IDENTIFICATION OF ALPHA-GLUCOSIDASE, ANTI-OXIDANT, AND TOXICITY ASSESSMENT OF *PSYCHOTRIA MALAYANA* JACK LEAVES USING METABOLOMICS APPROACH

BY

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Pharmaceutical Chemistry)

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FEBRUARY 2022

ABSTRACT

Psychotria malayana Jack belongs to Rubiaceae family and known in Malaysia as "meroyan sakat/salung". It is widely available in Malaysia and other Southeast Asian countries where it is traditionally used to manage diabetes. However, its folk claims as an antidiabetic agent and proper metabolites profiling are yet to be carried out to further confirm its role as an efficacious antidiabetic herbal remedy. Therefore, this study was aimed to evaluate the anti-diabetic activity of this plant extracts (0, 25, 50, 75, and 100% v/v methanol–water) through *α*-glucosidase (AG) inhibitory assay as well as toxicity determination using zebrafish embryos/larvae (*Danio rerio*) model. The extracts were also assessed for antioxidant activity to further confirm its antidiabetic potential. The AG inhibitors of the plant were identified using gas and liquid chromatography fitted with mass spectrometry (GCMS and LCMS, respectively) and nuclear resonance spectroscopy (NMR) based metabolomics approach. While the ligand-protein interaction was elucidated through molecular docking study. Furthermore, a validated analytical technique was also developed applying a Fourier Transform Infrared Spectroscopy (FTIR) fingerprint and utilizing an orthogonal partial least square (OPLS). The methanol extract possessed the highest AG inhibitory activity (IC_{50} 2.83 ± 0.32 µg/mL). In addition, methanol extract showed potent insulin sensitizing and antioxidant activities. A total of eight putative bioactive compounds were identified namely 1,3,5-benzenetriol (**1**); palmitic acid (**2**); cholesta-7,9(11)-diene-3-ol (**3**); 1-monopalmitin (**4**); *β*-tocopherol (**5**); *α*-tocopherol (**6**); 24-epicampesterol (**7**); and stigmast-5-ene (**8**) using GCMS-based metabolomics approach. Furthermore, five putative bioactive compounds, namely 1-monopalmitin (4), 4-hydroxyphenylpyruvic acid (**10**), 5′-hydroxymethyl-1′-(1, 2, 3, 9-tetrahydro-pyrrolo (2, 1-*b*) quinazolin-1-yl)-heptan-1′-one (**11**), *α*-terpinyl-*β*-glucoside (**12**), and machaeridiol-A (**13**) were identified through LCMS-based metabolomics approach. Additionally, using NMR-based metabolomics, two putative bioactive compounds were identified, namely 4-hydroxyphenylpyruvic acid (**10**) and glutamine (**14**). Docking results of all thirteen putative bioactive compounds showed moderate to high binding affinities (-5.5 to -10.0 kcal/mol) towards the active site of the enzymatic protein. Several residues, namely ASP352, HIE351, GLN182, ARG442, ASH215, SER311, ARG213, GLH277, GLN279, PRO312, HIE280, and GLU411 established hydrogen bond in the docked complex. The OPLS model developed by FTIR and validated using six external samples of same plant sepcies and potentially predicted the AGI activity of all extracts. Therefore, it can be suggested to be used as a tool in the plant's quality control. The median lethal concentrations (LC_{50}) of 0%, 25%, 50%, 75% and 100% MeOH extract were found to be toxic (252.45, 119.40, 81.28, 64.71, and 37.50 µg/mL, respectively). Conclusively, thirteen putative AG inhibitors from *P. malayana* were identified through metabolomics approach in this study and methanol extract is better to use based on its potency and safety. All of these findings might prove helpful for this plant to be used as a promising anti-diabetic medicine in the future.

