

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

BY

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ABSTRACT

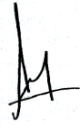
Psychotria malayana Jack belongs to Rubiaceae family and known in Malaysia as “meroyan sakit/salung”. It is widely available in Malaysia and other Southeast Asian countries where it is traditionally used to manage diabetes. However, its folk claims as an antidiabetic agent and proper metabolites profiling 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 ($IC_{50} 2.83 \pm 0.32 \mu\text{g/mL}$). 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-tetrahydropyrrolo (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 species 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 $\mu\text{g/mL}$, 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

تتمي *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. قيمت الفعالية المضادة للأكسدة للمستخلصات أيضاً 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 المستقبلات. في حين وضع تفاعل الربيطة والبروتين من خلال دراسة الإلتحام الجزئي. إلى جانب ذلك، طورت تقنية تحليلية مصادقة باستخدام مطيافية فورييه لتحويل الأشعة تحت الحمراء بصمة الإصبع (FTIR) وباستخدام المربع الأصغري الجزئي المتعامد (OPLS). أظهر مستخلص الميثانول أعلى نشاطاً مثبطاً لأنزيم ألفا غلوكوزيداز ($IC_{50} 0.32 \pm 2.83$ ميكروغرام / مل). بالإضافة إلى ذلك، أظهر مستخلص الميثانول فعالية عالية لتحسيس الأنسولين ومضادات الأكسدة. حددت ثمانية مركبات نشطة بيولوجياً بالمحمل وهي 1,3,5-بنزينيتريول (1); حمض البالميتيك (2); كوليستا-9,7 (11) -ديين-3-أر (3); 1-مونوبالميتين (4); β -توكوفيرول (5); ألفا توكوفيرول (6); 24-إبيكامبيستيروول (7); ستيغمست-5-اين (8) باستخدام نهج المستقبلات GCMS. إلى جانب ذلك، خمسة مركبات نشطة بيولوجياً مفترضة وهي 1-مونوبلمتتين (4,4), 4-حمض هيدروكسي فينيل بيروفيك (10), 5-هيدروكسي ميثيل-1 (1, 2, 3, 9-رباعي هيدرو-بيرولو (21-ب) فوينازولين-1-ي ل-أحادي الهبتان (11), الفا-تريينيل-بيتا-جلوكوسايد (12), ماشاريديول-أ (13) تم تحديدها من خلال نهج المستقبلات LCMS. بالإضافة إلى ذلك، حدد مركبين نشطين بيولوجيين باستخدام نهج المستقبلات NMR وهما 4-حمض الهيدروكسي فينيل بيروفيك (10) والجلوتامين (14). أظهرت نتائج دراسة الإلتحام الجزئي لجميع الثلاثة عشر مركب النشطة بيولوجياً المفترضة قابلية ارتباط متوسطة إلى عالية (5.5- إلى 10.0- كيلو كالوري / مول) تجاه الموقع الفعال للبروتين الإنزيمي. العديد من الرواسب وهي ASP352, HIE351, GLN182, ARG442, ASH215, SER311, ARG213, GLH277, GLN279, PRO312, HIE280, GLU411 شكلت رابطة هيدروجينية في المعقد الملتحم جزئياً. 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 المميئة (LC 50) ل 0%, 25%, 50%, 75%, 100% من مستخلص الميثانول وُجد أنها سامة (81.28, 64.71, 119.40, 252.45, 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 المستقبلات 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

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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.

