COMMERCIALIZATION OF *HERICIUM* AND EVALUATION OF ITS BIOACTIVE COMPOUNDS

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DOCTOR OF PHILOSOPHY
IN
BIOLOGICAL SCIENCE

SCHOOL OF SCIENCE
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Didsanutda Gonkhom

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ABSTRACT

Agaricomycetes Doweld (phylum Basidiomycota) produce a diverse of basidiocarps. These fruiting bodies encompass a wide range of forms, including agaricoid (gilled mushrooms), boletoid (poroid with central stipe), cantharelloid (chanterelle-like), clavarioid (coral-like), cyphelloid (small cup- or tube-shaped), corticioid (crust-like), secotioid (partially enclosed), gasteroid (enclosed), phalloids (stinkhorns), hydnoid (tooth fungi), and stereoid (leathery shelf-like). Notable examples include agarics (e.g., *Agaricus*), chanterelles (*Cantharellus*), polypores (*Trametes*, *Ganoderma*), puffballs (*Lycoperdon*, *Scleroderma*), bird's nest fungi (*Cyathus*), and false truffles (*Rhizopogon*), among others.

In addition, species of the genus *Hericium*, which belong to the hydnoid type due to their characteristic tooth-like spore-bearing structures, are widely valued for their culinary and medicinal properties. These fungi are known to produce a range of bioactive compounds with neuroprotective, antioxidant, and immunomodulatory effects. Species of the genus *Hericium* are currently the most important cultivated mushrooms. By contrast, members of the genus *Scleroderma* Pers. (1801), which are gasteroid fungi that form puffball-shaped fruiting bodies, are not typically consumed, but play an important ecological role. As ectomycorrhizal symbionts, various species of *Scleroderma* are commonly used in forestry and nursery practices to inoculate tree seedlings, enhancing nutrient uptake and promoting plant growth through mycorrhizal associations.

Hericium is a genus of edible fungi known for its medicinal efficacy. Both mycelium and basidiomata contain a variety of nutrients and bioactive compounds,

such as polysaccharides, erinacines, and hericenones. Recent and emerging research has highlighted its relevance in the support of human health, with studies indicating antioxidant, anti-cancer, anti-diabetic, hypolipidemic, anti-inflammatory, antimicrobial, antiviral, and hepatoprotective properties. Over the past decade, many studies have been done on cultivation of *Hericium* species to produce enough basidiomata, due to their rarity in the natural habitats. The purpose of this study was to improve cultivation methods, including indoor-outdoor cultivation and submerged culture methods, health-enhancing applications, economic importance, and industrial applications of *Hericium*.

In preparation for artificial cultivation of these mushrooms in Thailand, optimization of mycelial growth was carried out on different agar culture media, for various conditions (including temperature, pH, cereal grains and agricultural waste, carbon sources, nitrogen sources and the ratio of media components). For this study, three strains of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, and MFLUCC 21-0020) were grown on OMYA medium, at 25 °C and at a pH of 4 to 4.5, while one strain of *H. erinaceus* (MFLUCC 21-0021) the growth optimum was observed on CDA medium at 25 °C and pH 5.5. The optimal growth for *H. coralloides* (MFLUCC 21-0050) was observed on MYPA medium, at 30 °C and pH 5.5. All five strains presented higher mycelial growth on wheat grain. Molasses and yeast extract, as carbon and nitrogen sources, respectively, promoted higher growth rates, with a C/N ratio of 10:1 yielding the most favorable results.

Four strains of *Hericium erinaceus* (MFLUCC 21-0018, MFLCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) were grown under the optimization condition for cultivation and proximate analysis. The mushroom was cultivated on three different substrate treatments, designed using a completely randomized design (CRD), harvested as fresh fruiting bodies, dried at 40 to 45 °C, and the total yield was calculated. We pulverized the dried fruiting body for proximate composition and analyzed it according to standard procedures. The result showed that all *H. erinaceus* strains in three different substrate treatments produced mature fresh fruiting bodies when the temperature was 18 to 24 °C, while the second substrate treatment was under conditions of the sawdust bag content 77% of para rubber sawdust, 15% of red

sorghum, 3% of rice bran, 2% of yeast powder, 1% of lime (CaO), 1% of gypsum ($CaSO4 \bullet 2H_2O$), and 1% of molasses produced a high yield of 85.79–123.7 grams/bag and 12.95–19.58% of biological yield. Proximate analysis of the dried mushroom powder showed high levels of protein content between 15.30% and 19.56%. The cultivation of *H. erinaceus* in Thailand is a significant achievement, as this type of mushroom is generally valued for its nutritional and therapeutic properties.

For *Hericium coralloides*, the result demonstrated a successful fruiting body formation on all three substrates, yielding 141.40, 138.20 and 142.50 grams/bag, respectively. Proximate analysis of the dried mushroom powder revealed a protein content of 18.81 g/100 g. Three compounds were isolated and purified from the fruiting bodies, with ergosterol showing moderate cytotoxic activity against A549, SW-480 and Huh-7 cancer cell lines, with IC_{50} values of 4.6, 4.2 and 5.2 μg/ml, respectively. Ergosterol also inhibited colony formation and migration in these cell lines. Compared to standard chemotherapeutic agents such as doxorubicin, whose IC_{50} values typically fall within the low micromolar range, ergosterol showed lower potency. However, it exhibited minimal toxicity to normal mammalian cells, with IC50 values >100 μM in non-cancerous lines, suggesting a favorable selectivity profile. Although ergosterol is a common fungal metabolite and its cytotoxicity is relatively weak, *H. coralloides* remains a potential source of bioactive compounds that warrant further investigation.

Genus *Scleroderma* belong to the Gasteroid within the order Boletales (phylum Basidiomycota) and are characterized by the development of basidiospores inside enclosed basidiomata, without forcible discharge from the basidia. Commonly known as earth balls, species of *Scleroderma* are regarded inedible or toxic, and consumption is strongly discouraged due to reports of gastrointestinal symptoms such as nausea and vomiting. Additionally, these fungi exhibit medicinal properties through the production of bioactive compounds. However, there have been some concerns about the edibility and potential toxicity of this mushroom. In Asia, 25 species of *Scleroderma* have been documented, with eleven species identified in Thailand based on morphological evidence. This aims to provide insights into the taxonomy, distribution, life cycle, and cultivation of *Scleroderma* species found in Thailand. We have been collecting fresh specimens in Thailand and report three undescribed species and one new record for the

country. These species were characterized by photographs of freshly collected basidiomes and photographs, and their macro and microscopic features were compared with those of the known species of *Scleroderma*. Additionally, we generated DNA sequence data for four loci, including the nuclear ribosomal internal spacer region (ITS), the large subunit ribosomal RNA gene (LSU), the translation elongation factor 1-alpha gene (TEF1-α), and the second largest subunit gene RNA polymerase II (RPB2). We constructed a multi-locus phylogeny to confirm the taxonomical placements.

Keywords: Bioactive Compound, Cultivation, Hericiaceae, *Scleroderma*, Taxonomy

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

%	Percent
&	And
°C	Degree centigrade
°F	Degrees fahrenheit
^{13}C	Carbon-13
^{1}H	Protium
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AD	Alzheimer's disease
AEHE	Administration of the aqueous extract of *H. erinaceus*
AOAC	Association of Official Analytical Collaboration
B.E.	Biological efficiency
BDNF	Brain-derived neurotrophic factor
BHT	Butylated hydroxytoluene
BI	Bayesian Inference
bp	base pairs
BYPP	Bayesian Posterior Probabilities
C	Carbon
CDA	Carrot dextrose agar
CI	Consistency index
CLSI	Clinical and Laboratory Standards Institute
cm.	Centimeter
CMA	Corn meal agar
CTAB	Cetyltrimethyl ammonium bromide
DENV-2	Dengue virus serotype 2
diam.	Diameter
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazil

ABBREVIATIONS AND SYMBOLS

dwb	Dry weight basis
EAEs	Ethyl acetate extraction solvent
ECM	Ectomycorrhizal
EM	Effective Microorganism
et al.	And others
EtOH	Ethanol
eV	Electric Vehicle
EWEs	Water extraction solvent
FBS	Fetal bovine serum
g	Gram
g/kg	Grams per kilogram
h	Hour
H_2O	Water
HCl	Hydrochloric acid
HEO-A	*Hericium erinaceus* oligosaccharide
HEPES	Hydroxyethyl piperazineethanesulfonic acid
HI	Homoplasy index
HIV	Human immunodeficiency virus
HMA	Mueller Hinton agar
HPLC	High-performance liquid chromatography
HZI	Helmholtz Centre for Infection Research
I	Identity
IC_{50}	Half maximal inhibitory concentration
ITS	Internal transcribed spacer
kcal	Kilocalories
kg	Kilogram
KH_2PO	Potassium phosphate
KOH	Potassium Hydroxide

ABBREVIATIONS AND SYMBOLS

kV	Kilovolt
L/min	Liter per minute
LC-MS	Liquid Chromatography - Mass Spectrometry
LC–QTOF–MS	Liquid chromatography coupled to quadrupole–time-of-flight–mass spectrometry
LSU	Large subunit region
m/*z*	Mass-to-charge ratio
MBC	Minimal bactericidal concentration
MbIII	Metmyoglobin
MCMC	Markov Chain Monte Carlo sampling
MEA	Malt extract agar
MEM	Minimal essential medium
MeOHEs	Methanol extraction solvent
MFC	Minimum fungicidal concentration
MFLUCC	Mae Fah Luang University culture collection
mg	Milligram
mg/mL	Milligrams per milliliter
$MgSO_7$	Magnesium sulfate
MHz	Megahertz
MIC	Minimum inhibitory concentration
min	Minutes
ML	Maximum Likelihood
mL	Millilitre
mm	Millimeter
mM	Millimolar
mm i.d.	Inner Diameter
MMN	Medium modified Melin- Norkrans
MP	Maximum parsimony

ABBREVIATIONS AND SYMBOLS

MTT	3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyl tetrazolium bromide
MYPA	Malt yeast peptone agar
N	Nitrogen
N/A	not available
NaOH	Sodium hydroxide
neg	Negative
NGF	Nerve growth factor
nm	Nanometer
NMR	Nuclear magnetic resonance
OMA	Oat meal agar
OMYA	Oat meal yeast agar
PAUP	Phylogenetic Analysis Using Parsimony
PBS	Phosphate-buffered saline
PCDL	Personal Compound Database and Library
PCR	Polymerase chain reaction
PD	Parkinson's disease
PDA	Potato dextrose agar
PDYA	Potato dextrose yeast agar
pH	potential of hydrogen
pos	Positive
PP	Posterior probabilities
psi	Pound per square inch
Q	Quotient
QC	Query cover
R	Run/Round
RC	Rescaled consistency index
RI	Retention index

ABBREVIATIONS AND SYMBOLS

RPB2	Polymerase II second largest subunit
rpm	Revolutions Per Minute
Rt	Retention time
SDA	Sabouraud dextrose agar
sp.	Species
STZ	Streptozotocin
TBR	Tree-bisection reconnection
Tef1-α	Elongation factor 1-alpha
TL	Tree Length
TLC	Thin-layer chromatography
UpM	Umdrehung pro Minute
UV/Vis	Ultra-violet visible
V	Volt
v/v	Volume by volume
w/v	Weight in volume
W1	Pre-dried weight
W2	Weighed accurately
wt	Weight
wwb	Wet weight basis
α	alpha
µg	Microgram
µL	Microlitre
µm	Micrometre

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction to Basidiomycota

Basidiomycota R.T. Moore is a large division (phylum) of fungi, many of the species of Basidiomycota known as macrofungi or mushrooms (Krik et al., 2008). The subphyla of Basidiomycota are 20 classes, 77 orders, 297 families, and 2130 genera (He et al., 2024). The generation of sexual spores known as basidiospores in specialized cells known as basidia is a characteristic of Basidiomycota (Oberwinkler, 2012). They are very important decomposers, symbionts, and pathogens, these fungi are largely in charge of breaking down organic matter and are vital to ecosystems (Sudharsan et al., 2023).

1.2 Introduction to Agaricomycetes

Agaricomycetes Doweld, several generally referred to as "mushrooms" including agaricoid, boletoid, cantharelloid, clavarioid, cyphelloid, corticioid, secotioid, gasteroid, phalloids, hydnoid, stereoid, and other forms (Oberwinkler, 2014; Kuees & Navarro-Gonzalez, 2015; He et al., 2024; Stępień & Lalak-Kańczugowska, 2021) that produce a diverse of basidiocarps. Sexual spores, or basidiospores, are created on the specialized cells called basidia, which are characteristic of fungi in the phylum Basidiomycota, including, but not limited to members of the class Agaricomycetes (Oberwinkler, 2012). They play an essential role in ecosystems, primarily as decomposers, but also as symbionts and pathogens (Mattos-Shipley et al., 2016). Many species within Agaricomycetes are of significant economic, ecological, and medicinal importance (Azeem et al., 2020). There is a broad ecological impact through their activities in the destruction of wood and the ectomycorrhizal characteristics of forest trees (Hibbett et al., 2014).

Agaricomycetes mushrooms have been demonstrated to provide significant culinary and medicinal benefits, offering both nutritional richness and therapeutic value through bioactive compounds (Venturella et al., 2021; Bhatia & Yadav, 2024). A large number of edible mushrooms belong to the Agaricomycetes family, *Cantharellus cibarius* (chanterelle mushroom) and *Tricholoma matsutake* (matsutake mushroom), as well as cultivated saprotrophs such as *Agaricus bisporus* (white button, portobello mushroom), *Pleurotus ostreatus* (oyster mushroom), *Lentinula edodes* (shiitake mushroom) and *Russula vesca* (bare toothed Russula) and which include wild-collected ectomycorrhizal (ECM) species such as *Boletus edulis* (porcini mushroom) and *Boletus badius* (bay bolete) (Hibbett et al., 2014). In addition, the important edible and medicinal mushrooms of the genus *Ganoderma lucidum* (reishi or lingzhi), *Hericium erinaceus* (lion's mane), both of which are edible (Van der Berg, 2018).

1.3 Introduction to Genus *Hericium*

Mushrooms of the genus *Hericium* Pers. (1794), which belongs to the class Agaricomycetes, order Russulales, and family Hericiaceae (Figure 1.1). *Hericium* is known as 'Houtou', which means 'monkey head'. In Japan, *H. erinaceus* is named 'Yamabushitake', which means 'mountain priest'. It is also known as 'Lion's mane, 'Bear's head', 'Hog's Head Fungus', 'White beard', 'Old Man's beard', 'Pom Pom' and 'Bearded tooth' in other parts of the world (Thongbai et al., 2015). For artificial cultivation in a different part of the world (Zied &Pardo-Giménez, 2017), the species of genus *Hericium* are not among the most widely cultivated mushrooms globally, but *Hericium* are increasingly recognized for their culinary and medicinal value and are gaining importance in commercial cultivation (Royse et al., 2017). However, China is the largest producer; production in Thailand is low because it is not widely known.

The genus name *Hericium* refers to the hedgehog-like appearance of the fruiting body, while the species name *erinaceus* is derived from Latin for "hedgehog" and contains 26 species shown in Table 1.1 (Species Fungorum, 2024). They are found mainly in Europe and North America, but also occur in Asia, South America (e.g.,

Argentina), New Zealand, Latin America, and parts of Africa (Hallenberg et al., 2013; Thongbai et al., 2015; Roncero-Ramos et al., 2021; Gibertoni et al., 2020).

Domain: Eukaryota
Kingdom: Fungi
Phylum: Basidiomycota
Subphylum: Agaricomycotina
Class: Agaricomycetes Doweld
Order: Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David
Family: Hericiaceae Donk
Genus: *Hericium* Pers.

Source Kirk (2008)

Figure 1.1 Classifications of *Hericium*

Table 1.1 List of *Hericium* species

No.	Name
1	*Hericium abietis* (Weir ex Hubert) K.A. Harrison.
2	*Hericium agaricinum* Hoffm.
3	*Hericium americanum* Ginns.
4	*Hericium asiaticum* Koga & Thorn.
5	*Hericium barbatum* Pers.
6	*Hericium bembedjaense* Jumbam & Aime.
7	*Hericium bharengense* K. Das.
8	*Hericium botryoides* S. Ito & Otani.
9	*Hericium cardium* Pers.
10	*Hericium carolinense* Koga & Thorn.
11	*Hericium cirrhatum* (Pers.) Nikol.
12	*Hericium clathroides* (Pall.) Pers.
13	*Hericium commune* Roques

Table 1.1 (continued)

No.	Name
14	*Hericium coralloides* (Scop.) Pers.
15	*Hericium erinaceus* (Bull.) Pers.
16	*Hericium fimbriatum* Banker.
17	*Hericium fimbrillatum* (Iwade) R. Sugaw.
18	*Hericium flagellum* (Scop.) Pers.
19	*Hericium mori* Opiz.
20	*Hericium novae-zealandiae* (Colenso) Chr.A. Sm. & J.A. Cooper.
21	*Hericium nudicaule* Pers.
22	*Hericium ophelieae* B. Van der Merwe & K. Jacobs
23	*Hericium ptychogasteroides* Nikol.
24	*Hericium rajchenbergii* Robledo & Hallenb.
25	*Hericium rajendrae* U. Singh & K. Das
26	*Hericium yumthangense* K. Das

1.4 Introduction to Genus *Scleroderma*

Scleroderma Pers. is a puffball type of gasteromycete, commonly known as earth balls, belonging to the family Sclerodermataceae, Boletales, Agaricomycetes Doweld of Basidiomycota (Figure 1.2) (Jeffries, 1999). There are 46 species of *Scleroderma* (He et al., 2019), and Index Fungorum (2024) reported 73 species type *S. verrucosum* (Bull.) (Table 1.2). Pers. Nevertheless, a revised key of the genus *Scleroderma* was conceived for the identification of 25 Asian species, which included two indeterminate species (Sims et al., 1995). In Thailand, only 11 species of *Scleroderma* have been reported (Gonkhom et al., 2025), as provided in Table 1.3.

The peridium (outer wall) of *Scleroderma* species is notably thick and tough, with surface textures ranging from smooth to warted or nodular, making its morphological characteristics, particularly peridium thickness and ornamentation, key taxonomic features (Pratiwi et al., 2022). At maturity, the peridium of *Scleroderma* are splits irregularly over the upper part of the basidiocarp, exposing the dark mucous

membrane beneath (Ammirati, 1985). The spores are produced in small brownish-purple, pea-shaped bodies called peridioles, which are initially surrounded by wall-like clusters of white hyphae (Miller & Miller, 1988). These peridioles disintegrate as the fruiting body matures, and when the peridium splits open, only a powdery mass of dark spores is visible. The spores are roughly spherical with tubercles or reticulate ornamentation, thick-walled, and brown.

The species of the genus *Scleroderma* are ectomycorrhizal fungi of shrubs and trees and have a worldwide distribution (Miller & Miller, 1988). Certain *Scleroderma* species, such as *S. citrinum* and *S. verrucosum* are used as inoculant symbionts to colonize tree seedlings in nurseries and promote their growth (Chen et al., 2006).

Domain: Eukaryota
Kingdom: Fungi
Phylum: Basidiomycota R.T. Moore
Subphylum: Agaricomycotina Doweld
Class: Agaricomycetes Doweld
Order: Boletales E.-J. Gilbert
Family: Sclerodermataceae Corda
Genus: *Scleroderma* Pers.

Source Jeffries (1999)

Figure 1.2 Classifications of *Scleroderma*

The genus *Scleroderma* includes several species, which are toxic and generally not considered edible (Watling, 2006). In fact, they are often confused with edible boletes or puffballs due to their similar appearance, but they pose significant health risks (Winkler, 2022).

Table 1.2 Lists of *Scleroderma* species according to the Index fungorum, 2025

No.	Name
1	*Scleroderma albidum* Pat.
2	*Scleroderma anomalosporum* Baseia
3	*Scleroderma arenicola* Zeller
4	*Scleroderma areolatum* Ehrenb.
5	*Scleroderma australe* Massee
6	*Scleroderma bermudense* Coker
7	*Scleroderma bougheri* Trappe
8	*Scleroderma bovista* Fr.
9	*Scleroderma bulla* R.
10	*Scleroderma camassuense* M.P. Martín
11	*Scleroderma capeverdeanum* M.P. Martín
12	*Scleroderma cepa* Pers.
13	*Scleroderma chevalieri* Guzmán
14	*Scleroderma chrysastrum* G.W. Martin
15	*Scleroderma citrinum* Pers.
16	*Scleroderma coelatum* (Pat.) Sacc. & P. Syd.
17	*Scleroderma columnare* Berk. & Broome
18	*Scleroderma congolense* Demoulin & Dring
19	*Scleroderma cyaneoperidiatum* Watling & K.P. Sims
20	*Scleroderma dictyosporum* Pat.
21	*Scleroderma dingjieense* Ke Wang
22	*Scleroderma duckei* B.D.B. Silva
23	*Scleroderma dunense* B.D.B. Silva
24	*Scleroderma echinatum* (Petri) Guzmán
25	*Scleroderma echinosporites* Rouse
26	*Scleroderma endoxanthum* Petch
27	*Scleroderma erubescens* Z.W. Ge
28	*Scleroderma flavidum* Ellis & Everh.
29	*Scleroderma floridanum* Guzmán

Table 1.2 (continued)

No.	Name
30	*Scleroderma franceschii* Macchione
31	*Scleroderma furfuraceum* Rebriev & Zvyagina
32	*Scleroderma furfurellum* Zeller
33	*Scleroderma griseobrunneum* Ke Wang
34	*Scleroderma guzmanii* Ortiz-Rivero
35	*Scleroderma hakkodense* Kobayasi
36	*Scleroderma hypogaeum* Zeller
37	*Scleroderma laeve* Lloyd
38	*Scleroderma leptopodium* Pat. & Har.
39	*Scleroderma lycoperdoides* Schwein.
40	*Scleroderma macrosporum* (G. Cunn.) J.A. Cooper
41	*Scleroderma mayama* Grgur.
42	*Scleroderma mcalpinei* (Rodway) Castellano
43	*Scleroderma meridionale* Demoulin & Malençon
44	*Scleroderma michiganense* (Guzmán) Guzmán
45	*Scleroderma minutisporum* Baseia
46	*Scleroderma multiloculare* Dring & Rayss
47	*Scleroderma nastii* Raut
48	*Scleroderma nitidum* Berk.
49	*Scleroderma pantherinum* Mattir.
50	*Scleroderma paradoxum* G.W. Beaton
51	*Scleroderma patagonicum* Nouhra & Hern. Caff.
52	*Scleroderma poltaviense* Sosin
53	*Scleroderma polyrhizum* (J.F. Gmel.) Pers.
54	*Scleroderma pseudostipitatum* Petch1
55	*Scleroderma radicans* Lloyd
56	*Scleroderma reae* Guzmán
57	*Scleroderma rhodesicum* Verwoerd
58	*Scleroderma sapidiforme* Sosin

Table 1.2 (continued)

No.	Name
59	*Scleroderma schmitzii* Demoulin & Dring
60	*Scleroderma separatum* Z.W. Ge
61	*Scleroderma septentrionale* Jeppson
62	*Scleroderma sinnamariense* Mont.
63	*Scleroderma squamulosum* Z.W. Ge
64	*Scleroderma stellenbossiense* Verwoerd
65	*Scleroderma suthepense* Kumla, Suwannar. & Lumyong
66	*Scleroderma texense* Berk.
67	*Scleroderma torrendii* Bres.
68	*Scleroderma tuberoideum* Speg.
69	*Scleroderma uruguayense* (Guzmán) Guzmán
70	*Scleroderma verrucosum* (Bull.) Pers.
71	*Scleroderma vinaceum* Z.W. Ge, R. Wu & L.R. Zhou
72	*Scleroderma xanthochroum* Watling & K.P. Sims
73	*Scleroderma yunnanense* Y. Wang
74	*Scleroderma zenkeri* Henn.

Table 1.3 Lists of recoded *Scleroderma* from Thailand

No.	Name	Province	Ref.
1	*S. areolatum* Ehrenb.	Songkhla	Vasun (1998)
2	*S. bovista* Fr.	Chanthaburi	Teerawat (2007), Utis (1999)
3	*S. cepa* Pers.	Unknown	Dissing (1963)
4	*S. citrinum* Pers.	Mae Hong Son, and Songkhla	Anong et al. (2008), Dissing (1963), Ellingsen (1982), Niwat (2010), Teerawat (2002), Utis (1999), Vasun (1998)
5	*S. dictyosporum* Pat.	Chanthaburi	Teerawat (2007)
6	*S. flavidum* Ellis & Everh.	Chanthaburi	Anong et al. (2008), Teerawat (2007)
7	*S. lycoperdoides* Schwein.	Sisaket	Chalermpongse (1992)
8	*S. polyrhizum* (J.F. Gmel.) Pers.	Phetchabun	Anong et al. (2008), Niwat (2010)
9	*S. sinnamariense* Mont.	Chiang Mai	Ellingsen (1982), Kittima (2008), Niwat (2010), Ruksawong (2001), Wanida (1999)
10	*S. suthepense* Kumla, Suwannar. & Lumyong	Chiang Mai	Kumla et al. (2013)
11	*S. verrucosum* (Bull.) Pers.	Songkhla, and Chiang Mai	Anong et al. (2008), Teerawat (2007)

1.5 Introduction to cultivation of *Hericium*

Hericium are becoming more and more well-liked as both edible and therapeutic fungi (Kumar & Kushwaha, 2023). Lion's Mane is a notable addition to the culinary and supplement markets because of its remarkable appearance (it resembles a white, spiky ball of fur) and its cognitive and neuroprotective properties (Docherty et al., 2023). Although *Hericium* need certain growing circumstances, they can be successfully grown at home or on a commercial scale with the right methods.