خلاصة البحث

تنمي *Psychotria malayana* Jack إلى فصيلة الفويات والمعروف في ماليزيا باسم "meroyan sakat/salung" وهو متوفر على نطاق واسع في ماليزيا ودول جنوب شرق آسيا الأخرى حيث يستخدم تقليدياً لعلاج مرض السكري. ومع ذلك, فإن ادعاءاته الشعبية بأنه عامل مضاد للسكري و التنميط المناسب للمستقلبات لم يتم بعد وذلك لتأكيد دوره كعلاج عشبي فعال مضاد للسكري. لذلك هدفت هذه الدراسة إلى تقييم الفعالية المضادة للسكري لمستخلصات هذا النبات (0 ,25, 50, 75, 100٪ حجم/ حجم ميثانول-ماء) من خلال مقايسة مثبط أنزيم ألفا غلوكوزيداز وكذلك تحديد السمية باستخدام نموذج الأجنة / اليرقات لدانيو المخطط. قييمت الفعالية المضادة للأكسدة للمستخلصات أيضًا للتأكيد على فعاليتها المضادة للسكري. تم تحديد مثبطات أنزيم ألفا غلوكوزيداز للنبات باستخدام كروماتوغرافيا الغاز والسائل المجهزة بمطياف الكتلة (GCMS و LCMS على التوالي) والتحليل الطيفي بالرنين النووي (NMR) القائم على نهج المستقلبات. في حين وضح تفاعل الربيطة والبروتين من خلال دراسة الإلتحام الجزيئي. إلى جانب ذلك, طورت تقنية تحليلية مصادقة باستخدام مطيافية فورييه لتحويل الأشعة تحت الحمراء بصمة الإصبع (FTIR) وباستخدام المربع الأصغرى الجزئي المتعامد (OPLS). أظهر مستخلص الميثانول أعلى نشاطاً مثبطاً لأنزيم ألفا غلوكوزيداز (50 2.83 ± 0.32 IC50 ميكروغرام / مل). بالإضافة إلى ذلك, أظهر مستخلص الميثانول فعالية عالية لتحسيس الأنسولين ومضادات الأكسدة. حُددت ثمانية مركبات نشطة بيولوجياً بالمجمل وهي 1,3,5- بنزينيتريول (**1**); حمض البالمتيك (**2**); كوليستا-7،9 (11) -دبين-3-رأ (**3**);1-مونوبالميتين (**4**); β- توكوفيرول (**5**); ألفا توكوفيرول (**6**); 24-إبيكامبيستيرول (**7**); ستيقمست-5-اين (**8**) باستخدام نهج المستقبات GCMS. إلى جانب ذلك, خمسة مركبات نشطة بيولوجيًا مفترضة وهي 1- مونوبلمتين (**4**),4- حمض هيدروكسي فينيل بيروفيك (**10**),5′-هيدروكسي ميثيل -1 ′ - (1 , 2 ,3 ,9- رباعي هيدرو-بيرولو (,21-ب) قوينازولين-1-ي ل- أحادي الهبتان (**11**), الفا-تربينيل-بيتا-جلوكوسايد (**12**), ماشاريديول-أ (**13**) تم تحديدها من خلال نهج المستقبات LCMS. بالإضافة إلى ذلك, حدد مركبين نشطين بيولوجيين بيولوجيًا باستخدام نهج المستقبات NMRوهما 4-حمض الهيدروكسيفينيل بيروفيك (**10**) والجلوتامين (**14**). أظهرت نتائج دراسة الإلتحام الجزيئي لجميع الثلاثة عشر مركب النشطة بيولوجيًا المفترضة قابلية ارتباط متوسطة إلى عالية (5.5- إلى 10.0- كيلو كالوري / مول) تجاه الموقع الفعال للبروتين الإنزيمي. العديد من الرواسب وهي ARG213, SER311, ASH215, ARG442, GLN182, HIE351, ASP352 GLU411, HIE280, PRO312, GLN279, GLH277, شكلت رابطة هيدروجينية في المعقد الملتحم جزيئياً. طور نموذج OPLS بواسطة FTIR وتم التحقق من مصداقيته باستخدام ست عينات خارجية من نفس أنواع النبات ومن المحتمل أن تتنبأ بنشاط ال AGI العام لجميع المستخلصات. لذلك يمكن أن يُقترح استخدامه كأداة في مراقبة الجودة للنباتات. متوسط التركيزات المميتة (LC 50) ل 0٪, 25٪, 50٪, 75٪,100٪ من مستخلص الميثانول وُجد أنها سامة (252.45, 119.40, 64.71,81.28 ,37.50 ميكروغرام / مل, على التوالي). على أي حال , من خلال الكشف وإزالة المواد السامة, يمكن تطوير هذا النبات كعامل مفيد لمكافحة مرض السكري في المستقبل. بشكل قاطع, تم التعرف على ثلاثة عشر من مثبطات أنزيم ألفا غلوكوزيداز المفترضة من *P. malayana* من خلال نهج المستقبات في هذه الدراسة و من الأفضل استخدام مستخلص الميثانول بناءً على فعاليته وأمانه. قد تكون كل هذه النتائج مفيدة لاستخدام هذا النبات كدواء واعد لعلاج مرض السكري في المستقبل.

