



UNIVERSITI PUTRA MALAYSIA

**GC-MS-BASED METABOLITES PROFILING OF
COSMOS CAUDATUS KUNTH LEAVES POSSESSING
ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY**

NEDA JAVADI

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UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

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By

NEDA JAVADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Master of Science

June 2014

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DEDICATION

I would like to dedicate this project to all those who have helped me to complete it.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**GC-MS-BASED METABOLITES PROFILING OF *COSMOS*
CAUDATUS KUNTH LEAVES POSSESSING
ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY**

By

NEDA JAVADI

June 2014

Chairman: Associate Professor Faridah Binti Abas, PhD

Faculty: Food Science and Technology

A large number of plant metabolites have provided as an incomparable chemical source for drug development. *Cosmos caudatus*, which is known as *Ulam raja*, is one of the herbal plants used in Malaysia. This plant has been used traditionally to enhance vitality.

The current study focused on the evaluation of the α -glucosidase inhibitory activity of different ethanolic extracts of *Cosmos caudatus* (*C. caudatus*). Six series of extracted samples including water, 20%, 40%, 60%, 80%, and 100% ethanol (EtOH) were utilized. The IC₅₀ values for these six series of extracts from 13.7 to 298 μ g/mL. The highest α -glucosidase inhibitory activity was obtained from EtOH extract which was comparable to quercetin and more potent than acarbose. In contrast, water extract exhibited the lowest activity. To identify and profile the chemical compositions of the samples, gas chromatography-mass spectrometry (GC-MS) was employed. GC-MS combined with orthogonal partial least-squares analysis (OPLS) was applied to detect antidiabetic activity of *C. caudatus*. The OPLS showed an obvious and remarkable separation into six clusters representing the six different ethanolic extracts. Therefore, GC-MS was possible to be combined with MVDA for identification of compounds that inhibited α -glucosidase activity. Catechin, α -linolenic acid, α -d-glucopyranoside and vitamin E were identified and indicate the potential antidiabetic activity of this herb.

In the second part of this study, *C. caudatus* samples were subjected to seven different storage times (0, 2, 4, 6, 8, 10 and 12 h) at room temperature before

extraction and α -glucosidase inhibitory activity were determined for the respective samples. The IC_{50} values ranged from 12.6 to 40.9 $\mu\text{g}/\text{mL}$. α -Glucosidase inhibitory activity for the first group (fresh) was the highest with an IC_{50} value of 12.6 $\mu\text{g}/\text{mL}$, which was better than that of quercetin. After 12 h of storage, the extract exhibited the lowest activity with an IC_{50} value of 40.9 $\mu\text{g}/\text{mL}$, which is still better than that of acarbose. As a model experiment, GC-MS of the extracts obtained from the *Ulam raja* was correlated with the α -glucosidase inhibition activity with OPLS analysis to determine the antidiabetic compounds. A profound chemical change in the primary and secondary metabolites was observed. In the first group, catechin, α -tocopherol (vitamin E), benzoic acid, cyclohexen-1-carboxylic acid, myo-inositol, stigmasterol, and lycopene were observed. High quantities of primary metabolites including sugars, such as sucrose, α -d-galactopyranose and turanose were observed in samples stored for a long period of time (12 h). This study may provide guidance in the determination of pharmacological mechanism as well as the development of medicinal preparations, nutraceuticals or functional foods from *C. caudatus* for diabetes and related symptoms.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**METABOLIT PROFIL BERASASKAN GCMS KE ATAS EKSTRAK
DAUN *COSMOS CAUDATUS KUNTH* YANG MEMPUNYAI
AKTIVITI PERENCATAN ENZIM ALFA GLUKOSIDASE**

Oleh

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Sebilangan besar metabolit tumbuhan telah digunakan sebagai sumber kimia yang tiada tandingan untuk pembangunan dadah. *Cosmos caudatus*, yang dikenali sebagai *Ulam raja*, adalah salah satu tumbuhan herba yang digunakan di Malaysia. Tumbuhan ini telah digunakan secara tradisional untuk meningkatkan daya hidup.