Hericium erinaceus has health advantages, including neuroprotective qualities (Docherty et al., 2023). Growing them at home or on a commercial scale can be very fulfilling. For mushroom lovers, cultivating *Hericium* is a viable and fulfilling undertaking, although it requires careful attention to detail, particularly with respect to temperature, humidity, and substrate (Chutimanukul et al., 2023). *Hericium* mushrooms are an excellent species to cultivate for both culinary and medicinal uses, regardless of whether they are grown on logs, sawdust blocks, or other substrates (Gonkhom et al., 2021).

The selection of superior mushroom spawn and a suitable growing medium are the first steps in the production, grain or sawdust spawn are the two most common forms of spawn media (Borah et al., 2019). Although sawdust spawn is used more frequently to inoculate sawdust or wood-based substrates, grain spawn is easier to inoculate into larger substrates (Mbogoh et al., 2011). *Hericium* mushrooms grow best on wood substrates with a moisture content of about 60 to 65 %, the substrate must be pasteurized or sterilized before inoculation to eliminate any competing microorganisms that can prevent mushroom culture from growing (Stamets, 2011). For most substrates, pasteurization; heating the material to 140 ° F or 60 °C for approximately an hour, typically 250 ° F or 121 ° C for 30 minutes to an hour with sawdust or wood chips substrate (Stamets, 2011).

After the substrate has been prepared and cooled, inoculate it with the *Hericium* spawn. For the mycelium to properly colonize the substrate after inoculation, it must be incubated in a warm and dark atmosphere. The temperature for mycelium growth between 65 and 75 ° F (18–24 ° C), humidity between 80 and 90 %, duration for the

colonization process can take 2 to 4 weeks depending on the type of substrate and environmental conditions (Gonkhom et al., 2024). Fruiting body procedures, *Hericium* mushrooms prefer cooler temperatures than during the colonization phase with temperatures around 60–70 °F (15–21 °C), high humidity levels (85–95 %), require fresh air to initiate fruiting and indirect light to help trigger the formation of fruit bodies (Gonkhom et al., 2024). After the fruiting bodies develop for the first time, it typically takes two to three weeks for *Hericium* mushrooms to mature. When their spines (the white, hair-like structures) are long and pointed, but before they begin to turn yellow or brown, these should be harvested (Grace & Mudge, 2015).

Additionally, *Hericium* mushrooms can be grown in logs in addition to sawdust and wood-based substrates. Although slower, this technique may be perfect for growing plants outdoors (Stamets, 2011). *Hericium* spawn can be inoculated into fresh hardwood logs, including oak, maple, or birch, for log cultivation. The spawn is placed in holes drilled into the logs, which are then sealed with wax (Cotter, 2015). For roughly six months to a year, the logs are incubated in a humid, shaded area. After colonization, the logs can be placed in a cool and shaded spot outside. In the right season, which usually occurs late fall to early spring, *Hericium* mushrooms will bear fruit (Cotter, 2015).

1.6 Introduction to the Significance of *Hericium*

Hericium species are valued both as gourmet edible mushrooms and for their medicinal properties. In China, they are commonly consumed and have a long-standing reputation in traditional medicine for promoting digestive and neurological health (Thongbai et al., 2015).

Lion's Mane is frequently compared to crab or lobster because of its mild, seafood-like flavor (Charge, 2024). With its distinct and spongy texture, it is frequently used in vegetarian and vegan recipes in place of meat. It is frequently used in soups, steamed, or in mushroom "steaks" (Romine, 2018). Although many edible fungi are nutritionally valuable, *Hericium* mushrooms stand out for their unique combination of dietary fiber, bioavailable protein, B-vitamins, and essential minerals, particularly

potassium and zinc, as well as their notable antioxidant and neuroprotective compounds (Dimopoulou et al., 2022).

Hericium species are prized not for generic polysaccharides or phenolics, but for their unique secondary metabolites, phenolic terpenoids (hericenones) in fruiting bodies and cyathane diterpenoids (erinacines) in mycelia, which have demonstrated neurotrophic, neuroprotective and anti-inflammatory activities in both in *vitro* and in *vivo* studies (Thongbai et al., 2015; Abdelshafy et al., 2022).

The medicinal value of *Hericium* was highly valued because the therapeutic properties, including *Hericium*'s neuroprotective properties, are widely established. According to studies, it might encourage the synthesis of nerve growth factor (NGF), which supports nerve cell regeneration, cognitive function, and brain health (Yow et al., 2021). Its potential to treat neurodegenerative diseases including Parkinson's and Alzheimer's is being investigated (Rai et al., 2021; Badalyan & Rapior, 2021; Szućko-Kociuba et al., 2023). *Hericium* may improve mood by encouraging healthy neuron activity and reducing inflammation in the brain, which may help with anxiety and sadness (Limanaqi et al., 2020; Ratto, 2022).

Another remarkable activity of *Hericium* mushrooms is anticarcinogenic (Nam, 2005), antitumor (Kim et al., 2011), immunomodulatory activities (Wu et al., 2018), gastroprotective activity (Wang et al., 2018; Hou et al., 2022), antioxidant activity (Chutimanukul et al., 2023), antioxidative (Kushairi et al., 2019), hepatoprotective activities (Zhang et al., 2012), hypolipidemic activity (Jang et al., 2017), hypoglycemic activity (Chaiyasut & Sivamaruthi, 2017), anti-fatigue (Liu et al., 2015), anti-aging activities (Tripodi et al., 2022), anti-inflammatory activities (Xie et al., 2022), immune activity on polysaccharides (He et al., 2017; Kim et al., 2014; Zhu et al., 2019).

1.7 Research Objectives

1.7.1 To develop ways to cultivate selected strains of *Hericium*

1.7.2 To investigate the proximate composition, anti-microbial, anti-oxidants, cytotoxicity of selected strains of *Hericium*.

1.7.3 To isolate, structurally elucidate, and compare the difference between secondary metabolites from mycelium versus fruiting bodies from the *Hericium*.

1.7.4 To explore the potential of bioactive compounds from a selected strain of *Hericium* species for anticancer activity.

1.7.5 To study the morphology and phylogeny of selected strains of *Scleroderma*.

1.8 Research Contents

1.8.1 Chapter 1 (General Introduction)

Chapter 1 is the general introduction, which provides a background of Basidiomycota, characteristics of the genus *Hericium* and genus *Scleroderma*, check lists of *Scleroderma* mushrooms in Thailand, and the objectives of this research and the outline of the thesis.

1.8.2 Chapter 2 (Literature Reviews)

1.8.2.1 Provides the cultivation, health-enhancing applications, economic importance, industrial and pharmaceutical applications of *Hericium erinaceus.*

1.8.2.2 Provides the economic importance of *Scleroderma* mushroom including debility, cultivation, bioactive compound. Also, the ecology and distribution and the taxonomy of *Scleroderma* species from Thailand were reported.

1.8.3 Chapter 3 (General Material and Methodology)

Chapter 3 provides an overview of the material and methodology of the thesis, collection sites, sample collection and morphological identification, phylogenetic analysis of *Hericium* and *Scleroderma* mushrooms, cultivation test of selected strains of *Hericium* mushrooms with statistical analysis. In addition, nutritional analysis, mushroom extraction, purification, identification and screening of biological activity of *Hericium* species will be shown.

1.8.4 Chapter 4 (Cultivation of Selected Strains of *Hericium*)

This chapter provides the optimal conditions on media, temperature, pH, carbon source, nitrogen source, carbon and nitrogen ration for mycelial growth, spawn preparation, beg cultivation of *Hericium erinaceus* and *H. coralloides* in Thailand.

1.8.5 Chapter 5 (Secondary Metabolites of Selected Strains of *Hericium*).

Chapter 5 provides the secondary metabolite of selected strains of *Hericium* from liquid media fermentation, solid media fermentation (wheat), and dried fruiting bodies of selected strains of *Hericium*.

1.8.6 Chapter 6 (Screening of Biological Activity of *Hericium*)

This chapter provides the bioactive compound of selected strains of *Hericium* for antimicrobial, antifungal, antioxidant, cytotoxic, and anticancer were provided.

1.8.7 Chapter 7 (Taxonomy and Phylogeny of Genus *Scleroderma*)

Taxonomy and phylogeny of selected strain of three new species and one new record of *Scleroderma* was provided in this chapter; which are three new species included *Scleroderma longistipes*, *S. microcarpum*, and *S. magnisporum,* as well as *S. separatum* reported as a new record.

1.8.8 Chapter 8 (Overall Conclusion)

All of the results are summarized in this chapter of the thesis, along with the study's benefits and limitations, recommendations for additional research, and a list of my related publications.

CHAPTER 2

LITERATURE REVIEW

2.1 Genus *Hericium*

Hericium species are commonly considered as traditional food and traditional folk medicines in China (Shao et al., 2019). They are native to North America and commonly found in temperate forests in East Asia and India (Das et al., 2011), recent discoveries have extended their known range to include parts of Africa, such as Cameroon, Southern Africa and the Afrotemperate zones of South Africa, and also regions in Latin America, notably southern South America, highlighting a broader, more global distribution than previously recognized (Jumbam et al., 2019; Van der Merwe et al., 2023).

They are rarely found in European countries, but are common in Japan and North America. *Hericium* species have a variety of common names, e.g., Lion's Mane mushroom, Hóutóugū, Yamabushitake, Monkey's Head, Pom Pom, Bear’s Head, Hog’s Head Fungus, Whitebeard, Old-man’s Beard and Bearded Tooth (Thongbai et al., 2015; Sangtitanu et al., 2020).

Hericium is a genus of edible and medicinal mushrooms that belongs to the family Hericiaceae, order Russulales, and class Agaricomycetes (Kirk et al., 2008; He et al., 2019). *Hericium* comprises 26 species with 73 taxon names listed in Index Fungorum (Index Fungorum, 2025; accessed on July 2, 2025). The basidiomata in this genus are white and fleshy, growing on dead trees or dried woods, the basidiomata are similar to fragile iced thorns which either hang from a branch, supporting the framework or as a tough unbranched cushion of tissue (Gonkhom et al., 2022). The dangling spines easily identify a mature specimen (Ouali et al., 2020). The spines are arranged by clusters or often in rows (Park et al., 2004). Identifying immature specimens can be more difficult since they tend to start as a single clump and develop their branches as they age (Pegler, 2003). They have no caps and consist of spiny spherical to ellipsoid amyloid spores that are either smooth or covered with very fine

warts (Kuo, 2014). Their hyphae are gloeopleurous and filled with oil drops (Kuo, 2014).

Hericium has been extensively studied for cultivation and medical purposes. Historically, the first strains of *Hericium* were cultivated in China and belonged to the species *H. erinaceus* (Suzuki & Mizuno, 1997), which later became the commercial *Hericium* strain for cultivation (Sawant, 2021). *Hericium* species grow slowly and inhabit the top of beech or oak trunks in pairs, in wild forests (Jiang et al., 2014). The hardwood sawdust is the most suitable substrate for *H. erinaceus* in order to attain a high yield in mushroom cultivation (Hu et al., 2008). Atila (2019) reported that a reduction in lignin content concentrations with low cellulose of various sawdust had a positive effect on *Hericium* productivity.

Most *Hericium* species have long been examined for medicinal compounds. Several bioactive compounds from *Hericium* were found to have therapeutic potential for immune-stimulating effects (Chen et al., 2017; Sheng et al., 2017). *Hericium* is a great source of new therapeutic compounds and has been found to have effects on nerves and the brain (Chong et al., 2020). Most of the neurotrophic compounds have effects on the human nerve cell, and neurogenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Zhang et al., 2016; Ratto et al., 2019; Chong et al., 2020; Ryu et al., 2021). There have been many reports on the bioactive secondary metabolites, such as phenols, polyketides, terpenes (De Silva et al., 2013), polysaccharides, lipopolysaccharides, glycoproteins, pyrone, alkaloids, terpenoids, steroids, and non-ribosomal peptides (Keong et al., 2007; Chen et al., 2017).

The indoor-outdoor cultivation methods, health benefits, economic significance, and industrial applications of *Hericium*.

2.1.1 Cultivation of *Hericium*

2.1.1.1 Cultivation methods for mushroom production and biomass

The adaptability of wild *Hericium* growth is not entirely clear, but it depends on the microbiological condition of the substrate surface, basic nutrition, and environmental restrictions (Bruhn et al., 2000). However, there are two methods to grow *Hericium* species, including outdoor and indoor cultivation. Some *Hericium* species have been commercially cultivated, including *H. erinaceus*, *H. abietis*, and *H. americanum* (Xiao & Chapman, 1997; Hassan, 2007; Sokół et al., 2015; Atila et al.,

2017; Bunroj et al., 2017). Ko et al. (2005) reported that the fruiting bodies of *H. americanum*, *H. coralloides*, and *H. erinaceus* are usually produced on a wood sawdust substrate. Additionally, sawdust substrates from various deciduous trees are also considered the main substrate for commercial cultivation (Oei, 2016).

2.1.1.2 Outdoor cultivation

The first wide scale cultivation of *Hericium* originated in China (Sokół et al., 2015). Traditional outdoor cultivation is carried out seasonally in the shade of a tree by making mushroom beds on unprocessed logs. The wood chips overgrown with the *Hericium* mycelium were inoculated into dry logs or fresh tree stumps and incubated with high humidity, after which the mycelium will grow on the substrate (Ahmadi & Farsi, 2017). The *Hericium* mycelia can grow on sawdust of *Acer* species (maple), *Mangifera indica* (mango), *Populus* sp. (populus), *Psidium guajava* (common guava) and *Quercus* sp. (oak) (Pathmashini et al., 2008; Stamets, 2011). Several studies have recommended wood from coniferous tree species, including *Pinus taeda* and *P. ponderosa* as the substrate composition for *Hericium* cultivation (Croan, 2004). Outdoor *Hericium* production produces good yield on logs of *Acer* sp. (maple), *Fagus* sp. (beech), *Quercus* sp. (oak), *Ulmus* sp. (elm), and other hardwoods (Stamets, 2011; Grace & Mudge, 2015). The mushroom totem inoculation method can also be used for the outdoor cultivation of logs or stumps using sawdust and spawn (Grace & Mudge, 2015; Soderberg, 2019).

2.1.1.3 Indoor cultivation

Most of the demands for *Hericium* from the commercial market are fulfilled through indoor cultivation in sawdust and wood bags (Grace & Mudge, 2015). Current research is dedicated to indoor cultivation systems, largely because indoor systems are more lucrative and can be studied more efficiently (Bruhn et al., 2000).

An intensive cultivation method needs to be used in order to obtain high yields of good quality (Dai & Dong, 2014). Most of the intensive cultivation for this mushroom is typically done in bottles or bags (Imtiaj & Rahman, 2008). The culture substrate needs to be sterilized, and it must be made from a heat-resistant material such as polypropylene (Ko et al., 2005; Sokół et al., 2015). Several reports have proved that the use of agricultural by-products as supplements increases the mycelial growth of *Hericium* (Suwanno et al., 2019). Xiao and Chapman (1997) reported that conifer

sawdust supplemented with wheat bran, calcium sulfate and sugar was the main substrate for the growth of *H. abietis*. Zhang (2000) found that the favored substrate for *H. erinaceus* mycelium growth contains mainly corncobs and cotton chaffs supplemented with wheat bran, corn meal, gypsum, and sugar. Furthermore, Bunroj et al. (2017) reported that para rubber sawdust supplemented with rice bran, gypsum, dolomite, yeast, leucaena leaf meal, magnesium sulphate and EM solutions (Effective Microorganism), as effective for production of *Hericium* basidiomata. Moreover, other substrates reported to be beneficial for *H. americanum* mycelial growth include oak sawdust, olive press cake, and cottonseed hulls (Atila et al., 2017).

2.1.2 Mushroom Harvesting and Production

Mushroom cultivation mostly uses plastic bags with small holes so as the basidiomata are able to easily come off, thus reducing the loss of production during harvesting operations (Stamets, 2011). It usually takes 33–40 days for the primordia to appear and 10 days after the primordia appearance, the first flush of *Hericium* fruiting bodies can be harvested (Ko et al., 2005). Several *Hericium* cultivation experiments, the cultivation bags are capable of producing fruiting bodies for around 3 to 4 flushes per bag (Bunroj et al., 2017). Moreover, mushroom production depends on environmental factors including climate factors, especially the temperature inducer of the effect of the mushroom product (Andrew et al., 2018). *Hericium* should be harvested when the thorns are very long, but before the tops of the fruit mass weaken and become noticeably yellow or pink (Adamant, 2019). Picking mushrooms in this fashion makes the harvesting process faster. The relative humidity in the growing room should be reduced to 80 % for 4–8 hours before harvesting in order to reduce surface moisture and extend shelf life (Stamets, 2011).

2.1.3 Submerged Culture: Cultivation Methods for the Production of Bioactive Compounds

The submerged culturing of *Hericium* can produce bioactive secondary metabolites that might not be found in their basidiomata (Elisashvili, 2012). Critical conditions for the optimization of bioactive compounds include monitoring of nutrient consumption and respiration (Thongbai et al., 2015). Several studies have tried to ascertain the best conditions for growing and producing mushrooms so that the fungi produce higher biomass, thus increasing the availability of more essential bioactive

metabolites (Lee et al., 2004). Wolters et al. (2015) reported that the submerged cultivation of *H. erinaceus* is the best method to produce erinacine C. The final inoculation ratio 5:10 (v/v) at pH 7.5 of 100 mM hydroxyethyl piperazineethanesulfonic acid (HEPES), produces a high biomass of erinacine C at 2.73 gram/liter.

Nevertheless, many studies have also shown that some important secondary metabolites are also present in basidiomata of *H. erinaceus* growing on different substrates, such as artificial media and cheap agricultural substrates (Kulisic et al., 2004; Hu et al., 2008; Malinowska et al., 2009; Cui et al., 2010; Lee et al., 2010; Zhang et al., 2012). Wittstein et al. (2016) reported that the production of a bioactive compound of *H. coralloides* could be used basidiomata by homogenizing mechanically and stirring with acetone and then separating biomass by filtration. With the increasing demand for bioactive compounds from this mushroom, its cultivation for fruiting bodies is much needed. At present, there are still studies being done on mycelial cultivation so as more extracts are needed to meet the needs of consumers.

2.1.4 Health-enhancing Applications of Mushrooms

2.1.4.1 Mushroom nutrient

Hericium is a good source of dietary supplements in nutritional and medical food (Fernandes et al., 2021). In general, mushrooms contain 90% of water and 10% of dry matter (Ho et al., 2020). The composition of mushrooms depends on the growing medium and cultivation conditions (Badalyan, 2003). *Hericium* is a relatively good source of several nutrients including free amino acids, especially glutamic acid, and numerous volatile compounds (Friedman, 2015). In addition, *Hericium* contains sugar, fat content, and protein content in fruiting bodies (Ulziijargal & Mau, 2011). The basidiomata contains various amounts of nutritional macro-micro elements as well as low amounts of potentially toxic elements (Friedman, 2015; Atila, 2019). The nutritional content depends not only on environment and maturation stage but also on the species (Table 2.1).

Table 2.1 Nutrient contents of the basidiomata of *Hericium*

Properties	***Hericium* species**		
	H. americanum	***H. coralloides***	***H. erinaceus***
Ash (g/ 100g)	6.4	9.31	3.49
Carbohydrates (g/ 100g)	-	81.06	79.36
Calcium (g/ 100g)	0.026	0.0044	0.0013
Dietary fiber (%)	-	44.28	41.32
Energy (kcal/ 100 g)	-	394.67	374.79
Fat (g/ 100g)	-	2.38	1.75
Iron (g/ 100g)	4.95–7.22	0.00677	0.0203
Mannitol	-	3.86	5.63
Magnesium (g/ 100g)	0.063−0.133	0.0085	0.1230
Phosphorus (g/ 100g)	0.99–2.12	-	0.0012
Potassium (g/ 100g)	2.66–3.58	1.188	0.0044
Protein (g/ 100g)	8.5–23.7	15.4	36.4
Sodium (g/ 100g)	0.134–0.178	0.586	0.0012
Total sugar	-	10.79	23.63
Trehalose	-	0.68	0.54

Source Atila et al. (2017), Heleno et al. (2015), and Stamets (2011)

In addition, *Hericium* has a high average nutritional profile (Atila et al., 2021). The health-promoting properties responsible for the mushroom's nutritional value include amino acids, proteins, carbohydrates, fatty acids, vitamins, and minerals (Friedman, 2015).

2.1.4.2 Medicinal Property

Hericium has long been considered to have medicinal value. However, these mushrooms have vast prospects as a source of medicinal compounds (Elkhateeb et al., 2019). These have been investigated *in vivo* and *in vitro* model systems (Chen et al., 2019). Many bioactive substances with immunomodulatory effects have been isolated from this mushroom (Sheng et al., 2017). These include high-molecular-weight compounds such as polysaccharides and low-molecular-weight compounds such as

polyketides (Thongbai et al., 2015). The typical bioactive compounds isolated from *Hericium* include pyrone and alkaloids, terpenoids, sterols, and nonribosomal peptides (Table 2.2).

Hericium is a great source of novel therapeutic compounds (Chong et al., 2020). Most of the neurotrophic compounds have positive effects on the human nerve cell and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Prion disease, Motor neurone disease, Huntington's disease, Spinocerbellar ataxia, and Spinal muscular atrophy (Zhang et al., 2016; Ratto et al., 2019; Chong et al., 2020). Ryu et al. (2021) reported that hericerin and isohericerinol A increase and regulate the number of neurons by nerve growth factor synthesis and brain-derived neurotrophic factors, in combination with synaptophysin. hericerin and isohericerinol A can promote neuron differentiation and neuron growth, which may be useful for both preventive and therapeutic use in neurodegenerative diseases (Li et al., 2018).

Table 2.2 The typical bioactive compounds isolated from *Hericium*

***Hericium* species**	**Compounds**	**References**
H. coralloides	corallocins A−C; hericerin; [5-(2E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-hydroxy-6-methoxy-1-isoindoline D-Arabinitol; erinapyrones A, B, C; erinaceolactone A, C; erinacines A, B, C, E, F, G, H, I, S, Z1; erinacerin M; erinacol; hericerin; herierin III; hericanal A; hericene A; hericenones A, B, C, D, E, F, G, H;	Wittstein et al. (2016)

Table 2.2 (continued)

***Hericium* species**	**Compounds**	**References**
H. erinaceus	isohericerin; isohericerinol A; methyl linoleate; orsellinaldehyde; polyphenols; polysaccharides; N-De phenylethyl isohericerin; 11-O-acetylcyathin A3; 1-D-arabinitol-monolinoleate; 2-chloro-1,3-dimethoxy-5-methylbenzene; 4-chloro-3,5-dihydroxy-benzaldehyde; 4-[30,70-Dimethyl-20,60-octadienyl]-2-formyl-3-hydroxy-5-methyoxybenzylalcohol	Miyazawa et al. (2012), Wolters et al. (2015), Sokół et al. (2015), Chen et al. (2017), Rupcic et al. (2018), Ryu et al. (2021)
H. flagellum	erinacine Z2; erinacines A, B, C, E, F; CJ14.258	Rupcic et al. (2018)

Furthermore, the compounds have been traditionally used in China for the prevention and treatment of human diseases. The important assignment is to describe the bioactive compounds of *Hericium* and their medicinal properties. It has been demonstrated that this mushroom possesses anti-oxidant (Jiang et al., 2019), anti-cancer (Younis, 2017), anti-diabetic (Wu & Xu, 2015), anti-hyperglycemic (Yao et al., 2021), hypolipidemic (Liang et al., 2013), anti-inflammatory (Hetland et al., 2020), anti-microbial (Vamanu & Voica, 2017), anti-viral (Liu, 2019), anti-fungal (Song et al., 2020), and hepatoprotective properties (Wang et al., 2019).

2.1.4.3 *Hericium* as an antioxidant

Miles and Chang (2004) reported that *Hericium* prevents oxidation damage to cell DNA. According to Jiang et al. (2019), the effects of antioxidants are diverse, which may be due to external factors such as different components under different extraction conditions having different antioxidant mechanisms. Atila et al. (2018)

reported that the addition of olive cakes to the sawdust substrates for *Hericium* cultivation resulted in noticeable effects of its antioxidant activity. Moreover, Atila et al. (2018) recommended the cottonseed husks and olive cakes with wheat bran to increase the yield of *Hericium*.