APPROVAL PAGE

The thesis of Tanzina Sharmin Nipun has been approved by the following:

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Tanzina Sharmin Nipun

Signature .... Date 04/02/2022 ....

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This thesis is dedicated to my beloved father, Mohammed Rezaul Karim Chowdhury, who is my inspiration to complete this journey, my loving mother, Selina Akter, who has suffered from type-2 diabetes mellitus. Not to forget, to my husband and dearly-loved daughter, for nursing me to fulfil this dream with their unconditional supports and love, billions of thanks to both of you.

ACKNOWLEDGEMENTS

All glory is due to Allah, the Almighty, whose Grace and Mercies have been with me throughout the duration of my programme. Although, it has been tasking, His Mercies and Blessings on me ease the herculean task of completing this thesis.

I am most indebted to my supervisor, Assoc. Prof. Dr. Alfi Khatib, whose enduring disposition, kindness, promptitude, thoroughness and friendship have facilitated the successful completion of my work. I put on record and appreciate his detailed comments, useful suggestions and inspiring queries which have considerably improved this thesis. His brilliant grasp of the aim and content of this work led to his insightful comments, suggestions and queries which helped me a great deal. Despite his commitments, he took time to listen and attend to me whenever requested. I would be quite impossible to finish the thesis on time without his kind support.

I am also grateful and thankful to my co-supervisor, Assoc. Prof. Dr. Qamar Uddin Ahmed, Asst. Prof. Dr. Irina Elina Ridzwan, and Asst. Prof. Dr. Zalikha Ibrahim, whose support and cooperation contributed to the outcome of this work and for that, I will be forever grateful.

I would also like to thank the Bangabandhu Science and Technology Fellowship Trust, Ministry of Science and Technology of Bangladesh for a PhD fellowship that enables me to dream and to pursue this dream successfully.

Lastly, my gratitude goes to my loving parents, lovely daughter, beloved husband and my younger brother and sister; for their love, prayers, understanding and endurance while away as well as heartiest thanks to my colleagues, Md. Abdur Rashid Mia, May Kyaw, Batoul Allalam, Al'aina Yuhainis Firus Khan, and Sharifah Nurul Akilah, who have been of a great support and positive vibe throughout this journey and not to forget all my other colleagues and lab mates. I am also thankful to the staff of KOP, CREAM, and NMPC, who supported me throughout my whole Ph.D. journey. I humbly dedicate my thesis work to all of them.

Once again, we glorify Allah for His endless mercy on us one of which is enabling us to successfully round off the efforts of writing this thesis. Alhamdulillah.

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LIST OF ABBREVIATIONS

2-NBDG	2-[N-(7-Nitrobenz-2-Oxa-1,3-Diazol-4-yl)Amino]-2-Deoxy-D-Glucose
AACE	American Association of Clinical Endocrinologists
ACD	Advanced Chemistry Database
ADG	α -D-Glucose
AGI	α-Glucosidase Inhibition
AG	α-Glucosidase
ASP	Aspartic Acid
ATR	Attenuated Total Reflectance
ARG	Arginine
CDF	Computable Document Format
DM	Diabetes Mellitus
DMSO	Dimethyl Sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
ESI	Electrospray Ionization
FTIR	Fourier Transform Infrared Spectroscopy
FRAP	Ferric Reducing Antioxidant Power
GCMS	Gas Chromatography-Mass Spectrometry
GDM	Gestational Diabetes Mellitus
GLU	Glutamic Acid
GLH	Protonated Glutamic Acid
GLN	Glutamine
HIV-1	Human Immunodeficiency Virus-1
hpf	Hour Post-Fertilization
HIS	Histidine
IC_{50}	Half-maximal Inhibitory Concentration
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
IR	Infra-Red Spectroscopy
JOD	Juvenile Onset Diabetes
KOP	Kulliyyah Of Pharmacy
LCMS	Liquid Chromatography-Mass Spectrometry
LC	Lethal Concentration
LD_{50}	Median Lethal Dose
MS	Mass Spectrometry
MVDA	Multivariate Data Analysis
MeOH	Methanol
MTT	3-(4,5-Dimethylthiazole-2-Yl)-2,5-Diphenyltetrazolium Bromide
MSTFA	N-Methyl-N-(Trimethylsilyl)Trifluoroacetamide
NIST	National Institute of Standards and Technology
NPs	Natural Products
NMR	Nuclear Magnetic Resonance Spectroscopy
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NO	Nitric Oxide
OPLS	Orthogonal Partial Least Square

