



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERISATION OF COMPOUNDS FROM
'NAGA BUANA' (*PHYLLANTHUS PULCHER*) AND 'SIMILIT
MATINGGI' (*CASEARIA CAPITELLATA*) AND THEIR CYTOTOXIC
EFFECTS ON CANCER CELL LINES**

GURURAJ BAGALKOTKAR

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LINES**

By

GURURAJ BAGALKOTKAR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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Dedicated to My Dearest Parents



Abstract of 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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Phyllanthus pulcher from the family of Euphorbiaceae is used as a traditional medicine in Malaysia to treat stomachache and ulceration. *Casearia* species from the family of Flacourtiaceae are traditionally used as an antiseptic, cicatrizant and topical anesthetic agent. In a preliminary cytotoxic screening, *P. pulcher* and *C. capitellata* extracts were found to display remarkable activities against various *in vitro* tumour cell lines. On the basis of the screening results and literature review of the plants, *P. pulcher* and *C. capitellata* were selected for phytochemical investigations to identify compounds with cytotoxic activities.

The investigations of the active dichloromethane extract of aerial parts from *P. pulcher* by a combination of different chromatographic techniques led to the isolation of three new natural products, bis(6-methylheptyl)phthalate (**190**), phyllanthal-A (**192**) and 3,4-dihydroxy-5-methoxy-3',4',5'-trihydroxyoxepino-chromene-2-one (**196**) and five other known compounds including stigmast-5-en-3-ol-oleate (**189**), diisobutyl adipate (**195**), β -



sitosterol (**193**), 7-tridecanone (**194**) and β -sitosteryl- β -D-glucopyranoside (**191**). The isolation of stigmast-5en-3ol-oleate (**189**) is the first report from the genus *Phyllanthus*. All isolated compounds were tested for their cytotoxic activities against three human tumour cell lines: MCF-7 (breast), DU-145 (prostate) and H460 (lung). Compounds, **189**, **190**, **191** and **192** were found to exhibit significant antitumour activity against MCF-7 cells with the IC₅₀ values of 17.6, 69.2, 54.1 and 47.7 μ M, respectively. Among all the isolated compounds from *P. pulcher* aerial parts only the phyllanthal-A (**192**) exhibited cytotoxic activity against DU-145 cells with the IC₅₀ value of 20.5 μ M. Whereas all the isolated compounds from *P. pulcher* aerial parts failed to exhibit cytotoxic effect against H460 cells even at the highest concentration of 100 μ M.

Investigation on the active dichloromethane extract of *P. pulcher* roots resulted in the isolation of two new compounds, 12(13)-dehydro-3 α -acetoxyolean-28-oic acid (**199**) and lupanol acetate (**200**) and three other known compounds including 3 α -acetoxy-25-hydroxyolean-12-en-28-oic acid (**197**), glochidone (**198**) and glochidonol (**201**). 3 α -acetoxy-25-hydroxyolean-12-en-28-oic acid (**197**) which was isolated for the first time in this genus showed cytotoxic activity against MCF-7 and DU-145 cell lines. Whereas **201** exhibited potent antitumour activity against all the tested cell lines with the IC₅₀ values ranging 17.0 - 30.0 μ M. Among all the isolated compounds from *P. pulcher* only the **201** exhibited antitumour activity against H460 cells with the IC₅₀ value of 21.7 μ M.

Investigation on the bioactive phytochemicals of *C. capitellata* stem bark extracts yielded 7 α -acetoxy-hop-12(13)-en-11-one (**202**), 5-methoxy-7-hydroxycoumarin (**203**), 5-methoxy-7- β -D-glucopyranosylcoumarin (**204**), 5,7-dimethoxycoumarin (**205**), casearine-A (**206**) and casearine-B (**207**). The isolation of **206** and **207** has never been

reported from natural products before, whereas the isolation of **202**, **203**, **204**, and **205** is the first report from this genus. Among all the isolated compounds, **206** and **207** exhibited significant antitumour activity against MCF-7 and DU-145 cell lines with the IC₅₀ values ranging 5.0-14.0 μM. **206** and **207** also exhibited cytotoxic activity against H460 cells with the IC₅₀ values of 77.0 and 56.3 μM, respectively.

The structures of all isolated compounds were elucidated with the help of chemical and modern spectroscopic techniques (UV, IR, MS, ¹H NMR, ¹³C NMR, DEPT, COSY, HSQC, and HMBC).

In conclusion, some of these natural products, especially **189**, **192**, **201**, **206**, and **207**, are potential lead molecules for future antitumour studies to discover potential clinical candidates.



