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

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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 analayzed 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-methoxylbenzyl 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-methoxylbenzyl 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 termpatan 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
α	Alpha
ANOVA	Analysis of variance
ApoE4	Apolipoprotein E4
ATCC	American Tissue Culture Collection
β	beta
Ca^{2+}	Calcium ion
CO_2	Carbon dioxide
$CHCl_3$	Chloroform
cm	Centimeter
° C	Degree celcius
$CDCl_3$	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
λ	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
µg/ml	Microgram per mililitre
µM	Micromolar
mg/ml	Miligram per mililitre
ml	Mililitre
mm	Milimetre
min	Minute
ng/ml	Nanogram per mililitre
nm	Nanometer
NGF	Nerve Growth Factor
NO	Nitric oxide
N	Normality

NMR	Nuclear magnetic resonance spectroscopy
%	Percentage
PTFE	Polytetrafluoroethylene
KH_2PO_4	Potassium hydrogen phosphate
psi	Pounds per square inch
±	Plus-minus
RP	Reverse phase
rpm	Rotation per minute
Na_2HPO_4	Disodium hydrogen orthophosphate
$NaHCO_3$	Sodium bicarbonate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
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