

IDENTIFICATION OF COMPONENTS IN EXTRACTS  
OF *HERICIUM ERINACEUS* (BULL.: FR.) PERS THAT  
STIMULATE *IN VITRO* NEURITE OUTGROWTH OF  
NG108-15

WONG YUIN TENG

FACULTY OF SCIENCE  
UNIVERSITY OF MALAYA  
KUALA LUMPUR

2012

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NG108-15**

**WONG YUIN TENG**

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## ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: Wong Yui Teng (I.C/Passport No: 840601-05-5384 )

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Identification of components in extracts of *Hericium erinaceus* (Bull.: Fr.) Pers that stimulate *in vitro* neurite outgrowth of NG108-15

Field of Study: Mushroom Nutraceutical

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## ABSTRACT

*Hericium erinaceus*, locally known as cauliflower mushroom, and elsewhere as lion's mane mushroom, Houtou (monkey head mushroom), Yamabushitake and Harisenbon (balloon fish), is an edible mushroom. It is well known for its medicinal and nutritional values. *Hericium erinaceus* is reported to have good anti-tumor properties and nerve tonic effects. Although *H. erinaceus* is a temperate mushroom, it has been successfully cultivated in Malaysia. However, there are very few reported studies on the chemical constituents that stimulate the neurite outgrowth for the locally cultivated species.

The crude aqueous ethanol extract of *H. erinaceus* and its fractionated extracts (hexane, ethyl acetate and water) were evaluated for their effect in stimulating the neurite outgrowth using neural cell line NG108-15 whilst the Nerve Growth Factor (NGF) was used as the positive standard. The crude aqueous ethanol extract of *H. erinaceus* showed 15.0 % increase in neurite outgrowth at the concentration of 10.0 µg/ml. However, the crude aqueous ethanol extract showed decreased neurite growth as the dose was increased. The hexane, ethyl acetate and water fractions showed an increase in neurite outgrowth when the dose was increased exponentially (10.0, 25.0, 50.0 and 100.0 µg/ml). Maximum stimulation of neurite outgrowth was recorded with ethyl acetate fraction with 68.5 % increase compared to negative control followed by hexane fraction with 65.2 % increase.

The combined fraction of hexane and ethyl acetate was further subjected to flash column chromatography. Among the 7 isolated fractions (fraction E1-E7), fraction E1 and fraction E2 show relatively higher neurite stimulation activity compared to other fractions. Maximum stimulation was recorded as 160.6 % increase and 149.1 %

increase compared to negative control at the concentration of 100 µg/ml for fraction E1 and fraction E2 respectively.

The chemical compositions of the fraction E1 of *H. erinaceus* were analyzed by GCMS. Four components were identified from fraction E1 comprising about 80.5 % of the total. Fraction E1 was made up of ethyl palmitate (29.8 %), ethyl stearate (2.3 %), ethyl oleate (18.6 %) and ethyl linoleate (29.9 %). Further isolation of fraction E2 using preparative TLC and HPLC gave subfraction sub4b\_4 and subfraction sub4b\_6. Subfraction sub4b\_4 showed better neurite stimulation activity compared to subfraction sub4b\_6 with 187.1 % increase in comparison.

The chemical compositions of subfraction sub4b\_4 and sub4b\_6 were analyzed by NMR and LC/MS/MS. The components identified from subfraction sub4b\_4 were hericenone C (and its isomer) and 4-(3',7'-dimethyl-5'-oxo-2',6'-octadienyl)-2-formyl-3-hydroxy-5-methoxybenzyl oleate (and its isomer). On the other hand, subfraction sub4b\_6 comprised of hericenone C, 4-(3',7'-dimethyl-5'-oxo-2',6'-octadienyl)-2-formyl-3-hydroxy-5-methoxybenzyl oleate and a phenolic component attached to the fatty ester side chain contained 26 carbons with 3 double bonds.

## ABSTRAK

*Hericium erinaceus*, dikenali sebagai cendawan bunga kobis di Malaysia dan cendawan “lion’s mane”, Houtou (cendawan kepala monyet), Yamabushitake dan Harisenbon di tempat lain. Cendawan ini boleh dimakan dan ia terkenal dari segi nilai perubatan dan nutrisi. Kebelakangan ini, laporan saintifik menunjukkan *H. erinaceus* mempunyai nilai anti-tumor yang baik dan sebagai tonik terhadap saraf. Walaupun *H. erinaceus* merupakan cendawan yang ditanam di kawasan sederhana tetapi kini berjaya ditanam di Malaysia yang beriklim tropika. Walaubagaimanapun, tidak banyak terbitan laporan tempatan yang melaporkan tentang komposisi kimia dalam *H.erinaceus* yang ditanam secara tempatan dalam rangsangan pertumbuhan saraf.

Ekstrak mentah akueus etanol dan fraksi-fraksi (heksana, etil asetat dan air) dari *H.erinaceus* telah diselidik dalam rangsangan pertumbuhan saraf pada sel saraf NG108-15 dan NGF digunakan sebagai kawalan positif. Ekstrak mentah akueus ethanol menunjukkan peningkatan sebanyak 15.0 % dalam pertumbuhan saraf pada kepekatan 10.0 µg/ml. Walaubagaimanapun, peningkatan kepekatan ekstrak mentah akueus ethanol akan menyebabkan penurunan dalam pertumbuhan saraf. Fraksi hexana, etil asetat dan air akan menyebabkan peningkatan dalam pertumbuhan saraf apabila kepekatan fraksi-fraksi ditingkatkan secara eksponen (10.0, 25.0, 50.0 and 100.0 µg/ml). Pertumbuhan maksimum saraf direkodkan oleh fraksi etil asetat dengan 68.5 % peningkatan berbanding dengan kawalan negatif dan diikuti oleh fraksi hexana dengan 65.2 % peningkatan berbanding dengan kawalan negatif.