2.1.4.4 *Hericium* as anti-cancer

Cancer is the world's leading cause of death (Younis, 2017). *Hericium* has many medicinal properties, such as anti-cancer activity (Blagodatski et al., 2018). Younis (2017) reported that polar extracts from *H. erinaceus* could be a good natural anti-cancer compound source. Moreover, *Hericium* extracts (HTJ5 and HTJ5A) have anti-hepatic and are effective against liver cancer, colon cancer, and gastric cancer in vitro and tumor xenografts bearing in mice in vivo (Blagodatski et al., 2018). This compound has potential as an anti-cancer agent for the treatment of gastrointestinal cancer, used alone and/or in combination with clinical chemotherapy (Li et al., 2014).

2.1.4.5 Anti-diabetic, anti-hyperglycemic, and hypolipidemic properties of *Hericium*

Liang et al. (2013) reported that the administration of the aqueous extract of *H. erinaceus* (AEHE) in streptozotocin (STZ) induced diabetic rats, resulting in lower blood glucose levels and higher insulin levels in the blood. The AEHE treatment also reduces fat disorders (Liang et al., 2013). The AEHE of *H. erinaceus* management increased the activities of catalase, superoxide dismutase, glutathione peroxidase, and glutathione level, and reduced malondialdehyde level in the liver tissue (Liang et al., 2013).

2.1.4.6 *Hericium* as an anti-inflammatory agent

Hetland et al. (2020) reported that *Hericium* caused a decrease in inflammatory cytokines, oxidative stress, and changed intestinal microorganisms, thus leading to the anti-allergic mechanism in maintaining the balance of T helper cells. *Hericium* induced anti-inflammatory mechanisms also include cytokines that cause inflammation, nerve growth that prevents the death of neurons in the ischemic brain (Lee et al., 2014), growth of beneficial intestinal microbiota protecting against inflammatory bowel disease-induced mucosa damage, and improving host immunity (Diling et al., 2017), regulating oxidative stress through signaling pathways that attenuate colitis (Ren et al., 2018).

2.1.4.7 *Hericium* as an anti-microbial agent

The anti-microbial activity of *Hericium* is diverse due to the different species and their ability to inhabit diverse ecological niches with a variety of nutrients and physiological and biological conditions (Sheng, 2017). Kim et al. (2019) reported that the anti-microbial activity of *H. erinaceus* was highly effective against *Staphylococcus aureus*, *Salmonella enteritidis*, *Vibrio parahaemolyticus*, and *Escherichia coli* at 2.5 mg/mL or above. Hence, its extract can be used as food and a natural antimicrobial agent in the diet of pathogenic bacteria.

2.1.4.8 *Hericium* as an anti-viral agent

Ellan et al. (2019) reported anti-viral activity of *H. erinaceus*. The mushroom extract showed very prominent anti-dengue virus serotype 2 (DENV-2) activity (Ellan et al., 2019). Wang & Ng (2004) found that the low molecular weight laccase from the dried fruiting body of *H. erinaceus* showed anti-viral activity against human immunodeficiency virus (HIV) and HIV-1.

2.1.5 Economical Importance

Since ancient times, *Hericium erinaceus* has been a popular species due to its nutritional value and traditional medicinal benefits in China (Khan et al., 2013). *Hericium* has high importance and potential to improve many parts of human life (Valverde et al., 2015). In general, *Hericium* is saprobic, hence utilizing organic and agricultural wastes are recommended (Marshall & Nair, 2014). *Hericium* cultivation in some countries is done on a commercial level, earning a handsome income for the growers and farmers (Scherr et al., 2004). The employment generated through cultivation and its associated allied activities is also immense (Jha, 2006). Increasing the value of *Hericium* in terms of quality products is another economic avenue (Üstün et al., 2018).

Hericium is economically important, since the mushrooms are valuable resources for agricultural, food, and medicinal applications (Park et al., 2004). Ergothioneine accumulates at higher levels in mycelia than in fruiting bodies of economically important mushroom species (Lee et al., 2009). Therefore, the mycelial medium is an effective way to increase the anti-oxidant property of economically important mushroom species (Lee et al., 2009). The search for new sources of bioactive products from *Hericium* is still being done to date (Antunes et al., 2020).

2.1.6 Industrial and Pharmaceutical Applications

The mycelia of *Hericium erinaceus* are rich in erinacines and could be potential candidates in promoting positive brain and nerve health-related activities (Li et al., 2018). Zhang et al. (2017) and Tzeng et al. (2018) reported that *H. erinaceus* powder reduced short-term memory impairment and visual recognition, and neuron generation of the hippocampus of Alzheimer's disease in a mouse model. Also, *Hericium* has been used to treat cognitive impairment in Parkinson's disease (Kuo et al., 2016; Trovato et al., 2016). In addition, Rascher et al. (2020) reported that the cyathane diterpenoid erinacine C of the genus *Hericium* induces expression of the neurotrophins NGF and BDNF in glial cells, also erinacine C also promotes ETS-dependent transcription in astroglial cells, which may play a role in regulating germination and regeneration in the central nervous system.

Commercial health products made from *Hericium* are commonly used for health care and to promote learning and memory (Sokół et al., 2015). A large list of health care products that contain *H. erinaceus* extract as a medicinal ingredient has been introduced for promoting human health. There are patented health products, meal replacement powders, chewable tablets, and solid beverages containing the compounds of *H. erinaceus* that improve human health without side effects (He et al., 2017).

Only clinical study reported the intake of *Hericium* as local drugs, herbs, and in medicinal cuisine (Limanaqi et al., 2020). The pharmacological activities, including anti-allergic, anti-bacterial, anti-fungal, anti-inflammatory, anti-oxidative, anti-viral, cytotoxic, immunomodulating, antidepressive, anti-hyperlipidemic, anti-diabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective, and hypotensive activities (Venturella et al., 2021). In attempts to assess the impact on cognitive function for clinical research, Yamabushitake was used at 750 mg/day (available as a 250 mg tablet, three times daily for 16 weeks). The tablet contains 96% of dry powder of Yamabushitake (Mori et al., 2009). Wang et al. (2014) reported that the *Hericium* tablet was administered 3 g/day for 16 weeks, and 5 g/day of fruiting bodies pulp broth was eaten. Many of them possess health-promoting properties, notably benefiting the brain, heart, and gastrointestinal system. Bioactive compounds like hericenones and erinacines stimulate nerve growth factor (NGF) synthesis, supporting neuroprotection and cognitive function. In addition, Hericium

polysaccharides exhibit antioxidant activity, improve lipid metabolism, and modulate gut microbiota (Friedman, 2015; Mori et al., 2009). Chiu et al. (2018) reported that *Hericium* may help relieve mild symptoms of anxiety and depression and can reduce the impact of chronic illness.

2.2 Genus *Scleroderma*

Scleroderma Pers. belongs to Sclerodermataceae with *Scleroderma verrucosum* (Bull.) Pers. as a type species (He et al., 2019). This genus is distributed worldwide in temperate, subtropical, and tropical regions (Sims et al., 1997; He et al., 2019). Several morphological and molecular studies have confirmed the systematic position of *Scleroderma*, placing it in the suborder Sclerodermatineae within the Boletales (Binder & Bresinsky, 2002; Hughey et al., 2000; Louzan et al., 2007). Most lineages within this suborder are recognised as ectomycorrhizal taxa (Binder & Hibbett, 2006; Watling, 2006). The taxonomy of the genus *Scleroderma* is understudied, particularly in tropical Africa (Sanon et al., 1997) and in Asia (Farmer & Sylvia, 1998; Sims et al., 1999). There are 202 records with 121 species of *Scleroderma* in the Index Fungorum database (www.indexfungorum.org). However, only 46 species were accepted by He et al. (2019).

A revised key of the genus *Scleroderma* was considered for the identification of 25 species in Asia (Sims, 1995; Sanon et al., 2009). In Thailand, only eleven species of *Scleroderma* based on morphology have been reported, namely *S. areolatum* Ehrenb., *S. bovista* Fr., *S. cepa* Pers., *S. citrinum* Pers., *S. dictyosporum* Pat., *S. flavidum* Ellis & Everh., *S. lycoperdoides* Schwein., *S. polyrhizum* (J.F. Gmel.) Pers., *S. sinnamariense* Mont., *S. verrucosum* (Bull.) Pers. (Chandrasrikul, 2011) and *S. suthepense* Kumla, Suwannar. & Lumyong (Kumla et al., 2013).

Due to their accessibility, *Scleroderma* species are good candidates as symbionts for inoculation in afforestation initiatives involving pine and eucalyptus trees (Dell, 2002; Chen, 2006). Typically, the well-known species are *S. citrinum* and *S. verrucosum*, which are found in tropical areas (Cortez et al., 2011).

This chapter is an overview of the *Scleroderma* species, emphasizing their economic significance due to their edible nature, ease of cultivation, production of bioactive substances, and traditional medicinal applications. Moreover, it also tackles the taxonomy and distribution of *Scleroderma* species that are present in Thailand.

2.2.1 Economic Importance of *Scleroderma*

Scleroderma species have numerous ecological and economic significance. Mycorrhizal interactions exist between a number of *Scleroderma* species and plants and trees (Jeffries, 1999). They are essential for nutrient intake and can enhance the development and health of plants (Bradshaw, 2000). They also help increase the productivity of crops, forests, and the overall ecosystem (Wu et al., 2023).

Certain species of *Scleroderma* have been reported to be edible and used in medicinal development (Guzmán et al., 2013). Some substances derived from *Scleroderm*a species have therapeutic uses, although not explored as extensively as some other fungi (Menezes Filho et al., 2022). Research on their bioactive ingredients could lead to the development of new drugs or supplements (Kour et al., 2022). Although *Scleroderma* species possess economic significance due to their edibility, cultivation potential, and bioactive compounds, their utilization for consumption or broader applications remains limited.

2.2.1.1 Edibility

Scleroderma does not have much food value. Although in the early years, McIlvaine and Macadam (1902) claimed that all species were edible when young, this has been proven to be wrong. Many species of *Scleroderma* have been noted to be quite poisonous (Hall, 2003; Schmid et al., 1992; Sims, 1995). In fact, symptoms of poisoning can occur within an hour after eating, such as loss of consciousness, nausea, severe abdominal pain, vomiting, perspiration, generalised tingling sensations, spasms, cramps, paralysis, and anaphylactic shock (Hall, 2003; Schmid et al., 1992; Sims, 1995). Among the 25 species described worldwide, four were documented to be highly poisonous, namely *S. albidum*, *S. areolatum*, *S. cepa*, and *S. citrinum* (Van Der Sar et al., 2005; Rasalanavho et al., 2019). However, other authors claimed that some species of *Scleroderma* are safe to consume. *Scleroderma sinnamariense*, *S. polyrhizum*, and *S. verrucosum* are considered edible in Nepal (Christensen et al., 2008). Moreover, the edibility of *S. flavidum* was also verified (Wang, 2004; Li et al., 2021).

2.2.1.2 Cultivation

There is limited literature on the cultivation of this genus, as the species are thought to be poisonous. To date, only five species have been documented to grow under laboratory conditions (Table 2.3). *Scleroderma citrinum* was found to grow well in an axenic culture at 30 °C (Ingleby et al., 1985). *Scleroderma* sp. was found to have fast growth at 28 ºC and pH 7.5 on agar medium modified Melin- Norkrans (MMN media) with xylose. It was also found to grow rapidly in nutrient medium with NH_4^+ as an inorganic nitrogen source (Lazarević, 2013).

Scleroderma sinnamariense can also be grown under laboratory conditions. In the study of Siri-In et al. (2014), among the different culture media tested, fungal host agar was the best medium for optimal mycelium growth and high biomass yield. *S. sinnamariense* was able to grow at 30 °C. The optimal pH for mycelial growth was 5.0. This strain also produced indole-3-acetic acid and siderophores in pure culture, compounds commonly synthesized by many fungi; notably, indole-3-acetic acid functions as a plant hormone rather than having direct physiological effects in humans (Siri-In et al., 2014). Siri-In et al. (2014) provide valuable information for mycelial cultivation in Thailand.

Mycelial growth of *S. verrucosum* was also observed in the laboratory (Putra et al., 1999). The three-week-old culture was placed on a synthetic agar medium covered with cellophane and incubated at 24 °C in the dark. The growth in the plates was found to range from 0.9 to 36 mm after 4 weeks of incubation. For experiments with non-stirred liquid medium, the inoculum was grown on the surface of 100 ml of MMN medium in 250 ml Erlenmeyer flasks at pH 5−6 and maintained at 24 °C, shaken gently for 20 s at least once every 3 days, and then daily to ensure oxygenation during exponential growth (Putra et al., 1999). Although some chemically unique pigments have been isolated from the fruiting bodies of *Scleroderma* species, these compounds have not been demonstrated to possess any significant bioactivity (Zhou & Liu, 2010). Therefore, current interest in cultivating *Scleroderma* should be viewed more in the context of taxonomic, ecological, or chemical novelty rather than as a source of pharmacologically relevant compounds. In Thailand, field cultivation has not been documented yet; thus, further studies are necessary.

Table 2.3 *Scleroderma* species grown under laboratory conditions

Species of *Scleroderma*	**Media for mycelial growth**	**References**
Scleroderma sp.	MMN medium with xylose	Lazarević (2013)
(JQ685726)	MMN medium with NH_4+	
S. sinnamariense	MMN medium	Siri-In et al. (2014)
(CMUS01)	MMN liquid medium	
S. verrucosum (MH13)	MMN medium	Putra et al. (1999)

2.2.1.3 Bioactive compounds

Some species of *Scleroderm*a show bioactive properties in laboratory studies. They possess secondary metabolites with bioactivities. *Scleroderma nitidum* and *S. cepa,* for instance, can be used as an anti-inflammatory and hemostatic agent (Nascimento et al., 2011; Guzmán et al., 2013). *Scleroderma polyrhizum* has been found to have anti-inflammatory and hemostatic properties. It can also be used to stop the bleeding of external wounds by applying the spore dust to wounds (Guzmán et al., 2013). In addition, *S. nitidum* has anti-inflammatory and immunomodulatory activities (Nascimento et al., 2011).

Among the species of *Scleroderma*, *S. citrinum* is the most studied for its bioactivities (Entwistle & Pratt, 1968; 1969). The methanol extract of *S. citrinum* exhibited weak antiviral activity against *Herpes simplex* virus type 1 (IC_{50} = 15 µg/mL) and minimal activity against *Myobacterium tuberculosis* H37Ra (MIC = 100 µg/mL) (Guzmán & Ovrebo, 2000; Liu, 1984).

Many compounds have been isolated from *Scleroderma* species. Various pigments such as xerocomic acid, badione A, norbadione A, and sclerocitrin (Velíšek & Cejpek, 2011; Winner et al., 2004) (Figure 2.1).

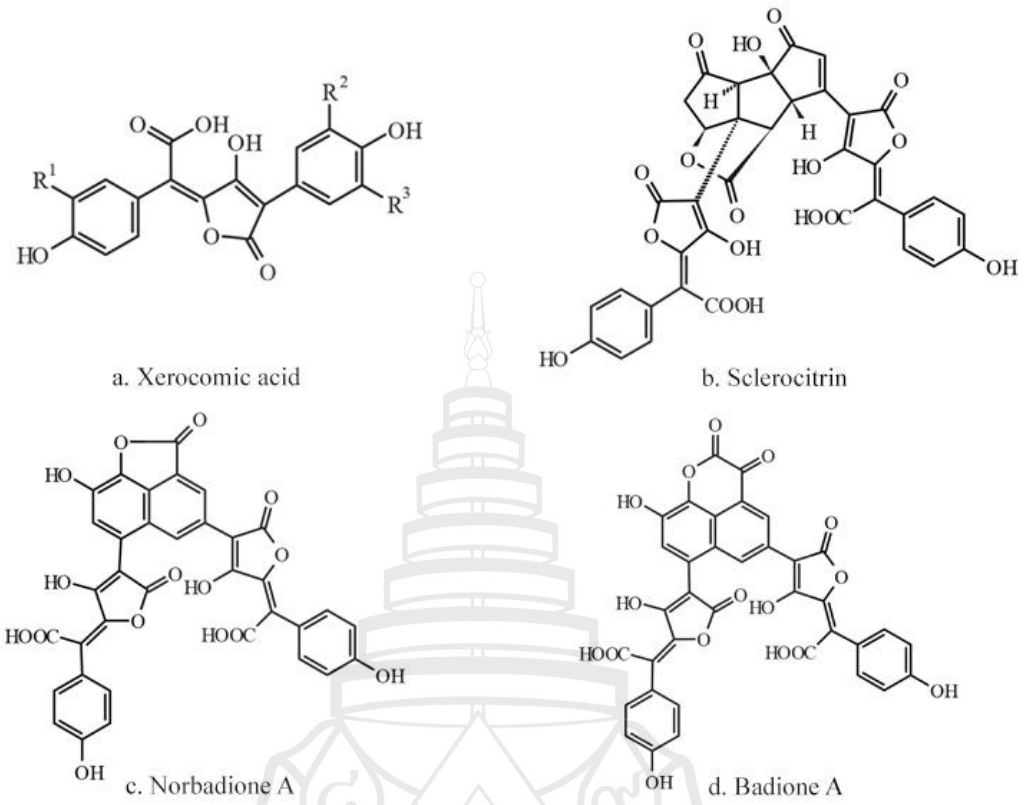
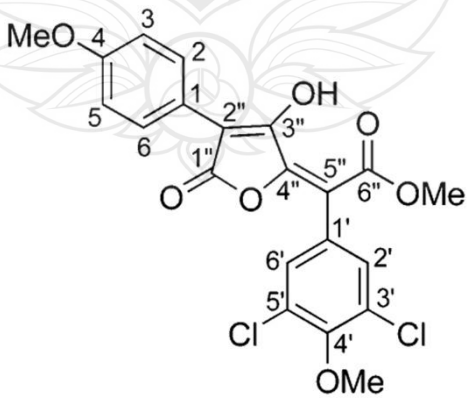


Figure 2.1 Structures of various pigments isolated from *Scleroderma* species

Pulvinic acid dimers, a family of pigments characteristic of Boletales fungi, have been isolated from *S. citrinum* (Winner et al., 2004) (Figure 2.2). Purification of the extract yielded a novel pulvinic acid derivative alongside three known derivatives of this pigment class (Van Der Sar et al., 2005).



methyl-3¢,5¢-dichloro-4,4¢-di-O-methylatromentate

Figure 2.2 Structure of pulvinic acid

Scleroderma nitidum has also been reported to produce polysaccharides and glucans with anti-inflammatory potential (Nascimento et al., 2011). Two lanostane triterpenoids were also isolated from *Scleroderma* UFSMSc1, namely sclerodol A and B (Figure 2.3). Lanostanes are a relevant group of lanosterol-derived tetracyclic triterpenoids that possess important biological and pharmacological properties, such as potential anticancer, antimicrobial, anti-inflammatory, and antiviral activities (Morandini et al., 2016).

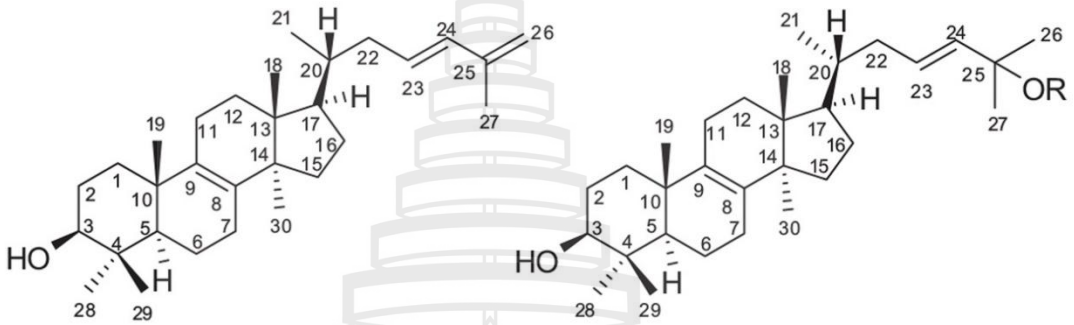


Figure 2.3 Structures of lanostane-type triterpenes compounds. Sclerodol A (1), Sclerodol B (2), and lanostane tripenoid (3)

In general, *Scleroderma* fungi play a significant role in enhancing human well-being and promoting environmental sustainability, despite their economic importance not being as firmly established as that of certain other organisms. This is attributed to their ecological functions and potential applications in areas such as bioremediation, agriculture, and medicine.

2.2.2 Ecology and Distribution

Scleroderma is ectomycorrhizal, form symbiotic establish symbiotic partnerships with plant roots, especially those of trees. *Scleroderma* has a worldwide distribution in tropical, subtropical, and temperate regions (Siri-In et al., 2014) and boreal areas (Wang et al., 2020), often in association with both deciduous and coniferous trees. However, in Asia, *Scleroderma* was commonly found from temperate regions in East Asia like Japan, China, and Korea (Wang et al., 2020; Cho et al.; 2022) to tropical areas in Southeast Asia, including Thailand, Malaysia, Indonesia, and

Vietnam (Naksuwankul et al., 2022; Amira, 2018; Turjaman, 2018; Truyen & Patacsil, 2017). Also found in South Asia, like India, Nepal, and Sri Lanka (Pradhan et al., 2011; Kathmandu, 2020), to Central Asia in Kazakhstan and Uzbekistan (Temreshev, 2019; Zoirjon et al., 2023) (Figure 2.4).

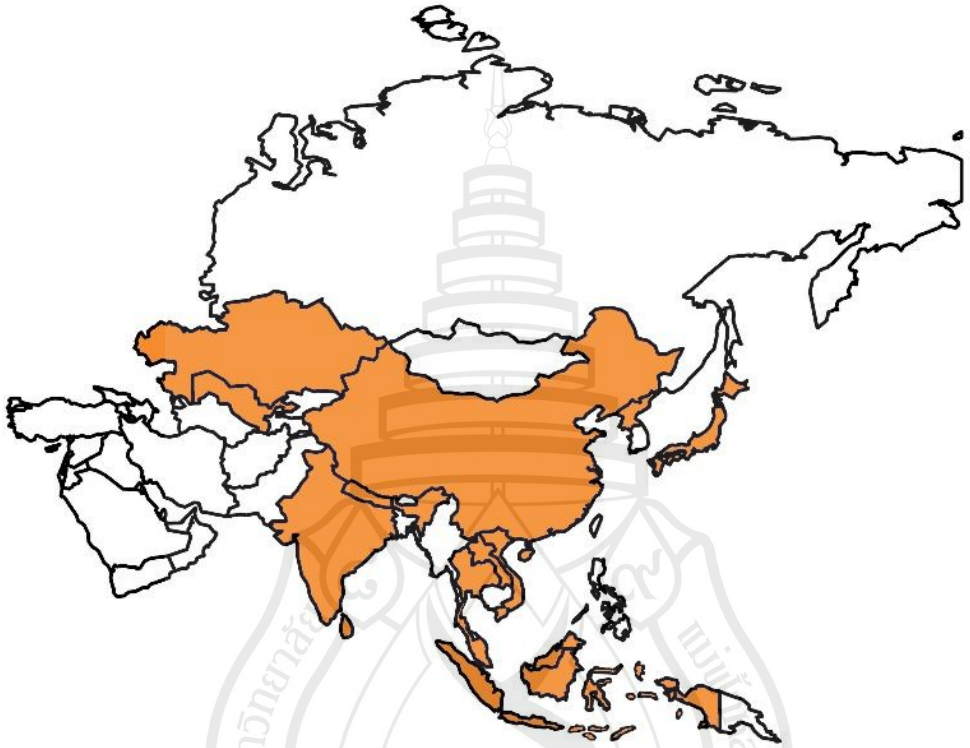


Figure 2.4 Map of the distribution of S*cleroderma* in Asia

Scleroderma species grow on the soil, forming ectomycorrhizal associations with several trees or shrubs such as Pinaceae (*Abies*), Betulaceae (*Betula*), Polygonaceae (*Coccoloba*), Myrtaceae (*Eucalyptus*), Nothofagaceae (*Nothofagus*), Pinaceae (*Pinus*), Salicaceae (*Populus*), and Fagaceae (*Quercus*) (Morris et al., 2008). *Scleroderma* basidiomata are communal in all species, but sometimes they are caespitose or fasciculose and hypogeous or subhypogeous to epigeous in immature stages, such as *S. areolatum*, *S. cepa*, *S. citrinum*, and *S. bovista*. However, some species such as *S. columnare*, *S. hypogaeum*, *S. mexicana*, and *S. sinnamariense* participate with Caesalpinaceae, Dipterocarpaceae, or Phyllanthaceae trees (Guzmán et al., 2013). There were 11 species of *Scleroderma* in Thailand (Chandrasrikul, 2011; Kumla et al., 2013) (Table 1.3).

