UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL ACTIVITIES AND DETERMINATION OF STILBENOIDS FROM EXTRACTS OF *GNETUM GNEMON* L. (MANINJAU)

DAYANA WAZIR

FBSB 2011 35

BIOLOGICAL ACTIVITIES AND DETERMINATION OF STILBENOIDS FROM EXTRACTS OF *GNETUM GNEMON* L. (MANINJAU)

By

DAYANA WAZIR

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

February 2011

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

BIOLOGICAL ACTIVITIES AND DETERMINATION OF STILBENOIDS FROM EXTRACTS OF *GNETUM GNEMON* L. (MANINJAU)

By

DAYANA WAZIR

February 2011

Chair : Assoc. Prof. Dr. Radzali Muse, PhD

Faculty : Faculty of Biotechnology and Biomolecular Sciences

Gnetum gnemon or known as maninjau (Malaysia) , which belongs to genus of *Gnetum,* order of *Gnetales* and family of *Gnetaceae* is a dioecious, evergreen tree that are widely cultivated in Southeast Asia. The seeds are usually cooked as crackers and well known among Indonesian while leaves, shoots and fibres are used as 'ulam' and ropes. Although some studies has done on Gnetacae family but studies specifically on *Gnetum gnemon* are very limited since the researchers only concentrate on isolation of the compound and limited to certain solvents. Analyses on biological activities of *G. gnemon* were done to determine the total phenolic, antioxidant, antimicrobial and anti-tyrosinase of the plant. The determination of stilbenoids using high-performance liquid chromatography (HPLC) was done since most of the family of this plant contained high amount of stilbenoids and this plant may have the potential to contain the same compounds. Four parts of *Gnetum gnemon* were used in this study, which were leaf, bark, twig, and seed of the plant.

All parts were extracted in methanol, ethanol, hexane, chloroform and hot water using reflux technique. The total phenolic content of the plant extracts were determine by using Folin-Ciocalteu method. The results demonstrated that the bark from hot water extract showed the highest total phenolic which was 10.71 ± 0.008 mg GAE/ FDW, while the lowest was seed from chloroform extract which was 2.15 ± 0.006 mg GAE/ FDW. The antioxidant activity of the plant extracts were determined by using DPPH and FRAP assays. The DPPH results showed that all plant extracts demonstrated weak free radical scavenging activity tested at final concentration of 300 µg/ml. In contrast, the methanolic twig extract showed strong reducing power activity (FRAP) with 83.55± 1.05 %, while the hot water seed extract showed the least activity with 41.86 ± 4.22 % tested at final concentration of 300 µg/ml. There were no correlation between total phenolics and both antioxidant assays tested based on the results obtained. Anti-tyrosinase activity of the plant extracts were determined by using mushroom tyrosinase enzymatic assay. The results showed that hot water bark extract demonstrated moderate anti-tyrosinase activity with 57.78 ± 2.13 %, while other plant extracts demonstrated weak anti-tyrosinase activity tested at final concentration of 200 µg/ml. In addition, four Gram-positive bacteria (*Bacillus subtilis, Micrococcus luteus, Enterococcus avium* and *Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomomas aeruginosa* and *Shigella sonnei*) were tested for antimicrobial activity of the plant extracts. The results showed that ethanolic twig extract demonstrated the highest antimicrobial inhibition among all samples tested which were 4.33 mm upon antimicrobial activity upon Gram-positive bacteria (*Staphylococcus aureus*) at concentration of 20 mg/ml . On the other hand, the anti-fungal activities of the plant extracts were evaluated upon *Aspergillus terreus,*

Penicillum notatum, Mucor, Ganoderma lucidum and *Tricoderma harzianum*.The results showed that the hot water extract of seed showed moderate anti-fungal activity which were 1.60 mm upon *G. lucidium*. Resveratrol, piceatannol and ptereostilbene were used as the stilbenoids standard in this study. The HPLC results showed that piceatannol was the highest in the methanolic seed extract (2.62 mg), while the resveratrol was the lowest in hexanoic bark extracts (0.03 mg). Overall, from the result obtained, the stilbenoids compounds in the samples extract did not contribute to the antioxidant activity. However, they show a potential activity upon antimicrobial and anti-tyrosinase activities since the result are quite promising. Further reserach is needed to identify the active compound from this plant and their phyto-pharmaceutical studies.

