1,2-dithio-bis-nitrobenzic acid
EDTA	: Ethylenediamine tetraacetic acid
FBG	: Fasting Blood Glucose
FCR	: Folin Ciocalteu Reagent
FeCl ₃	: Ferric Chloride
GAE	: Gallic Acid Equivalents
GLP-1	: Glucagon-like Peptide-1
GLUT-2	: Glucose Transporter 2
GLUT-4	: Glucose Transporter 4
GR	: Glutathione Reductase
GSH	: Reduced Glutathione
GSSG	: Oxidized Glutathione
GST	: Glutathione-S-Transferase
GPX	: Glutathione Peroxidase
H ₂ O ₂	: Hydrogen peroxide
H ₂ SO ₄	: Sulfuric Acid
H & E	: Haematoxylin and Eosin
HbA _{1c}	: Glycosylated Haemoglobin
I	: Iodine
IC ₅₀	The half maximal of inhibitory concentration

IDDM	: Insulin Dependent Diabetes Mellitus
IDF	: International Diabetes Federation
IGT	: Impaired Glucose Tolerance
IUCN	: International Union for Conservational of Nature
K+	: Potassium
KI	: Potassium Iodide
LPO	: Lipid Peroxidation
M	: Molar
Min	: Minute
MDA	: Malondialdehyde
MNT	: Medical Nutrition Therapy
MOH	: Ministry of Health
Na₂HPO₄.2H₂O	: Di-sodium hydrogen phosphate dehydrate
NaH₂PO₄	: Sodium dihydrogen phosphate
NADPH	: B-nicotinamide adenine dinucleotide phosphate reduced
NaNO₃	: Sodium nitrite
NaOH	: Sodium hydroxide
NCD	: Non-Communicable Disease
NIDDM	: Noninsulin Dependent Diabetes Mellitus
NIH	: National Center for Complementary and Integrative Health
NHMS	: National Health and Morbidity Survey
NH₃	: Ammonia
OADs	: Oral Antidiabetic Drugs
OGTT	: Oral Glucose Tolerance Test
PBS	: Phosphate Buffer Saline
PMS	: Post Mitochondrial Supernatant
QOL	: Quality of Life
ROS	: Reactive Oxygen Species
SD	: Sprague Dawley
Sec	: Second
SEM	: Standard error of the mean
SGLT-2	: Sodium Glucose Transporter 2

SOD	: Superoxide Dismutase
SPSS	: Statistical Package for the Social Science
SSA	: Sulfosalicylic Acid
STZ	: Streptozotocin
SUR-1	: Sulphonylurea 1
TBA	: Thiobarbituric Acid
TCA	: Trichloroacetic Acid
TFC	: Total Flavonoids Content
TPC	: Total Phenolic Content
UiTM	: Universiti Teknologi MARA
UMS	: Universiti Malaysia Sabah
UV	: Ultraviolet
UV-Vis	: Ultraviolet Visible
V	: Volume
WHO	: World Health Organization



CHAPTER 1

INTRODUCTION

1.0 Introduction

Diabetes mellitus (DM) is a complex and chronic endocrine disorder involving a metabolic disorder of carbohydrate, fat, and protein (Mohan, Jesuthankaraj, & Ramasamy Thangavelu, 2013). Chronic hyperglycemia is the character of the disorder due to a relative or absolute lack of insulin secretion or insulin actions.

In a major advance, 2015, the World Health Organization (WHO) carried out a first WHO global report on diabetes. It was estimated that 422 million adults were living with diabetes in 2004 compared to 108 million in 1980 which is 74.4% increment. The disease is deadly, silent killer, and costly because it involves high expenses for its medication (Saleh, Azahari, & Ismail, 2013).

DM incidences have soared worldwide and are currently increasing. In Malaysia, DM is worrying with the highest prevalence as listed at the 10th rank in the world. Malaysia faces a massive task to provide a comprehensive management of this disorder. It shows that the number of citizens at the age ranged between 18 years to 79 years had the diabetes keep arising.