2.2.3 Taxonomy

Scleroderma has received many other common names, such as ‘earthball’ and ‘poison pigskin puffball’. Numerous kinds of *Scleroderma* species have been proposed, based on the physical traits of their basidiomes and basidiospores (Sims et al., 1995; Guzmán et al., 2013; Kumla et al., 2013). The listed taxa are documented in Thailand, with their descriptions drawn from various sources.

2.2.3.1 *Scleroderma* Pers., Syn. meth. fung. (Göttingen) 1: xiv, 150 (1801)

Synonymy:

=*Actigea* Raf., Précis Découv. Trav. Somiologiques Palermo: 52 (1814)

=*Actinodermium* Nees, Syst. Pilze (Würzburg): 135 (1816) [1816-17]

=*Caloderma* Petri, Malpighia 14: 136 (1900)

=*Goupilia* Mérat, Nouv. Fl. Environs Paris, Edn 3 1: 91 (1834)

=*Lycoperdastrum* P. Micheli, Nov. pl. gen. (Florentiae): 219, tab. 99 (1729)

=*Mycastrum* Raf., Ann. Bot. (Desvaux) 1: 236 (1813)

=*Neosaccardia* Mattir., Annali Fac. Med. vet. Torino 56: 32 (1921)

=*Nepotatus* Lloyd, Mycol. Writ. 7(Letter 75): 1355 (1925)

=*Phlyctospora* Corda, in Sturm, Deutschl. Fl., 3 Abt. (Pilze Deutschl.) [7](19-20): 51 (1841)

=*Pirogaster* Henn., Hedwigia 40(Beibl.): (27) (1901)

=*Pompholyx* Corda, in Sturm, Deutschl. Fl., 3 Abt. (Pilze Deutschl.) 3(12): 51 (1834)

=*Sclerangium* Lév., Annls Sci. Nat., Bot., sér. 3 9: 130 (1848)

=*Stella* Massee, J. Mycol. 5(4): 185 (1889)

=*Sterrebekia* Link, Mag. Gesell. naturf. Freunde, Berlin 8: 44 (1816) [1815]

=*Veligaster* Guzmán, Mycologia 61(6): 1117 (1970) [1969]

The following description is from Guzmán et al. (2013), with a few adaptations to modern terminology.

Basidiome with a massive, compact basal mass of mycelium, globose, subglobose, pyriform, sessile, pseudostipitate, or with a well-developed stipe, leathery to very hard when dry. Exoperidium, which develops by the basidiome growth that tears and digests hyphae, has dry, thin, silky smooth cracked, scaly, or wrapped with small to large scales, constantly with a membranaceous wrap on the base of the globose

basidiome or in the upper portion of the stipe, and occasionally at/on the apex of the basidiome. The endoperidium is thin, and the gleba is protected by a membrane. Frequently rufescent exoperidium and endoperidium are both. White, soon purple or dark greyish-brown or reddish-brown, at first with tramal plates, later with thin whitish or yellowish filaments, Gleba subfleshy to leathery, compact, ultimately dusty. Dehiscence through an irregularly lacerated apical pore or by breaking the apical portion of the basidiome. Hymenium undeveloped. Absent from capillitium. When immature, basidiospores are subglobose, seamless, with a visible apiculus, echinulated or subreticulated to reticulated, and have a thick wall. Basidia are pyriform, 4-6 (-8) spored, thin- or thick-walled, hyaline, and release their basidiospores early when they are still juvenile. Taste and smell are generally harsh and rubbery.

Habitat: On soil, ectomycorrhizal, epigeous or hypogeous fern stipes, and occasionally rotting wood. Tropical, subtropical, and temperate species.

2.2.3.2 *Scleroderma areolatum* Ehrenb., Sylv. mycol. berol. (Berlin): 27 (1818) (Figure 2.5; a).

The following description is from Nouhra et al. (2012), with a few adaptations to modern terminology.

Basidiome 2-4 cm diam, subglobose to pyriform, rounded on the top, sessile, or with a short pseudostipe, rhizomorphs accumulate at base of the basidiocarp. Surface smooth, and the apical section of the brown polygonal scales is clearly dark. When peridium is stretchy at an immature stage, it becomes yellowish white and then becomes paler as it ages. Some specimens are yellow. Dehiscence occurs when the top section ruptures or forms an uneven apical whole form. Fresh peridium is 800-1000 mm thick, divided into two layers; exoperidium is thin and discontinuous. With KOH, the thin-walled, intertwined, brownish to hyaline hyphae in the upper layer change color from yellow to reddish brown. The endoperidium is an 8 m wide, thick-walled, pseudoparenchymatic structure made of hyaline hyphae. Basidia none observed. At maturity, the gleba becomes powdery, brownish violet to dark olivaceous, and has many trama veins that are yellowish. Basidiospore globose, yellowish brown in KOH, echinulate, densely packed, (10–)11–16(–17) µm diam, ornamentation.

Habitat: On soil, growing under *Pinus radiata*, *P. elliottii*, *Cedrus* sp., *Quercus* sp. and *Betula* sp.

2.2.3.3 *Scleroderma bovista* Fr., Syst. Mycol. (Lundae) 3(1): 48 (1829)

Synonymy:

= *Scleroderma verrucosum* subsp. bovista (Fr.) Šebek, Sydowia 7(1-4): 177 (1953)

= *Scleroderma verrucosum* var. bovista (Fr.) Šebek, Fl. ČSR, B-1, Gasteromycetes: 570 (1958)

= *Tuber fuscum* Corda, Icon. fung. (Prague) 1: 25 (1837)

= *Scleroderma fuscum* (Corda) E. Fisch., in Engler & Prantl, Nat. Pflanzenfam., Teil. I (Leipzig) 1(1**): 336 (1898)

= *Scleroderma lycoperdoides* var. *reticulatum* Coker & Couch, Gasteromycetes E. U.S. Canada (Chapel Hill): 170 (1928)

= *Scleroderma citrinum* var. *reticulatum* (Coker & Couch) Guzmán, Ciencia Méx. 25: 204 (1967)

(Figure 2.5;b).

The following description is from Nouhra et al. (2012) and Siri-In et al. (2014), with a few adaptations to modern terminology.

Basidiome 2–7 cm globose to subglobose, accumulating soil particles in a brief basal cluster. Near the top, the surface is smooth, scaly, or finely fractured; the scales are up to 3 mm large and uneven in shape. The peridium is rather stretchy when fresh, whitish to light brown or pale yellowish brown, with cracking of the upper surface. Gleba greyish green with yellowish trama veins that become powdery at maturity, constituted by spores, nurse cells, and clamped hyphae. Basidia none observed. Basidiospore globose, dark yellowish brown in KOH, reticulate with spines, (11–)12–14(–16) mm diam, including ornamentation.

Habitat: On soil, growing under *Pinus radiata*, *P. elliottii*, *Cedrus* sp., *Quercus* sp., and *Betula* sp.

2.2.3.4 *Scleroderma cepa* Pers., Syn. meth. fung. (Göttingen) 1: 155 (1801)

Synonymy:

= *Scleroderma cepa* var. *erythraeum* Sacc., Malpighia 23: 233 (1916)

= *Scleroderma cepioides* Gray, Nat. Arr. Brit. Pl. (London) 1: 582 (1821)

= *Scleroderma verrucosum* var. cepa (Pers.) Maire, Treb. Mus. Ciènc. nat. Barcelona, sér. bot. 15(no. 2): 112 (1933)

= *Scleroderma vulgare* var. *cepa* (Pers.) W.G. Sm., Syn. Brit. Basidiomyc.: 480 (1908)

(Figure 2.5;c).

The following description is from Guzmán et al. (2013), with a few adaptations to modern terminology.

Basidiomes 2–3 cm in diam, globose or subpyriform, sessile, or pseudostipitate. Exoperidium 1–2 mm thick, white, whitish, or yellowish to orangish-yellow, smooth to coarsely cracked. Endoperidium is whitish to yellowish. Stellate dehiscence with 6–8 lobes or through an unpredictably shaped fissure in the upper peridium. Gleba white to violaceous brown, typically rubescent in context. Occasionally has a rubbery smell and flavor. Clamp connections are absent. Basidia 18–25 x 8.5–10 µm, with 4 sterigmata, pyriform, and hyaline. Basidiospores (7–) 8-13 (–14) µm diam., echinulated, spines 1–2 µm high. Hyphae of the endoperidium 3–7 (–10) µm wide, thin-walled.

Habitat: On the ground, in gardens and parks as well as in Quercus, Pinus-Quercus, or mesophytic forests.

2.2.3.5 *Scleroderma citrinum* Pers., Syn. meth. fung. (Göttingen) 1: 153 (1801)

Synonymy:

= *Scleroderma aurantiacum* sensu Carleton Rea (1922), Ramsbottom (1953), non-Linnaeus (Sp. Pl., 1753); fide Checklist of Basidiomycota of Great Britain and Ireland (2005)

= *Scleroderma aurantium* sensu auct.; fide Checklist of Basidiomycota of Great Britain and Ireland (2005)

= *Lycoperdon aurantium* sensu auct.; fide Checklist of Basidiomycota of Great Britain and Ireland (2005)

= *Scleroderma vulgare* Hornem., Fl. Danic. 10: tab. 1969, fig. 2 (1819)

= *Scleroderma vulgare* var. *macrorhizum* Fr., Syst. mycol. (Lundae) 3(1): 47 (1829)

= *Scleroderma macrorhizum* (Fr.) Wallr., Fl. crypt. Germ. (Norimbergae) 2: 404 (1833)

Scleroderma aurantium var. *macrorhizum* (Fr.) Šebek [as 'macrorrhizum'], Sydowia 7(1-4): 170 (1953)

= *Scleroderma vulgare* subsp. macrorrhizon (Wallr.) Sacc. [as 'macrorhizon'], in Berlese, De Toni & Fischer, Syll. fung. (Abellini) 7(1): 135 (1888)

= *Scleroderma vulgare* var. *novoguineense* Henn., Bot. Jb. 18(4 (Beibl. 44)): 37 (1894)

= *Scleroderma vulgare* var. *bogoriense* Henn. & E. Nyman, in Hennings in Warburg, Monsunia 1: 159 (1899)

= *Scleroderma vulgare* var. *aurantiacum* Bull. ex W.G. Sm., Syn. Brit. Basidiomyc.: 480 (1908)

= *Scleroderma aurantium* var. *aurantiacum* (Bull. ex W.G. Sm.) Rea, Brit. basidiomyc. (Cambridge): 49 (1922)

Common name:

= Pigskin poison puffball

(Figure 2.5;d).

The following description is from Anong (2008) and Soytong et al. (2014), with a few adaptations to modern terminology.

Basidiomes approximately 2−6 cm across, high 2−3 cm, nearly round when young, depressed at maturity, yellow brown, covered with tough raised warts which may have center darker brown, thick rind, white, spitted into irregular lobes in age, the mass of the spore white, solid and firm, soon becoming marble and publishing black spores out, then the gleba powdery at maturity. Clamp connections none observed. Basidia none observed. Basidiospore 8−12 μm, round, with fine spines and netlike ridges, spore print blackish brown.

Habitat: single to many on the ground near stumps and logs of deciduous trees or on soil, associated with pine forest (Quercus), ectomycorrhiza.

Edibility: inedible, bitter, poisonous.

2.2.3.6 *Scleroderma dictyosporum* Pat., Bull. Soc. mycol. Fr. 12(3): 135 (1896)

The following description is from Teerawat (2007), with a few adaptations to modern terminology.

Basidiomes 7−12 mm in diam, ovoid, rooted at the base by an abundance of white branching rhizomorphs, and covered in irregular brown granular warts 0.2 mm in diam. Peridium is 0.5 mm thick, delicate, yellowish white, and rippling. Dark greyish brown gleba. Basidiospores are 7−8.5 m in diam, globose, extremely coarsely pustulate-reticulate, and brown in color.

Habitat: Solitary on soil.

2.2.3.7 *Scleroderma flavidum* Ellis & Everh., J. Mycol. 1(7): 88 (1885)

Synonymy:

= *Actigea multifida* Raf., Précis Découv. Trav. Somiologiques (Palermo): 52 (1814)

= *Scleroderma flavidum* f. multifidum (Raf.) De Toni, in Berlese, De Toni & Fischer, Syll. fung. (Abellini) 7(1): 139 (1888)

= *Scleroderma flavidum* var. *fenestratum* Cleland, Trans. Roy. Soc. S. Australia 47: 75 (1923)

(Figure 2.5;e).

The following description is from Anong (2008), with a few adaptations to modern terminology.

Basidomes approximately 2−5 cm, pear-shaped to nearly round, golden yellow, cracked above and on the sides into small to large pieces with brownish scales, apex rupturing at maturity, peridium tough, thin, open irregularly, soil, attached by yellow threads and joined into a yellow stalk-like base. Clamp connections none observed. Basidia none observed. Basidiospore 7−10 µm round, with 1−2 µm long spines, spore print purple black.

Habitat: single or group on bare soil, lawn, or parks.

Edibility: edible when young.

2.2.3.8 *Scleroderma lycoperdoides* Schwein., Schr. naturf. Ges. Leipzig 1: 61 [35 of repr.] (1822)

Synonymy:

= *Bovistella lycoperdoides* (Schwein.) Lloyd, mycol. writ. (Cincinnati) 2(Letter 23): 280 (1906)

(Figure 2.5;f).

The following description is from Coker (1974), with a few adaptations to modern terminology.

The appearance of the basidiome surface is about the same as in the strain, from which it differs in that the mature spore mass is olive in color, that the spores are firmly reticulated rather than just spiny, and that the root is more fragile and less large. Although the color may be slightly deeper and the markings may be less obvious than is typical for the species, these traits are too variable to be highlighted. The difference in basidiospore and the color of the greba are highly distinct, and the latter is readily visible at moderate magnification. The bright golden olivaceous matrix remains when the black olivaceous spores are shook out. Spherical, 10–13 μm long, highly reticulate, and spiky spores.

Habitat: open woodlands' moist sand.

2.2.3.9 *Scleroderma polyrhizum* (J.F. Gmel.) Pers., Syn. meth. fung. (Göttingen) 1: 156 (1801)

Synonymy:

= *Lycoperdon polyrhizum* J.F. Gmel. [as 'polyrhizon'], Syst. Nat., Edn 13 2: 1464 (1792)

= *Sclerangium polyrhizum* (J.F. Gmel.) Lév. [as 'polyrhiza'], Annls Sci. Nat., Bot., sér. 3 9: 130 (1848)

= *Actigea sicula* Raf., Précis Découv. Trav. Somiologiques (Palermo): 52 (1814)

= *Scleroderma geaster* var. *siculum* (Raf.) Sacc., in Berlese, De Toni & Fischer, Syll. fung. (Abellini) 7(1): 139 (1888)

= *Scleroderma geaster* Fr., Syst. mycol. (Lundae) 3(1): 46 (1829)

= *Sclerangium geaster* (Fr.) Lév., Annls Sci. Nat., Bot., sér. 3 9: 131 (1848)

= *Scleroderma geaster* var. *socotranum* Henn., Bull. Herb. Boissier 1: 100 (1893)

= *Scleroderma primigenium* Bianchi, Bollettino della Società naturalisti 'Silvia Zenari' 12(no. 58): 35 (1986)

(Figure 2.5;g).

The following description is from Anong (2008), with a few adaptations to modern terminology.

Basidiomes 3−6 cm, round, sometimes irregularly lobed. Peridium thick 1−2 mm, brownish, surface roughened or cracked to scale with age, tough, eventually splitting into star-shaped rays. Endoperidium is thin, brownish, becomes blackened, and becomes empty with age. Clamp connections present. Basidia none observed. Basidiospores 7−12 µm, round, warted, spore print purple brown.

Habitat: On sandy soils in pine forest.

Edibility: Edible when young.

2.2.3.10 *Scleroderma sinnamariense* Mont., Annls Sci. Nat., Bot., sér. 2 14: 331 (1840)

(Figure 2.5;h).

The following description is from Ruksawong (2001) and Siri-In et al. (2014), with a few adaptations to modern terminology.

Basidiomes 8−10 cm hemispherical or subglobal. Exoperidium thick when fresh, leathery, verrucose, yellowish to lemon-yellow, and brown to dark scales. Endoperidium thin, yellowish. Sessile, rhizoid, white gleba when young become dark brown to back at maturity and pulverulent. Clamp connections present. Basidia none observed. Basidiospores 7−9 µm, globose to subglobose with short spines.

Habitat: On sandy soils in pine forest.

Edibility: No report of it being edible.

2.2.3.11 *Scleroderma suthepense* Kumla, Suwannar. & Lumyong, Mycotaxon 123: 2 (2013)

(Figure 2.5;i).

The following description is from Kumla et al. (2013), with a few adaptations to modern terminology.

Basidiomes globose or subglobose 1.1−3.5 cm in diam., 1.0−3.9 cm high, well-developed rhizomorphs, white to yellow, 0.5−1.2 cm long. Peridium 0.5−1.0 mm thick when fresh, leathery, partially smooth surface with scattered, small, and thin scales, greyish yellow to greyish brown, consisting of two layers. The exoperidium consists of cylindrical, thick-walled, yellowish to brown hypha up to 8.0 µm diam, with scattered clamp connections, turning reddish brown with KOH. The endoperidium consists of cylindrical, thick-walled, hyaline hyphae up to 6.0 µm diam, with clamp connections. Gleba, when mature, is dark greyish brown on the back and pulverulent.

Basidia none observed. Basidiospores globose to subglobose, strongly reticulate with spines, 8.0−13.0 µm in diam. including ornamentation, spine 1.0−2.5 µm in length, dark yellowish brown in water or KOH, and not changing in Melzer's reagent.

Habitat: Terrestrial on sandy loam, under *Prunus cerasoides* in a dipterocarp forest.

2.2.3.12 *Scleroderma verrucosum* (Bull.) Pers., Syn. meth. fung. (Göttingen) 1: 154 (1801)

Synonymy:

= *Lycoperdon verrucosum* Bull., Hist. Champ. Fr. (Paris) 1(1): 157 (1791)

= *Lycoperdon defossum* sensu Sowerby; fide Checklist of Basidiomycota of Great Britain and Ireland (2005)

= *Scleroderma verrucosum* var. *maculatum* Peck, Ann. Rep. Reg. N.Y. St. Mus. 53: 848 (1901)

= *Scleroderma cepa* var. *maculatum* (Peck) Lloyd, Mycol. Writ. (Cincinnati) 6(Letter 63): 950 (1920)

= *Scleroderma maculatum* (Peck) Lloyd, Mycol. Writ. (Cincinnati) 6(Letter 65): 1058 (1920)

= *Scleroderma verrucosum* var. *fascirhizum* Šebek, Sydowia 7(1-4): 179 (1953)

= *Scleroderma verrucosum* var. *violascens* Herink, Sydowia 7(1-4): 176 (1953)

= *Scleroderma verrucosum* var. *angustistipitatum* Dissing & M. Lange, Bull. Jard. bot. État Brux. 32: 394 (1962)

(Figure 2.5;j).

The following description is from Anong (2008), with a few adaptations to modern terminology.

Basidiome 5−10 cm, round, shortly pseudostipitate. Peridium thin, with scales, irregularly spitted apex, yellowish pale brown to brown, with stalk-like base 3−8 cm, concolored with basidiome, attached to the ground by root-like threads. Basidiospores 8−10 µm, round, with short spines, spore print purple brown.

Habitat: Gregarious on the ground in the woods, ectomycorrhiza.

Edibility: Edible when young.

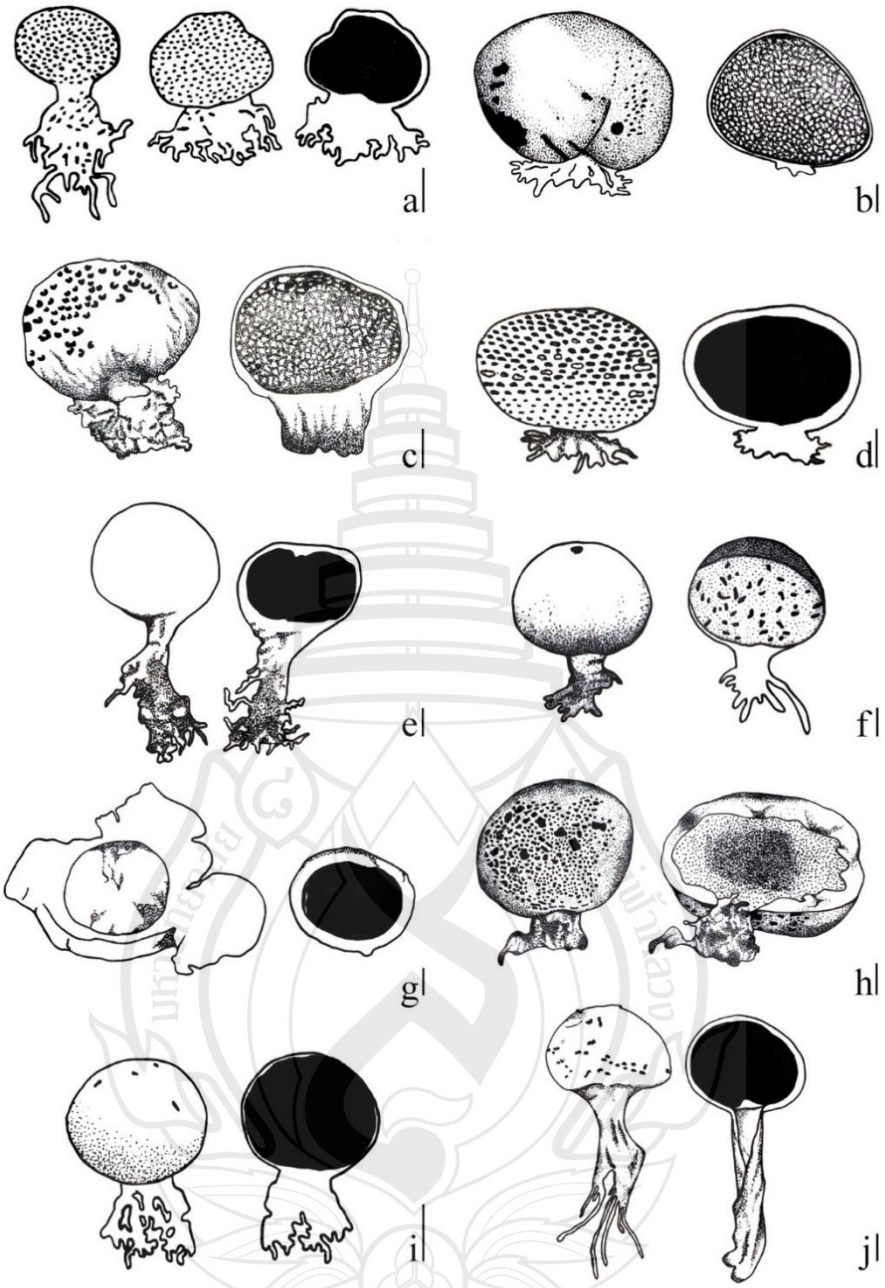


Figure 2.5 Basidiomata of *Scleroderma* species. a, *S. areolatum* (redrawn from Nouhra et al., 2012). b, *S. bovista* (redrawn from Nouhra et al., 2012). c, *S. cepa* (redrawn from Guzmán et al., 2013). d, *S. citrinum* (redrawn from Anong, 2008). e, *S. flavidum* (redrawn from Anong, 2008). f, *S. lycoperdoides* (redrawn from Coker 1974). g, *S. polyrhizum* (redrawn from Anong, 2008). h, *S. sinnamariense* (redrawn from Ruksawong, 2001). i, *S. suthepense* (redrawn from Kumla, et al. 2013). j. *S. verrucosum* (redrawn from Anong, 2008). Scale bars = 1 cm.

2.2.4 Perspectives

To date, Thailand has documented eleven species of *Scleroderma*. Certain species, like *S. citrinum*, are notable for their bioactive properties. However, research on cultivating *Scleroderma* in Thailand is limited, as the edibility of *Scleroderma* is not extensively studied. Further investigation into the taxonomy and bioactive properties of *Scleroderma* in Thailand is essential to determine its potential as a bioactive compound source. Additionally, refining cultivation techniques is necessary to ensure a reliable source for future utilisation of species known for their bioactivities.

CHAPTER 3

GENERAL MATERIAL AND METHODOLOGY

3.1 Sample Collection and Morphological Identification

3.1.1 Collection of *Hericium*

Fresh fruiting bodies of *Hericium* were collected and purchased from the markets. Specimens were examined, photographed, described, and dried using a food dehydrator. Two cultures of a selected strain of *Hericium* were brought from the Department Microbial Drugs, Helmholtz Centre for Infection Research (HZI), Germany. The method for obtaining cultures is well-established and routinely used. These methods are detailed in Stamets (1983, 2000). The fungal isolates in our study were deposited at Mae Fah Luang University (MFLUCC) culture collection and other international culture collections.

3.1.2 Collection of *Scleroderma*

The specimens had been collected at selected locations in Thailand during the rainy seasons of 2019–2024 (Figure 3.1, Table 3.1). The forest and the mushroom substrate or habitat were noted in the field type, and the samples were photographed.