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

**PEMENCILAN DAN PENCIRIAN SEBATIAN DARIPADA ‘NAGA BUANA’
(*PHYLLANTHUS PULCHER*) DAN ‘SIMILIT MATINGGI’ (*CASEARIA
CAPITELLATA*) DAN KESAN SITOTOKSIK KEATAS SEL KANSER**

Oleh

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Phyllanthus pulcher berasal daripada famili Euphorbiaceae digunakan sebagai ubat tradisional di Malaysia untuk merawat sakit perut dan ulser. Spesies *Casearia* daripada famili Flacourtiaceae digunakan secara turun-temurun sebagai antiseptik, cicatrizant dan agen pelali (anestetik). Kajian awal sitotoksik mendapati ekstrak kasar *P. pulcher* dan *C. capitellata* boleh merencat aktiviti pertumbuhan perbagai titisan sel kanser. Oleh yang demikian, *P. pulcher* dan *C. capitellata* telah dipilih untuk kajian lanjut berdasarkan keputusan sitotoksik *in vitro* dan kajian.

Kajian terhadap ekstrak diklorometana *P. pulcher* dengan menggunakan kombinasi perbagai teknik kromatografi yang berbeza telah berjaya memencilkan tiga sebatian semulajadi yang baru: bis(6-metilheptil)phtalat (**190**), pillantal-A (**192**) dan 3,4-dihidroksi-5-metoksi-3',4',5'-trihidroksooxepino-kromene-2-one (**196**). Lima lagi sebatian yang pernah dipencilkan daripada alam semulajadi iaitu stigmast-5en-3ol-oleat



(**189**), diisobutil adipat (**195**), β -sitosterol (**193**), 7-tridekanon (**194**), β -sitosteril- β -D-glucopiranosid (**191**). Penemuan stigmast-5-en-3-ol-oleat (**P-1**) adalah buat kali pertama dalam genus *Phyllanthus*. Kesemua sebatian yang dipencilkan kemudian digunakan untuk penentuan aktiviti sitotoksik. **189**, **190**, **191** dan **192** telah menunjukkan keputusan yang signifikan terhadap titisan sel MCF-7 dengan nilai IC₅₀ 17.6, 69.2, 54.1 dan 47.7 μ M masing-masing. Daripada kesemua sebatian yang dipencilkan daripada bahagian aerial *P. pulcher*, hanya **192** sahaja yang memberi kesan sitotoksik terhadap DU-145 sel. Dengan nilai IC₅₀ 20.5 μ M manakala semua sebatian terpencil daripada bahagian aerial *P. Pulcher* gagal mempanerkan kesan sitotoksik terhadap sel H460 walaupun pada kepekatan 100 μ M.

Dalam penyelidikan fitokimia bahagian akar *P. pulcher* pula, ekstrak diklorometana telah berjaya memencilkan Dua (2) sebatian baru iaitu 12(13)-dehidro-3 α -acetoksiolean-28-oik asid (**199**) dan lupanol asetat (**200**). Manakala tiga sebatian yang pernah dilanjutkan diketahui iaitu 3 α -acetoksi-25-hidroksiolean-12-en-28-oik asid (**197**), glochidon (**198**) dan glochidonol (**201**) juga telah dijumpai dalam ekstrak ini. Penemuan 3 α -acetoksi-25-hidroksiolean-12-en-28-oik asid (**197**) merupakan buat kali pertama dalam genus ini dan didapati aktif dalam merencat pertumbuhan sel MCF-7 dan DU-145 sel. PR-5 adalah yang paling poten antara lima sebatian ini dimana nilainya adalah IC₅₀ 17.0 - 30.0 μ M.

Bagi pemencilan sebatian bioaktif daripada *C. Capitellta*, silica gel kolumn kromatografi digunakan untuk pemencilan 7 α -acetoksi-hop-12(13)-en-11-one (**202**), 5-metoksi-7-hidroksikoumarin (**203**), 5-metoksi-7- β -D-glukopiranosilkoumarin (**204**), 5,7-dimetoksikoumarin (**205**), kasearin-A (**206**) dan kasearin-B (**207**). Sebatian **206** dan **207** yang dipencilkan tidak pernah dilaporkan dalam penyelidikan hasilan semulajadi sebelum

ini. Manakala, penemuan **202**, **203**, **204**, dan **205** merupakan buat kali pertama dalam genus ini. Daripada kesemua sebatian yang dipencilkan, hanya **206** dan **207** memberikan aktiviti yang ketara terhadap ujian sitotoksik dengan nilai IC_{50} 5.0 - 14.0 μ M.

Struktur kesemua sebatian telah ditentukan dengan menggunakan teknik kimia dan spektroskopi moden ((UV, IR, MS, 1H NMR, ^{13}C NMR, DEPT, COSY, HSQC, dan HMBC).

Secara kesimpulannya, sebahagian daripada sebatian semulajadi ini, terutamanya **189**, **192**, **201**, **206**, dan **207** berpotensi dalam penyelidikan yang selanjutnya untuk penemuan ubat antikanser.

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I certify that an Examination Committee met on **14-02-2007** to conduct the final examination of Gururaj Bagalkotkar on his Doctor of Philosophy thesis entitled “Isolation and Characterization of Cytotoxic Antitumour Compounds from *Phyllanthus pulcher* and *Casearia capitellata*” 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 degree at UPM or other institutions.

GURURAJ BAGALKOTKAR

Date: 14 MAY 2007



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