



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF
KAEMPFERIA ANGUSTIFOLIA, K. ROTUNDA, SPERMACOCE
ARTICULARIS AND S. EXILIS**

NEOH BEE KEAT.

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AND *S. EXILIS***

By

NEOH BEE KEAT

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Mohd Aspollah Hj. Sukari, PhD

Faculty: Science

Kaempferia angustifolia, *K. rotunda* (Zingiberaceae), *Spermacoce articularis* and *S. exilis* (Rubiaceae) were chosen since they have long been used traditionally in medicinal treatment. In this research, isolation and separation work had been carried out using various solvents and chromatographic methods. The structure of these compounds were determined by using spectroscopic methods such as infrared (IR), nuclear magnetic resonance (^1H , ^{13}C and 2D- NMR), mass spectrometry (MS) and by comparison with the data reported previously. The crude extracts and some of the isolated compounds were subjected to the biological activity test: antioxidant, anti-microbial tests, cytotoxic screening and larvicidal activity.



Extracts from *Kaempferia angustifolia* have yielded a new compound angustifolienol (58), together with seven other compounds; crotepoxide (33), β -sitosterol (44), boesenboxide (34), 2'-hydroxy-4, 4', 6'-trimethoxychalcone (36), 6-methylzeylenol (59), and sucrose (60), and zeylenol (35). Fractionation of extracts from *K. rotunda* has yielded five pure compounds; crotepoxide (33), tetracosanoic acid (61), benzoic acid (62), stigmasterol (45) and β -sitosterol (44). Both tetracosanoic acid (61) and benzoic acid (62) are new to species. Extracts from *S. articularis* gave two pure compounds, identified as ursolic acid (63) and stigmasterol (45). While the extracts from *S. exilis* gave four compounds, benzo[g]isoquinoline-5,10-dione (64), stigmasterol (45), hexadecanoic acid (65) and ursolic acid (63). Phytochemical studies on later species had never been reported before.

Acetylations of new compound, angustifolienol (58) gave two derivatives, triacetoxiangustifolienol (66) and diacetoxiangustifolienol (67). Both are new compounds, and have never been reported previously either as natural product or synthesized compounds.

The major constituents of the essential oil of *K. angustifolia* was DL- camphor (20.57 %), while the major constituent of *K. rotunda* was benzyl benzoate (49.84 %). The major constituent of the essential oil of *S. articularis* was phytol (10.31 %), while the major constituent of *S. exilis* was oleic acid (20.9 %).



Cytotoxic screening showed that all the crude extracts of *K. angustifolia* and *K. rotunda* were not cytotoxic against HL-60 cell line. However, pure compounds of *K. angustifolia* showed strong activity. All crude extracts of *S. articularis* and *S. exilis* (except hexane extract of *S. exilis*) and ursolic acid (63) were strongly cytotoxic towards HL-60 and MCF-7 cell lines. All extracts and most of the pure compounds were subjected to four strains of microbes (MRSA, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*) in the antimicrobial test. Pet. ether extracts from *Kaempferia* species, all extracts from *S. exilis* and boesenboxide (34) were active towards some microbes. In antioxidant assay, *K. rotunda* was more active than *Kaempferia angustifolia* while both *Spermacoce* species were moderately active. Besides that, some of the compounds from *Kaempferia angustifolia* were subjected to the antioxidant assay and showed stronger antioxidant activity than α -tocopherol. In addition, all plants also showed moderate to low larvicidal activity.



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

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Kaempferia angustifolia, *K. rotunda* (Zingiberaceae), *Spermacoce articularis* dan *S. exilis* (Rubiaceae) telah dipilih sebagai bahan kajian kerana telah lama digunakan sebagai ubatan tradisional. Dalam kajian ini, kerja pemencilan dijalankan menggunakan pelbagai pelarut dan kaedah kromatografi. Struktur sebatian dikenalpasti dengan kaedah spektroskopi seperti inframerah (IR), resonans magnet nukleus (^1H , ^{13}C and 2D NMR) dan spektroskopi jisim (MS) dan perbandingan dengan data yang telah dilaporkan. Ujian biocerakinan seperti ujian antioksidan, ujian antimikrob, ujian sitotoksik dan ujian aktiviti larva telah dilakukan ke atas kesemua ekstrak dan sebahagian sebatian tulen.

