

**PHYTOCHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF
CURCUMA AERUGINOSA ROXB., *C. OCHRORHIZA* VAL. AND
ANDROGRAPHIS ASCULATA NEES.**

By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of
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PHYTOCHEMICAL CONTITUENTS AND BIOLOGICAL ACTIVITY OF
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Tropical plants have been acknowledged to be the source of variety of forest products that could be exploited as potential pharmaceutical, natural insecticides, oils, foods and other industrial goods. Out of these only a small proportion has been systematically investigated. This research has been carried out to investigate the chemical constituents and bioactivities of *Curcuma aeruginosa*, *Curcuma ochrorhiza* and *Andrographis asculata*.

In this present study, the rhizomes of *Curcuma aeruginosa* and *Curcuma ochrorhiza* and also the leaves of *Andrographis asculata* were investigated and have resulted in the isolation of eight pure compounds and three mixtures. The structures of the pure

compounds were elucidated by using spectroscopic experiments namely NMR, MS and IR, while the constituents of the mixtures were identified using GC-MS technique.

Detailed chemical studies on *Curcuma aeruginosa* have yielded three sesquiterpenes, curcumenol (**10**), zedoarol (**6**) and isocurcumenol (**43**) and phytosterol mixtures containing stigmasterol (**52**) and α -sitosterol (**53**). Isocurcumenol (**43**) was isolated for the first time from this plant species, whereas the occurrences of other compounds have been reported previously. Meanwhile investigations on *Curcuma ochrorhiza* have afforded three sesquiterpenes, zederone (**13**), zerumbone (**46**) and furanodienone (**2**), two triterpenes namely stigmasterol (**52**) and α -sitosterol (**53**) and mixtures of long chain compounds comprising of tricosanoic acid (**54**) and ester of tetradecanoic acid (**55**). Zerumbone (**46**) and furanodienone (**2**) were isolated for the first time from this species. Studies on *Andrographis asculata* have yielded α -sitosterol (**53**) as the major constituent and mixtures of docosanoic acid (**56**), hexadecanoic acid (**57**), tetradecanoic acid (dodecyl ester) (**58**) and hexacosanoic acid (**59**).

Cytotoxic tests were performed using HL-60 cell lines and CEM-SS cell lines. The crude chloroform extract of *Curcuma aeruginosa* and crude hexane extract of *Curcuma ochrorhiza* were considered being active against CEM-SS cell lines with IC_{50} values 6 μ g/mL. Meanwhile, pure compounds isolated from *Curcuma ochrorhiza*, zederone (**13**) and zerumbone (**46**) were found to be very active against CEM-SS and HL-60 cell lines.

As for antimicrobial test, crude chloroform extract of *Curcuma aeruginosa* were mildly active against four bacteria used in the test, while crude methanol extract of *Andrographis asculata* exhibited mild activity against *Bacillus substillis*. In addition, two isolated compounds isocurcumenol (**10**) and zerumbone (**46**) were found to be moderately active against some of the microbes.

The antioxidant activity of all extracts from *Curcuma aeruginosa* were close to or lower than quarcetin, and this suggest that the crude extract posses antioxidant activity. The results also showed the same pattern with all crude extracts of *Curcuma ochrorhiza*. Two pure compounds from *Curcuma aeruginosa* were also subjected to the screening of antioxidant tests and the results showed curcumenol (**10**) and isocurcumenol (**43**) did posses antioxidant activity. Meanwhile, the crude hexane and CHCl_3 extract of *Andrographis asculata* also showed antioxidant activity, while the crude methanol extract was not active.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan ijazah Master Sains.

SEBATIAN FITOKIMIA DAN UJIAN AKTIVITI BIOLOGI BAGI *CURCUMA AERUGINOSA* ROXB., *C. OCHRORHIZA* VAL. DAN *ANDROGRAPHIS ASCULATA* NEES.

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Tumbuhan tropika telah dikenalpasti sebagai sumber kepelbagaian hasil hutan yang boleh dimajukan di dalam bidang farmaseutikal, bahan racun serangga semulajadi, minyak, makanan dan beberapa barangan industri. Hanya sebilangan kecil tumbuhan tropika yang telah dikaji secara sistematik. Oleh itu, kajian in dijalankan bagi mengenalpasti sebatian kimia dan juga aktiviti biologi, tiga tumbuhan telah dipilih iaitu *Curcuma aeruginosa* , *Curcuma ochrorhiza* dan *Andrographis asculata*.

