



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS FROM ANNONA MURICATA (LINN.)
AND GARCINIA MANGOSTANA (LINN.) AND THEIR BIOLOGICAL
ACTIVITIES**

SHAARI BIN DAUD.

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AND THEIR BIOLOGICAL ACTIVITIES**

By

SHAARI BIN DAUD

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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Chairman : Associate Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Chemical studies were carried out on *Annona muricata* L. (Annonaceae) and *Garcinia mangostana* L. (Guttiferae). The chemical investigations covered acetogenin, triterpenoids and xanthenes. These compounds were isolated using common chromatographic techniques and were identified by using spectroscopic technique such as NMR, MS, IR and UV.

Biological activities for both plants were also evaluated for their antimicrobial activities against four pathogenic bacteria and four fungi, cytotoxic activity against CEM-SS and HL-60 cell lines and larvicidal activity against *Aedes aegypti*. Larvicidal activity data have never been reported before.



Annona muricata L. (stem bark) have afforded one acetogenin, solamin and two triterpenoids, stigmasterol and sitosterol. Meanwhile studies on *Garcinia mangostana* L. (bark) have provided a new xanthone, mangosharin (2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone) together with six other xanthones, α -mangostin, β -mangostin, garcinone D, xanthone 1, 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone and mangostanol and one triterpenoid, friedelin.

Cytotoxic tests were performed using CEM-SS cell line and HL-60 cell line. The crude hexane extract of *Annona muricata* L. gave a significant activity with an IC₅₀ value of 0.8 μ g/ml against CEM-SS cell line while the crude ethyl acetate (EA) extract also gave a significant activity with an IC₅₀ value of 0.5 μ g/ml but against HL-60 cell line. The crude hexane extract of *Garcinia mangostana* L. is also considered to be active against the CEM-SS cell-line with an IC₅₀ value of 17 μ g/ml. Other than that, the pure compounds α -mangostin, mangostanol and garcinone D also gave significant activities with IC₅₀ values of 5.5, 9.6 and 3.2 μ g/ml against CEM-SS cell line, respectively.

The antimicrobial activity test was also carried out using four pathogenic bacteria, namely, Methicillin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*. However, most of the crude extracts gave only moderate or weak activity. The pure compound α -mangostin gave weak activity against *Salmonella typhimurium* and medium inhibition against *Bacillus subtilis*.



The larvicidal tests were performed against the larvae of *Aedes aegypti* using the WHO (1981) standard procedures with slight modifications. The crude hexane and ethyl acetate extracts of *Annona muricata L.* showed moderate toxicity towards the larvae by giving LC₅₀ values of less than 100 µg/ml. The crude hexane and ethanol extracts of *Garcinia mangostana L.* gave weak activities against the larvae with LC₅₀ values of more than 150 µg/ml while the crude ethyl acetate extract showed moderate activity with an LC₅₀ value of 30.1 µg/ml. Larvacidal activity on the pure compound, α-mangostin gave a LC₅₀ value of 19.4 µg/ml.

No activity was recorded for the antifungal activity tests.



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sebagai memenuhi keperluan untuk ijazah Master Sains

**KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA
ANNONA MURICATA (LINN.) DAN *GARCINIA MANGOSTANA (LINN.)***

Oleh

SHAARI BIN DAUD

April 2005

Pengerusi : Profesor Madya Gwendoline Ee Cheng Lian, PhD

Fakulti : Sains

Kajian kimia telah dijalankan terhadap *Annona muricata L.* (Annonaceae) dan *Garcinia mangostana L.* (Guttiferae). Kajian kimia terperinci merangkumi jenis sebatian seperti asetogenin, tritepenoid dan xanthone. Sebatian-sebatian ini diasingkan dengan menggunakan pelbagai teknik kromatografi dan dikenalpasti dengan menggunakan teknik spektroskopi seperti NMR, MS, IR dan UV.

Aktiviti biologi untuk kedua-dua tumbuhan juga dinilai untuk aktiviti microbial terhadap empat bacteria dan empat fungi, aktiviti sitotoksik terhadap sel CEM-SS dan sel HL-60 dan aktiviti larva terhadap *Aedes aegypti*. Data untuk aktiviti larva tidak pernah direkodkan sebelum ini.

