UNIVERSITI PUTRA MALAYSIA

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OILS FROM Goniothalamus AND Xylopia (ANNONACEAE)SPECIES

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By

SITI HUMEIRAH BINTI ABDUL GHANI

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

March 2012

In the name of ALLAH, the Beneficent, the Merciful

This thesis is dedicated to my beloved:

- Dad (Ab Ghani bin Sudin)
- Mom (Salmiza binti Ujang@ Baharin)
- Brothers (Mohd Qayyum & Zhafri)
- Supervisors (DR Puad, DR Muhajir, DR Nor Azah)
- Datuk DR Latiff, DR Rasadah, Pn. Wan Rahmah, Pn Liza Ismail, Pn Ruziah Ripin, Che Rohani and also all Staff of Natural Products Division, FRIM
- Friends
- Zulkifli Anuar (Ajoi)

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

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March 2012

Chairman : Associate Professor Mohd Puad Bin Abdullah, PhD

Faculty : Biotechnology and Biomolecular Sciences

The use of plant essential oil in daily care products, cosmetics and fragrances has increasingly gained attention in recent years. However, detailed studies on the oil especially in the aspects of chemical compositions, biological activity, safety and efficacy as a raw material for daily care products are limited thus limiting its commercial potential.

The essential oils of five plant species from the family Annonaceae, namely *Goniothalanus macrophyllus*, *G. tortilipetalus*, *Xylopia malayana*, *X. fusca* and *X. elliptica* were studied and their chemical compositions were analyzed by Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS). The leaf oil from *X. malayana* (1.39%) which was obtained from a water distillation produced the highest yield of essential oil. The chemical compositions of the essential oils from both

species were analyzed by comparing their Retention Indices (RI) with the literature values, by comparing their mass spectral data with the mass spectral database from the published or by co-chromatography with authentic samples. This is the first report on the chemical compositions, antimicrobial activities and cytotoxicity effects of the essential oils from *G. tortilipetalus, X. fusca* and *X. elliptica*. The chemical constituents of the oils differed from species to species but were mainly of monoterpenoids and sesquiterpenoids. A total of 239 components were identified, representing 42.5% to 91.6% of the total oils. The main monoterpenoids were α-pinene, linalool, terpinen-4-ol, 1,8-cineole and α-terpineol while geranyl acetate, geraniol, bicyclogermacrene and spathulenol are the major sesquiterpenoids identified from the oils.

The essential oils together with eight standards: α-pinene, linalool, geraniol, geranyl acetate, terpinen-4-ol, limonene, 1,8-cineole and β-pinene were evaluated for their antimicrobial properties against nine microorganisms including Gram positive and Gram negative bacteria, and yeast by using the broth microdilution method. The essential oils exhibited antimicrobial activities (MIC values between 156-2500 µg/ml) against all of the microorganisms tested, except for *Pseudomonas aeruginosa* and *Escherichia coli*. The standards tested exhibited weak antimicrobial activities (MIC values >5000 µg/ml) against all of the microorganisms tested except for α-pinene.

The cytotoxicity of the oils was evaluated against the human normal Chang liver and human normal skin fibroblast cell lines. The essential oils from the twig of *X. elliptica* and the leaf of *G. tortilipetalus* were found to be non-cytotoxic towards the Chang liver cell line even at higher concentrations (IC_{50} >1000 μg/ml). The other oils, however, exhibited moderate to weakly cytotoxic activity against both cell lines with the IC_{50} values ranged from 97.7 μg/ml to 877.0 μg/ml. The study revealed the potential use of the essential oils from ariel parts of *Xylopia* and *Goniothalamus* species as a new ingredient for various practical applications.

Key Word: Annonaceae; Essential oil; Antimicrobial activity; Cytotoxicity activity

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KOMPONEN KIMIA DAN AKTIVITI BIOLOGI TERHADAP MINYAK PATI DARI SPESIS *Xylopia* DAN *Goniothalamus* (ANNONACEAE)

Oleh

SITI HUMEIRAH BINTI ABDUL GHANI

Mac 2012

Pengerusi : Profesor Madya Mohd Puad Bin Abdullah, PhD

Fakulti :Bioteknologi dan Sains Biomolekul

Penggunaan minyak pati daripada tumbuhan beraroma dalam produk penjagaan harian, barangan kosmetik dan haruman semakin mendapat perhatian masyarakat kebelakangan ini. Namun demikian, kajian terperinci mengenai minyak pati khususya dari segi komposisi kimia, aktiviti biologi, keselamatan serta keberkesanannya sebagai bahan mentah dalam produk penjagaan harian adalah sangat sedikit sekaligus menghadkan penggunaannya secara komersial.