Pengasingan komponen daripada gabungan fraksi heksana dal etil asetat menggunakan kaedah kromatografi kolum kilat menghasilkan 7 fraksi (fraksi E1-E7). Fraksi E1 dan E2 menunjukkan aktiviti pertumbuhan saraf yang lebih tinggi jika berbanding dengan fraksi-fraksi lain. Peningkatan pertumbuhan maksimum sebanyak

160.6 % dan 149.1 % direkodkan oleh fraksi E1 dan E2 pada kepekatan 100µg/ml berbanding kawalan negatif.

Komposisi kimia fraksi E1 bagi *H. erinaceus* dianalisis dengan menggunakan GCMS. Empat komponen telah dikenalpasti daripada fraksi E1 dan komponen-komponen tersebut adalah terdiri daripada 80.5 % daripada keseluruhan fraksi E1. Komponen yang terkandung dalam fraksi E1 adalah etil palmitat, etil stearat, etil oleat dan etil linoleat. Subfraksi sub4b\_4 dan subfraksi sub4b\_6 adalah hasil daripada isolasi fraksi E2 dengan menggunakan preparatif TLC dan HPLC. Subfraksi sub4b\_4 menunjukkan aktiviti pertumbuhan saraf yang lebih baik daripada subfraksi sub4b\_6 iaitu 187.1 % peningkatan pada kepekatan 100 µg/ml jika dibandingkan dengan kawalan negatif.

Komposisi kimia subfraksi sub4b\_4 dan sub4b\_6 dianalisis dengan menggunakan NMR dan LC/MS/MS. Komponen-komponen yang dikenalpasti daripada subfraksi sub4b\_4 termasuk hericenone C (dan isomernya) dan 4-(3',7'-dimetil-5'-oxo-2',6'-octadienil)-2-formil-3-hidroksi-5-metoksibenzil oleat (dan isomernya). Identifikasi subfraksi sub4b\_6 menunjukkan kehadiran hericenone C, 4-(3',7'-dimetil-5'-oxo-2',6'-octadienil)-2-formil-3-hidroksi-5-metoksibenzil oleat dan satu komponen fenolik yang mengandungi rantai ester asid lemak yang mempunyai 26 karbon dan 3 ikatan dubel.

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## LIST OF SYMBOLS AND ABBREVIATIONS

Ac	Acetone
AD	Alzheimer's disease
ADFM	Alzheimer's Disease Foundation Malaysia
ADI	Alzheimer's Disease International
$\alpha$	Alpha
ANOVA	Analysis of variance
ApoE4	Apolipoprotein E4
ATCC	American Tissue Culture Collection
$\beta$	beta
$\text{Ca}^{2+}$	Calcium ion
$\text{CO}_2$	Carbon dioxide
$\text{CHCl}_3$	Chloroform
cm	Centimeter
$^{\circ}\text{C}$	Degree celcius
$\text{CDCl}_3$	Deuterated chloroform
Da	Dalton
DLPE	Dilinoleoyl-phosphatidylethanolamine
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle's Medium
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
GC-MS	Gas Chromatography-Mass Spectroscopy
g	Gram
g/l	Gram per litre
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA

HPLC	High-performance liquid chromatography
hr	Hour
HIV	Human immunodeficiency virus
HCl	Hydrochloric acid
HAT	Hypoxanthine- aminopterine- thymidine
kg	Kilogram
$\lambda$	Lambda
<	Less than
LC/MS/MS	Liquid chromatography-mass spectrometry
L	Litre
LDL	Low-density lipoprotein
m/z	Mass-to-charge ratio
MHz	Megahertz
mRNA	Messenger RNA
MeOH	Methanol
$\mu\text{g/ml}$	Microgram per millilitre
$\mu\text{M}$	Micromolar
$\text{mg/ml}$	Miligram per millilitre
ml	Mililitre
mm	Milimetre
min	Minute
$\text{ng/ml}$	Nanogram per millilitre
nm	Nanometer
NGF	Nerve Growth Factor
NO	Nitric oxide
N	Normality

NMR	Nuclear magnetic resonance spectroscopy
%	Percentage
PTFE	Polytetrafluoroethylene
$\text{KH}_2\text{PO}_4$	Potassium hydrogen phosphate
psi	Pounds per square inch
$\pm$	Plus-minus
RP	Reverse phase
rpm	Rotation per minute
$\text{Na}_2\text{HPO}_4$	Disodium hydrogen orthophosphate
$\text{NaHCO}_3$	Sodium bicarbonate
$\text{NaCl}$	Sodium chloride
$\text{NaOH}$	Sodium hydroxide
$\text{Na}^+$	Sodium ion
TMS	Tetramethylsilane
TLC	Thin layer chromatography
USP-NF	The United States Pharmacopeia–National Formulary
UPLC	Ultra pure liquid chromatography
UV	Ultraviolet
v/v	Volume per volume
w/v	weight per volume