Rizom bagi spesies *Curcuma aeruginosa* dan *Curcuma ochrorhiza* dan juga daun *Andrographis asculata* telah dikaji. Lapan sebatian tulen dan tiga sebatian campuran

telah dikenalpasti. Struktur sebatian-sebatian ini telah dicirikan melalui ujian spektroskopi NMR, MS dan IR.

Kajian terperinci bagi spesies *Curcuma aeruginosa* telah menghasilkan tiga seskuiterpena iaitu, curcumenol (**10**), zedoarol (**6**) dan isocurcumenol (**43**) serta campuran sitosterol iaitu stigmasterol (**52**) dan α -sitosterol (**53**). Isocurcumenol (**43**) merupakan sebatian yang belum pernah dihasilkan sebelum ini bagi spesies *Curcuma aeruginosa*, manakala sebatian yang lain pernah dilaporkan sebelum ini. Kajian bagi spesies *Curcuma ochrorhiza* pula menghasilkan tiga seskuiterpena iaitu, zederon (**13**), zerumbon (**46**) dan furanodienon (**2**), dua triterpena iaitu, stigmasterol (**52**) dan α -sitosterol (**53**) dan juga campuran sebatian rantai panjang iaitu, asid tricosanoik (**54**) dan asid tetradecanoik (**55**) (tetradesil ester). Zerumbon (**46**) dan furanodienon (**2**) merupakan sebatian yang diperolehi bagi pertama kali untuk spesies ini. Bagi spesies *Andrographis asculata* pula menunjukkan kehadiran satu sebatian triterpena iaitu, α -sitosterol (**53**) dan campuran sebatian rantai panjang iaitu, asid docosanoik (**56**), asid heksadecanoik (**57**), asid tetradecanoik (dodesil ester) (**58**) dan asid heksacosanoik (**59**).

Ujian sitotoksik telah dijalankan melalui penggunaan sel HL-60 dan CEM-SS. Ekstrak mentah kloroform bagi *Curcuma aeruginosa* dan heksana bagi *Curcuma ochrorhiza* boleh dianggap aktif ke atas sel CEM-SS dengan nilai IC_{50} 6 μ g/mL. Manakala bagi dua sebatian tulen iaitu zederon (**13**) dan zerumbon (**46**) juga dikenalpasti sebagai aktif terhadap sel kanser CEM-SS dan HL-60.

Bagi ujian antimikrob pula, ekstrak mentah kloroform *Curcuma aeruginosa* menunjukkan aktiviti terhadap empat mikrob yang digunakan dalam ujian ini. Ekstrak metanol *Andrographis asculata* pula menunjukkan aktiviti yang rendah terhadap mikrob *Basillus substillis*. Selain ekstrak mentah, dua sebatian tulen iaitu isocurcumenol **(43)** dan zerumbon **(46)** juga diuji dan didapati kedua-dua sebatian ini aktif terhadap beberapa mikrob yang digunakan dalam ujian ini.

Aktiviti antioksidan pula menunjukkan kesemua ekstrak mentah bagi *Curcuma aeruginosa* rendah atau hampir dengan quarcetin, melalui pemerhatian ini dapat disimpulkan bahawa ekstrak mentah bagi spesies *Curcuma aeruginosa* menunjukkan aktiviti antioksidan. Keputusan yang sama juga diperolehi bagi kesemua ekstrak mentah *Curcuma ochrorhiza*. Dua sebatian daripada spesies *Curcuma aeruginosa* juga telah dijalankan ujian antioksidan ini, dan keputusan menunjukkan sebatian tulen ini iaitu, curcumenol **(10)** dan isocurcumenol **(43)** juga menunjukkan aktiviti antioksidan. Ekstrak heksana dan kloroform spesies *Andrographis asculata* dianggap aktif antioksida, manakala ekstrak metanol pula didapati tidak aktif antioksida.

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I certify that an Examination Committee has met on 10th February 2006 to conduct the final examination of Suhaila binti Md. Saad on her Master of Science thesis entitled “Phytochemical Constituents and Biological Activity of *Curcuma aeruginosa* Roxb., *C. ochrorhiza* Val. and *Andrographis asculata* Nees.” 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:

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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 other degree at UPM or other institutions.

SUHAILA MD. SAAD

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