UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF *Haplophyllum laeviusculum* C.C. TOWNS. AND *H. villosum* (BYE.) DON.

PARIMAH PARHOODEH

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By

PARIMAH PARHOODEH

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

August 2010

Dedicated

To my husband Behrooz

You are every thing for me, without your love and understanding
I would not be able to make it…

To my father and my mother

You introduced me to the joy of reading from birth
enabling such a study to take place today…

To my children Hamed and Reihaneh

You are two angels who made my life wonderfully
happy and sprinkle joys in my heart…

ABSTRACT

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

CHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF *Haplophyllum laeviusculum* C.C. TOWNS. AND *H. villosum* (BYE.) DON.

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Chairman : Professor Mawardi Rahmani, PhD

Faculty : Science

The genus *Haplophyllum* from the Rutaceae family is made up of about 70 species distributed mainly around the Mediteranean countries and thirty of them are found in Iran. Chemical literature survey revealed that the genus contains various interesting secondary metabolites such as alkaloids, lignans, coumarins and triterpenes. In this study two species of the plant, *Haplophyllum laeviusculum* and *Haplophyllum villosum* collected from West Azarbayejan province in Iran, were investigated in detailed. Literature search indicated that there have been no previous reports on chemical compounds and biological activities of these two plants.

A chemical study on the aerial parts of the plants has resulted in the isolation and structural elucidation of ten compounds. These compounds were isolated by different

chromatographic techniques such as gravity column chromatography, vacuum chromatography, Thin Layer Chromatography (TLC), Preparative Thin Layer Chromatography (PTLC) and Chromatotron. The structures of these compounds were identified by using spectroscopic methods which are UV, IR, NMR, MS and also by comparison with previous work. The crude extracts and isolated compounds were tested for antioxidant activities by using DPPH (1, 1-Diphenyl- 2-pircrylhydrazyl) free radical scavenging activity.

Detailed chemical studies on *Haplophyllum laeviusculum* afforded two triterpenoids (γ-sitosterol **(62)** and campesterol **(63)**) and three alkaloids, skimmianine **(5)**, lunamarine **(64)** and ribalinidine **(65)**). Meanwhile extraction and separation of the aerial parts of *Haplophyllum villosum* gave one alkaloid, haplamine **(67))**, two lignans eudesmin **(66)** and eudesmin A **(70)**) and two coumarins, scopoletin **(69)** and umbelliferone **(68)**.

Both methanol crude extracts of these two species possess strong antioxidant activities against DPPH stable free radical. The ethyl acetate crude extracts of *Haplophyllum laeviusculum* and chloroform crude extracts of *Haplophyllum villosum* showed moderate activities against DPPH stable free radical test with IC_{50} values of less than 400 µg/ml. Other crude extracts and isolated compounds such as skimmianine **(5)**, lunamarine **(64)** and ribalinidine **(65),** eudesmin **(66)** and haplamine **(67),** showed weak radical scavenging activities.

ABSTRAK

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

KANDUNGAN BAHAN KIMIA DAN AKTIVITI ANTIOKSIDAN TUMBUHAN *Haplophyllum laeviusculum* C.C. TOWNS. DAN *H. villosum* (BYE.) DON.

Oleh

PARIMAH PARHOODEH

Ogos 2010

Pengerusi : Profesor Mawardi Rahmani, PhD

Fakulti : Sains

Genus *Haplophyllum* dari famili Rutaceae mengandungi lebih kurang 70 spesies terdapat di sekitar kawasan Mediterranean dan tiga puluh darinya ditemui di Iran. Kajian rujukan mendapati bahawa tumbuhan ini mengandungi berbagai jenis metabolit sekunder seperti alkaloid, lignan, koumarin dan terpena. Dalam kajian ini, dua species tumbuhan, *Haplophyllum laeviusculum* dan *Haplophyllum villosum* yang dikumpulkan dari kawasan Barat Azarbayejan di Iran, telah dikaji dengan mendalam. Kajian rujukan juga mendapati tidak terdapat sebarang laporan lepas mengenai kandungan kimia dan aktiviti biologi terhadap kedua-dua spesies ini.

Kajian kimia terhadap bahagian atas tumbuhan ini telah berjaya memisahkan dan pengenalan struktur sepuluh sebatian. Sebatian ini telah dipencilkan dengan menggunakan berbagai teknik pemisahan kromatografi seperti kromatografi kolum, kromatografi vakum, kromatografi lapisan tipis, kromatografi lapisan tipis penyediaan dan kromatotron. Sebatian yang dipencil telah ditentukan strukturnya dengan menggunakan kaedah spektroskopi seperti UV, IR, NMR, MS dan juga perbandingan dengan kajian sebelum ini. Ekstrak mentah dan sebatian tulen telah dikaji kesan aktiviti antioksidan dengan menggunakan DPPH (1,1-difenil-2-pikrilhidrazil).

Kajian mendalam terhadap *Haplophyllum laeviusculum* telah berjaya menghasilkan dua terpenoid, (γ-sitosterol **(62)** dan kampesterol **(63)**) dan tiga alkaloid (skimmianine **(5)**, lunamarine **(64)** dan ribalinidine **(65)**). Manakala pengekstrakan dan pemencilan bahagian atas *Haplophyllum villosum* telah memberikan satu alkaloid (haplamine **(67)**), dua lignan (eudesmin **(66)** dan eudesmin A **(70))** dan dua koumarin (scopoletin **(69)** dan umbelliferone **(68)**).

Kedua-dua ekstrak metanol dari dua tumbuhan ini telah menunjukan kesan aktiviti antioksidan yang kuat terhadap radikal bebas DPPH yang stabil. Ekstrak etil asetat mentah *Haplophyllum laeviusculum* dan ekstrak mentah klorofom *Haplophyllum villosum* menunjukan kesan aktiviti antioksidan sederhana terhadap radikal bebas DPPH yang stabil dengan nilai IC_{50} kurang dari 400 μg/ml. Ekstrak mentah yang lain dan sebatian tulen yang dipencilkan seperti skimmianine **(5)**, lunamarine **(64)** dan ribalinidine **(65),** eudesmin **(66)** dan haplamine **(67),** menunjukan kesan antioksidan yang lemah.

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APPROVAL

I certify that an Examination Committee has met on 25 August 2010 to conduct the final examination of Parimah Parhoodeh on her Master of Science thesis entitled "Chemical Constituents and Antioxidant Activities of *Haplophyllum laeviusculum* C.C. Towns. and *H. villosum* (Bye.) Don." 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 relevant degree.

Members of the Examination Committee are as follows:

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Date: 25 August 2010

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree Master of Science. The members of the Supervisory Committee were as follows:

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Date: 25 November 2010

DECLARATION

I declare that the 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 and is not concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

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PARIMAH PARHOODEH

Date : 25 August 2010

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