ANTIDIABETIC ACTIVITY OF *AQUILARIA MALACCENSIS* (AGARWOOD) LEAVES EXTRACTS

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MASTER OF SCIENCE (BIOTECHNOLOGY)

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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Science (Biotechnology).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Diabetes mellitus adalah disebabkan oleh penurunan pengambilan glukosa dalam darah oleh sel dan metabolisme. Objektif tesis ini adalah untuk mengkaji mengenai kesan aktiviti antidiabetes dari ekstrak metanol dan air dari daun *Aquilaria malaccensis*. Ia dijalankan melalui kaedah *in vitro* dan *in vivo*. Dalam kajian *in vitro*, dua kaedah enzimatik yang dijalankan adalah perencatan α-glucosidase dan α-amylase dimana kesan perencatan dari ekstrak metanol dan ekstrak air dengan kepekatan dari 100 hingga 1000μg/ml berbanding dengan Acarbose. Kajian toksik akut dan toksik sub-kronik telah dijalankan untuk melihat kesan toksik dengan dos maksimum 2g/kg selama 14 hari ke atas tikus, manakala dalam sub-kronik, dua kepekatan iaitu 250mg/kg dan 500mg/kg ekstrak telah diberi kepada tikus setiap hari selama 28 hari. Dalam kajian *in vivo* dengan menggunakan tikus diabetes yang diinduksi dengan streptozotocin (STZ) pemerhatian dibuat keatas kesan 500mg/kg *Aquilaria malaccensis* ekstrak metanol dan ekstrak air dalam menurunkan paras glukosa darah berbanding dengan Metformin. Ekstrak metanol dan ekstrak air *Aquilaria malaccensis* menunjukkan aktiviti perencatan α-glucosidase dengan nilai IC_{50} 428.92 dan 425.09μg/ml, berbanding IC_{50} Acarbose iaitu 402.06μg/ml. Begitu juga dengan aktiviti perencatan α-amylase, kedua-dua ekstrak ini menunjukkan nilai IC_{50} metanol dan ekstrak air adalah sebanyak 752.98 dan 771.53μg/ml berbanding Acarbose, 584.93μg/ml. Kajian ketoksikan akut *Aquilaria malaccensis* ekstrak metanol dan ekstrak air menunjukkan bahawa ia tidak menyebabkan kesan toksik pada tikus dimana dos maut (LD_{50}) untuk semua ekstrak adalah lebih tinggi daripada 2g/kg. Kajian sub-kronik dilakukan selama 28 hari di mana tikus *Sprague Dawley* jantan dewasa dirawat dengan ekstrak pada kepekatan 250mg/kg dan 500mg/kg. Serum darah dianalisis untuk profil buah pinggang dan fungsi hati tiada perubahan signifikan dan kekal dalam julat normal. Dalam kajian *in vivo* menggunakan tikus diabetes STZ, rawatan dengan ekstrak metanol dan air *Aquilaria malaccensis* dengan 500 mg/kg berat selama 5 hari menunjukkan penurunan yang signifikan dalam glukosa darah dengan peratusan kesan penurunan glukosa pada 57.08% dan 55.48% menurun masing-masing berbanding metformin, 68.79%. Analisis biokimia pada tikus diabetes yang dirawat dengan kedua-dua ekstrak menunjukkan bahawa ekstrak tidak meningkatkan kerosakan pada protein serum. Ia menunjukkan bahawa ekstrak ini tidak menghasilkan sebarang kerosakan yang ketara di dalam organ dalaman; hati dan buah pinggang. Secara keseluruhan ekstrak metanol dan ekstrak air *Aquilaria malaccensis* mempunyai potensi untuk menurunkan tahap glukosa darah tanpa membahayakan haiwan dan mempunyai potensi untuk digunakan sebagai maklumat tambahan dalam pengurusan diabetes mellitus dan boleh membangunkan standard perubatan untuk diabetes mellitus.