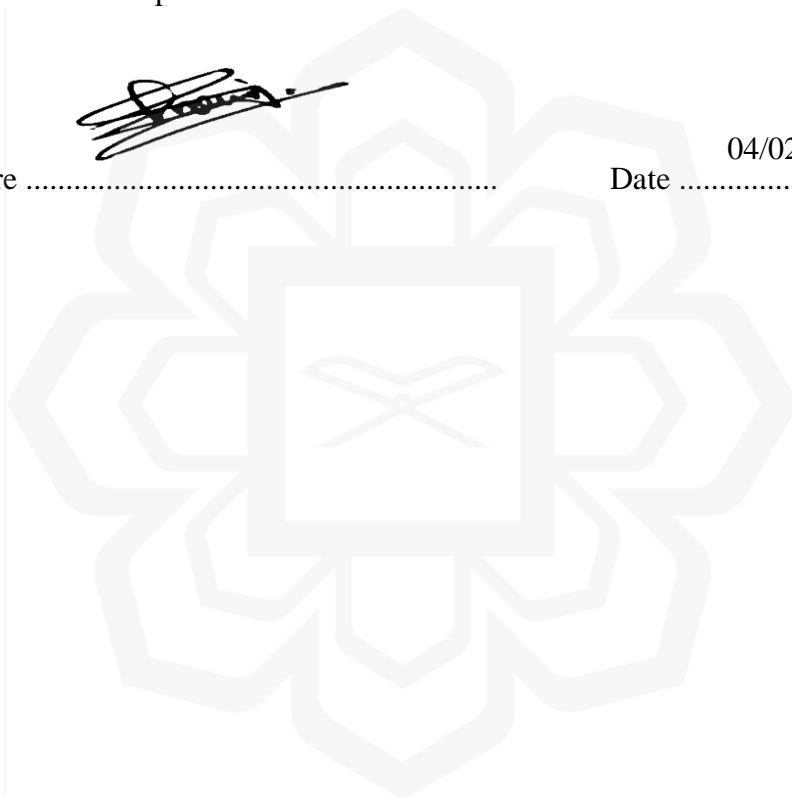
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**EVALUATION OF ANTI-DIABETIC ACTIVITIES AND
TOXICITY OF *PSYCHOTRIA MALAYANA* JACK LEAVES AND
CHARACTERIZATION OF ITS BIOACTIVE COMPOUNDS**

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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.

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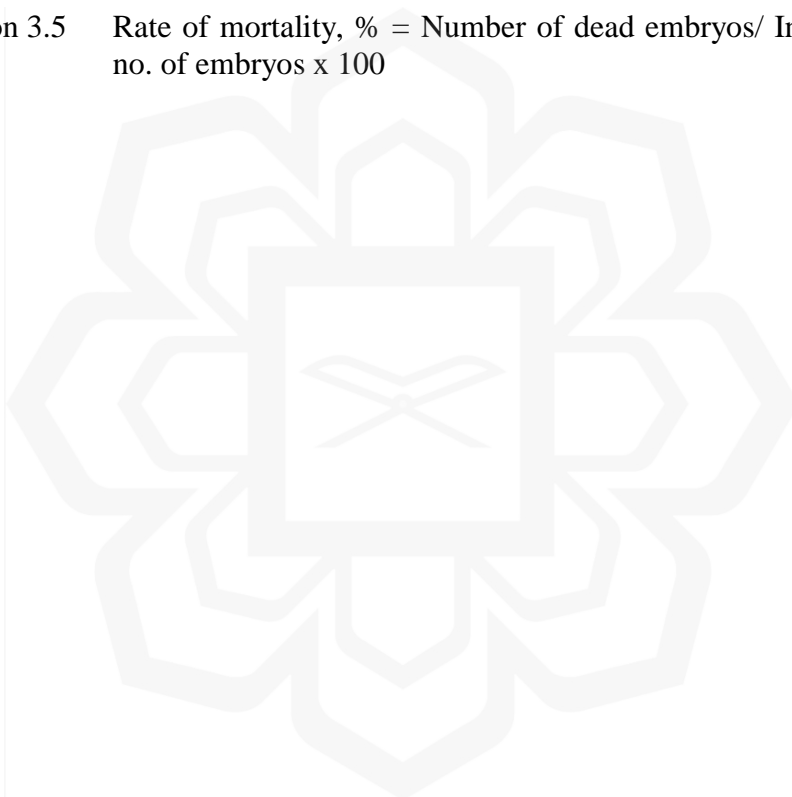
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LIST OF ABBREVIATIONS

| | |
|------------------|--|
| 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 | <i>p</i> -Nitrophenyl- <i>p</i> -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 | Ultraviolet |
| 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,