UNIVERSITI TEKNOLOGI MARA

PHYTOCHEMICAL CONSTITUENTS AND ANTIDIABETIC PROPERTIES OF *Asystasia gangetica* (L.) T. ANDERSON SUBSP. *micrantha* AND *Bouea macrophylla* GRIFF

ISNA ATHIRAH BINTI OTHMAN

Thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy
(Science)

Faculty of Applied Sciences

October 2024

ABSTRACT

The phytochemical investigations were conducted on two plants: *Asystasia gangetica* (L.) T. Anderson subsp. *micrantha* (Acanthaceae) and *Bouea macrophylla* Griff. (Anacardiaceae). This study aims to isolate and elucidate the secondary metabolites from the two plants as mentioned, propose the plausible biosynthetic pathway of the isolates, and assess the isolated compounds' cytotoxicity and glucose uptake activities. The isolation process was conducted by using chromatographic techniques such as Vacuum Liquid Chromatography (VLC), Thin Layer Chromatography (TLC), column chromatography (CC), and High-Performance Liquid Chromatography (HPLC). The structures of the isolated compounds were elucidated employing various spectroscopic analyses, namely infrared (IR), ultraviolet-visible (UV-Vis), mass spectrometer (MS) and nuclear magnetic resonance (NMR) spectroscopies, and comparison with the previous literature studies. Eleven known compounds were isolated from the leaves of *A. gangetica*, including three monoterpenes, which are loliolide, dehydrololiolide and dehydrovomifoliol; two cinnamic acid derivatives including ferulic acid and methyl caffeates; two flavones namely chrysoeriol and chrysoeriol-4'-glycoside; a glycosidic tyrosol namely salidroside; two phenylethanoids namely verbascoside and forsythiaside as well as two iridoids namely 3''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol and 4''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol. Ten flavonoids were obtained from the twigs of *B. macrophylla*, which include six flavanonols: garbanzol, fustin, taxifolin, astilbin, isoastilbin and neoastilbin; one flavonol, which is resokaempferol; one flavanol characterized as catechin; and two flavandiol known as mollisacacidin and guibourtacacidin. Meanwhile, the purification steps on the leaves of *B. macrophylla* have successfully yielded six pure compounds, including three flavonols, which are myricitrin and mearnsitrin, as well as one newly elucidated compound namely myricetin-3-*O*-(2''-*O*-galloyl)-a-rhamnopyranoside. A tannic acid, penta-*O*-galloyl glucoside, and triterpene, lupeol, were also isolated. This is the first report describing and elucidating the stated compounds from *B. macrophylla*. Selected isolated compounds were tested for cytotoxicity against C2C12 muscle cell lines and glucose uptake assay. None of the compounds exhibited toxicity. Significant glucose uptake activities ($p < 0.05$) were observed for salidroside (2.12-fold at 6.25 µg/mL), chrysoeriol (1.79-fold at 1.562 µg/mL), and verbascoside (1.79-fold at 1.562 µg/mL) isolated from *A. gangetica*. From *B. macrophylla*, significant activities were found for myricitrin (3.10-fold at 1.56 µg/mL), mearnisitrin (1.58-fold at 1.56 µg/mL), and astilbin (1.28-fold at 3.13 µg/mL). Among all tested compounds, myricitrin exhibited the highest glucose uptake activity. All compounds showed comparable activities to the standard rosiglitazone. Additionally, the study proposed biosynthetic pathways for the newly identified compounds and thoroughly discussed their Structure-Activity Relationship (SAR). The SAR analysis revealed that antidiabetic activity is influenced by the number of hydroxyl groups, the presence of a catechol moiety, and a C-4 ketonic group. Flavanols showed higher activity compared to other flavonoids. The position of methoxy groups affects electron-donating properties and interactions with biological targets. In conclusion, this study isolated several bioactive compounds with significant glucose uptake activity, providing insights into their potential as antidiabetic agents and laying the groundwork for further drug development.

ACKNOWLEDGEMENT

All praise is due to Allah for granting me the strength and patience to overcome the challenges throughout this journey. I wish to express my deepest gratitude to my supervisor, Prof. Dr. Norizan Ahmat, for her unwavering guidance, wisdom, motivation, and the countless opportunities she provided me during my PhD studies.

I also extend my sincere appreciation to the staff at the Faculty of Applied Sciences and the Atta-ur-Rahman Institute for their cooperation and invaluable assistance during my research. A special note of thanks goes to Zuriati binti Zahari for her exceptional guidance in the field of biology.

To my colleagues and friends, particularly Prof. Dr. Norizan's students, and the members of Phytochemistry Lab 1 and the Atta-ur-Rahman Institute: your support was a pillar of strength during this challenging and often solitary journey. Your encouragement enabled me to persevere with courage and patience.

I dedicate this PhD degree to my late father, Othman bin Hassan, and my mother, [redacted], whose unwavering support, inspiration, and prayers have guided me to this achievement. I am also profoundly grateful to my beloved husband, Abdul Hadi bin Hussin, and my sons, Umar Nawfal and Adam Luthfi, for their endless love, sacrifices, and steadfast support throughout this endeavor.

Finally, I extend my heartfelt gratitude to my entire family for their immense help and prayers. This achievement is dedicated to all of you. May Allah shower each of you with abundant blessings and love.

TABLE OF CONTENTS

CHAPTER 1
INTRODUCTION

1.1 Research Background

Type 2 Diabetes Mellitus (T2DM) stands as a considerable global health challenge due to its increasing prevalence and intricate connections with conditions such as obesity, liver disease, and cardiovascular disorders. The World Health Organization (WHO) reports that around 422 million individuals worldwide have diabetes, mainly concentrated in low- and middle-income countries. Every year, 1.5 million deaths are directly attributed to diabetes. The gravity of the global diabetes situation is further emphasized by The IDF Diabetes Atlas, 10th edition, estimating that 537 million people currently live with diabetes, with a projected increase to 700 million by 2045. The annual toll of over 4 million deaths underscores the urgent need for effective prevention and management strategies globally, affecting diverse demographics, communities, and continents.

Malaysia exhibits a significant prevalence of prediabetes and diabetes, with the occurrence of diabetes being linked to both periods and advancing age (Akhtar et al., 2022). In this country, diabetes is experiencing a significant upsurge, presenting a substantial health challenge. The National Diabetes Registry Report 2013 – 2019, prepared by the Disease Control Division, Ministry of Health Malaysia, reveals alarming statistics. By the end of 2019, 1,614,363 patients were enrolled in the registry, with 897,421 active diabetes patients. Almost all were diagnosed with Type 2 Diabetes Mellitus (T2DM), while Type 1 Diabetes Mellitus (T1DM) and other forms of diabetes were minimal at 0.62% and 0.09%, respectively. The high prevalence of undiagnosed diabetes, reaching 49.0% in 2021, suggests potential gaps in healthcare awareness and diagnostic services, imposing a large economic burden. The projected diabetes-related health expenditure is estimated to reach 6,317.4 million USD in 2045, significantly damaging healthcare resources. Overall, the number of cases and the prevalence of diabetes have steadily increased over the past few decades. The persistent challenges in managing T2DM, marked by prolonged high blood sugar levels, underscore the necessity for alternative treatment approaches.