3.1.3 Morphological Identification

Mushrooms were identified from macro characters being recorded at the collection and micro characters being recorded later from dried material. Macromorphological characters were described according to fresh and dried basidiomes, including the structure of fruiting bodies and also the morphological characters, such as tooth and context. The naming of original colours is based on the Methuen Handbook of Colour, 3rd ed. (Kornerup & Wanscher, 1978). The micromorphological analysis was document pertinent anatomical structures and were carried out in the laboratory as time permits. Specimens are photographed using a Canon EPS 18-55 mm camera. Microscopic characters are observed and photographed, mounted in water or in 5% KOH using a Motic SMZ-171 microscope, fitted with a Nikon Eclipse Ni, DS-Ri2 digital camera. All measurements (e.g. basidia, basidiospore

size), the dimensions of at least 50 basidiospores per collection were measured inside view, the size averages are given in the description, while the quotient (Q) of length and width average was calculated to indicate the basidiospore shape using Tarosoft Image Frame Work software and images use for figures are processed with Adobe Photoshop CS3 Extended v.10.0 software (Adobe Systems, USA) (Figure 3.2).

The samples were stored in plastic bags with silica gel after being dried for 24 hours in hot, dry air. The specimens were deposited in Mae Fah Luang University's (MFLU) herbarium.

Figure 3.1 Map of gathering locations from Thailand of *Scleroderma* species

Figure 3.2 Macro-micro morphological characteristics of *Scleroderma*. A B. immature basidiomata. C. scale on the peridium surface. D. cut the side of the peridium. E F. basidiospore. Scale bars: A, B = 1 cm. C, D = 0.5 cm. E, F = 10 µm.

Table 3.1 Collection sites of *Scleroderma* species in this study

Collection sites
Chiang Mai, northern Thailand
Pong Deuad village, Mae Taeng district, Chiang Mai province, Thailand
Pha Deng village, Mare Taeng district, Chiang Mai province, Thailand
Tha Pha village, Mae Taeng district, Chiang Mai province, Thailand
Mae Kam Pong village, Mae On district, Chiang Mai Province, Thailand
Chiang Rai, northern Thailand
Mae Fah Luang University Campus, Muang district, Chiang Rai province, Thailand

Table 3.1 (continued)

Collection sites
Chiang Rai, northern Thailand
Pha Ngae village, Pa Daed district, Chiang Rai province, Thailand
Mae Lao village, Vieng Chiang Rung district, Chiang Rai province, Thailand
Doi Mae Sa Long community forest, Doi Mae Sa Long Village, Mae Fah Luang district, Chiang Rai province, Thailand
Thad Village, Mae Fah Luang district, Chiang Rai province, Thailand
Nang Lae Nai Village, Muang district, Chiang Rai province, Thailand
Mukdahan, northeast Thailand
Nong Iandong village, Kham Cha-E district, Mukdahan province, Thailand
Na Lak village, Dongluang district, Mukdahan province, Thailand
Na Sameng village, Dontan district, Mukdahan province, Thailand
Roi Et, northeast Thailand
Phu Khao Thong village, Pho Chai district, Roi Et province, Thailand
Pha Namyoi, Nong Phok district, Roi Et province, Thailand
Karasin, northeast Thailand
Na Krai village, Kuchinnarai district, Karasin province, Thailand
Surat Thani, southern Thailand
Klong Sra, Kanjanadij district, Surat Thani province, Thailand
Krung Ching, Nopphitam district, Surat Thani province, Thailand
Ban Song, Vieng Sra district, Surat Thani province, Thailand
Nakhon Si Thammarat, southern Thailand
Sirivong, Lansaka district, Nakhon Si Thammarat province, Thailand
Than Yong village, Thung Song district, Nakhon Si Thammarat province, Thailand

3.2 DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted directly from the basidiome using the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, China) or extracted from mycelia grown on PDA or MEA for 2 weeks. Also, DNA extraction was carried out using CTAB lysis buffer and phenol-chloroform as outlined by Jeewon et al. (2003). Primers ITS1 and ITS4 were used for the nrITS1, 5.8S, and nrITS2 regions; primers LR0R and LR5 for the large subunit region (LSU); primers fRPB2-6F and fRPB2-7R for polymerase II second largest subunit (rpb2) region; primers EF1-983F and EF1-1567R for Tef1-α region. DNA samples were checked for purity and integrity by gel electrophoresis. Polymerase chain reaction (PCR) amplification was amplified in a Thermal controllable Cycler DNA amplification using multigene and PCR conditions, followed by common protocols (Table 3.2). Sequencing and Alignment: The DNA strands were sequenced in an automated sequencer following the manufacturer's protocols. The sequences were blasted to an online website to find regions of local similarity between sequences for species confirmation, and all new sequences were deposited in GenBank. The sequences were checked against existing sequences at GenBank, and related sequences were obtained for the analysis.

3.3 Phylogenetic Analysis

The most closely related taxa are determined using nucleotide BLAST searches online in GenBank (http://www.ncbi.nlm.nih.gov/). All sequences are aligned using MAFFT v7.110 online program (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley, 2013). The alignments are checked, and uninformative gaps are minimized manually is necessary for BioEdit 6.0.7 (Hall, 2004). Maximum Likelihood (ML), maximum parsimony (MP), and Bayesian Inference (BI) are used in analyses with individual data from each partition in addition to the combined aligned dataset. Intron or variable sequences are excluded from all analyses.

Maximum Likelihood (ML) analysis is run in RAxML (Stamatakis, 2014) implemented in raxmlGUI v.0.9b2 (Silvestro & Michalak, 2010) with 1000 rapid

bootstrap replicates using the GTR+ GAMMA model of nucleotide substitution. Maximum Likelihood bootstrap values (MLBP) equal to or greater than 60% are given above each node.

Parsimony analysis was carried out with the heuristic search option in PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 2002). Alignment gaps were treated as missing characters in the analysis of the combined data set, where they appeared in relatively conserved regions. The branch-swapping algorithm performs the heuristic search option with 1000 random sequence additions and tree-bisection reconnection (TBR). Maxtrees are set up at 1000. Descriptive tree statistics for parsimony, such as Tree Length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) are included in the analysis. The robustness of the most parsimonious tree is estimated based on 1000 bootstrap replications with every 100 replicates of random stepwise addition of taxa.

The model of nucleotide substitution was performed by using MrModeltest 2.2 (Nylander, 2004) for each gene. Posterior probabilities (PP) (Rannala & Yang, 1996; Zhaxybayeva & Gogarten, 2002) are determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist, 2001). Six simultaneous Markov chains are run from random trees for 5000000 generations, and trees are sampled every 1000th generation. The first 20% of trees are discarded as the burn in-phase, and the remaining trees are used for calculating posterior probabilities in the majority rule consensus tree (the standard deviation of split frequencies was reached to 0.01) (Ariyawansa et al., 2013). Bayesian Posterior Probabilities (BYPP) with those equal to or greater than 0.90 are given below each node. Phylogenetic trees were drawn using Treeview v. 1.6.6. The sequences are deposited in GenBank and the alignments in TreeBASE.

Phylograms were visualized with FigTree v1.4.0 program (Rambaut, 2012) and reorganized in Microsoft PowerPoint (2007) and Adobe Photoshop CS6. Sequences derived in this study were deposited in GenBank, and the alignments in TreeBASE (www.treebase.org).

Table 3.2 Partial gene regions, primers, and PCR amplification conditions for *Scleroderma* species used in this study

Gene	Primer		PCR protocol					
	Forward	Reverse	Initial Denaturation	Denaturation	Annealing	Extension	Final extension	Reference
ITS	ITS1	ITS4	95 °C, 3 min 1 cycle	94 °C, 40 sec	54 °C, 40 sec 35 cycles	72°C, 1 min	72°C, 08 min 1 cycle	White et al. (1990)
LSU	LROR	LR5	95 °C, 3 min 1 cycle	94 °C, 40 sec	50 °C, 40 sec 35 cycles	72°C, 2 min	72°C, 08 min 1 cycle	Vilgalys and Sun (1994)
RBP2	fRPB2-5F	fRPB2-7CR	95 °C, 3 min 1 cycle	94 °C, 40 sec	54 °C, 40 sec 35 cycles	72°C, 1 min	72°C, 08 min 1 cycle	Liu et al. (1999)
Tef1-α	EF1-983F	EF1-1567R	95 °C, 3 min 1 cycle	94°C, 40 sec	54 °C, 40 sec 35 cycles	72°C, 1 min	72°C, 08 min 1 cycle	Rehner and Buckley (2005),
ATP-6	512F	783R	95 °C, 3 min 1 cycle	94 °C, 40 sec	54 °C, 40 sec 35 cycles	72°C, 1 min	72°C, 08 min 1 cycle	Carbone and Kohn (1999)

3.4 Cultivation of *Hericium*

3.4.1 Mushroom Isolation

Fresh fruiting bodies of *Hericium* were obtained from the Thai Royal project shop, Chiang Rai, Thailand. Pure culture of *Hericium* was obtained from Kunming Institute of Botany, Kunming, China, by plating sterile tissues of the mycelial context onto PDA; and two culture collections of *Hericium* from the Institute of Department Microbial Drugs, Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany, include *H*. coralloides (STMA 14278) and *H*. *erinaceus* (STMA 14279) which were isolated from basidiomes provided by the commercial mushroom growing company Pilzgarten GmbH, Fabrikstraße 12, 27389 Helvesiek, Germany, by plating sterile tissues of the mycelial context onto YMG agar, and the culture are deposited at the culture collection of the DSMZ, Braunschweig, Germany. The fungal isolates in our study were deposited at Mae Fah Luang University (MFLUCC) culture collection (Figure 3.3).

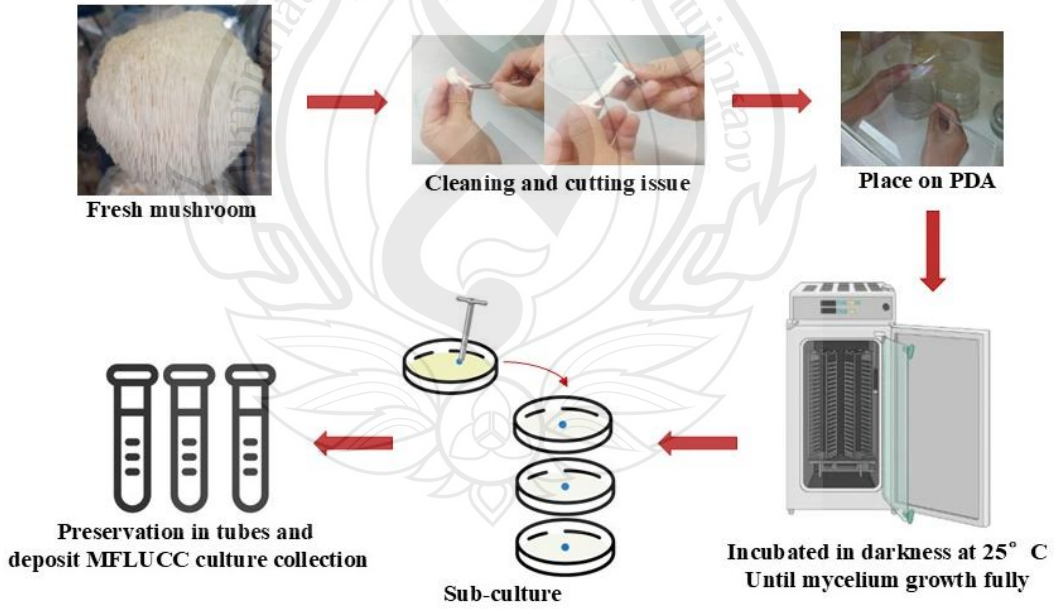


Figure 3.3 Process of cultural isolation from a fresh specimen

3.4.2 Optimization of Culture Conditions for Mycelial Growth

Nine different culture media were used in this study, namely carrot dextrose agar (CDA), corn meal agar (CMA), malt extract agar (MEA), malt yeast peptone agar (MYPA), oat meal agar (OMA), oat meal yeast agar (OMYA), potato dextrose agar (PDA), potato dextrose yeast agar (PDYA), and sabouraud dextrose agar (SDA). Mycelia discs were cut from the advancing margin of 15–day–old pure cultures and were placed at the center of each medium (5 mm diam), and incubated in darkness at 25 °C for 15 days. After incubation, the total yield of mushroom mycelium dried weight was harvested at day 15 by boiling the mycelium culture and which was then dried at 45 °C. The experiment was carried out in triplicate (Figure 3.4).

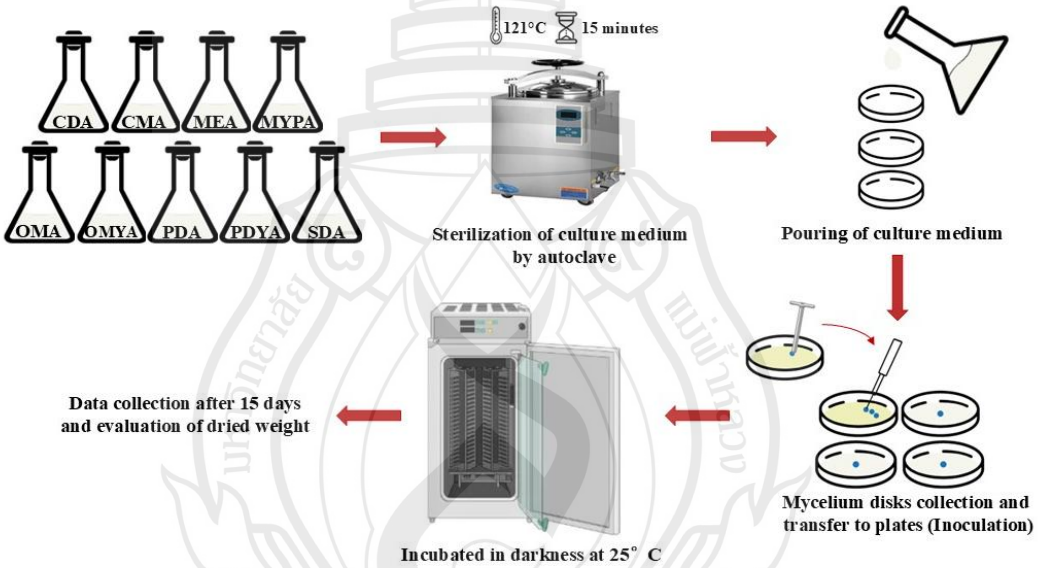


Figure 3.4 Process of optimal media test

3.4.3 Optimization of Temperature for Mycelium Growth

The optimum medium for growth was used as the basis medium to evaluate the impact of different temperatures (16 °C, 20 °C, 25 °C, 30 °C, and 35 °C) and incubated in darkness. After incubation, the mushroom mycelium dried weights were harvested at day 15. The experiment was carried out in triplicate (Figure 3.5).

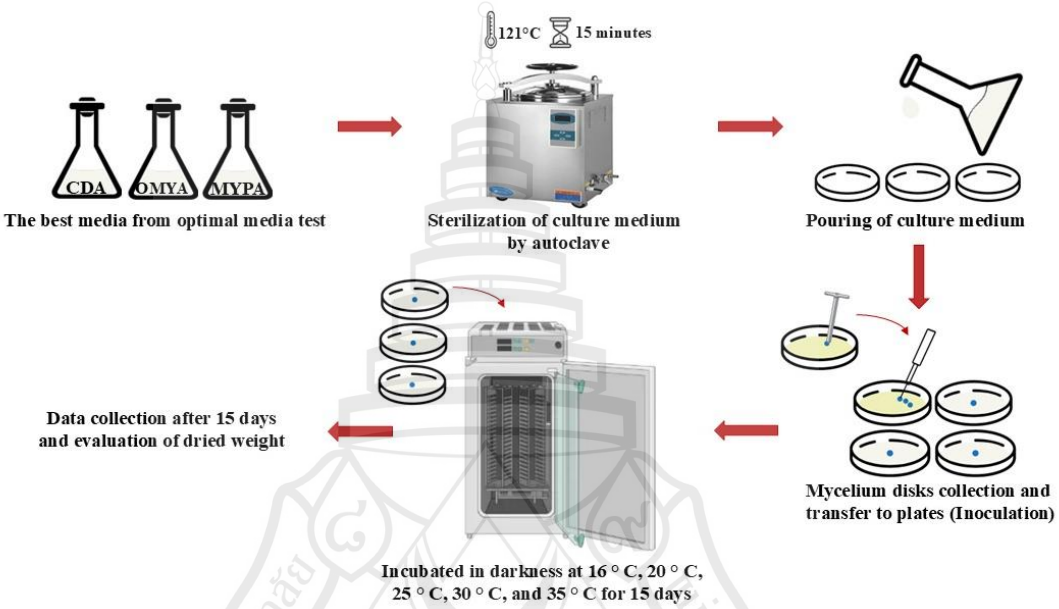


Figure 3.5 Process of temperature test

3.4.4 Optimization of pH for Mycelium Growth

The experiment used a liquid medium state to evaluate the optimal pH. The best medium broth was adjusted to a pH of 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, and 8 with 1M NaOH and 1M HCl. The pH range is measured using a digital pH meter before autoclaving. 100 mL of the liquid medium found previously to be optimum for growth was inoculated with active mycelia (discs 0.5 mm in diameter) of the different mushroom strains, and was incubated in a shaker at 25 °C, 120 rpm for 14 days. The dried mycelium biomass was recorded after 15 days. The experiments were carried out in triplicate (Figure 3.6).

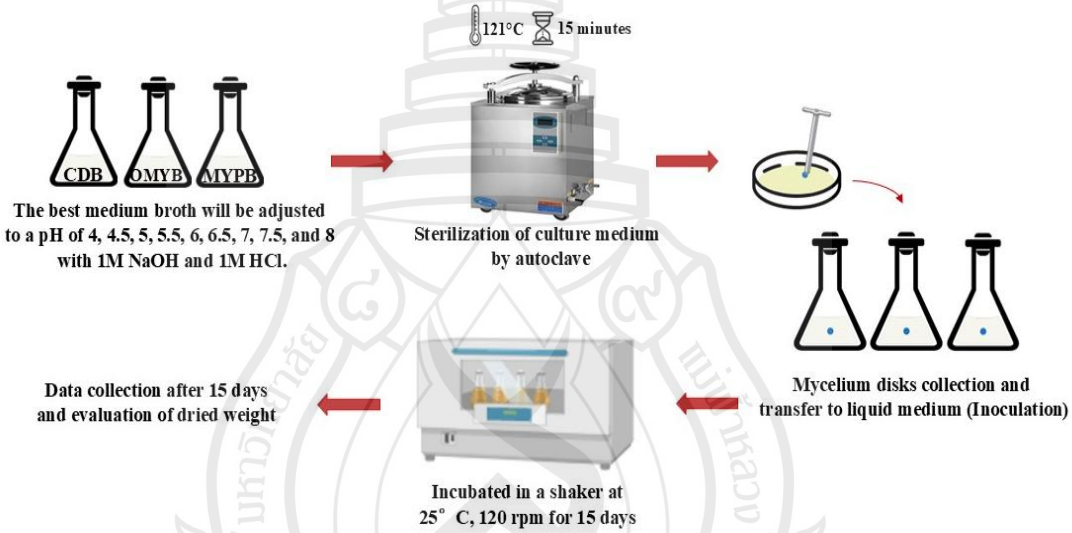


Figure 3.6 Process of pH test

3.4.5 Optimization of Different Cereal Grains and Agricultural Substrates for Mycelium Growth

Fifteen substrates were used to determine the best spawn production, eleven types of cereal grains, including *Avena sativa* (oat), *Coix lacryma*–jobi Linn. (millet), *Hordeum vulgare* L. (barley), *Oryza sativa* (brown rice), *Oryza sativa* L. (rice berry), *Oryza sativa* L. ssp. indica (rice), *Oryza sativa* var. glutinosa (sticky rice), *Sorghum bicolor* (L.) Moench (red sorghum), *Triticum aestivum* L. (wheat), *Vigna radiata* (mung bean), *Zea Mays* L. (corn seed), and four types of agricultural wastes including *Cocos nucifera* Linn. (coir), *Morinda coreia* Ham. (bagasse), *Oryza sativa* L. ssp. indica (rice straw, and paddy). Each substrate was washed and soaked overnight (12–14 hrs), boiled for 10–15 mins, and allowed to cool down in order to keep the moisture content at 50–70%. Fifty grams of each medium were filled into the media test tubes, autoclaved at 121 °C for 15 minutes. After being left to cool down for 24 hrs, the media are inoculated with 5 plugs of the selected strain of Agaricomycetes active mycelium. All media tubes are incubated at room temperature, and the mycelia growth length was recorded every two days until fully colonized (21 days) (Figure 3.7).

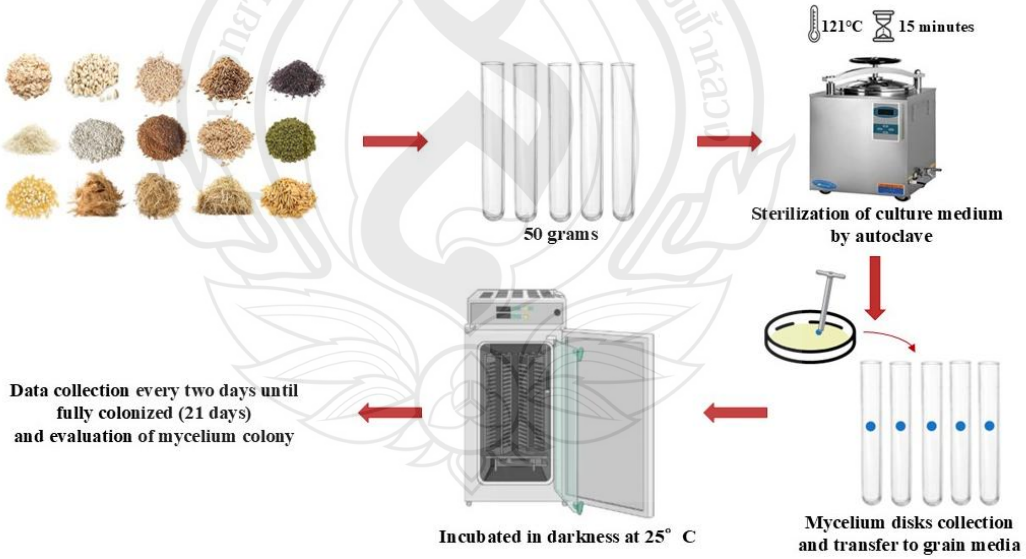


Figure 3.7 Process of optimum grain/agricultural waste media for spawn production

3.4.6 Optimization of Carbon Sources for Mycelium Growth

To screen for a favorable carbon source, the following tests were performed using basal media supplemented with nine different carbon sources, including dextrose, fructose, glucose, glycine, lactose, maltose, molasse, sucrose, and xylose. The basal media is composed of 20 g of tested carbon source, 0.05 g of $MgSO_7H_2O$, 0.46 g of KH_2PO, 1.0 g of K_2HPO, 120 µg of Thiamine HCL, 20 g of agar, and 1,000 ml of distilled water. The basal medium was adjusted to pH 6 before sterilization. After sterilization, the active mushroom mycelial plug (5 mm diam..) of each strain, placed at the center of basal media containing one of ten carbon sources and incubated in the dark for 15 days at 25 ℃. After incubation, the mycelial growth was recorded and measured. The experiment was carried out in triplicate (Figure 3.8).

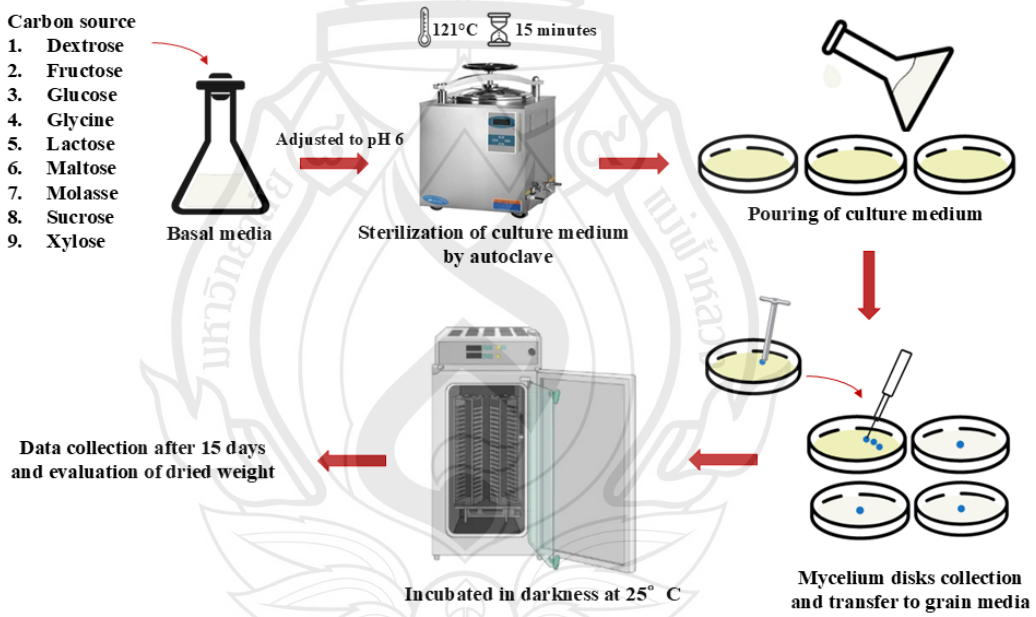


Figure 3.8 Process of carbon sources for mycelium growth

3.4.7 Optimization of Nitrogen Source on Mycelial Growth

Nine different nitrogen sources: ammonium chloride, ammonium nitrate, malt extract, peptone, potassium nitrate, sodium chloride, sodium nitrate, urea, and yeast extract were tested with the basal media supplement. Twenty grams of each nitrogen source were added to the basal media and adjusted to pH 6 before sterile. An active mushroom mycelial plug (5 mm diam.) was placed at the center of the basal media containing each nitrogen source, incubated in darkness for 15 days at 25 ℃. After 15 days of incubation, the mycelia were recorded and measured. The experiment was carried out in triplicate (Figure 3.9).