OECD	Organization Of Economic Co-Operation And Development
PBS	Phosphate Buffer Saline
PCA	Principal Component Analysis
PC	Principal Components
PDB	Protein Data Bank
pH	Potential Hydrogen
PLS	Partial Least Square
PNPG	*p*-Nitrophenyl-*p*-D-Glucopyranoside
PPAR-γ	Peroxisome Proliferator-Activated Receptor-Gamma
PHE	Phenylalanine
PRO	Proline
Q-ToF	Quadrupole Time-of-Flight
RMSEE	Root Mean Square Error of Estimation
RMSECV	Root Mean Square Error of Cross-Validation
RMSD	Root Mean Square Deviation
ROS	Reactive Oxygen Species
SD	Standard Deviation
SER	Serine
TPTZ	2,4,6-Tris(2-pyridyl)-s-Triazine
T1DM	Type-1 Diabetes Mellitus
T2DM	Type-2 Diabetes Mellitus
TZDs	Thiozodinedones
2D	Two-Dimensional
3D	Three-Dimensional
TYR	Tyrosine
UV	Ultraviolate
VAL	Valine
WHO	World Health Organization

LIST OF SYMBOLS

α	Alpha
Å	Angstrom
β	Beta
cm	Centimetre
Da	Dalton
°C	Degree Celsius
δ	Delta
g	Gram
kcal/mol	Kilocalorie Per Mole
kg	Kilogram
L/min	Liter Per Minute
L	Litre
µm	Micrometre
µg	Microgram
µL	Microliter
mg	Milligram
mL	Millilitre
mM	Millimolar
M	Molar
min	Minute
mg AAE/g	Milligram of Ascorbic Acid Per Gram
m/z	Mass Per Charge
nm	Nanometre
%	Percent
psi	Pound Per Square Inch
ppm	Parts Per Million
v/v	Volume Per Volume
w/v	Weight Per Volume
w/w	Weight Per Weight

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Medicinal herbs are being extensively used in the pharmaceutical sector as the vital sources of drugs. Nature has offered us an enormous abundance of natural products. Natural products are well known as the key sources of new medicines and therapeutic agents. Natural products are revealed via trials and errors to manage various unknown diseases. The scientific evidence on the bioactivity of plants provide the beneficial information to develop the new therapeutic agents. The research on the identification of the bioactive compounds from various medicinal plants started after discovering morphine from poppy in 1806 (Yang et al., 2016; Zhang et al., 2020). Around the world, 80% of the people are using medicinal plants to manage or prevent the sickness. But majority of them are unaware of their appropriate use and safety of these plants which may arise serious health problems (Adib-Hajbaghery & Rafiee, 2018; Tugume & Nyakoojo, 2019). Therefore, it is very important to collect the scientific proof on the quality, bioactivity, and toxicity of the medicinal plants.

The long-term metabolic disease produced by hyperglycemia is known as diabetes mellitus (DM), referring to a condition where a consistently high blood sugar level leads to an imbalance of tissue homeostasis. Various complications may develop if diabetes is not well managed. Myopia, glaucoma, and retinal detachment may be caused by diabetic retinopathy (Roglic, 2016). The World Health Organization (WHO) has reported that diabetic retinopathy, heart attacks, kidney failure, strokes, and lower limb amputation are all common complications of diabetes. Diabetes is the leading cause of blindness in the world, accounting for 2.6 percent of all cases. Apart from this,