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ISOLATION OF *KAEMPFERIA ANGUSTIFOLIA* ROSC., *ALPINIA CONCHIGERA* GRIFF. AND *CURCUMA MANGGA* VAL. AND VAN ZIJP. PHYTOCHEMICALS AND THEIR BIOACTIVITIES

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By

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ISOLATION OF *KAEMPFERIA ANGUSTIFOLIA* ROSC., *ALPINIA CONCHIGERA* GRIFF. AND *CURCUMA MANGGA* VAL. AND VAN ZIJP. PHYTOCHEMICALS AND THEIR BIOACTIVITIES

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Chairman: Professor Mohd. Aspollah Hj. Sukari, PhD

Faculty: Science

Zingiberaceae is one of the most important herbaceous families found in tropical forests. In this research, *Kaempferia angustifolia* Rosc. (*Kunci pepet*), *Alpinia conchigera* Griff. (*lengkuas ranting*) and *Curcuma mangga* Val. & Van Zijp. (mango turmeric) were selected for phytochemical and bioactivity studies. Several classes of chemical constituents have been isolated and characterized spectroscopically. This included cyclohexane epoxides, chalcones, terpenes, phenylpropanoids and diarylheptanoids.

From *Kaempferia angustifolia*, crotepoxide **(29)**, boesenboxide **(30)**, 2'-hydroxy-4,4',6'-trimethoxychalcone **(31)**, zeylenol **(28)**, 6-methylzeylenol **(32)**, abiet-8(14)-enepenta-6,7,9,11,13-ol (kaempfolienol) **(84)**, (24*S*)-24-methyl-lanosta-9(11),25-dien-3β-ol **(80)**, pipoxide **(25)** were isolated besides β-sitosterol **(82)** and its glycoside **(83)**. Similarly, β-sitosterol-3-*O*-β-D-glucopyranoside **(83)** was also obtained from the rhizomes of *Alpinia conchigera* along with *p*-

hydroxycinnamaldehyde **(55)**, 1'-acetoxychavicol acetate **(33)** and *trans-p*-coumaryl diacetate **(53)**. Furthermore, β-sitosterol **(82)**, curcumin **(59)**, demethoxycurcumin **(60)** and 12,17-epoxy-3β,17-dihydroxylabda-13-en-16,15-olide (curcumangganol) **(94)** were yielded from the *Curcuma mangga* extract. The structure of zeylenol **(28)** was modified by using chemical reactions to give zeylenol diacetate **(89)**, zeylenol triacetate **(90)** and zeylenol epoxide **(91)** derivatives. *p*-Hydroxycinnamaldehyde **(55)** was also derivatized to *p*-methoxycinnamaldehyde **(92)** and *p*-acetoxycinnamaldehyde **(93)**.

5*S*,6*S*,7*S*,9*S*,10*S*,11*R*,13*S*-Abiet-8(14)-enepenta-6,7,9,11,13-ol (Kaempfolienol) **(84)** and epimeric 12,17-epoxy-3β,17-dihydroxylabda-13-en-16,15-olide (curcumangganol I and II) **(94)** were isolated as new compounds. The stereostructure of compound **84** was determined on the basis of its single crystal X-ray crystallography. Compounds **80**, **83** and (+)-**25** were firstly described from the respective species. Structures of all the isolated phytochemicals and structurally-modified compounds were elucidated by using various spectroscopic methods [infrared (IR), mass spectrometry (MS) and nuclear magnetic resonance (NMR)] and by comparison with the previous literature.

Crude extracts, isolated constituents and derivatives were subjected to cytotoxic screening against four cancer cell lines (HL-60, HT-29, MCF-7 and HeLa) as well as antimicrobial testing. Crude extracts of *Kaempferia angustifolia* were not active in the cytotoxic assays, with IC_{50} values more than 30 µg/mL. Isolated phytochemicals from this species exhibited different degrees of inhibitions against the cancer cell lines tested, with 2'-hydroxy-4,4',6'-trimethoxychalcone **(31)** and (24*S*)-24-methyl-