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

AKTIVITI BIOLOGI DAN PENENTUAN STILBENOIDS DARIPADA EKSTRAK *GNETUM GNEMON* L. (MANINJAU)

Oleh

DAYANA WAZIR

Februari 2011

Pengerusi: Prof. Madya Radzali Muse, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Gnetum gnemon atau lebih dikenali sebagai maninjau (Malaysia) , yang tergolong dari genus Gnetum, order Gnetales dan famili Gnetaceae merupakan pokok yg mempunyai organ reproduktif jantan dan betina pada pokok yang berbeza ini banyak di tanam di Asia Tenggara. Bijinya sering dimasak sebagai emping yang terkenal di kalangan masyarakat Indonesia manakala daun, pucuk dan fibernya sering dibuat 'ulam' dan tali. Walaupun beberapa kajian di jalankan pada famili Gnetacae tetapi kajian secara terperinci terhadap *Gnetum gnemon* adalah terhad memandangkan kajian lebih tertumpu kepada pengasingan sebatian dan pelarut sahaja. Analisis aktiviti biologi *Gnetum gnemon* telah dijalankan untuk menentukan total phenol, antioksidan, antimikrobial dan anti-tyrosinase tumbuhan tersebut. Penentuan sebatian stilbenoids dilakukan dengan menggunakan kaedah ' high-performance liquid chromatography' (HPLC) memandangkan kebanyakan famili dari tumbuhan ini mempunyai kandungan stilbenoid yang tinggi maka *Gnetum gnemon* berpotensi

untuk mempunyai kompoun tersebut. Empat bahagian *Gnetum gnemon* iaitu daun, kulit kayu, ranting dan biji telah digunakan di dalam kajian ini. Semua bahagian tersebut telah diekstrak menggunakan pelarut metanol, etanol, heksan, kloroform dan air panas dengan menggunakan kaedah 'refluks'. Kandungan total fenol ekstran sampel telah ditentukan menggunakan reagen 'Folin-Ciocalteu'.Keputusan menunjukkan kulit kayu kayu yang diekstrak dari air panas menunjukkan total fenol yang tinggi iaitu 10.71 ± 0.008 mg GAE/ FDW manakala yang paling rendah adalah daripada biji yang diekstrak dari kloroform iaitu 2.15 ± 0.006 mg GAE/ FDW. Aktiviti antioksidan di dalam sampel ekstrak ditentukan melalui ujian DPPH dan FRAP. Ujian DPPH menunjukkan semua sampel ekstrak memberi keputusan yang rendah di dalam kepekatan 300 µg/ml. Ranting yang diekstrak di dalam pelarut metanol memberi bacaan yang tinggi di dalam ujian FRAP iaitu 83.55± 1.05% manakala sampel biji yang diekstrak di dalam air panas memberi bacaan yang rendah iaitu 41.86 ± 4.22 % di dalam kepekatan 300 µg/ml. Ujian total fenol dan aktiviti antioksida tidak berkait antara satu sama lain berdasarkan pada keputusan yang didapati. Ujian anti-tyrosinase ditentukan dengan menggunakan esei 'mushroom tyrosinase enzymatic'. Ujian menunjukkan kulit kayu yang diekstrak di dalam air panas menunjukkan keputusan yang sederhana iaitu 57.78 ± 2.13 %, manakala semua sampel ekstrak memberi bacaan yang rendah terhadap ujian ini di dalam kepekatan 200 µg/ml. Empat bakteria Gram-positif (*Bacillus subtilis, Micrococcus luteus, Enterococcus avium* and *Staphylococcus aureus*) dan empat bakteria Gram-negatif (*Escherichia coli, Klebsiella pneumoniae, Pseudomomas aeruginosa* and *Shigella sonnei*) telah diuji untuk menentukan aktiviti antimikrob didalam ekstrak tumbuhan ini. Sampel ranting yang diekstrak didalam pelarut etanol memberi nilai perencatan yang paling tinggi iaitu 4.33 mm di dalam sampel yang berkepekatan