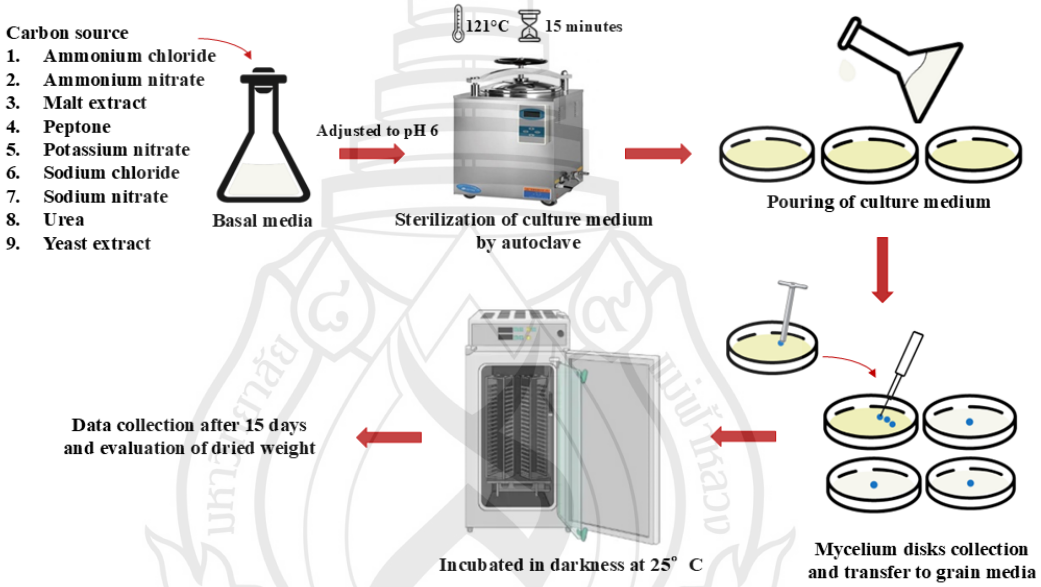


Figure 3.9 Process of nitrogen sources for mycelium growth

3.4.8 Optimization of Carbon/Nitrogen (C/N) Ratio

Mycelium growth was measured in basal media that was mixed with 2% molasses (w/v) as a carbon source, then mixed continually with yeast as the nitrogen source. The C/N ratio was adjusted to 1:1, 5:1, 10:1, 1:5, and 1:10 in each medium, adjusted to pH 6 before sterilization. A 5 mm diameter of active mycelium was plugged at the center of the basal media mixed with a carbon and nitrogen source, incubated in darkness at 25 ℃. After 15 days of incubation, the mycelia growth was recorded, measured, and the mycelia were harvested, and the yield was measured. The experiment was carried out in three replicates (Figure 3.10).

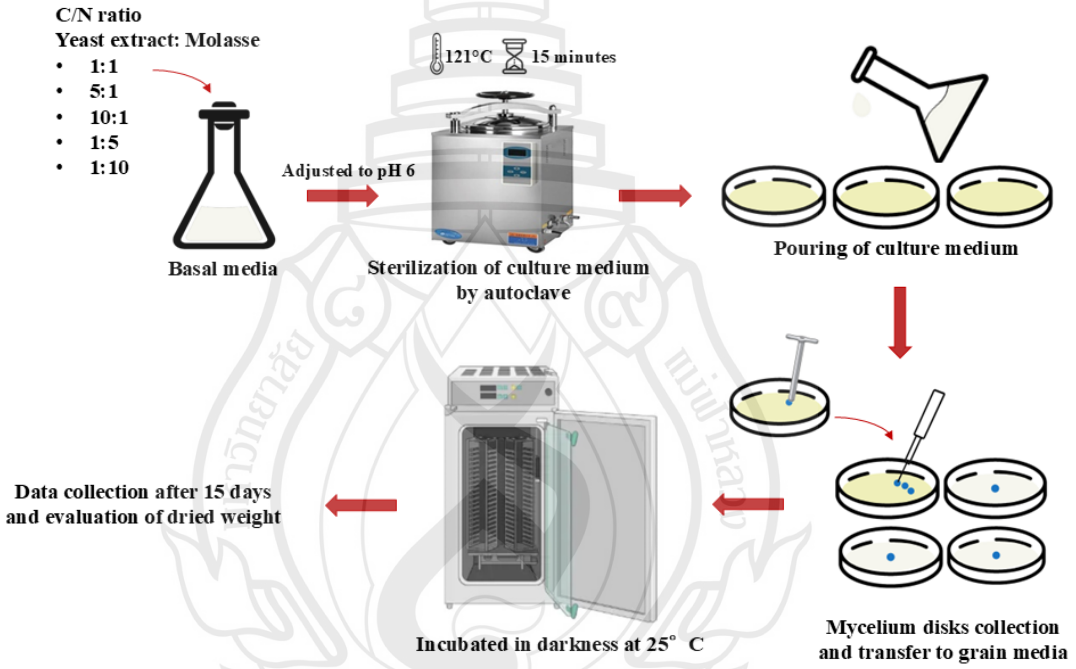


Figure 3.10 Process of carbon/nitrogen ratio for mycelium growth

3.4.9 Data Collection and Statistical Analysis

Data were collected for the optimal mycelial growth based on culture media, temperature, pH, cereal grains/ agricultural substrate, carbon and nitrogen source, and C/N ratio. The diameter of the mycelia (cm), mycelium morphology, and dry weight were measured. The optimum growth parameter data were carried out using statistical analysis programs with triplicate. Data were compared to obtain a mean separation performed using Duncan's multiple test ($p < 0.05$) followed by post–hoc tests, and expressed in a one–way ANOVA analysis using the SPSS program (Statistics Package for Social Sciences).

3.4.10 Fruiting Test

3.4.9.1 Spawn production

Cereal grain media/ agricultural waste was used as spawn production substrates. Spawn tubes were contained 40 grams of each medium and inoculated with mycelial plugs of selected strains of *Hericium* sp. The cultures were incubated at 25–35 ℃. The experiments were carried out in triplicate. The growth rate data of the spawn cultures were collected.

3.4.10.2 Bag cultivation

The bag cultivation was used with rubber sawdust and other agricultural waste to cultivate the selected strains of *Hericium* sp. The medium was contained in polypropylene bags with about 600 g of medium, then capped with a plastic ring and lid. The bags are sterilized at 121 °C for 30 minutes or at 90–100 °C for 3 h (Figure 3.11). After the temperature cooled to 25 °C or room temperature, the spawn is inoculated into the bag's media. The bags are kept at room temperature with 70-90% humidity in order to produce fruiting bodies. The experiment was carried out in triplicate. The fruiting bodies, including those with open and closed caps, were manually harvested, counted, and weighed daily (Figure 3.12).

Figure 3.11 Process of preparing the substrate for mushroom cultivation

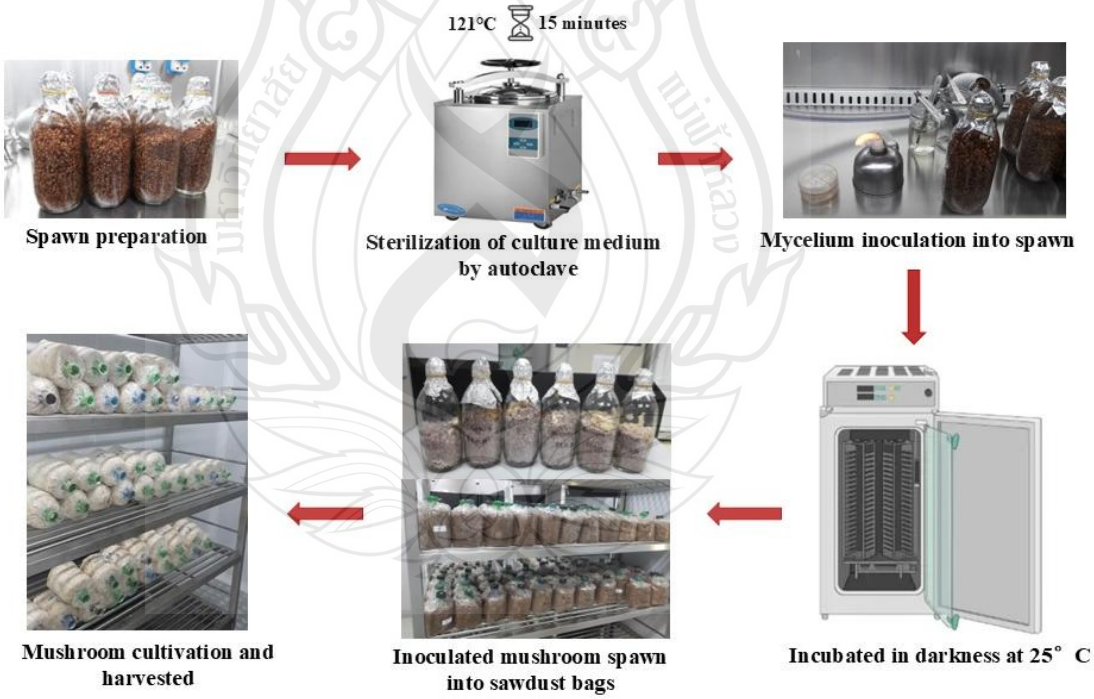


Figure 3.12 Process of preparing spawn and inoculation mycelium to bags of substrates

3.4.11 Statistical Analysis for Fruiting Test

For the fruiting test trial, the fruiting bodies of three species of *Hericium* sp. were manually harvested, counted, and weighed daily. Yield data and biological efficiency (B.E.) were calculated as reported by Stamets (2005) as follows:

$$\text{BE\%} = \frac{\text{Fresh fruiting body (g)}}{\text{Dry weight of medium substrate}} \times 100$$

Yield data was defined as the total weight of fresh mushroom per kilogram of the substrate (Royse, 2010; Llarena-Hernández et al., 2011; Thongklang et al., 2014),

3.5 Nutritional Analysis of *Hericium*

Proximate analysis is one of the most common analyses for nutritional testing. The fruiting bodies of selected strains of *Hericium* sp. were dried for 24 hours at 45 ºC and powdered using a blender.

3.5.1 Moisture Content Analysis

Moisture content analysis was done by the oven drying method following Nielsen (2003). Pre-dried 6 disposable aluminum pans at 100 ºC for 24 hours. Around 3 g of the powdered mushroom sample was placed in each pan and weighed accurately. Samples were put in an oven and dried at 103 ± 2 ºC for 18 hours. After drying, the samples were put in the desiccator to lower the temperature and weighed. The percentage of moisture and dry matter was calculated as follows:

$$\text{\% Moisture} = \frac{\text{wt of } H_2O \text{ in sample}}{\text{wt of wet sample}} \times 100$$

$$\text{\% Moisture} = \frac{(\text{wt of wet sample} + \text{pan}) - (\text{wt of dried sample} + \text{pan})}{(\text{wt of wet sample} + \text{pan}) - (\text{wt of pan})} \times 100$$

$$\text{\% Dry matter} = 100 - \text{\% moisture}$$

3.5.2 Ash Content Analysis

Ash refers to the inorganic matter remaining after the complete oxidation of organic matter in mushroom samples. Ash content analysis was done by the dry ashing method following Jame (1995). Six crucibles were pre-heated at 525 ºC for 24 hours. Three grams of the mushroom powdered sample were placed in each crucible and weighed accurately. The crucibles were put in a muffle furnace and the samples were dried at 525 ºC for 4 hours. After drying, samples were stored in a desiccator and weighed accurately. The percentage of ash on wet weight basis (wwb) and dry weight basis (dwb) was calculated as follows: (Hyde, 2020).

$$\% \text{ Ash (wwb)} = \frac{\text{wt of ash}}{\text{wt of sample}} \times 100$$

$$\% \text{ Ash (wwb)} = \frac{(\text{wt of ashed sample} + \text{wt of crucible}) \; - \; (\text{wt of crucible})}{(\text{wt of wet sample} + \text{crucible}) \; - \; (\text{wt of crucible})} \times 100$$

$$\% \text{ Ash (dwb)} = \frac{\% \text{ ash (wwb)}}{(100 - \% \text{ moisture})} \times 100$$

3.5.3 Fat Content Analysis

The fat content was determined by the continuous Soxhlet method using an organic solvent following Nielsen and Carpenter (2017). Six extraction thimbles were prepared. Around 3 g of the sample was weighed and placed in each thimble. The thimbles were placed in a Soxhlet extractor. Pre-dried extraction cups were weighed and labeled. They were placed in the Soxhlet extractor. Exactly 70 mL of petroleum ether was put in the set-up by using the dispenser. The program was set following the manual guidelines of the equipment. After finishing the process, the extraction cups were put into the oven to dry at 105 ºC for 2 hours. The extraction cups were then stored in the desiccator to cool and later weighed. The percentage of fat (wwb and dwb) was calculated as follows:

$$\% \text{ Fat (wwb)} = \frac{(\text{wt of cup} + \text{fat}) - (\text{wt of cup})}{(\text{wt of wet sample})} \times 100$$

$$\% \text{ Fat (dwb)} = \frac{\% \text{ fat (wwb)}}{(100 - \% \text{ moisture})} \times 100$$

3.5.4 Protein Content Analysis

The protein content of mushroom samples was determined by using the Kjeldahl method following Nielsen (2017). Digestion was started by turning on the digestion block and setting the temperature to 420 ºC. Six digestion tubes were prepared. Approximately 1 g of the sample was weighed and recorded, then placed in each digestion tube. Exactly 5 g of catalyst and 12 mL of concentrated sulfuric acid were placed in each tube with a sample. The digestion tubes were arranged in the rack and placed into the digestion block, then the exhaust system was turned on. The digestion process was completed at about 45 minutes or until the samples became clear. The samples were taken from the digestion block and cooled. The samples were diluted with 20 mL of distilled water.

The distillation process was done following the manual of the distillation equipment. An appropriate volume of boric acid (25 mL) was dispensed into the receiving flask. The receiving flask was placed on the distillation system and submerged in the boric acid solution. In the distillation process, NaOH solution (50 mL) was delivered to the tube. The steam generator was set to 4 minutes to distill the sample. The color of boric acid was then changing from red to green. The same procedures were applied to all 6 tube samples.

The titration process was done by using a standardized hydrochloric acid (HCl) solution in 6 sample replicates in tubes and 1 blank. The normality of HCl was recorded. Methyl red indicator (5 drops) was added to each tube, then titrated with the standardized HCl solution. The color was changed from green to pink. The volume of HCl titrant used was recorded. The percent nitrogen and percent protein were calculated using the formula below. The conversion factor to be used for nitrogen to protein was 6.25.

$$\% \text{ N (wwb)} = \frac{\text{Normality HCl}}{1000} \times \frac{\text{Corrected acid vol. (ml)}}{\text{wt of sample (g)}} \times 14 \left(\frac{\text{g of N}}{\text{mol}}\right) \times 100$$

$$\% \text{ Protein (wwb)} = \% \text{ N} \times \text{Protein Factor}$$

Remark:

Corrected acid vol.= (ml std. acid used for sample) – (ml std. acid used for blank)

3.5.5 Crude Fiber Content Analysis

Crude fiber is composed of cellulose, hemicellulose, and lignin, the residue after chemical digestion with hot sulfuric acid (1.25% w/v) and hot sodium hydroxide (1.25% w/v). The crucibles to be used were pre-dried (W1) and weighed. One gram of a powdered sample was accurately weighed and placed in the crucible. The crucible was placed in the Fibertec cold extraction unit. Exactly 25 mL of acetone was added to the crucible and left for 10 minutes to filter. This process was repeated three times and then washed with water. The crucibles were placed in the Fibertec hot extraction unit and added with 150 mL hot 1.25% w/v sulfuric acid, diatomaceous earth, and filter aid. Four drops of n-octanol were added to prevent foaming and heated to a boil for 30 minutes. The acid was filtered and washed 3 times with hot distilled water. The crucible was again put in the Fibertec hot extraction unit and added with 150 mL hot 1.25% w/v sodium hydroxide, and followed the previous process with sulfuric acid. The crucible was then placed in the Fibertec cold extraction unit and filled with 25 mL of acetone, then filtered for 10 minutes, repeatedly 3 times.

The solvent was then evaporated and the crucibles were dried at 130 ºC for 2 hours. The crucible was cooled in the desiccator and weighed accurately (W2). The

sample in the crucible was then ashed at 525 ± 25 ºC for 4 hours. It was then cooled in the desiccator and weighed (W3). The calculation for the percentage of crude fiber (wwb) is as follows:

$$\% \text{ Crude fiber (wwb)} = \frac{(W2 - W3)}{W1} \times 100$$

Where:

W1 = Sample weight (g)
W2 = Weight of crucible + residue (g)
W3 = Weight of crucible + ash (g)

3.5.6 Carbohydrate Content Analysis

The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Fat} + \% \text{ Protein} + \% \text{ Ash} + \% \text{ Fiber})$$

3.6 Screening and Investigation of the Secondary Metabolite Production of the *Hericium*

3.6.1 Preparation of the Culture

3.6.1.1 Preparation of pre-cultures

The preparation of pre-cultures is necessary for inoculation of the main cultures. Therefore, the first step is to prepare some agar plates (YM6.3) with the relevant strain. The cultivation of the pre-cultures is also used to check the growth ability of the strains, and it enables a relatively sufficient growth of fungal material in order to inoculate the main cultures. To inoculate the pre-cultures, a piece of mycelium is placed in the center of a new agar plate with the aid of a spatula. This is incubated at 23 °C for about two weeks (depending on growth characteristics) in the incubation room. Before the main cultures are inoculated, the plates should be covered at least two-thirds.

3.6.1.2 Preparation of main cultures

To inoculate the main cultures, five roundels should be used, trepanned from the plate with a cork borer (Ø7 mm, marked in red). With the aid of sterile transferring loops, they are transferred into a 500 mL flask filled with 200 mL of media. Depending on the instructions, several flasks with different media are used. They are placed on the shaker (140 rpm) in the incubation room and incubated at 23 ºC for 7 to 40 days (168-960 h) or longer. Different strains require different cultivating times, and the growth period may vary and even take up to four weeks or more. The advantage of the main cultures is that there is much more fungal material grown in the flask then on the plates at the same time.

3.6.2 Screening-break-up

The screening is broken up when the glucose in the flask is consumed to zero. When the screening is finished and the strains are harvested, there are 10 mL of the culture is transferred into a 15 mL-falcon tube. This tube is used to determine the data explained later.

3.6.2.1 Measuring glucose

This procedure is used to determine how much glucose is implemented by the metabolism of the fungus. The glucose is measured with the aid of a test strip. The application of the test strip is shown in the accompanying manual. The colour change of the strip shows the content of glucose in mg/dl. If the content of glucose is still higher than zero, the main culture should be further incubated.

3.6.2.2 Measuring of Value

When measuring the pH-value, the pH-meter must always be calibrated. Most culture media have a slightly acidic pH between pH 5 and pH 7.

3.6.3 Separation of Mycelium and Supernatant

Separate the mycelium from the supernatant, use a feeding bottle with a suction filter (vacuum filtration), or use the centrifuge or gaze for separating. Also, the centrifuge has to split the culture into a few centrifuge vials and centrifuge them for 30 min at 4000 rpm. After that, discard the supernatant carefully over a filter.

3.6.4 Reconditioning of the Supernatant

Mix the supernatant with the same volume of ethyl acetate in a separating funnel, then shake it in the separating funnel for 10 to 15 times. This procedure is

separating the lipophilic substances from the hydrophilic substances, in which the hydrophilic substances (aqueous/lower phase). In the organic phase (upper phase) was always water remaining. For removing it were used some water-free sodium sulphate ($Na2SO_4$) was used because it is hydroscopic. Therefore, the organic phase was transferred into a round-bottom flask, and a funnel with a folded filter, including some water-free sodium sulphate. Then were evaporated the solvent was evaporated in a rotary evaporator.

3.6.5 Reconditioning of the Mycelium

The same volume of acetone adds to the mycelium, shake it, and put it in the ultrasonic bath for 10-30 minutes. Then add the acetone into a round-bottom flask over a filter, remove the acetone in the rotary evaporator until only some aqueous phase is remaining. Fill up the volume to 50-100 mL. This volume was shaken in a separation funnel with ethyl acetate. Follow the method for reconditioning of the supernatant, then the solvent was evaporated in a rotary evaporator.

3.6.6 Store Temporarily

The samples should be stored dry in -20 ºC or at least at 4 ºC.

3.6.7 HPLC and NMR Analysis

The protocol of HPLC analysis was followed by Thongbai et al. (2013). Peaks in the crude extract were compared with the bioactive compound library and also relying on the mass spectrum in the positive ESI mode, as well as its characteristic UV/Vis chromophore and retention time (Rt). Active fractions are further analysed by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy to elucidate the structure of active compounds.

3.7 Screening of Biological Activity of *Hericium*

3.7.1 Preparation of the Mushroom Extract

Extraction of mushroom: Mycelia were soaked in ethyl acetate, methanol, and water, respectively. For mycelia sample was soaked in 400 ml of solvent, and then left for 24 h at room temperature (25 ± 2 ℃). Each portion was filtered using Whatman filter paper. The filtrates were collected in different beakers and labeled accordingly. The filtrates were evaporated to dryness in a steady air current for about 24 h in previously weighed evaporation dishes (porcelain dishes). After evaporation, the dishes were re-weighed, and the differences in weights before and after evaporation were calculated (Trease & Evans, 1994). The extracts (residues) were stored (4 °C) in a clean sterile container for further use. Yields of essential oils and ethyl acetate extract obtained were calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of fresh citrus peel}} \times 100$$

3.7.2 Anti-microbial Assay

3.7.2.1 Agar disc diffusion

The anti-microbial assay was performed by the agar diffusion method (Bauer et al., 1966). The surface of Mueller Hinton agar (HMA) plate was inoculated by streaking with the swab containing the inoculum. The disc (6 mm) was saturated with each of the mushroom extracts, allowed to dry, and were placed on the surface of the agar plate. The plates were incubated for 18-14 h at 37 ℃. Anti-microbial activities were determined by measuring the diameter of the zone of inhibition. The negative control is 10% Dimethyl Sulfoxide (DMSO), and ampicillin, gentamycin, and vancomycin are used as the positive controls.

3.7.2.2 Minimum inhibitory concentration (MIC)

The crude extracts dissolved in DMSO were prepared to a final concentration of 1280 µg/mL. Minimum inhibitory concentrations (MICs) were determined by the micro broth dilution method according to the Clinical and Laboratory Standards Institute recommendation (CLSI, 2009) in MHB for bacteria. Concisely, the crude extracts with DMSO solutions were prepared as two-fold dilutions, with a final

concentration ranging between 2.5-1280 µg/mL. Each well was inoculated with bacterial suspension at 0.5 McFarland. After incubation at 37 ℃ for 18-24 h. After incubation, 10 µl resazurin (6.75 mg/mL) was added to all wells and incubated at 37 ℃ for another 4 hrs. Colour changes were observed and recorded. The lowest concentration the color change is considered the MIC.

3.7.2.3 Minimal bactericidal concentration (MBC) or minimum fungicidal concentration (MFC)

To determine MBC/ MFC, 1 µL of each well from the 96-well plate was a simply streaked on MHA using a micropipette tip. After 24 h incubation, the concentration at which no visible growth was seen was recorded as the MBC/MFC.

3.7.3 Anti-oxidants Assay

Anti-oxidant activity of the extracts was assayed by the $DPPH^+$, 2,2-diphenyl-1-picrylhydrazil (DPPH), and ABTS methods.