lanosta-9(11),25-dien-3β-ol **(80)** demonstrating the most prominent cytotoxic activities (IC_{50} < 8 μg/mL) against certain cell lines. As for *Alpinia conchigera*, non-polar and semi-polar extracts showed strong cytotoxic properties against human promyelocytic leukaemia (HL-60), human breast cancer (MCF-7) and human cervical cancer (HeLa) cell lines. 1'-Acetoxychavicol acetate **(33)** and *p*-hydroxycinnamaldehyde **(55)** displayed a broad spectrum of anticancer effects against the tested cell lines. In addition, the chloroform extract of *Curcuma mangga* and isolated compounds **59**, **60** and **94** also exhibited potent cytotoxic activities in the study.

In antimicrobial screening test, extracts and constituents of *Kaempferia angustifolia* were not active. On the other hand, the crude hexane, chloroform and ethyl acetate extracts of *Alpinia conchigera* were strongly active against *Salmonella choleraesuis* in the antimicrobial assay. In antifungal screening test, hexane and ethyl acetate extracts of *Alpinia conchigera* showed slight inhibition on *Saccharomyces cerevisiae*. 4- Hydroxycinnamaldehyde **(55)** showed a remarkable activity in the antimicrobial testing with a diameter of inhibition zone > 20 mm. The chloroform-soluble fraction of *Curcuma mangga* and demethoxycurcumin **(60)** showed negative results in the antimicrobial and antifungal screenings but curcumangganol **(94)** from the extract demonstrated a moderate inhibitory activity towards *Bacillus subtilis* (B_{29}).

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

PEMENCILAN SEBATIAN FITOKIMIA DARIPADA *KAEMPFERIA ANGUSTIFOLIA* ROSC., *ALPINIA CONCHIGERA* GRIFF. DAN *CURCUMA MANGGA* VAL. DAN VAN ZIJP. SERTA KEAKTIFAN BIOLOGINYA

Oleh

TANG SOOK WAH

April 2011

Pengerusi: Profesor Mohd. Aspollah Hj. Sukari, PhD

Fakulti: Sains

Zingiberaceae merupakan salah satu daripada famili herba yang paling penting dalam hutan tropika. Dalam kajian ini, *Kaempferia angustifolia* Rosc. (*Kunci pepet*), *Alpinia conchigera* Griff. (*lengkuas ranting*) dan *Curcuma mangga* Val. & Van Zijp. (kunyit mangga) telah dipilih untuk kajian fitokimia dan bioaktiviti. Kandungan kimia daripada beberapa kelas sebatian telah dipencilkan dan dikenalpasti dengan teknik spektroskopi. Ini termasuklah sikloheksana epoksida, kalkon, terpena, fenilpropanoid dan diarilheptanoid.

Kaempferia angustifolia telah menghasilkan krotepoksida **(29)**, boesenboksida **(30)**, 2'-hidroksi-4,4',6'-trimetoksikalkon **(31)**, zeylenol **(28)**, 6-metilzeylenol **(32)**, abiet-8(14)-enapenta-6,7,9,11,13-ol (kaempfolienol) **(84)**, (24*S*)-24-metil-lanosta-9(11),25-dien-3β-ol **(80)**, pipoksida **(25)**, β-sitosterol **(82)** dan β-sitosterol-3-*O*-β-D-glukopiranosida **(83)**. β-Sitosterol-3-*O*-β-D-glukopiranosida **(83)** juga diperoleh daripada rizom *Alpinia conchigera* selain *p*-hidroksisinamaldehid **(55)**, 1'-

asetoksikavikol asetat **(33)** and *trans-p*-koumaril diasetat **(53)**. Di samping itu, β-sitosterol **(82)**, kurkumin **(59)**, demetoksikurkumin **(60)** dan 12,17-epoksi-3β,17-dihidroksilabda-13-en-16,15-olida (curcumangganol) **(94)** juga didapati daripada ekstrak *Curcuma mangga*. Struktur sebatian zeylenol **(28)** telah diubahsuai dengan menggunakan tindak balas kimia untuk menghasilkan zeylenol diasetat **(89)**, zeylenol triasetat **(90)** and zeylenol epoksida **(91)**. *p*-Hidroksisinamaldehid **(55)** juga digunakan untuk mengsintesiskan *p*-metoksisinamaldehid **(92)** and *p*-asetoksisinamaldehid **(93)**.