Annona muricata L. (kulit batang) telah menghasilkan satu asetogenin, solamin dan dua tritepenoid, stigmasterol dan sitosterol. Sementara itu, kajian terhadap kulit pokok

Garcinia mangostana L. telah memberikan satu xanthone baru, mangoshaarin (2,8-dihydroxy-6-methoxy-5-(3-methylbut-2-enyl)-xanthone bersama dengan enam xanthone yang lain, α -mangostin, β -mangostin, garcinone D, xanthone 1, 1,6-dihydroxy-3,7-dimethoxy-2-(3-methylbut-2-enyl)-xanthone dan mangostanol dan satu triterpenoid, friedelin.

Ujian sitotoksik telah dijalankan dengan menggunakan sel CEM-SS dan HL-60. Ekstrak mentah heksana dari *Annona muricata L.* memberikan aktiviti yang kuat dengan nilai IC_{50} 0.8 $\mu\text{g/ml}$ terhadap sel CEM-SS manakala ekstrak mentah etil asetat juga menunjukkan aktiviti yang kuat dengan nilai IC_{50} 0.5 $\mu\text{g/ml}$ tetapi terhadap sel HL-60. Ekstrak heksana mentah dari *Garcinia mangostana L.* dianggap sebagai aktif terhadap sel CEM-SS dengan nilai IC_{50} 17 $\mu\text{g/ml}$. Selain daripada itu tiga sebatian tulen iaitu α -mangostin, mangostanol dan garcinone D menunjukkan aktiviti yang kuat dengan nilai IC_{50} 5.5, 9.6 dan 3.2 $\mu\text{g/ml}$ masing-masing terhadap sel CEM-SS.

Aktiviti antimikrob dijalankan dengan menggunakan empat jenis bakteria iaitu Methicillin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*. Bagaimanapun, kebanyakan ekstrak mentah yang di uji dengan bakteria-bakteria ini hanya memberikan keaktifan yang sederhana dan lemah. Sebatian tulen α -mangostin menunjukkan aktiviti yang lemah terhadap bakteria *Salmonella typhimurium* dan sederhana terhadap *Bacillus subtilis*.

Ujian larva telah dijalankan dengan menggunakan larva jenis *Aedes aegypti* mengikut prosedur-prosedur piawai WHO (1981) dengan sedikit pengubahsuaian. Ekstrak mentah heksana dan etil asetat daripada *Annona muricata L.* menunjukkan toksik yang sederhana terhadap larva dengan memberi nilai LC_{50} kurang daripada 100 $\mu\text{g/ml}$. Ekstrak mentah heksana dan etanol daripada *Garcinia mangostana L.* memberikan aktiviti yang lemah terhadap larva dengan nilai LC_{50} lebih daripada 150 $\mu\text{g/ml}$ manakala ekstrak mentah etil asetat menunjukkan toksik yang sederhana dengan nilai LC_{50} 30.1 $\mu\text{g/ml}$. Aktiviti larva pada sebatian tulen, α -mangostin memberi nilai LC_{50} 19.4 $\mu\text{g/ml}$.

Tiada sebarang aktiviti yang direkodkan untuk ujian antifungi.

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LIST OF ABBREVIATIONS

α	alpha
β	beta
δ	chemical shift in ppm
γ	gamma
μg	micro gram
br s	broad singlet
br t	broad triplet
^{13}C	carbon-13
CHCl_3	chloroform
CDCl_3	deuterated chloroform
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	dimethylsulfoxide
dt	doublet of triplet
EA	ethyl acetate
EIMS	Electron ionisation mass spectrometry
g	gram
GC	Gas Chromatography
GC-MS	Gas Chromatography- Mass spectrometry
^1H	proton



HETCOR	Heteronuclear Chemical Shift-correlation
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IC	Inhibition Concentration
IR	Infra Red
<i>J</i>	coupling constant in Hz
<i>L</i>	<i>LINN</i> .
l	litre
LC	Lethal Concentration
LD	Lethal Dose
m	multiplet
ml	mili litre
Me ₂ CO	acetone
MeOH	methanol
m.p.	melting point
MS	Mass Spectrum/Spectra/Spectrometer
NMR	Nuclear Magnetic Resonance
ppm	part per million
s	singlet
t	triplet
TLC	Thin Layer Chromatography



UV **Ultra Violet**

WHO **World Health Organization**

