UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *VITEX NEGUNDO* L. LEAVES

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the requirement for the degree of Doctor of Philosophy

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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *VITEX NEGUNDO* L. LEAVES

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Chairman : Associate Professor Azizah Abdul Hamid, PhD

Faculty : Food Science and Technology

This study was conducted to assess the biological activities of different extracts of *Vitex negundo* L. including antioxidant, antibacterial, antifungal and antiviral activity. In addition, profiling of bioactive compounds of the samples was also done. Antioxidant activity of essential oil, methanol and n-hexane extracts of *V. negundo* leaf were performed using 2,2-diphenyl-1-picrylhydrozyl (DPPH) free radical scavenging capacity, ferric ion reducing antioxidant power (FRAP) and β-carotene-linoleic acid assays. Results of the present study showed that methanol extract of *V. negundo* leaf exhibited significantly ($p < 0.05$) higher antioxidant activity than those of n-hexane extract and essential oil. The essential oil, methanol and n-hexane extracts of *V. negundo* leaf were also tested for antimicrobial activity against nineteen pathogenic bacteria and five pathogenic yeasts belonging to *Candida* family utilizing Agar disc diffusion,

minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). The essential oil showed antibacterial activity against 16 of 19 tested bacteria with inhibition zone ranging from 7 to 15 mm. The higher inhibition zone diameter for essential oil was that of *Vibrio cholera* and *Staphylococcus aureus* (15 mm) followed by *E. coli* with 14 mm inhibition zone. The methanol extract exhibited strong antibacterial activity against *Shigella dysenteriae* with 18 mm inhibition zone and MIC value of 3.12 mg/mL followed by that of *Vibrio cholerae* and *Klebsiella pneumoniae* with inhibition zone 14 mm and MIC value 6.25 mg/mL, respectively. Generally, the n-hexane extract showed lower antibacterial activity compared to that of methanol extract. However, the methanol extract and essential oil did not show any activity against all *Candida* strains tested. On the other hand, the n-hexane extract showed weak activity against *C. tropicalis* and *C. lusitaniae*. Antiviral activity of essential oil, methanol and n-hexane extracts of *V. negundo* leaf was evaluated against respiratory syncytial virus (RSV) and herpes simplex virus type 1 (HSV-1). Antiviral activities assay were performed in both intracellular and extracellular antiviral method. Methanol extract showed intracellular antiviral activity against both RSV and HSV-1 with SI value of 10.2, On the other hand, essential oil of *V. negundo* leaf showed intracellular antiviral activity against RSV and HSV-1 with SI value of 4.92 and 4.6, respectively. The n-hexane extract showed no activity against both RSV and HSV-1in both intracellular and extracellular manner. Chemical compositions of essential oil, methanol and n-hexane extracts of *V. negundo* leaf were determined using gas chromatography–mass spectrometry (GC/MS). The results of the study showed that major constituents of methanol extract of *V. negundo* were identified as linoleic acid methyl ester (11.26%), alpha isomethyl ionone (10.27%), ledol (7.31%) and

caryophyllene (4.13%), whereas the major constituents of that of n-hexane extract were tetracontane (22.98%), tetrapentacontane (21.42%), hexacontane (3.35%), hexatriacontane (2.61) and beta-Thujene (2.04%). Also, the results of the present study showed that the major constituent of essential oil of *V. negundo* leaf were identified as α—thujene (44.47%), α-terpinene (10.3%), ɣ-terpinene (4.78%), 4-terpinenyle acetate (3.56%) and citronellol (2.51%) was identified as major monoterpenes, and also, viridiflorene (4.13%), bisabolene (3.97%), citronellol (2.51%), viridiflorol (2.05%), caryophyllene (1.73%), and cadinol (1.17%) were identified as major sesquiterpenes.

The results of this study showed that *V. negundo* extracts possess effective bioactive compounds with broad spectrum of activity against a panel of pathogenic bacteria that are responsible for infection diseases. Also of methanol extract of *V. negundo* has shown antiviral activities against RSV, whereas the essential oil showed antiviral activity against RSV and HSV-1. These biological properties could be due to bioactive compounds in this plant. This medicinal plant may represent new source of antimicrobial activity and probably explain the reason for the use of the plant by the locals in treating a number of infectious diseases.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untak ijazah Doktor Falsafah

AKTIVITI ANTIMIKROB DAN ANTIOKSIDAN *VITEX NEGUNDO* L. DAUN

Oleh

Mohsen Zargar

Jun 2012

Pengerusi : Profesor Madya Azizah Abdul Hamid, PhD

Fakulti : Sains Dan Teknologi Makanan

Kajian ini telah dijalankan untuk menguji aktiviti-aktiviti biologi seperti antioksida, antibakteria, antifungi dan antivirus terhadap ekstrak-ekstrak *Vitex negundo* L. yang berbeza. Selain itu, sebatian bioaktif di dalam setiap sampel juga telah diprofilkan. Aktitivi antioksida terhadap ekstrak metanol, n-hexane dan minyak pati dari daun *V. negundo* telah diuji melalui ujian kapasiti pengaut radikal bebas 2,2-diphenyl-1-picrylhydrozyl (DPPH), kuasa antioksida menurunkan ion ferik (FRAP) dan beta karotena-asid linoleik asai. Keputusan kajian telah menunjukkan bahawa ekstrak metanol dari daun *V. negundo* menunjukkan aktiviti antioksida yang tinggi ($p < 0.05$) berbanding ekstrak n-hexane dan minyak patinya. Minyak pati , ekstrak metanol dan n-hexane dari daun *V. negundo* juga telah diuji aktiviti antimikrobiologinya terhadap 19 bakteria patogenik dan 5 standard yis patogenik yang tergolong dalam keturunan Candida melalui ujikaji cakera resapan, minimum kepekatan bantut (MIC) dan minimum