Based on the Diabetes Country Profiles–Malaysia (2016) by WHO, insulin, metformin, and sulphonylurea are the common medicines available in the primary care facilities. These synthetic drugs may be associated with many adverse effects. Low blood sugar, upset stomach, skin rash, kidney complications, tiredness or dizziness, bloating and diarrhea, weight gain, weight loss, liver disease, and anemia are the examples of the synthetic drugs effects.



Treatments or management of DM is very important. Plant and plant products play a vital role in DM treatment and have been used throughout the world since ancient times. Folk and traditional medicine healing systems have been used globally as an antioxidant, antihyperglycemic, and antidiabetic properties from plants. *Galega officinalis* is the example of a traditional remedy for diabetes. It is a metformin derivative.

1.1 Problem Statement

DM is a serious metabolic disorder which is associated with long term damage, dysfunction, and organ failure. DM will cause microvascular complications (retinopathy, nephropathy, and neuropathy) and macrovascular complications (ischemic heart disease, stroke, and peripheral vascular disease). As a major public health concern in Malaysia, treating DM is costly and poses a burden to the individual and health system.

In the treatment of DM, a variety of plants with new therapeutic agents have been explored. In this study, the antidiabetic properties of *L. microphyllum* was screened. Ancient people used *L. microphyllum* for a various mode of uses. They use as a medicine to treat dysentery, tuberculosis, fever, cancer, joint and muscle pain, skin diseases, and others. *Lygodium flexosum*, *Hemionitis arifolia*, *Pteris vitata*, *Nephoelepsis*, *Adiantum philippense*, and *Cheilantes glauca* are the examples of ferns with an antidiabetic properties.

Local knowledge of *L. microphyllum* as an antidiabetic agent are poorly documented in scientific literature. There are many species of ferns reported containing antidiabetic properties. *L. microphyllum* can be potentially used for antidiabetic purpose. However, more work is needed to establish the mechanism (s) of antihyperglycemic action.

1.2 Significance of the Study

Increased prevalence of DM over the years is the main reason for this study. DM is a major public health problem and economic burden in the world including Malaysia.

Study on this area is important to provide scientific proof in order to justify the use of *L. microphyllum* as an antidiabetic and antihyperglycemic agents. The nature of this plant is easily grown in Malaysia and the medicinal values discovered may provide access to a medicine that is cheap, effective, and easily accessible.

1.3 Purpose of the Study

The study aims to access the anti-oxidative and antihyperglycemic activities of an aqueous extract of *L. microphyllum*. This investigation also to archive the following objectives:

- a. To evaluate the phytochemical constituents and anti-oxidative potential of *L. microphyllum*.
- b. To determine the *in vivo* efficiency of *L. microphyllum* against changes in the levels of blood glucose of alloxan-induced diabetic rats.
- c. To access the *in vivo* efficiency of *L. microphyllum* against manifestation of oxidative stress in alloxan-induced diabetic rats by measuring malonaldehyde (MDA) concentration, the end product of lipid peroxidation (LPO), and assessing total antioxidant power such as reduced glutathione and anti-oxidative enzyme.
- d. To evaluate the *in vivo* efficiency of *L. microphyllum* against histopathological alteration in the pancreas of alloxan-induced diabetic rats.

1.4 Scope

The study was conducted for 14 days treatment which the alloxan-induced diabetic rats were treated with different doses (400, 200, and 100 mg/kg of body weight) of an aqueous extract of *L. microphyllum*. Total antioxidant and anti-oxidative enzymes were determined in order to prove that *L. microphyllum* has a potential as an antidiabetic and antihyperglycemic agent.

1.5 Limitations

There are few limitations in this study:

- a. Ethics must be considered. When using animals in this study, negative-control test might be unethical.



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