CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF A LOCALLY GROWN *GANODERMA* SPECIES

by

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TO MY PARENTS

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ABSTRACT

Two locally grown *Ganoderma* species obtained from a mushroom farm in Semenyih, Malaysia were tentatively identified as *G. tsugae* and *G. lucidum* based on the morphology of basidiospores and cultural characteristics. Basidiospores of *G. tsugae* were 'rough' walled and had broad inter-wall pillars. Isolates of *G. tsugae* do not produce chlamydospores or primordia in culture and had an average growth rate of 6.8 mm/day at the optimal temperature range of 33-37°C.

In contrast, the basidiospores of *G lucidum* appeared 'smooth' walled, characterized by narrow, numerous inter-wall pillars. Isolates of *G. lucidum* produce chlamydospores and primordia in culture and had a higher average growth rate of 11.8 mm/day at a lower optimal temperature range between 28-33°C as compared to *G. tsugae*.

The fruit body of *G. tsugae* was investigated for its phytochemical content. Extraction followed by repeat chromatography of the hexane and methanol crude extracts resulted in the isolation of four compounds. The elucidation of structures were carried out by various spectroscopic methods.

Terpenoids content were found to be present in the highest levels, followed by alkaloids, saponins and flavonoids. Chemically guided fractionations based on TLC patterns of terpenoids allowed four compounds (GM 1.1, GM 2.2, GM 3.6.5 and

GM4.3) to be isolated from the hexane crude extract. These compounds were identified as stellasterol (GM 3.6.5), ergosterol (GM 4.3), di-(2-ethylhexyl) phthalate (DEHP) and a new brominated ergosta-type sterol (GM 1.1)

All the fractions collected from chromatography of both hexane and methanol crude, together with the four isolated compounds were used to test for antibacterial and antifungal activity. Using 6mm-paper disc diffusion method, all fractions showed weak (7.0 to 8.9 mm) to moderate (9.0 to 10.9 mm) activity against one or more of the test microorganisms. *Candida albicans* was the most susceptible organism, with 35% of the fractions showing moderate activity against this opportunistic pathogen.

DEHP showed a moderate broad-spectrum inhibition against the Gram-positive bacteria, *Bacillus cereus* and *Bacillus subtilis* and two fungi, *Candida albicans* and *Schizosaccharomyces pombe*. The other three ergosta-type sterols only exhibited mild activity against one or more of the test microorganism. Based on the results obtained, the occurrences of the isolated compounds were discussed.

ABSTRAK

Dua spesies *Ganoderma* tempatan yang diperolehi daripada sebuah ladang cendawan di Semenyih, Malaysia telah dikenalpasti secara tentatif sebagai *Ganoderma tsugae* and *Ganoderma lucidum* berdasarkan morfologi basidiospora dan ciri-ciri kultur.

Dinding basidiospora *G. tsugae* adalah 'kasar' dan mempunyai 'inter-wall pillar' yang lebar. Pencilan *G. tsugae* juga didapati tidak menghasilkan klamidospora atau 'primordia' dalam kultur dan mempunyai purata kadar pertumbuhan 6.8 mm/hari pada suhu optimum 33-37°C.

Sebaliknya, dinding basidiospora *G. lucidum* adalah 'licin' dan mempunyai 'inter-wall pillar' yang sempit. Kultur pencilan *G. lucidum* juga menghasilkan klamidospora dan 'primordia', dan mempunyai purata kadar pertumbuhan yang lebih tinggi, iaitu 11.8 mm/hari pada suhu optimum yang lebih rendah, iaitu 28-33°C berbanding dengan *G. tsugae*.

Jasad buah *G. tsugae* digunakan untuk pengajian kandungan fitokimia. Ekstraksi dengan heksana dan metanol, diikuti oleh kromatografi berulang, berjaya menyaringkan empat sebatian. Struktur keempat-empat sebatian ini dikenalpasti dengan kaedah-kaedah spektroskopi.

Kandungan 'terpenoids' adalah tertinggi diikuti 'alkaloids', 'saponins' dan 'flavonoids'. Empat sebatian yang disaringkan daripada ekstrak heksana dikenalpastikan sebagai 'sellasterol' (GM 3.6.5), 'ergosterol' (GM 4.3), 'di-(2-ethylhexyl) phthlate' (DEHP) dan satu sebatian baru yang merupakan 'brominated sterol'.

Semua fraksi-fraksi dan kempat-empat sebatian yang disaringkan digunakan untuk menguji activiti antibakteria dan antikulat. Dengan menggunakan kaedah difusi kertas 6 mm, semua fraksi menunjukkan aktiviti lemah (7.0 – 8.9mm) sehingga sederhana (9.0 – 10.9 mm) terhadap satu atau lebih mikroorganisma yang diuji. *Candida albicans* merupakan organisma yang paling sensitif, dengan 35% daripada fraksi-fraksi menunjukkan activiti yang serdahana terhadap patogen oportunis ini.

DEHP pula menunjukkan aktiviti spektrum luas terhadap perencatan bacteria Gram-positif, *Bacillus cereus* dan *Bacillus subtilis*, dan dua kulat, *Candida albicans* dan *Schizosaccharomycetes pombe*. Tiga sterol yang lain pula hanya menunjukkan activiti yang lemah terhadap satu atau lebih mikroorganisma yang diujikan. Berdasarkan keputusan yang didapati, sebatian-sebatian yang wujud dibincangkan.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	percentage
aq	aqueous
$CDCL_3$	deuterated chloroform
$CHCl_3$	chloroform
conc	concentrated
COSY	Correlation Spectroscopy (homonuclear ^1H-^1H correlation spectroscopy)
d	doublet
dd	double doublet
DEPT	Distortionless Enhancement by Polarisation Transfer
EIMS	electron impact mass spectrum
g	gram
HETCHOR	Heteronuclear Correlation
Hex	Hexane
m	multiplet
MS	Mass Spectroscopy
m/z	mass/ charge
MeOH	methanol
mg	milligram
MHz	megahertz
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
q	quartet
s	singlet
TLC	Thin Layer Chromatography
TMS	trimethylsilane
vol	volume
w/w	weight/ weight