Minyak pati daripada lima spesis pokok keluarga Annonaceae iaitu *Goniothalamus macrophyllus, G. tortilipetalus, Xylopia malayana, X. fusca* dan *X. elliptica* telah dikaji dan komponen kimianya telah dianalisis menggunakan teknik Kromatografi Gas (KG) dan Kromatografi Gas/Spektrometer Jisim (KG/SJ). Proses penyulingan air terhadap sampel daun dari *X. malayana* telah menghasilkan peratusan minyak pati yang tertinggi iaitu sebanyak 1.39%. Komponen kimia minyak pati telah dikenal pasti melalui pengiraan dan perbandingan nilai Indeks Penahanan (RI) dengan nilai daripada literatur,

ko-kromatografi dengan sampel tulen serta perbandingan spekrum jisim yang diperolehi terhadap spekrum jisim rujukan. Hasil kandungan kimia, aktiviti antimikrob serta kesan sitoksiksiti daripada minyak pati sampel *G. tortilipetalus*, *X. elliptica* dan *X. fusca* adalah pertama kali dilaporkan dalam kajian ini. Analisis kimia menunjukkan bahawa komponen minyak pati kedua-dua spesis adalah berbeza dan terdiri daripada sebatian monoterpenoid dan seskuiterpenoid. Sebanyak 239 komponen telah dikenalpasti iaitu merangkumi sebanyak 42.5% hingga 91.6% daripada jumlah keseluruhan minyak pati yang dikenalpasti. Kumpulan monoterpena utama adalah α-pinena, linalol, terpinen-4-ol, 1,8-sineol dan α-terpineol manakala geranil asetat, geraniol, bisiklogermakrena dan spatulenol merupakan kumpulan sesquiterpena yang tertinggi dalam minyak yang dikaji.

Ujian antimikrob terhadap sembilan jenis mikroorganisma yang terdiri daripada bakteria Gram positif, Gram negatif dan yis telah dijalankan ke atas minyak pati serta lapan rujukan piawai iaitu α-pinena, linalol, geraniol, geranil asetat, terpinen-4-ol, limonena, 1,8-sineol, β-pinena melaui kaedah pencairan mikro berkaldu. Keputusan ujian antimikrob menunjukkan bahawa minyak pati mempunyai aktiviti perencatan (nilai MIC adalah diantara 156-2500 µg/ml) terhadap mikroorganisma yang diuji kecuali bakteria *Pseudomonas aeruginosa* dan *Escherichia coli*. Semua piawaian tunggal kecuali α-pinena menunjukkan aktiviti perencatan yang lemah (nilai MIC adalah >5000 µg/ml) terhadap semua mikroorganisma yang diuji.

Ujian sitotoksik telah dijalankan ke atas sel hati Chang dan sel kulit fibroblast. Minyak pati daripada ranting *X. elliptica* dan daun *G. tortilipetalus* didapati tidak menunjukkan kesan sitotoksik terhadap sel hati Chang walaupun pada tahap kepekatan yang tertinggi (IC_{50} >1000 µg/ml). Minyak pati daripada spesis yang lain menunjukkan kesan sitotoksik yang sederhana dan rendah terhadap kedua-dua sel yang diuji di mana nilai IC_{50} adalah secara meningkat iaitu dari 97.7 µg/ml hingga 877.0 µg/ml. Kajian ini menunjukkan bahawa minyak pati daripada spesis *Xylopia* dan *Goniothalamus* mempunyai potensi untuk diaplikasikan dalam menghasilkan barangan untuk kegunaan harian.

Kata Kunci: Annonaceae; Minyak Pati; Aktiviti Antimikrob; Aktiviti Sitotoksik

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I certify that a thesis Examination Committee has met on 5 Mac 2012 to conduct the final examination of Siti Humeirah Binti Abd Ghani on her Master Degree thesis entitled "Chemical compositions and biological activities of essential oils from *Goniothalamus* and *Xylopia* (Annonaceae) species" 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 Master of Science.

Members of the Examination Committee were as follows:

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Date:

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

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Date:

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 University Putra Malaysia or other institutions.

SITI HUMEIRAH BINTI ABDUL GHANI

Date: 5 March 2012

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