

ISOLATION AND BIOACTIVITY SCREENING OF
CHEMICAL CONSTITUENTS EXTRACTED
FROM *Gracilaria changii*

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Chairperson : Dr. Habsah Mohamed, Ph.D.
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Faculty : Science and Technology

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Gracilaria changii has been used to obtain the ethanol, diethyl ether, ethyl acetate, butanol extracts and polysaccharide. In this research, isolation and separation of chemical compounds have been carried out by various solvents and chromatographic methods. The structures of these pure compounds have been determined by various spectroscopic methods such as infrared (IR), ultra-violet (UV), nuclear magnetic resonance (^1H , ^{13}C and 2D NMR), mass spectrometry (MS) and by comparison with the data reported previously. The crude extracts, isolated pure compounds and synthetic compounds were subjected to the biological activities screening test such as antitumor, antibacterial, cytotoxic assay and TLC bioassay method for acetylcholinesterase inhibition assay.

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**ISOLATION AND BIOACTIVITY SCREENING OF CHEMICAL
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Gracilaria changii has been extracted and fractionated to obtain the ethanol, diethyl ether, ethyl acetate, buthanol extracts and polysaccharide. In this research, isolation and separation of chemical compounds have been carried out by various solvent and chromatographic methods. The structure of these pure compounds have been determined by various spectroscopic methods such as infrared (IR), ultra-violet (UV), nuclear magnetic resonance (^1H , ^{13}C and 2D NMR), mass spectroscopy (MS) and by comparison with the data reported previously. The crude extracts, isolated pure compounds and synthetic compounds were subjected to the biological activities screening test such as antioxidant, antibacterial, cytotoxic assay and TLC bioautographic method for acetylcholinesterase inhibition assay.

Four pure compounds have been isolated form the diethyl ether extract and was identified as cholest-5-en-3-ol, hexadecanoic acid, 2-hydroxypentadecanoic acid and cholesteryl myristate.

Cholesterol derivatives have been synthesized to study the biological activity. Three synthetic compounds were $5\beta,6\beta$ -epoxycholesterol, cholestan- $3\beta,5\alpha,6\beta$ -triol and cholestan-6-oxo-3,5-diol. The structures were determined by spectroscopic methods.

In antioxidant assay, all crude extracts, pure and synthetic compounds were not active. Almost all of the crude extracts, pure and synthetic compounds showed moderate activity in antibacterial assay except polysaccharide and one of the synthetic compound which is $5\beta,6\beta$ -epoxycholesterol. In cytotoxicity screening, diethyl ether extract showed moderate activity against HL 60 with $IC_{50} 27 \pm 5.2$ mg/mL. Buthanol extract also showed moderate activity against HL60 and MCF 7 cancer cell line with the $IC_{50} 21 \pm 6.5$ mg/mL and 28 ± 4.61 mg/mL respectively. Hexadecanoic acid isolated from diethyl ether extract also demonstrated the moderate activity with IC_{50} 0.082 μ M and 0.089 μ M. The synthetic compounds gave positive result in the cytotoxicity assay, which are cholestan- $3\beta,5\alpha,6\beta$ -triol and cholestan-6-oxo-3,5-diol with strong activity against both cancer cell line. The IC_{50} for cholestan- $3\beta,5\alpha,6\beta$ -triol against HL60 and MCF 7 cancer cell line are 0.005 μ M and 0.001 μ M respectively and the IC_{50} for cholestan-6-oxo-3,5-diol are 0.001 μ M and 0.005 μ M respectively. $5\beta,6\beta$ -epoxycholesterol showed moderate activity with IC_{50} 0.025 μ M and 0.035 μ M. In the TLC bioautographic method for acetylcholinesterase inhibition assay, methanol and diethyl ether extracts showed positive results. Among the pure compounds that exhibited positive results were hexadecanoic acid and 2-hydroxypentadecanoic acid. Only one synthetic compounds, $5\beta,6\beta$ -epoxycholesterol was weakly active in this assay.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk ijazah Sarjana Sains