5*S*,6*S*,7*S*,9*S*,10*S*,11*R*,13*S*-Abiet-8-enapenta-6,7,9,11,13-ol (Kaempfolienol) **(84)** dan epimer 12,17-epoksi-3β,17-dihidroksilabda-13-en-16,15-olida (kurkumangganol I dan II) **(94)** telah dipencilkan sebagai sebatian baru. Stereokimia bagi sebatian **84** telah ditentukan berdasarkan analisis kristallografi kristal tunggal sinar-X. Sebatian **80**, **83** dan (+)-**25** diasingkan daripada spesies berkenaan untuk kali pertama. Semua sebatian fitokimia dan sebatian yang diperoleh daripada sintesis organik telah dikenalpasti dengan pelbagai teknik spektroskopi seperti inframerah, spektrometri jisim, resonans magnet nukleus serta berdasarkan perbandingan dengan literatur dahulu.

Ekstrak mentah dan sebatian hasil telah dijalankan ujian sitotoksik ke atas empat sel kanser (HL-60, HT-29, MCF-7 dan HeLa) dan ujian antimikrob. Ekstrak *Kaempferia angustifolia* tidak aktif dalam ujian sitotoksik, dengan nilai IC_{50} melebihi 30 µg/mL. Sebatian fitokimia daripada spesies ini menunjukkan rangsangan yang berbeza terhadap sel kanser yang dikaji, dengan 2'-hidroksi-4,4',6'-trimetoksikalkon **(31)** dan (24*S*)-24-metil-lanosta-9(11),25-dien-3β-ol **(80)** menunjukkan aktiviti sitotoksik

yang lebih tinggi (IC_{50} < 8 µg/mL) terhadap sel kanser tertentu. Bagi *Alpinia conchigera* pula, ekstrak yang tidak polar dan sederhana polar menunjukkan rangsangan sitotoksik yang sangat kuat ke atas sel leukemia manusia (HL-60), sel kanser payudara manusia (MCF-7) serta sel servik manusia (HeLa). Di antaranya, sebatian 1'-asetoksikavikol asetat **(33)** dan *p*-hidroksisinamaldehid **(55)** menunjukkan kesan sitotoksik ke atas semua sel kanser yang dikaji. Selain itu, aktiviti sitotoksik ekstrak kloroform *Curcuma mangga* dan juga sebatian yang dipencilkan daripadanya iaitu **59**, **60** dan **94** mempamerkan aktiviti sitotoksik yang berpotensi dalam kajian ini.

Semua ekstrak dan sebatian daripada *Kaempferia angustifolia* tidak aktif dalam ujian antimikrob. Sebaliknya, ekstrak heksana, kloroform dan etil asetat bagi *Alpinia conchigera* sangat aktif terhadap *Salmonella choleraesuis*. Dalam ujian penyaringan antifungal, ekstrak heksana dan etil asetat bagi *Alpinia conchigera* menunjukkan aktiviti yang rendah ke atas *Saccharomyces cerevisiae*. *p*- Hidroksisinamaldehid **(55)** menunjukkan aktiviti yang menonjol dalam ujian antimikrob dengan diameter zon rencatan lebih daripada 20 mm. Ekstrak kloroform *Curcuma mangga* dan demetoksikurkumin **(60)** menunjukkan keputusan negatif dalam ujian penyaringan antimikrob dan antifungus tetapi sebatian kurkumangganol **(94)** mempamerkan aktiviti yang sederhana terhadap *Bacillus subtilis* (B_{29}).

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I certify that a Thesis Examination Committee has met on 12 April 2011 to conduct the final examination of Tang Sook Wah on her thesis entitled "Isolation of *Kaempferia angustifolia* Rosc., *Alpinia conchigera* Griff. and *Curcuma mangga* Val. and van Zijp. Phytochemicals and their Bioactivities" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

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This thesis was 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 were as follows:

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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 at any other institution.

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TANG SOOK WAH

Date: 12 April 2011

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