BIOLOGICAL ACTIVITY OF *HEDYOTIS* SPP. AND CHEMICAL CONSTITUENTS OF *HEDYOTIS CAPITELLATA*

By

ROHAYA AHMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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BIOLOGICAL ACTIVITY OF *HEDYOTIS* SPP. AND CHEMICAL CONSTITUENTS OF *HEDYOTIS CAPITELLATA*

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Chairman: Professor Md. Nordin Hj.Lajis, PhD

Institute: Bioscience

Hedyotis (Family, Rubiaceae) is a genus of erect decumbent or climbing herbs. The genus consists of some 180 species. They grow well on dry and sandy soil, along rivers and coasts and in the forests. There are 35 species recorded in Malaysia. Most of the species possess medicinal properties and are used by the Malay as well as the Chinese communities.

The methanolic extracts of seven *Hedyotis* species including *H. capitellata* (stems, leaves and roots), *H. dichotoma* (aerial parts and roots), *H. verticillata* (leaves and stems), *H. herbacea* (aerial parts), *H. pinifolia* (aerial parts), *H. corymbosa* (aerial parts) and *H. nudicaulis* (aerial parts) were screened for antioxidant, radical-scavenging, anti-inflammatory, cytotoxic as well as anti-bacterial properties using the ferric thiocyanate (FTC) and thiobarbituric acid (TBA), the diphenylpicryl hydrazyl (DPPH), the Griess assay, the MTT assay and the disc diffusion methods, respectively. The results showed that all of the extracts tested possess strong antioxidant potential. However, they are poor radical scavengers and nitric oxide

inhibitors. They are also found to be weakly cytotoxic and possess weak to moderate antibacterial properties. On the basis of the screening results and literature review, *H. capitellata* (stems) was selected for further phytochemical study.

Phytochemical investigation on the active fraction of the stems of *H. capitellata* plant yielded fifteen compounds. The structure of the compounds was elucidated based on spectroscopic techniques and comparison with literature values. Eight compounds are *new* furanoanthraquinones named capitellataquinone A-G and *epi*-capitellataquinone E. A new anthraquinone, 2,8-dihydroxy-1-methoxyanthraquinone together with rubiadin, alizarin 1-methyl ether, anthragallol 2-methyl ether, digiferruginol and scopoletin were also isolated. From the roots of the plant, lucidin 3-*O*-*β*-glucoside was also isolated. The isolation of an anthraquinone glycoside is a first for the genus.

Antioxidant assays on ten compounds including capitellataquinones A, B, E, F, *epi*-capitellataquinone E, rubiadin, alizarin 1-methyl ether, anthragallol 2-methyl ether, digiferruginol and scopoletin showed that capitellataquinone A and scopoletin possess strong antioxidant properties with percent inhibition of 94-96% compared to quercetin (98%). MTT cytotoxic assays of the same compounds tested showed weak cytotoxicity to most cell-lines with IC_{50} values of 24-40 μg/ml. However, alizarin 1-methyl ether was found to be selectively cytotoxic against MDA-MB-231 with an IC_{50} value of 8 μg/ml.

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

KEAKTIFAN BIOLOGI SPESIES *HEDYOTIS* DAN KONSTITUEN KIMIA *HEDYOTIS CAPITELLATA*

Oleh

ROHAYA AHMAD

Februari 2005

Pengerusi: **Profesor Md. Nordin Hj.Lajis, PhD**

Institut: **Biosains**

Hedyotis (Famili Rubiaceae) ialah genus tumbuhan herba yang tumbuh menegak atau memanjat. Genus ini terdiri daripada 180 spesies. Ia tumbuh dengan baik di tanah yang kering dan berpasir, di tepi pantai dan di dalam hutan. Terdapat 35 spesies yang direkodkan di Malaysia. Kebanyakan daripadanya mempunyai nilai perubatan dan digunakan oleh kaum Melayu dan Cina.