Ekstrak dari *K. angustifolia* menghasilkan satu sebatian baru angustifolienol (58), bersama tujuh sebatian lain; kroteposida (33), β - sitosterol (44), boesenboksida (34), 2'-hidroksi-4, 4', 6'-trimetoksikalkon (36), 6-metoksizeylenol (59), sukrosa (60) dan zeylenol (35). Pemencilan ekstrak- ekstrak dari *K. rotunda* menghasilkan lima sebatian tulen, krotepoksida (33), asid tetrakosanoik (61), asid benzoik (62), stigmasterol (45) dan β - sitosterol (44). Pemencilan kedua- dua sebatian asid tetrakosanoik (61) dan asid benzoik (62) belum pernah dilaporkan sebelum ini. Ekstrak dari *S. articularis* menghasilkan dua sebatian tulen iaitu asid ursolik (63) dan stigmasterol (45), sementara ekstrak *S. exilis* menghasilkan empat sebatian tulen iaitu benzo[g]isokuinolin-5,10-dion (64), stigmasterol (45), asid heksadekanoik (65) and asid ursolik (63). Kerja pemencilan ke atas *S. exilis* tidak pernah dilaporkan sebelum ini.

Proses pengasetatan ke atas sebatian baru angustifolienol (58) menghasilkan dua sebatian, triasetoksiangustifolienol (66) dan diasetoksiangustifolienol (67). Kedua- dua sebatian disyorkan sebagai sebatian baru dan tidak pernah dilaporkan sama ada sebagai hasil semulajadi atau sebatian hasil sintesis.

Sebatian utama dari minyak pati *K. angustifolia* adalah DL- kamfor dengan hasil 20.57 %, sementara sebatian utama *K. rotunda* adalah benzil benzoat dengan hasil 49.84 %. Sebatian utama dari minyak pati *S. articularis* adalah fitol dengan hasil 10.31 %, sementara sebatian utama *S. exilis* adalah asid oleik dengan hasil 20.93 %.

Ujian sitotoksik menunjukkan kesemua ekstrak dari *K. angustifolia* dan *K. rotunda* tidak aktif terhadap sel HL-60. Walau bagaimanapun, sebatian tulen dari *K. angustifolia* adalah aktif. Semua ekstrak *S. articularis* dan *S. exilis* (kecuali ekstrak heksana *S. exilis*) dan asid ursolik (63) sangat aktif terhadap sel HL-60 dan MCF-7. Semua ekstrak dan kebanyakan sebatian tulen disubjek kepada empat spesies mikrob (MRSA, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*) dalam ujian anti- mikrob. Ekstrak petroleum eter dari kedua-dua spesis *Kaempferia* kesemua ekstrak dari spesis *Spermacoce* dan boesenboksida (34) adalah aktif terhadap sebilangan mikrob dalam aktiviti antimikrob. Dalam ujian antioksidasi, *K. rotunda* adalah lebih aktif berbanding *K. angustifolia* sementara kedua-dua spesis *Spermacoce* adalah sederhana aktif. Sebahagian daripada sebatian tulen dari *Kaempferia angustifolia* telah dijalani ujian antioksidasi dan menunjukkan aktiviti yang lebih kuat daripada aktiviti α -tokoferol. Keempat –empat tumbuhan menunjukkan keaktifan yang rendah ke sederhana dalam ujian aktiviti larva.

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I certify that an Examination Committee met on 24th January 2006 to conduct the final examination of Neoh Bee Keat on her Doctor of Philosophy Thesis entitled “Chemical Constituents and Biological Activity of *Kaempferia angustifolia*, *K. rotunda*, *Spermacoce articularis* and *S. exilis*” 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 own original work except for quotations and citations which have been duly acknowledgement. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



NEOH BEE KEAT

Date: 02/03/06

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