

ABSTRACT

The study of phytochemical constituents on the bark of *Pseuduvaria Rugosa* from the family Annonaceae has resulted in the isolation and characterization of aporphine alkaloids, benzo[7]annulene and tripenoids. Six aporphine alkaloids have been isolated from the dichloromethane and methanol crude extract of which three of them are new compounds. The known alkaloids were liriodenine **1**, ouregidione **5**, and *N*-methylouregidione **11**, whereas the new alkaloids were 3-amino-1,2-dimethoxy-4,5-dioxoaporphine (pseuduvarine A) **36**, *N*-methyl-3-amino-1,2-dimethoxy-4,5-dioxoaporphine (pseuduvarine B) **37** and 3-hydroxy-1,2-dimethoxy-3,4-dioxoaporphine (pseuduvarine C) **38**. The aporphine alkaloids with a substituted amino group at position C-3 in ring A have never been discovered in any plant species before. One compound with the benzo[7]annulene skeleton has been isolated from the methanol extract in very miniscule amount. The structural elucidation of this compound was done based on the mass spectroscopy spectrum and from the fragmentation pattern, this compound was identified as 1-hydroxy-2,3,9-trimethoxy-9-methyl-5*H*-benzo[7]annulene-5,6,7,8,(9*H*)-tetraone or Pseuduvarin **39**. This compound is a novel compound and has never been isolated before. From the hexane extract, some of the compounds identified by GCMS were terpenoids and most of them were sesquiterpenes and diterpene including fatty acid esters. The major compounds found in the hexane extract were isoelemicin **40**, 1,2,3,4-tetramethoxy-5-(2-propenyl)benzene **41** and 3-hydroxy-1-propenyl-2-methoxyphenol **42**. The other compounds are elemicin **24**, spathulenol **29** and caryophyllene oxide **30**. The sterol compounds found were stigmasterol **43** and gamma sitosterol **44**. This thesis also investigated the antioxidant and cytotoxic activities of the crude extracts and isolated compounds from *Pseuduvaria Rugosa*. The crude extracts

and isolated compounds with sufficient quantities were tested for their radical scavenging activity using DPPH method and cytotoxic activity against selected cancer cell lines using the MTS and MTT assay. Preliminary bioactivity screening showed that the hexane, dichloromethane and methanol extracts of *Pseuduvaria rugosa* displayed weak to moderate activities in the radical scavenging activity but exhibited significant cytotoxic activities against seven cancer cell lines with more than 80% of cell death at 100 µg/ml. Ouregidione, *N*-methylouregidione and pseudovarine C showed moderate cytotoxic activity on seven cancer cell lines. Pseudovarine A and pseudovarine B showed potent cytotoxicity against human cancer cell lines, MCF7 (IC₅₀ : 0.9 µM, >50.0 µM), HepG2 (IC₅₀: 21.7 µM, 15.7 µM) and HL-60 cells (IC₅₀ : >50 µM, 12.4 µM).