Kajian ini memberi tumpuan kepada penilaian aktiviti perencatan α -glukosidase, ekstrak etanol yang berbeza dari *Cosmos caudatus* (*C. caudatus*). Enam siri sampel diekstrak termasuk air, 20%, 40%, 60%, 80%, dan 100% etanol (EtOH) telah digunakan. Nilai IC_{50} di antara 13.7-298 $\mu\text{g/mL}$. Nilai tertinggi aktiviti perencatan α -glukosidase telah diperolehi bagi ekstrak EtOH, nilai ini adalah sebaik kuercetin dan lebih baik daripada akarbose. Di samping itu, ekstrak air menunjukkan aktiviti yang paling rendah. Untuk mengenalpasti profil komposisi kimia sampel, gas kromatografi-spektrometri jisim (GC-MS) telah digunakan. GC-MS digabungkan dengan analisis data multivariat, ortogonal separa analisis kuasa dua terkecil (OPLS) telah digunakan untuk mengesan aktiviti antidiabetik ekstrak *C. caudatus*. OPLS menunjukkan pemisahan jelas kepada enam kelompok mewakili enam kepekatan etanol yang berbeza. Oleh itu, GC-MS boleh digabungkan dengan analisis data multivariat untuk mengenal pasti sebatian yang menghalang aktiviti α -glukosidase. Di samping itu, katekin, α -linolenik asid, α -D-glukopiranosida dan vitamin E telah dikenal pasti dan menunjukkan aktiviti antidiabetik potensi herba ini.

Dalam bahagian kedua kajian ini, sampel *C. caudatus* telah di simpan dalam tujuh masa penyimpanan yang berlainan (0, 2, 4, 6, 8, 10 dan 12 jam) pada suhu bilik

sebelumengekstrakan dan aktiviti perencatan α -glukosidase ditentukan untuk sampel masing-masing. Nilai IC_{50} antara 12.6 to 40.9 $\mu\text{g}/\text{mL}$. Aktiviti perencatan α -glukosidase untuk kumpulan pertama (segar) adalah yang tertinggi dengan nilai IC_{50} 12.6 $\mu\text{g}/\text{mL}$, yang mana lebih baik daripada kuercetin. Selepas 12 jam penyimpanan, ekstrak menunjukkan aktiviti yang paling rendah dengan nilai IC_{50} 40.9 $\mu\text{g}/\text{mL}$, nilai ini masih lebih baik daripada akkarbose. Sebagai model eksperimen, ekstrak yang diperoleh daripada *Ulam raja* yang berkait rapat dengan aktiviti perencatan α -glukosidase telah dianalisis menggunakan GC-MS digabung dengan analisis data multivariat untuk menentukan sebatian antidiabetik. Ortogonal PLS (OPLS) telah digunakan untuk menyiasat perubahan metabolomik. Perubahan kimia dalam metabolit rendah dan menengah diperhatikan. Dalam kumpulan pertama katekin, α -tokoferol (vitamin E), asid benzoik, myo-inositol, asid siklohexen-1-karboksilik, stigmasterol dan likopena telah dikenalpasti. Kuantiti yang tinggi metabolit utama termasuk gula, seperti sukrosa, α -d-galaktopiranosa dan turanosa diperhatikan dalam sampel yang disimpan untuk tempoh masa yang panjang. Kajian ini boleh memberi panduan dalam penentuan mekanisme farmakologi dan juga pembangunan persediaan perubatan, nutraseutikal atau makanan berfungsi untuk kencing manis dan gejala yang berkaitan.

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I certify that a Thesis Examination Committee has met on 23 June 2014 to conduct the final examination of Neda Javadi on her thesis entitled “GC-MS-based metabolites profiling of *Cosmos Caudatus Kunth* leaves possessing alpha-glucosidase inhibitory activity” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

Declaration by graduate student

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