kepekatan bunuh bakteria/fungi (MBC/MFC). Minyak pati telah menunjukkan diameter zon pembantutan aktiviti antibakteria berada di antara 7 hingga 15 mm terhadap 16 daripada 19 jenis bacteria yang diuji.. Diameter zon penbantutan yang terbaik bagi minyak pati adalah terhadap *Vibrio cholera* dan *Staphylococcus aureus* (15mm) diikuti *E.coli* dengan zon penbantutan sebanyak 14 mm. Ekstrak metanol pula menunjukkan aktiviti antibakteria yang kuat terhadap *Shigella dysenteriae* dengan zon pembantutan sebanyak 18 mm dan nilai MIC sebanyak 3.12 mg/ml diikuti oleh *Vibrio cholera* dan *Klebsiella pneumonia* dengan zon pembantutan sebanyak 14 mm dan nilai MIC 6.25 mg/mL. Keseluruhannya, ekstrak n-hexane telah menunjukkan aktiviti antibakteria yang rendah berbanding ekstrak metanol. Meskipun ekstrak metanol dan minyak pati gagal menunjukkan sebarang aktiviti terhadap strain Candida. Ekstrak n-hexane menunjukkan aktiviti yang lemah terhadap *C. tropicalis* dan *C. lusitaniae*. Aktiviti antivirus oleh minyak pati, ekstrak metanol dan n-hexane dari daun *V. negundo* telah dipraktiskan terhadap virus sinsitium respirasi (RSV) dan herpes simpleks jenis 1(HSV-1). Beberapa ujikaji aktiviti antivirus telah dijalankan iaitu ujikaji antivirus dalaman sel dan luaran sel. Ekstrak metanol telah menunjukkan aktiviti antivirus dalaman sel terhadap kedua-dua RSV dengan nilai SI 10.2. Manakala, minyak pati daun *V. negundo* pula menunjukkan aktiviti antivirus dalaman sel yang sederhana terhadap RSV dan HSV-1 dengan nilai SI 4.92 dan 4.6. Ekstrak n-hexane tidak menunjukkan sebarang aktiviti terhadap kedua-dua RSV dan HSV-1 secara dalaman sel atau luaran sel. Komposisi kimia di dalam minyak pati, ekstrak metanol dan n-hexane dari daun *V. negundo* telah dikenalpasti dengan menggunakan gas kromatografi jisim spektrofotometer (GCMS). Keputusan kajian menunjukkan bahan utama di dalam ekstrak metanol *V. negundo* adalah metil ester linoleik acid(11.26 %), alfa isometil ionone (10.27%), ledol (7.31%) dan caryophyllene

(4.13%), manakala bahan utama dalam ekstrak n-hexane adalah tetracontane (22.98%), tetrapetacontane (21.42%), hexacontane (3.35%), hexatriacontane (2.16%) dan beta Thujene (2.04%). Keputusan kajian ini juga telah mengenalpasti bahan utama di dalam minyak pati daun *V. negundo* iaitu α—thujene (44.47%), α-terpinene (10.3%), ɣ-terpinene (4.78%), 4-terpinenyle acetate (3.56%) dan citronellol (2.51%) telah dikenalpasti sebagai monoterpenes utama, dan juga viridiflorene (4.13%), bisabolene (3.97%), citronellol (2.51%), viridiflorol (2.05%), caryophyllene (1.73%), dan cadinol (1.17%) pula merupakan sesquiterpenes utama.

Keputusan kajian telah menunjukkan bahawa ekstrak *V. negundo* mengandungi banyak sebatian bioaktif yang berkesan terhadap bacteria-bakteria patogenik yang kebiasaannya merupakan penyebab kepada jangkitan penyakit. Ektrak metanol *V. negundo* ini juga menunjukkan aktiviti antivirus terhadap HSV-1 dan RSV. Ciri-ciri biologi ini berkemungkinan disebabkan oleh sebatian-sebatian bioaktif di dalam tumbuhan. Tumbuhan ubatan ini mungkin boleh dijadikan sebagai sumber baru bagi aktiviti antimikrobiologi dan berkemungkinan juga dapat menerangkan bagaimana tumbuhan ini bertindak balas dalam merawat pelbagai jenis penyakit berjangkit.

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I certify that an examination Committee has met on 7 June 2012 to conduct the final examination of Mohsen Zargar on his Doctor of Philosophy thesis entitled "Antimicrobial and antioxidant activities of *Vitex negundo* L. leaves'' 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 candidate be awarded the Doctor of Philosophy degree.

Members of the Thesis Examination Committee were as follows:

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Date: 23 July 2012

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as bellows:

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DECLARATION

I declare that the thesis is 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.

MOHSEN ZARGAR

Date: 7 June 2012

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