3.7.3.1 DPPH assay

Antioxidant activity was measured following the method of Brand-William et al. 1995. The crude extract (5 to 50 mg/mL) was used in this study. The mixture of each sample included 30 µL of the crude extract and 220 µL of the methanolic solution of DPPH, and was performed in 96-well microtiter plates. The mixture was incubated in the darkness at room temperature and measured absorbance was measured at 517 nm every 30 minutes for 2 hrs (by 3 replicates). Butylated hydroxytoluene (BHT) was used as a standard antioxidant. The DPPH radical scavenging activity percentage was calculated using the following formula.

$$\text{Scavenging effect (\%)} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100$$

Where:

A blank = Absorbance of the control solution, DPPH solution without the tested sample.

A sample = Absorbance of the test extract, DPPH solution with the tested sample.

3.7.3.2 ABTS assay

The radical scavenging activities of extracts were determined by using the ferryl myoglobin/ABTS protocol (Alzoreky & Nakahara, 2001). The stock solutions of 500 mM ABTS diammonium salt, 400 mM myoglobin, 740 mM potassium ferricyanide, and 450 mM H_2O2 were prepared in phosphate-buffered saline (PBS) (pH 7.4). Metmyoglobin (MbIII) was prepared by mixing equal amounts of myoglobin and potassium ferricyanide solutions. The reaction mixture (total volume 2 mL) contained the following substances (final concentrations in the reaction mixture): ABTS (150 mM), MbIII (2.5 mM), 16.8 mL of the sample, and 978 mL PBS. The reaction was initiated by adding 75 mM H_2O2 (330mL), and the lag time in seconds, before the absorbance of $ABTS^+$ at 734 nm began to increase was recorded. The calibration curve was plotted with lag time in seconds versus the concentration of the standard antioxidants (L-ascorbic acid or Trolox).

3.7.4 Cytotoxicity Assay

The mushroom extracts from the 3 different solvents were tested for cytotoxic activity against larynx carcinoma (Hep-2) and breast carcinoma (MCF-7) cell lines. The cells were maintained in a minimal essential medium (MEM; Sigma Aldrich) as monolayers in Petri dishes (100 × 15 cm) at 37 °C in a water-jacketed double-door incubator (Shellab, Sheldon Manufacturing, Inc., Cornelius, OR, USA) under 5% CO_2. To count the number of cells, 1 mL of trypsin/EDTA (ethylene-diaminetetra-acetic acid) solution was added to the culture flask (with 25 cm^2 of surface area) containing the monolayer cells to dislodge the cells. A hemocytometer was then used to determine the cell number. Cells were suspended in MEM medium at 106 /mL and dispensed into 96-well tissue culture plates at 100 µL/well. For the cytotoxicity test, each mushroom extract was made into 6 different concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.56 µg/mL and then dispensed into 96-well plates at 50 µL per well. After incubating for 24 hours, the numbers of viable cells were determined by the trypan blue dye exclusion method14 by absorbance at 450 nm using an ELISA plate reader (Sunrise™ by TECAN Inc., Männedorf, Switzerland). The cytotoxicity of the extracts was determined by the percentages of viable cells remaining after the treatment by the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{ODt}}{\text{ODc}} \times 100$$

Where: ODt and ODc = The means of optical densities of wells with treated and untreated cells, respectively.

3.7.5 Anti-cancer Assay

3.7.5.1 Preparation of cell cultures

Adenocarcinoma human alveolar basal epithelial cells (A549), hepatocellular carcinoma (Huh-7), and cellosaurus cells (SW-480) were obtained from American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in Dulbecco's Modified Eagle Medium with 10% (v/v) fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA, USA), and 1 × antibiotic and anti-mycotic solution (Gibco) at 37 °C under humidified 5% CO_2.

3.7.5.2 Preparation of selected compound

The selected six pure compounds were used for this experiment. Selected compound 1 is soluble in ethanol (EtOH), forming a clear and colorless solution, but is poorly soluble in DMSO. Compounds 2, 3 are highly soluble in dimethyl sulfoxide (DMSO), forming a clear and colorless solution.

3.7.5.3 MTT assay

Cells were grown in a 96-well plate with 7×10^3 cells/well for 24 h. Then, they were treated with varying concentrations (0–50 µg/mL) of six pure compounds and incubated for 72 h. The 3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyl tetrazolium bromide (MTT) solution (5 mg/mL) was added to all test wells and incubated for 2 h at 37 °C. After removing the culture supernatant, DMSO was added to dissolve the dark blue crystals, and the solution was thoroughly mixed. Optical density was measured at 570 nm using a microplate reader (Cytation 5; Biotek, Winooski, VT, USA). The percentages of cell viability and IC_{50} values for 7R-AMDL and doxorubicin were calculated using GraphPad Prism (version 8.0.1; San Diego, CA, USA).

3.7.5.4 Clonogenic cell survival assay

Cells were grown in 96-well plates with 7×10^3 cells/well for 24 h. Then, the cells were incubated with compound 3 (0, 5, or 10 µg/mL) for 24 h. After treatment, the cells were trypsinized, reseeded in 12-well plates with 1000 cells/well, and cultured in complete medium for 6 days. Finally, the attached cells were fixed with cold absolute

methanol for 15 min and stained with 0.5% (w/v) crystal violet at room temperature overnight. Excess crystal violet dye was washed off with tap water before the plates were left to air-dry. The cell colonies on the plate were counted under a light microscope and photographed.

3.7.5.5 Wound healing assay

A wound healing assay was performed to test cell migration. 2×10^5 cells were seeded into 6-well plates and maintained overnight at 37 °C in the atmosphere of incubators with 5% CO_2 for 24 h. The monolayer cells were scratched with a 200 μL pipette tip on the bottom of each plate after the cells reached 100% confluence. Then, the cell debris was washed away with PBS, and the remaining cells were cultured in serum-free medium with compound 6 at the 0, 5, and 10 μg/mL for 24 h. At 0 h and 24 h after scratching, the gap area width was captured using a Zeiss Primovert inverted microscope (Carl Zeiss Microscopy GmbH) and analyzed using Image J Fiji software to measure the wound area.

CHAPTER 4

CULTIVATION AND NUTRITIONAL ANALYSIS OF *HERICIUM*

4.1 Introduction of *Hericium* cultivation

Hericium Pers. (Basidiomycota) species are saprotrophs that grow in the wood of angiosperm trees, especially Fagaceae (Boddy, 2016). *Hericium* is a genus of the Hericiaceae, Russulales, and 71 records were reported in the Index Fungorum. The characteristics were distinctive by shaggy spines with a furry appearance form; some were round balloons when young to the mature stage, but can age to a yellow or tan hue (Meuninck & Littlefield., 2017).

Hericium erinaceus is generally known as “Lion's Mane” and occurs naturally in deciduous forests (Jumbam et al., 2019). It has a long tradition as an edible mushroom, but it was later developed to be used medicinally (Thongbai et al., 2015). This mushroom is widely recognized as a highly nutritious food and is used for medicine (Jayachandran et al., 2017), although it is common in Asia, Europe, and North America (Boddy, 2016; Reis et al., 2017).

Commonly, *Hericium* delicate taste is often compared to seafood, such as crab or lobster (Thongbai et al., 2015; Sholyavei et al., 2020), combined with its nutritional value. Nutritional properties of this mushroom contented macronutrient, vitamins that contains significant levels of B vitamins, including B1 (thiamine), B2 (riboflavin), B3 (niacin), and B5 (pantothenic acid), minerals provide essential minerals such as potassium, zinc, iron, and selenium (Heleno et al., 2015), make it a valuable addition to both the diet and potential therapeutic applications (Lazur et al., 2024). *Hericium* contains high amounts of ash, protein, carbohydrate, fiber, minerals, vitamins, unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid, and carotenoids, all of which are considered food supplements (Reis et al., 2017; Valverde et al., 2015). *Hericium erinaceus* contains high amounts of ash, protein, carbohydrate, fiber, minerals, vitamins, unsaturated fatty acids, phenolic compounds, tocopherols,

ascorbic acid, and carotenoids, all of which are considered food supplements (Reis et al., 2017; Valverde et al., 2015). Several reports demonstrated the medicinal properties of *H. erinaceus*, suggesting that it can be an adjunct drug to immunotherapy, stimulating activity in the synthesis of nerve growth factors that could have a preventive and ameliorative effect on age-related neurological dysfunctions such as Alzheimer's disease and Parkinson's disease (Li et al., 2018). Furthermore, antifibrotic, antiinflammatory, antidiabetic, antibacterial activity, antitumor, anti-HIV, antimalarial, antioxidant activity, blood sugar-lowering, cholesterol-lowering, and liver protective properties of *Hericium* have previously been documented (Thongbai et al., 2015; Smith et al., 2002; Genkinger et al., 2004; Barros et al., 2007; Wong et al., 2019; Alves et al., 2012; Abdullah et al., 2012; De Silva et al., 2013). Despite its growing popularity due to its wonderful taste and nutritional content, as well as its therapeutic effects (Liu et al., 2019), *H. erinaceus* is not frequently grown in Thailand.

Hericium is highly popular and expensive in Europe, with a price range of about $8−36 per pound (Lejeune, 2024). Therefore, artificial cultivation methods have been created for a variety of edible mushrooms in response to increasing demand. However, the production of fruiting bodies and mycelia in an artificial culture medium has been proven problematic (Chang & Wasser, 2017). Most cultivable mushrooms have specific requirements for log growth (Grace & Mudge, 2015). Currently, mushroom production is not only a lucrative source of revenue for farmers, but it also appears to be a popular nutritious meal for consumers. Fruitbodies of *Hericium erinaceus*, and *H. coralloides*, also known *H. ramosum* are produced prodigiously in culture and are the best-flavored. *H. abietis*, associated with conifers, is more difficult to cultivate (Stamets, 2011).

The cultivation of edible mushrooms. Many substrate/agricultural wastes can be used for cultivation, for example, rice straw with casing, sawdust (*Albiza saman*), paddy straw, wheat straw, leaves, coffee pulp, tea leaves, banana leaves (Rizal et al., 2016; Kumla et al., 2013; Randive, 2012; Shah et al., 2004; Kamthan & Tiwari, 2017). Agricultural wastes should be explored for alternative cultivation. However, *Hericium* cultivation was believed to have evolved from keen observations of those obtained in the wild (Sokol et al., 2016). According to Figlas et al. (2007), sawdust has proven to be a good and cost-effective substrate for the growth of *H. erinaceus*. This was agreed to by Xiao and Chapman (1997), who reported a successful indoor cultivation of *H.*

abietis and *H. erinaceus* using conifer sawdust. According to Gerbec et al. (2007), *H. erinaceus* was cultivated on a beech sawdust substrate. Hassan (2007) also used hardwood sawdust to cultivate *H. erinaceus* in Egypt. Gerbec et al. (2015) reported fungal growth in a horizontal stirred tank reactor where the cultivation substrate consisted of beech sawdust, paddy millet, and hulled millet.

Currently, few studies in Thailand have created methods for cultivating *Hericium*, even though cultivation of *Hericium* has been the topic of substantial research in China and Japan (Sokol et al., 2016; Mizuno, 1999; He et al., 2017; Spelman et al., 2017). Moreover, many research studies have provided insights into the characteristics of various edible mushroom species in Thailand; however, there need to be more knowledge concerning *Hericium*. Furthermore, Thailand has conducted some research studies on the antibacterial, antifungal, and antioxidant activities of the available wild extract of *Hericium*. Therefore, this study aimed to investigated the effects of media, temperature, pH, cereal grain and agricultural substrate source, carbon and nitrogen sources, and media component ratio on the mycelial growth, develop cultivation methods, identify appropriate substrate treatments to promote the growth of *Hericium erinaceus* and *H. coralloides*, and investigate the nutritional characteristics of the resulting *H. erinaceus* and *H. coralloides* fruiting bodies.

4.2 Materials and Methods

4.2.1 Fungal Strains

All five strains were used in this study. The *H. erinaceus* and *H. coralloides* isolation was following Section 3.4.1 of Chapter 3 (General Material and Methodology). The culture was maintained in sterilized potato dextrose agar (PDA) medium, incubated at 25 °C for 15 days, and stored at 4−7 °C for future use.

4.2.2 Phylogenetic Analysis

The DNA extraction and PCR amplification were following Section 3.2 of Chapter 3 (General Material and Methodology).

All sequences were assembled in SeqManTM II expert sequence analysis software (DNASTAR). ITS1 and ITS4 sequences of *H. coralloides* strain MFLUCC

21-0050 and ITS5 and ITS4 of *H. erinaceus* strains MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021 from this study were extracted from the whole ITS amplicon sequence using ITS. Moreover, the ITS1 + ITS2 were blasted against the GenBank database to check for similarities with other sequences derived from *Hericium*. The phylogenetic tree was constructed using maximum likelihood (ML) analyses using the Cipres Science Gateway. The reliability of the tree topology was evaluated by bootstrap analysis of 100 replicates using *Pseudowrightoporia crassihypha* (KM107873) and *Wrightoporiopsis amylohypha* (KM107877) sequences as the outgroup. All obtained sequences were submitted to the GenBank database under the accession numbers, and other reference sequences were downloaded from GenBank (Table 4.1).

Table 4.1 Details of the selected taxa of *Hericium* used in the phylogenetic analyses

Species	Isolate/voucher	Country	GenBank	Reference
Hericium alpestre	NH 13240	Russia	AF506457	Larsson & Larsson (2003)
H. americanum	DAOM F-21467	Canada	AF506458	Larsson & Larsson (2003)
H. coralloides	NH 282	Sweden	AF506459	Larsson & Larsson (2003)
H. coralloides	FCUG 426	France	JQ716935	Hallenberg et al. (2013)
H. coralloides	MFLUCC 21-0050	Germany	MZ379513	This study
H. erinaceus	MFLUCC 21-0018	Thailand	MZ342890	This study
H. erinaceus	MFLUCC 21-0020	Thailand	MZ342961	This study
H. erinaceus	MFLUCC 21-0019	China	MZ342907	This study
H. erinaceus	MFLUCC 21-0021	Germany	MZ343154	This study
H. erinaceus	NH 12163	Russia	AF506460	Larsson & Larsson (2003)
H. erinaceus	SCC 1	India	MT448853	Chakraborty & Acharya (Direct Submission)

Table 4.1 (continued)

Species	Isolate/voucher	Country	GenBank	Reference
H. erinaceus	HEZY ITS region	China	MW131237	Qi, J et al. (Direct Submission)
H. erinaceus	HE-01	Thailand	MW672510	Kimkong et al. (Direct Submission)
H. flagellum	N/A	Poland	MG649451	Kujawska et al. (Direct Submission)
H. rajchenbergii	FCUG GR1997	Argentina	JX403945	Hallenberg et al. (2013)
H. rajchenbergii	FCUG GR2041	Argentina	JQ716939	Hallenberg et al. (2013)
H. yumthangense	BSHC:KD-11-146	India	NR155021	Das et al. (Direct Submission)
H. yumthangense	Cui 10632	China	MH085971	Wang (Direct Submission)
Pseudowrightoporia crassihypha	Yuan 6247	China	KM107873	Chen & Dai (Direct Submission)
Wrightoporiopsis amylohypha	Yuan 3579	China	KM107877	Chen & Dai (Direct Submission)

4.2.3 Optimization for mycelial growth

4.2.3.1 Optimization of culture conditions for mycelial growth

The optimization of culture conditions for mycelial growth followed Section 3.4.2 of Chapter 3 (General Material and Methodology).

4.2.3.2 Optimization of temperature for mycelium growth

The optimization of temperature for mycelium growth was following Section 3.4.3 of Chapter 3 (General Material and Methodology).

4.2.3.3 Optimization of pH for mycelium growth

The optimization of pH for mycelium growth followed Section 3.4.4 of Chapter 3 (General Material and Methodology).

4.2.3.4 Optimization of different cereal grains and agricultural substrates for mycelium growth

The optimization of different cereal grains and agricultural substrates for mycelium growth followed Section 3.4.5 of Chapter 3 (General Material and Methodology).

4.2.3.5 Optimization of carbon sources for mycelium growth

The optimization of carbon sources for mycelium growth followed Section 3.4.6 of Chapter 3 (General Material and Methodology).

4.2.3.6 Optimization of nitrogen source on mycelial growth

The optimization of nitrogen source on mycelial growth was following Section 3.4.7 of Chapter 3 (General Material and Methodology).

4.2.3.7 Optimization of Carbon/Nitrogen (C/N) ratio

The optimization of the Carbon/Nitrogen (C/N) ratio for mycelial growth was following Section 3.4.8 of Chapter 3 (General Material and Methodology).

The data collection and statistical analysis were following Section 3.4.9 of Chapter 3 (General Material and Methodology).

4.2.4 Mushroom Cultivation

4.2.4.1 Spawn production

The spawn of each strain was prepared using red sorghum grains (*Sorghum bicolor*). The grains were cleaned and then boiled for 15 minutes, and 50 grams were placed in transparent glass bottles. The spawn medium bottles were sterilized at 15 psi at 121 °C for 15 minutes and cooled before inoculation (Grace & Mudge, 2015).

Inoculated by adding the active mycelial 2/4 of a 90 mm plate of the colonized PDA into the bottles of sterilized spawn media. Incubated at room temperature (25±2 °C) until the mycelium grew fully in the spawn medium.

4.2.4.2 Cultivation Test of *Hericium erinaceus* and *H. coralloides*

The preparation of the sawdust substrate for cultivating *Hericium erinaceus* and *H. coralloides* involved three different treatments (Table 4.2).

Table 4.2 The substrate treatment for *Hericium erinaceus* and *H. coralloides* cultivation

Substrate (%)	**Substrate Treatment number**		
	1	**2**	**3**
Cereal grain (red sorghum)	-	15	-
Gypsum ($CaSO_4 \cdot 2H_2O$)	-	1	0.4
Lime (CaO)	-	1	0.8
Magnesium sulfate ($MgSO_4$)	-	-	0.2
Molasses	-	1	-
Para rubber sawdust	95	77	88
Rice bran	3	3	9
Rice flour	-	-	0.8
Sugar	2	-	0.8
Yeast powder	-	2	-

Each substrate treatment was mixed well, and the moisture content was adjusted to 70%. To prepare the sawdust substrate bag, 600 grams of sawdust substrate were filled into polypropylene bags and sterilized at 15 psi at 121 °C for 40 minutes. After the temperature cooled, the active spawn (~ 3 grams) was transferred to each sterilized bag substrate. Subsequently, all sawdust bags were placed in complete darkness at room temperature (25±2 °C) for 30–40 days to facilitate the growth of mushroom mycelium. The experiment was carried out in five replications. When mycelial growth spread to full speed on the substrate of the bags, the bags were opened at 18−24 °C and the relative humidity was 90−95 % to produce the primordia in 17−28

days, followed by the development of fruiting bodies in 7−10 days. The fruiting bodies were collected daily and weighed for biomass.

4.2.4.3 Statistical Analysis for the Cultivation Test

Data collections were analyzed for the fresh weight of the fruiting bodies of flush 1, the time taken for flush 1, the number of flushes per bag, and the total yield of fruiting bodies from each strain. After harvest, the weight of fresh mushrooms and the number of fruiting bodies per bag were recorded for each swarm. Biological efficiency (BE%) was calculated as reported by Stamets (Stamets, 2011) as follows:

$$\text{BE\%} = \frac{\text{(Fresh fruiting bodies (g)}}{\text{(dry weight of medium substrate)}} \times 100$$

The dry weight of the mushroom was determined by placing the fresh mushroom in a hot air oven at 45–50 °C for 48 hours (Pewlong et al., 2019; Yang et al., 2019).

4.2.5 Proximate Composition Analysis

4.2.5.1 Sample preparations

The fruiting bodies were oven-dried at 45 to 50 °C for 48 hours. The dried fruiting bodies were ground into a powered form by using a blender. The dried mushroom powders were analyzed.

4.2.5.2 Nutritional Analysis of *Hericium erinaceus*

The nutritional Analysis of *Hericium erinaceus* was following Section 3.5 of Chapter 3 (General Material and Methodology).

4.2.5.3 Nutritional Analysis of *Hericium coralloides*

The dried mushroom powders were analyzed for ash, carbohydrates, crude fiber, energy, fat, moisture, and proteins were determined by the AOAC and the method of analysis for nutrition labeling procedures (Table 4.3).

Table 4.3 The methodology of nutrient contents

Proximate composition	Method
Ash	AOAC (2019) 923.03 and 920.153
Carbohydrate	Method of Analysis for Nutrition Labeling (1993), Chapter 6 Proximate and Mineral Analysis
Crude fiber	AOAC (2019) 978.10
Energy	Method of Analysis for Nutrition Labeling (1993), Chapter 6 Proximate and Mineral Analysis
Fat	AOAC (2019) 948.15
Moisture	AOAC (2019) 925.10 and 950.46
Protein	AOAC (2019) 991.20

4.2.5.4 Statistical Analysis for the Proximate Composition

The statistical analysis was carried out using IBM SPSS Statistics 26 software. For the *Hericium* grown on three different substrate treatments, a one-way ANOVA was used to test the significance of the difference between the means was determined by Duncan's multiple range tests at 95 % least significant difference ($p < 0.05$).

4.3 Results

4.3.1 Phylogenetic Analysis

According to the BLAST result of ITS1 + ITS2, the taxonomy of all studied strains with *Hericium erinaceus* and *H. coralloides* was confirmed. The ITS sequence of *H. coralloides* (MFLUCC 21-0050) presented high similarity to *H. coralloides* (99.67%) and *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) showed high similarity to *H. erinaceus* (99.53-99.84%) (Table 4.4). The ITS dataset included 20 sequences of seven *Hericium* species. The amplification of the ITS region showed fragments of approximately 600 base pairs (bp). The topologies of the phylogenetic trees built with maximum likelihood were similar and indicate that the studied specimen is a member of *H. coralloides* clade, which

shares 93% sequence identity, and *H. erinaceus,* which shares 82% sequence identity (Figure 4.1).

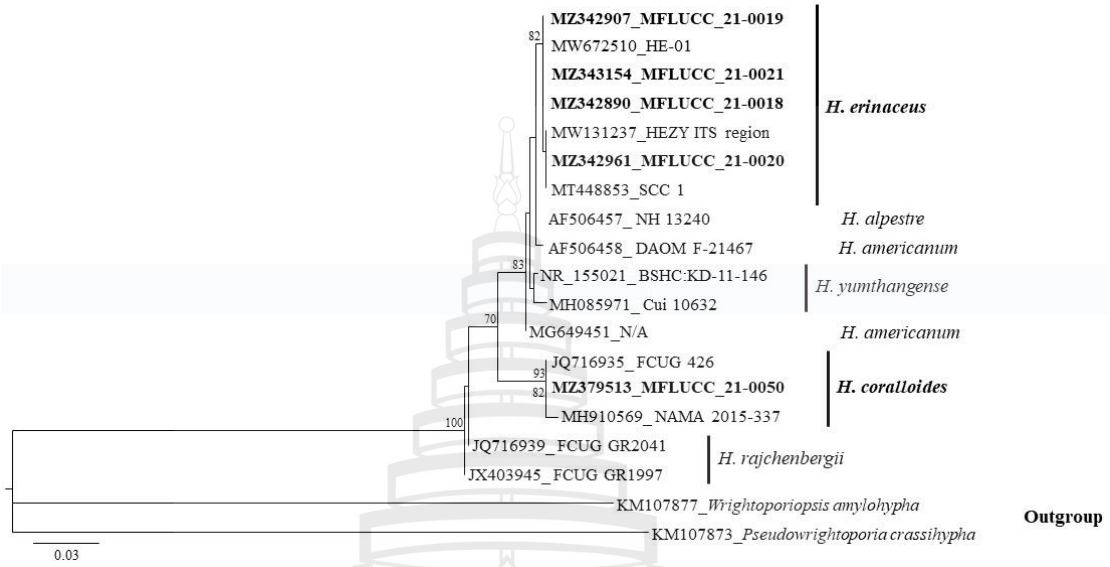


Figure 4.1 Maximum likelihood phylogenetic tree inferred from the internal transcribed spacer (ITS), including *Hericium coralloides*, *H. erinaceus*, and the related species. Bootstrap frequencies are equal to or greater than 70% and are shown above supported branches

Table 4.4 GenBank BLAST search results of ITS1 + ITS2 sequences of *Hericium* species from this study against the GenBank database (I, identity; QC, query cover)

Species	Voucher, GB accession no	Most similar ITS1 + ITS2 sequences in GenBank	ITS1	ITS2	ITS1 + ITS2	Original voucher	Locality	Reference
Hericium erinaceus	MFLUCC 21–0018, MZ342890	FJ810143, I = 99.53%, QC = 99%	180/180 (100%)	201/204 (99%)	381/384	dd08026	–	GenBank
H. erinaceus	MFLUCC 21–0020, MZ342961	FJ810143, I = 99.69%, QC = 100%	180/180 (100%)	202/204 (99%)	382/384	dd08026	–	GenBank
H. erinaceus	MFLUCC 21–0019, MZ342907	MT448853, I = 99.83%, QC = 100%	179/180 (99%)	204/204 (100%)	383/384	SCC 1	India	GenBank
H. erinaceus	MFLUCC 21–0021, MZ343154	KT693230, I = 99.84%, QC = 99%	180/180 (100%)	204/204 (100%)	384/384	B2_13319025	USA	Rajas et al. (2017)
H. coralloides	MFLUCC 21–0050, MZ379513	MZ159723, I = 99.67%, QC = 100%	183/184 (99%)	248/249 (99%)	431/433	K(M):250882	UK	GenBank

4.3.2 Optimization for Mycelial Growth of *Hericium erinaceus* and *H. coralloides*

4.3.2.1 Effect of favorable culture media on mycelial growth

The optimal agar media for mycelium growth of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) and *H. coralloides* (MFLUCC 21-0050) are shown in Table 4.5. *H. erinaceus* strains MFLUCC 21-0018 and MFLUCC 21-0020 were optimal in OMYA; strain MFLUCC 21-0019 was optimal in MYPA, OMYA, and MEA media; and CDA was suitable for *H. erinaceus* strain MFLUCC 21-0021. Moreover, *H. coralloides* strain MFLUCC 21-0050 grew the best on MYPA medium (Figure 4.2).