**PEMENCILAN AND PENCERAKINAN BIOAKTIVITI SEBATIAN KIMIA
YANG DIEKSTRAK DARIPADA *Gracilaria changii***

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Gracilaria changii telah diekstrak dan difraksikan untuk mendapatkan ekstrak methanol, dietil eter, etil asetat, butanol dan polisakarida. Dalam kajian ini, pemisahan dan pemencilan sebatian kimia dilakukan dengan menggunakan pelbagai pelarut dan kaedah kromatografi. Struktur sebatian tulen telah ditentukan dengan menggunakan beberapa kaedah spektroskopi seperti inframerah (IR), resonan magnet nukleus (^1H , ^{13}C dan 2D NMR), spektroskopi jisim (MS) dan perbandingan dengan data yang telah dilaporkan. Ekstrak mentah, sebatian tulen dan sebatian sintetik telah diuji aktiviti biologi seperti antioksidan, antibakteria, aktiviti kesitotoksikan dan kaedah bioautografik KLN untuk aktiviti perencatan enzim asetilkolinesterase.

Empat sebatian tulen telah dipencilkan daripada ekstrak dietil eter dan telah dikenalpastikan sebagai koles-5-en-3-ol, asid heksadekanoik, asid 2-hidroksipentadekanoik dan kolesteril miristat.

Terbitan kolesterol telah disintesis untuk ujian aktiviti biologi. Tiga sebatian sintetik ialah $5\beta,6\beta$ -epoksikolesterol, kolestan- $3\beta,5\alpha,6\beta$ -triol dan kolestan-6-oxo-3,5-diol. Struktur sebatian dikenapasti dengan menggunakan kaedah spektroskopi.

Bagi ujian antioksidan, kesemua ekstrak mentah, sebatian tulen dan sebatian sintetik adalah tidak aktif. Hampir kesemua ekstrak mentah, sebatian tulen dan sebatian sintetik sederhana aktif bagi antibakteria kecuali polisakarida dan salah satu sebatian sintetik, iaitu $5\beta,6\beta$ -epoxycholesterol.

Bagi aktiviti kesitosikan, ekstrak dietil eter menunjukkan aktiviti terhadap titisan sel kanser HL 60 dengan nilai $IC_{50} 27 \pm 5.2$ mg/mL. Ekstrak butanol juga menunjukkan aktiviti sederhana terhadap titisan sel kanser HL 60 dan MCF 7 dengan nilai $IC_{50} 21 \pm 6.5$ mg/mL dan 28 ± 4.61 mg/mL. Asid heksadekanoik yang dipencilkan daripada ekstrak dietil eter menunjukkan aktiviti sederhana terhadap kedua-dua titisan sel dengan nilai $IC_{50} 0.082 \mu\text{M}$ and $0.089 \mu\text{M}$. Sebatian sintetik menunjukkan keputusan positif bagi aktiviti ketoksikan. Kolestan- $3\beta,5\alpha,6\beta$ -triol dan kolestan-6-oxo-3,5-diol menunjukkan aktiviti yang tinggi terhadap kedua-dua titisan sel kanser. Nilai IC_{50} bagi kolestan- $3\beta,5\alpha,6\beta$ -triol terhadap HL60 dan MCF 7 masing-masing ialah $0.005 \mu\text{M}$ and $0.0012 \mu\text{M}$ dan nilai IC_{50} bagi kolestan-6-oxo-3,5-diol ialah $0.001 \mu\text{M}$ and $0.005 \mu\text{M}$. $5\beta,6\beta$ -epoksikolesterol menunjukkan aktiviti sederhana dengan nilai $IC_{50} 0.025 \mu\text{M}$ and $0.035 \mu\text{M}$. Bagi ujian kaedah bioautografik KLN untuk perencatan enzim asetilkolinesterase, ekstrak methanol dan dietil eter menunjukkan aktiviti yang positif. Di antara sebatian tulen yang menunjukkan keputusan positif ialah asid heksadekanoik dan asid 2-hidroksipentadekanoik. Cuma satu sebatian sintetik iaitu $5\beta,6\beta$ -epoxycholesterol adalah sederhana aktif bagi ujian ini.