ABSTRACT

Diabetes mellitus is defines clinically by hyperglycaemia or an abnormal increased glucose uptake. The purpose of this study was to assess the possible inhibitory effects of methanolic and aqueous leaves extract of *A. malaccensis* against α-glucosidase and α-amylase activities at concentrations ranged from 100 to 1000µg/ml compared with Acarbose, a commercial drug used in the clinical management of diabetes. Acute and sub-chronic toxicity studies were conducted to observe any toxic effects of *A. malaccensis* leaves extracts by administrating maximum dose of 2g/kg body weight for 14 days and two concentrations of 250mg/kg and 500mg/kg body weight daily for 28 days, respectively. *In vivo* study was conducted using STZ-induced diabetic rats model to evaluate the effects of administrating 500mg/kg body weight of methanolic and aqueous *A. malaccensis* leaves extracts on blood glucose level compared with standard drug, Metformin. *A. malaccensis* methanolic and aqueous leaves extracts exhibited potent inhibitory effects against α-glucosidase activity with IC_{50} values of 428.92 and 425.09µg/ml, respectively, compared to Acarbose, with IC_{50} values of 402.06µg/ml. Similarly, methanolic and aqueous *A. malaccensis* extracts showed dose dependant inhibitory effects against α-amylase activity with IC_{50} values of 752.98 and 771.53µg/ml, respectively compared with Acarbose, with IC_{50} value of 584.93µg/ml. Acute toxicity study of *A. malaccensis* methanolic and aqueous leaves extracts showed that the extracts did not exhibit any toxic effect in rats and is therefore, likely to be safe for consumption; oral lethal dose (LD_{50}) recorded for all extracts was greater than 2g/kg body weight. Blood serum was analysed for kidney profile and liver function; changes of these values were insignificant and remained within normal range. *In vivo* study using STZ-induced diabetic rats treated with *A. malaccensis* methanolic and aqueous leaves extracts at concentration of 500mg/kg body weight for 5 days showed that blood glucose level was significantly decreased by a percentage of 57.08% and 55.48%, respectively, compared with metformin, 68.79%. Assessment of biochemical parameters in diabetic rats treated with both extracts showed that the extracts did not inflict damage to serum protein; the results suggest that these extracts do not cause significant damage to the internal organs, particularly liver and kidney. These results suggest that *A. malaccensis* methanolic and aqueous extracts can potentially lower the blood glucose level in diabetic individual without inflicting harmful side effects. The extracts can be potentially used as an adjunct in the management of diabetes mellitus as well as development of standardized phytomedicine for diabetes mellitus.

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LIST OF SYMBOLS

^{o}C	degree Celcius
α	alpha
β	beta
%	percentage
IC_{50}	inhibitory concentration at 50%
µg/ml	microgram per millilitre
µM	micromolar
µg	microgram

LIST OF ABBREVIATIONS

Alb	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CK	creatine kinase
CREA	creatinine
CTLA4	cytotoxic T-Lymphocyte Associated Protein 4
DH_2O	distilled water
DMSO	dimethylsulfoxide
DNS	3.5-dinitrosalicyclic acid
e.g.	for example
EHD	extremely high dose
g	gram
g/day	gram per day
G6PD	glucose-6-phosphate dehydrogenase
Glob	globulin
GLUT4	glucose transporter type 4
HbA1c	glycated haemoglobin
HD	high dose
HDL	high density lipoprotein
i.e.	for example
IL2Ra	interleukin 2 receptor subunit alpha
kg	kilogram
LD	low dose
LDH	lactate dehydrogenase
LDL	low density lipoprotein
mg	milligram
mg/kg	milligram per kilogram
mg/ml	milligram per millilitre
min	minutes
ml	millilitre

mmol	millimole
mmol/L	millimole per litre
NaCl	Sodium chloride
$NaCO_3$	Sodium carbonate
nm	nanometre
O.D	optical density
pH	potential of hydrogen (pH value)
PLT	platelet
pNPG	para-nitrophenol-α-D-glucopyranoside
PTPN22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
RBC	red blood cells
rpm	rotation per minutes
S.E.M	standard error of the mean
spp	subspecies
STZ	streptozotocin
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TBil	total bilirubin
TC	total cholesterol
TG	triglyceride
TP	total protein
U	Unit
UA	uric acid
v/v	volume/volume
w/v	weight/volume
WBC	white blood cells

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