Ekstrak metanol tujuh spesies *Hedyotis* termasuk *H. capitellata* (daun, batang dan akar), *H. dichotoma* (atas dan akar), *H. verticillata* (atas dan batang), *H. herbacea* (atas), *H. pinifolia* (daun), *H. corymbosa* (atas) dan *H. nudicaulis* (atas) disaring untuk sifat antioksida, anti-inflamasi, sitotoksik dan anti-bakteria menggunakan biocerakinan ferrik tiosianat (FTC) dan asid tiobarbiturik (TBA), kaedah difenilpikrilhidrazil (DPPH), kaedah Griess, kaedah MTT dan kaedah pembauran cakera, masing-masing. Keputusan biocerakinan menunjukkan bahawa kesemua ekstrak mempunyai potensi antioksidaan yang kuat. Walau bagaimana pun, kesemuanya merupakan pemerangkap radikal dan perencat nitrik oksida yang lemah. Mereka juga didapati mempunyai sifat sititoksik yang sederhana serta

mempunyai sifat antibakteria lemah hingga sederhana. Berdasarkan keputusan penyaringan dan kajian literatur, *H. capitellata* (batang) telah dipilih untuk fitokimia.

Penyelidikan fitokimia ke atas fraksi aktif batang *H. capitellata* menghasilkan lima belas sebatian. Struktur kesemua sebatian dikenalpasti berdasarkan kaedah spektroskopi dan perbandingan dengan literatur. Lapan sebatian merupakan furanoantrakuinon baru yang dinamakan capitellataquinone A-G dan *epi*-capitellataquinone E. Satu antrakuinon baru, 2,8-dihidroksi-1-metoksiantrakuinon bersama dengan rubiadin, alizarin 1-metil eter, antragalol 2-metil eter, digiferuginol dan skopoletin juga berjaya dipencilkan. Daripada bahagian akar tumbuhan tersebut, lucidin 3-*O*-β-glukosida telah dipencilkan. Ini adalah kali pertama anthrakuinon glikosida dipencilkan daripada genus ini.

Cerakinan antioksidaan terhadap sepuluh sebatian termasuk capitellatakuinon A, B, E, F, *epi*-capitellatakuinon E, rubiadin, alizarin 1-metil ether, anthragallol 2-metil ether, digiferruginol dan skopoletin menunjukkan bahawa hanya capitellatakuinon A dan skopoletin menunjukkan sifat antioksidaan yang baik dengan peratus hindaran sebanyak 94-96% berbanding dengan quercetin (98%). Cerakinan sitotoksik MTT keatas sebatian yang sama menunjukkan sifat sitotoksik yang lemah terhadap kebanyakan titisan sel dengan nilai IC_{50} antara 24-40 μg/ml. Walau bagaimana pun, alizarin 1-metil eter didapati sitotoksik secara selektif terhadap MDA-MB-231 dengan nilai IC_{50} 8 μg/ml.

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I certify that an Examination Committee met on 22nd February 2005 to conduct the final examination of Rohaya Ahmad on her Doctor of Philosophy thesis entitled "Biological Activity of *Hedyotis* spp. and Chemical Constituents of *Hedyotis capitellata*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

GWENDOLINE EE CHANG LIAN Ph.D.
Associate Professor
Faculty of Science and Environmental Study
Universiti Putra Malaysia
(Chairman)

MAWARDI RAHMANI, Ph.D.
Professor
Faculty of Science and Environmental Study
Universiti Putra Malaysia
(Member)

MOHD ASPOLLAH HJ. SUKARI, Ph.D.
Associate Professor
Faculty of Science and Environmental Study
Universiti Putra Malaysia
(Member)

Walter C. Taylor, PH.D., DSC., O.A.M.
Emeritus Professor
School of Chemistry
University of Sydney
Australia
(Independent Examiner)

_____
GULAM RUSUL RAHMAT ALI, PH.D.
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

The thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

MOHD. NORDIN HJ. LAJIS, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

NOR HADIANI ISMAIL, PhD
Associate Professor
Faculty of Applied Science
Universiti Teknologi MARA
(Member)

DAUD A. ISRAF ALI, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Abdul Manaf Ali, PH.D.
Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

_____

AINI IDERIS, PHD
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I hereby 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 or concurrently submitted for any degree at UPM or other institutions.

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ROHAYA AHMAD

Date:

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