20mg/ml. Fungi seperti *Aspergillus terreus, Penicillum notatum, Mucor, Ganoderma lucidum* and *Tricoderma harzianum* digunakan di dalam ujian antifungus. Keputusan menunjukkan ekstrak biji yang diekstrak menggunakan air panas memberi aktiviti yang sederhana iaitu 1.60 mm terhadap fungus *Ganoderma lucidium*. Resveratrol, piceatannol dan pterostilbene digunakan sebagai rujukan utama stilbenoid di dalam kajian ini. Keputusan HPLC menunjukkan piceatannol memberi bacaan yang paling tinggi di dalam sampel biji yang diekstrak di dalam pelarut metanol (2.62 mg), sementara resveratrol memberi bacaan yang paling rendah di dalam sampel kulit kayu yang di ekstrak di dalam pelarut heksan (0.03 mg). Secara keseluruhan, aktiviti antioksidan di dalam sampel ekstrak tidak dipengaruhi oleh sebatian stilbenoid tetapi ianya mempunyai potensi terhadap aktiviti antimikrob dan anti-tyrosinase. Kajian lanjut perlu dijalankan untuk mengenalpasti sebatian yang mungkin mempunyai potensi di dalam bidang fito-farmaseutikal.

ACKNOWLEDGEMENT

All praises to the Almighty God, the Most Gracious and Merciful, who is omnipotent and all giving, for affording me the strength and determination to complete this study. I owe a great deal of gratitude to many people for their kind assistance and support at various stages of my research work. Words are insufficient to adequately express my sense of indebtedness to these individuals.

First and foremost, I would like to express my deepest appreciation to my supervisors, Associate Professor Dr. Radzali Muse, Dr. Syahida Ahmad, Prof. Dr. Maziah Mahmood, Associate Professor Dr. Yunus Shukor and Associate Professor Dr. Johari Ramli for their guidance, help, knowledge and encouragement and critical review towards the completion of my thesis. Without this invaluable help from these people, this level of success would have been unattainable.

I am sincerely thankful and greatly appreciation for all of the help, encouragement, guidance and friendship that I have gained during working with this project from the staff and members of Secondary Metabolite Lab (Lab 230), Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, and Institute of Bioscience, Universiti Putra Malaysia. Also special thanks to Universiti Putra Malaysia for their financial support, Pasca Siswazah Fellowship

Finally, I would like to express my heartfelt gratitude and appreciation to my family and friends for their advice, encouragement, understanding, support and prayers. May God bless you.

I certify that an Examination Committee has met on **4th February 2011** to conduct the final examination of **Dayana Wazir** on her Master Science thesis entitled **"BIOLOGICAL ACTIVITIES AND DETERMINATION OF STILBENOIDS FROM EXTRACTS OF *GNETUM GNEMON* L. (MANINJAU)"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) regulations 1981. The Commitee recommends that the student be awarded the relevant degree.

Members of the Examination Committee were as follows:

Norihan Mohd Salleh, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

Umi Kalsom Md Shah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

Norjahan Banu Mohamed Alitheen, PhD
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

A. Manaf Ali
Deputy Vice Chancellor
(Research and Innovation)
Universiti Darul Iman Malaysia
Malaysia

BUJANG KIM HUAT, PhD
Professsor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :

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

Radzali Muse, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

Syahida Ahmad, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

Maziah Mahamood, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

_____

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declared that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other instution.

_____

DAYANA WAZIR

Date : 7 February 2011

TABLE OF CONTENT