ABSTRAK

Kajian terhadap kandungan fitokimia ke atas bahagian batang dari pokok *Pseuduvaria rugosa* dari family Annonacea telah menghasilkan sebatian alkaloid dari aporphine, benzo[7]annulena and tripenoid. Sebanyak enam sebatian alkaloid telah diasingkan dari ekstrak diklorometana dan methanol dimana tiga adalah sebatian baru. Sebatian alkaloid yang sedia dikenalpasti adalah liriodenina **1**, ouregidion **5** dan *N*-metil ouregidion **11** manakala tiga sebatian alkaloid yang baru adalah 3-amino-1,2-dimetoksi-4,5-dioksoaporfina (pseuduvarina A) **36**, *N*-metil-3-amino-1,2-dimetoksi-4,5-dioksoaporfina (pseuduvarina B) **37** and 3-hidroksi-1,2-dimetoksi-3,4-dioksoaporfina (pseuduvarina C) **38** . Sebatian alkaloid aporphina dengan kumpulan amino di C-3 dalam ring A belum pernah ditemui dari mana-mana tumbuhan. Satu lagi sebatian telah diasingkan dengan jumlah yang sangat kecil . Pengenalan pasti struktur sebatian tersebut dibuat berdasarkan spektroskopi spectrum jisim dan paten fragmentasi , sebatian ini dikenali sebagai 1-hidroksi-2,3,9-trimetoksi-9-metil-5*H*-benzo[7]annulena-5,6,7,8,(9*H*)-tetraon atau pseuvarin **39**. Sebatian ini merupakan sebatian baru yang belum pernah ditemui. Daripada ekstrak heksana, sebatian yang dikenalpasti melalui GCMS adalah terpenoid, seskuiterpena dan diterpena termasuk asid lemak. Sebatian utama dalam ekstrak heksana adalah isoelemicin **40**, 1,2,3,4-tetrametoksi-5-(2-propenil)benzena **41** and 3-hidroksi-1-propenil-2-metoksifenol **42**. Sebatian lain adalah elemicin **24**, spathulenol **29** and cariofyllena oksida **30**. Sebatian sterol adalah stigmasterol **43** dan γ -sitosterol **44**. Thesis ini juga mengkaji aktiviti antioksidan dan sitotoksik dari ekstrak dan sebatian yang diasingkan dari *Pseuduvaria rugosa* dengan menggunakan kaedah DPPH , MTS dan MTT assay. Saringan awal menunjukkan ekstrak heksana, diklorometana dan metanol dari *Pseuduvaria rugosa* menunjukkan aktiviti tidak aktif dan sederhana aktif dalam aktiviti antioksidan tetapi menunjukkan

aktiviti yang kuat ke atas tujuh sel kanser dengan menyebabkan kematian sel lebih daripada 80% pada kepekatan 100 $\mu\text{g/mL}$. Ouregidion, *N*-metilouregidion dan pseuduvarina C menunjukkan keaktifan sitotoksik yang sederhana ke atas tujuh sel kanser. Pseuduvarina A dan pseuduvarina B menunjukkan keaktifan sitotoksi yang tinggi ke atas sel kanser MCF7 (IC50 : 0.9 μM , >50.0 μM), HepG2 (IC50: 21.7 μM , 15.7 μM) and HL-60 cells (IC50 : >50 μM , 12.4 μM).

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ABBREVIATION

CC	Column Chromatography
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
CDCl ₃	Deuterated chloroform
CHCl ₃	Chloroform
CD ₃ OH	Deuterated methanol
CH ₂ CL ₂	Dichloromethane
MeOH/CH ₃ OH	Methanol
CH ₃ COCH ₃	Acetone
HCl	Hydrochloric acid
NH ₃	Ammonia
CH ₃	Methyl group
C ₆ H ₁₂	Hexane
COSY	H-H Correlation Spectroscopy
δ	Delta value (chemical shift) in ppm
d	Doublet
dd	Doublet of doublet
dt	Doublet of triplet
ESI	Electrospray Ionization
FT-NMR	Fourier Transform NMR
g	Gram
GCMS	Gas Chromatogram-Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation

HSQC	Heteronuclear Single Quantum Correlation
Hz	Hertz
IR	Infrared
J	Coupling Constant (Hz)
λ_{\max}	Maximum wavelength
m	Metre
m/z	Mass to charge ratio
MHz	Mega Hertz
MS	Mass Spectrum
ml	Mililitre
mg/ml ⁻¹	Microgram per mililitre
nm	Nanometer
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
OCH ₃	Methoxyl group
OCH ₂ O	Methylenedioxy group
OH	Hydroxyl group
¹ H	Proton
¹³ C	13-Carbon
ppm	Parts per million
1D NMR	One dimensional NMR
2D NMR	Two-dimensional NMR
UV	Ultraviolet
IC ₅₀	Median inhibition concentration
OH	Hydroxyl
S	singlet

HPLC

High Performance liquid
Chromatography

COSY

^1H - ^1H Correlation Spectroscopy