4.3.2.2 Effect of temperature on mycelial growth

The optimal temperature of four strains of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) resulted in a dry weight maximum at 25 °C, while *H. coralloides* (MFLUCC 21-0050) showed a peak of dried weight at 30 °C, and mycelial growth was 16–35 °C. However, the statistical analysis indicated no significant differences in mycelial growth in the temperature range of 16–35 °C (Table 4.6).

4.3.2.3 Effect of pH on mycelial growth

The most favorable pH range for mycelial growth of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, and MFLUCC 21-0020) was pH 4–5, while *H. erinaceus* strain MFLUCC 21-0021 and *H. coralloides* strain MFLUCC 21-0050 grew most abundantly at pH 5.5 (Table 4.7).

4.3.2.4 Effect of cereal grain and agricultural substrate

All *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) were able to grow colonies well on coir, while *H. coralloides* (MFLUCC 21-0050) showed the most abundant colonies on wheat. However, the mycelium characteristics of *Hericium* on coir substrate had the appearance of being thinner than other spawn substrates (Table 4.8).

4.3.2.5 Effect of carbon sources on mycelial growth

Nine different carbon sources were tested for promoting mycelial growth of all *H. erinaceus* strains (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020,

and MFLUCC 21-0021) and *H. coralloides* (MFLUCC 21-0050) was higher on the basal medium supplemented with molasses (Table 4.9).

4.3.2.6 Effect of nitrogen sources on mycelial growth

The nitrogen source for mycelial growth of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) and *H. coralloides* strain MFLUCC 21-0050 was higher on the basal medium supplemented with yeast extract (Table 4.10).

4.3.2.7 Effect of media components ratio

A test for the ratio of media components suitable for the favorable growth of *H. erinaceus* and *H. coralloides* was observed using the basal culture medium, which was adjusted to a ratio of molasses and yeast extract of 1:1, 5:1, 10:1, 1:5, and 1:10, respectively. The most favorable media component ratio was 10:1 for both species as measured by peaks of the dry weight of mycelial growth (Table 4.11, Figure 4.3).

Table 4.5 Dry weight of mycelial growth on different culture media for 15 days (grams)

Culture media	*H. erinaceus*				*H. coralloides*
	MFLUCC 21–0018	**MFLUCC 21–0019**	**MFLUCC 21–0020**	**MFLUCC 21–0021**	**MFLUCC 21–0050**
CDA	0.1354 ± 0.0254^{ab}	0.0462 ± 0.0086^{cd}	0.0774 ± 0.0131^{bc}	0.1118 ± 0.0055^{a}	0.0873 ± 0.0005^{b}
CMA	0.0440 ± 0.0046^{ef}	0.0458 ± 0.0180^{cd}	0.0399 ± 0.0091^{de}	0.0373 ± 0.0068^{de}	0.0324 ± 0.0066^{d}
MEA	0.0739 ± 0.0089^{de}	0.0807 ± 0.0138^{a}	0.0573 ± 0.0289^{cd}	0.0547 ± 0.0076^{c}	0.0724 ± 0.0073^{bc}
MYPA	0.0761 ± 0.0049^{d}	0.0835 ± 0.0042^{a}	0.0769 ± 0.0067^{bc}	0.0526 ± 0.0025^{cd}	0.1119 ± 0.0180^{a}
OMA	0.1144 ± 0.0060^{bc}	0.0675 ± 0.0141^{abc}	0.0951 ± 0.0230^{ab}	0.0872 ± 0.0122^{b}	0.0872 ± 0.0105^{b}
OMYA	0.1496 ± 0.0340^{a}	0.0814 ± 0.0159^{a}	0.1085 ± 0.0199^{a}	0.0641 ± 0.0043^{c}	$0.7786 \pm 0 .0024^{b}$
PDA	$0.0637 \pm 0.0093d^{ef}$	0.0513 ± 0.0041^{bcd}	0.0254 ± 0.0009^{e}	0.0527 ± 0.0166^{cd}	0.0382 ± 0.0013^{d}
PDYA	0.0869 ± 0.0214^{cd}	0.0695 ± 0.0078^{ab}	0.0756 ± 0.0020^{bc}	0.0515 ± 0.0137^{cd}	0.0397 ± 0.0039^{d}
SDA	0.0364 ± 0.0069^{f}	0.0401 ± 0.0154^{d}	0.0601 ± 0.0161^{cd}	0.0226 ± 0.0034^{e}	0.0580 ± 0.0106^{c}

Note Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to the Duncan's multiple range test ($p < 0.05$).

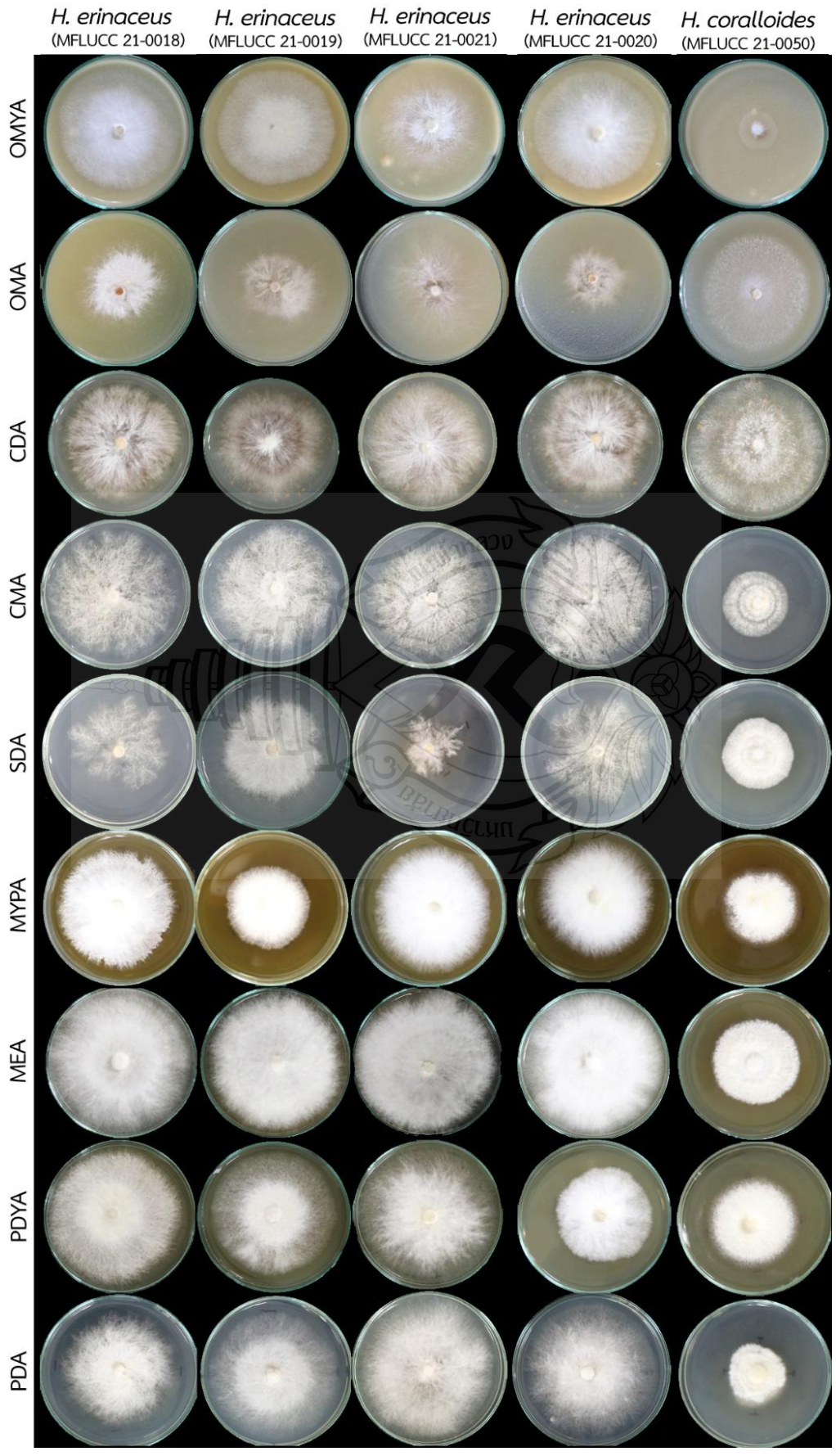


Figure 4.2 Morphology of mycelium of different *Hericium* strains on different culture media after 15 days of incubation

Table 4.6 Dry weight of mycelial growth at different temperatures after 15 days of incubation (grams)

Temperature	*H. erinaceus*				*H. coralloides*
(°C)	**MFLUCC 21–0018**	**MFLUCC 21–0019**	**MFLUCC 21–0020**	**MFLUCC 21–0021**	**MFLUCC 21–0050**
16	0.0729 ± 0.0286^{a}	0.2009 ± 0.0146^{b}	0.0660 ± 0.0067^{b}	0.1076 ± 0.0314^{a}	0.0739 ± 0.0234^{a}
20	0.0547 ± 0.0320^{a}	0.1870 ± 0.0276^{bc}	0.0980 ± 0.0120^{ab}	0.1126 ± 0.0533^{a}	0.0803 ± 0.0036^{a}
25	0.1316 ± 0.0781^{a}	0.2522 ± 0.0178^{a}	0.1403 ± 0.0447^{a}	0.1285 ± 0.0586^{a}	0.0522 ± 0.0060^{b}
30	0.0644 ± 0.0185^{a}	0.1354 ± 0.0399^{d}	0.0704 ± 0.0166^{b}	0.0823 ± 0.0088^{a}	0.0880 ± 0.0161^{a}
35	0.0925 ± 0.0127^{a}	0.1424 ± 0.0184^{cd}	0.1050 ± 0.0472^{ab}	0.1089 ± 0.0049^{a}	0.0842 ± 0.0047^{a}

Note Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to the Duncan's multiple range test ($p < 0.05$).

Table 4.7 Dry weight of mycelial growth on pH optimal condition after 2 weeks of incubation (grams)

pH	*H. erinaceus*				*H. coralloides*
	MFLUCC 21–0018	**MFLUCC 21–0019**	**MFLUCC 21–0020**	**MFLUCC 21–0021**	**MFLUCC 21–0050**
4	1.0110 ± 0.0770^{a}	0.8665 ± 0.2710^{a}	1.0252 ± 0.0904^{ab}	0.2388 ± 0.0116^{abc}	0.3021 ± 0.0231^{b}
4.5	0.9382 ± 0.0716^{ab}	0.9266 ± 0.0526^{a}	1.1797 ± 0.6005^{a}	0.2425 ± 0.0328^{abc}	0.3100 ± 0.0617^{b}
5	0.7612 ± 0.1538^{c}	0.5509 ± 0.0962^{ab}	0.6795 ± 0.0409^{abc}	0.2427 ± 0.0051^{abc}	0.2840 ± 0.2160^{b}
5.5	0.7450 ± 0.0503^{c}	0.4466 ± 0.0442^{bc}	1.0019 ± 0.2113^{ab}	0.2722 ± 0.0143^{a}	0.5256 ± 0.0542^{a}
6	0.8302 ± 0.0847^{bc}	0.3396 ± 0.2757^{bcd}	0.9706 ± 0.1192^{ab}	0.2545 ± 0.0431^{ab}	0.0159 ± 0.0112^{c}
6.5	0.5558 ± 0.0166^{d}	0.1299 ± 0.2116^{cd}	0.9243 ± 0.1836^{abc}	0.2534 ± 0.0139^{ab}	0.0047 ± 0.0011^{c}
7	0.3889 ± 0.0574^{e}	0.2084 ± 0.3270^{bcd}	0.8770 ± 0.2493^{abc}	0.1731 ± 0.0527^{d}	0.0311 ± 0.0241^{c}
7.5	0.7568 ± 0.0257^{c}	0.0106 ± 0.0063^{d}	0.3659 ± 0.3725^{c}	0.2028 ± 0.0202^{bcd}	0.0316 ± 0.0210^{c}
8	0.5105 ± 0.0426^{de}	0.3139 ± 0.3180^{bcd}	0.4724 ± 0.4144^{bc}	0.1915 ± 0.0097^{cd}	0.0109 ± 0.0056^{c}

Note Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to the Duncan's multiple range test ($p < 0.05$).

Table 4.8 Effect of different substrates of spawn on mycelium of *Hericium* growth after 21 days of incubation (centimeters)

Substrate	***H. erinaceus***				***H. coralloides***
media	**MFLUCC 21–0018**	**MFLUCC 21–0019**	**MFLUCC 21–0020**	**MFLUCC 21–0021**	**MFLUCC 21–0050**
Bagasse	3.1222 ± 0.6598^{bcd}	2.4666 ± 1.7306^{def}	1.0444 ± 0.1602^{f}	4.6987 ± 0.4309^{ab}	5.3062 ± 0.0588^{f}
Barley	3.7827 ± 0.1342^{bcd}	1.9211 ± 0.3224^{efg}	2.6876 ± 0.4223^{d}	3.1666 ± 0.4522^{d}	3.6247 ± 0.2075^{g}
Brown rice	4.6166 ± 0.3482^{abc}	3.8951 ± 0.7033^{abc}	4.4987 ± 0.2808^{b}	4.5518 ± 0.2537^{b}	5.2259 ± 0.2118^{f}
Coir	5.4741 ± 0.0669^{a}	5.0543 ± 0.4159^{a}	5.2592 ± 0.1201^{a}	5.2592 ± 0.1091^{a}	7.0086 ± 0.3483^{bc}
Corn	0^{e}	1.6382 ± 0.4443^{fg}	1.6580 ± 0.6804^{e}	2.0061 ± 0.3997^{f}	1.3172 ± 0.4042^{i}
Millet	2.7321 ± 0.8136^{d}	1.9382 ± 0.1955^{efg}	2.7617 ± 0.2093^{d}	2.5012 ± 0.3038^{ef}	2.8098 ± 0.5613^{h}
Mung bean	0.3666 ± 0.6351^{e}	0.9333 ± 0.1855^{gh}	0.9790 ± 0.0396^{f}	0.7358 ± 0.4138^{g}	0^{j}
Oat	0.4442 ± 0.1681^{e}	0^{h}	0^{g}	0^{h}	1.2889 ± 0.0694^{i}
Paddy	0.9382 ± 1.3735^{e}	0^{h}	0^{g}	0.9481 ± 0.0648^{g}	3.4099 ± 0.3109^{g}
Rice	2.9148 ± 2.4376^{d}	2.8629 ± 0.0582^{cde}	3.0506 ± 0.0466^{d}	3.8012 ± 0.1619^{c}	6.5321 ± 0.0815^{cd}
Rice berry	3.7469 ± 0.9967^{bcd}	4.7738 ± 0.6923^{a}	3.7321 ± 0.4277^{c}	4.8679 ± 0.4493^{ab}	7.2802 ± 0.1557^{b}
Rice straw	0.7481 ± 0.3429^{e}	0^{h}	0^{g}	0^{h}	1.0790 ± 0.0182^{i}
Sticky rice	3.0444 ± 0.4627^{cd}	3.4605 ± 0.2851^{bcd}	4.3086 ± 0.3623^{b}	2.7370 ± 0.4361^{de}	5.9049 ± 0.3702^{e}
Sorghum	4.0617 ± 0.1408^{abcd}	3.5747 ± 1.2822^{bcd}	2.4913 ± 0.5111^{d}	3.8086 ± 0.6654^{c}	6.2815 ± 0.0971^{de}
Wheat	4.7012 ± 0.0407^{ab}	4.2670 ± 0.4419^{ab}	3.9864 ± 0.2830^{bc}	4.6531 ± 0.4704^{ab}	7.8442 ± 0.5311^{a}

Note Values are the means ± SD of length of mycelial growth (centimeters). Values in the same letter differ significantly according to the Duncan's multiple range test ($p < 0.05$).

Table 4.9 Effect of carbon source of basal media on the mycelial growth of *Hericium* strains (gram)

Carbon source	***H. erinaceus***				***H. coralloides***
	MFLUCC 21–0018	**MFLUCC 21–0020**	**MFLUCC 21–0019**	**MFLUCC 21–0021**	**MFLUCC 21–0050**
Dextrose	0.0093 ± 0.0005^{b}	0.0302 ± 0.0093^{b}	0.0053 ± 0.0009^{b}	0.0113 ± 0.0020^{b}	0.0054 ± 0.0012^{b}
Fructose	0.0053 ± 0.0012^{b}	0.0455 ± 0.0151^{b}	0.0057 ± 0.0003^{b}	0.0051 ± 0.0007^{b}	0.0106 ± 0.0037^{b}
Glucose	0.0184 ± 0.0052^{b}	0.0213 ± 0.0123^{b}	0.0186 ± 0.0054^{b}	0.0173 ± 0.0013^{b}	0.0042 ± 0.0001^{b}
Glycine	0.0032 ± 0.0010^{b}	0.0055 ± 0.0019^{b}	0.0057 ± 0.0011^{b}	0.0022 ± 0.0004^{b}	0.0039 ± 0.0033^{b}
Lactose	0.0027 ± 0.0001^{b}	0.0108 ± 0.0072^{b}	0.0036 ± 0.0013^{b}	0.0020 ± 0.0004^{b}	0.0105 ± 0.0047^{b}
Maltose	0.0054 ± 0.0005^{b}	0.0108 ± 0.0045^{b}	0.0046 ± 0.0018^{b}	0.0037 ± 0.0006^{b}	0.0160 ± 0.0057^{b}
Molasse	0.2097 ± 0.0654^{a}	0.3384 ± 0.1088^{a}	0.1983 ± 0.0387^{a}	0.0930 ± 0.0300^{a}	0.0453 ± 0.0208^{a}
Sucrose	0.0060 ± 0.0019^{b}	0.0054 ± 0.0022^{b}	0.0041 ± 0.0011^{b}	0.0071 ± 0.0025^{b}	0.0146 ± 0.0041^{b}
Xylose	0.0042 ± 0.0006^{b}	0.0044 ± 0.0005^{b}	0.0037 ± 0.0032^{b}	0.0037 ± 0.0014^{b}	0.0149 ± 0.0097^{b}

Note Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to the Duncan's multiple range test ($p < 0.05$).

Table 4.10 Effect of nitrogen source of basal media on the mycelial growth of *Hericium* strains (grams)

Nitrogen source	***H. erinaceus***				***H. coralloides***
	MFLUCC 21–0018	**MFLUCC 21–0020**	**MFLUCC 21–0019**	**MFLUCC 21–0021**	**MFLUCC 21–0050**
NH_4Cl	0.0060 ± 0.0007^{cd}	0.0054 ± 0.0015^{d}	0.0055 ± 0.0025^{cd}	0.0025 ± 0.0001^{c}	0.0033 ± 0.0008^{cd}
NH_4NO_3	0.0026 ± 0.0011^{cd}	0.0030 ± 0.0014^{d}	0.0051 ± 0.0023^{cd}	0.0034 ± 0.0006^{c}	0.0033 ± 0.0008^{cd}
Malt extract	0.0314 ± 0.0091^{b}	0.0394 ± 0.0162^{b}	0.0153 ± 0.0037^{b}	0.0216 ± 0.0081^{b}	0.0112 ± 0.0006^{b}
Peptone	0.0078 ± 0.0014^{c}	0.0200 ± 0.0039^{c}	0.0053 ± 0.0027^{cd}	0.0063 ± 0.0022^{c}	0.0158 ± 0.0056^{b}
KNO_3	0.0031 ± 0.0005^{cd}	0.0061 ± 0.0029^{d}	0.0039 ± 0.0015^{cde}	0.0030 ± 0.0001^{c}	0.0105 ± 0.0086^{bc}
$NaNO_3$	0.0024 ± 0.0037^{cd}	0.0010 ± 0.0003^{d}	0.0013 ± 0.0002^{de}	0.0011 ± 0.0002^{c}	0.0027 ± 0.0026^{d}
Urea	0.0016 ± 0.0006^{cd}	0.0015 ± 0.0009^{d}	0.0064 ± 0.0021^{c}	0.0034 ± 0.0024^{c}	0.0024 ± 0.0015^{d}
Yeast extract	0.0936 ± 0.0051^{a}	0.0746 ± 0.0157^{a}	0.0500 ± 0.0045^{a}	0.0864 ± 0.0172^{a}	0.0942 ± 0.0055^{a}

Note Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to the Duncan's multiple range test ($p < 0.05$).

Table 4.11 Effect of carbon to nitrogen ratio on the mycelial growth of *Hericium* strains (grams)

Ratio	*H. erinaceus*				*H. coralloides*
	MFLUCC 21–0018	**MFLUCC 21–0020**	**MFLUCC 21–0019**	**MFLUCC 21–0021**	**MFLUCC 21–0050**
1:1	0.0836 ± 0.0424^{c}	0.0543 ± 0.0085^{b}	0.0327 ± 0.0135^{b}	0.1252 ± 0.0117^{a}	0.1510 ± 0.0313^{a}
1:5	0.0856 ± 0.0156^{c}	0.1081 ± 0.0464^{b}	0.0564 ± 0.0152^{b}	0.1087 ± 0.0129^{a}	0.1344 ± 0.0268^{a}
1:10	0.0732 ± 0.0192^{c}	0.0626 ± 0.0064^{b}	0.0334 ± 0.0071^{b}	0.0451 ± 0.0098^{b}	0.0557 ± 0.0017^{c}
5:1	0.1833 ± 0.0232^{b}	0.0963 ± 0.0308^{b}	0.0370 ± 0.0023^{b}	0.1166 ± 0.0166^{a}	0.0942 ± 0.0086^{b}
10:1	0.2349 ± 0.0310^{a}	0.3547 ± 0.0621^{a}	0.1133 ± 0.0260^{a}	0.1141 ± 0.0083^{a}	0.1537 ± 0.0198^{a}

Note Values are the means ± SD of mycelium dry weight (g). Values in the same letter differ significantly according to Duncan's multiple range test ($p < 0.05$).

Figure 4.3 Morphology of mycelium of different *Hericium* strains on basal media with varying carbon and nitrogen sources after 15 days of incubation

4.3.3 Cultivation of *Hericium erinaceus*

Four strains of *Hericium erinaceus* initiated primordia growth within 2–4 weeks (17–28 days) after opening the substrate treatment bags at the time when the mycelial fully grew. The first flush of fruiting bodies occurs in the next 7−10 days, with individual fruiting bodies exhibiting a fresh weight ranging from 17 to 74 grams each. The fruiting bodies are produced at 18−24 °C and a relative humidity of 90−95%.

In the three different substrates, the mushroom products had the highest fresh weight in the second substrate treatment. All *H. erinaceus* strains grown on the substrate treatments ranged from 63 to 74 g/600 g, with a B.E. of 16.11–27.18%. The average

weight of the mushrooms, the total yield, and the B.E. of the *H. erinaceus* strains were affected by growth on three different substrates. The total yield of the four strains of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) on the three substrates varied between 21.10 and 123.70 g/kg of substrate, while B.E. varied between 3.75% and 27.19% (Table 4.12). All strains of *H. erinaceus* show more favorable growth under the second substrate treatment than under the first and third substrate treatments.

Table 4.12 Comparison of the first flush yield of *Hericium erinaceus* stains in various substrates

Parameters	Treatment number	Strains			
		MFLUCC 21-0018	**MFLUCC 21-0019**	**MFLUCC 21-0020**	**MFLUCC 21-0021**
Fresh weight of fruiting bodies of flush 1 (grams)	1	43.68 ± 15.61^{ab}	55.38 ± 24.40^{ab}	41.61 ± 9.82^{ab}	56.89 ± 15.03^{a}
	2	69.09 ± 10.11^{a}	74.30 ± 10.46^{a}	63.45 ± 25.82^{a}	68.84 ± 14.06^{a}
	3	17.97 ± 28.89^{b}	28.49 ± 39.52^{b}	23.84 ± 33.60^{b}	25.87 ± 25.64^{b}
Time taken for flush 1 (days)*	1	73.00 ± 0.00^{b}	70.00 ±6.70^{b}	67.60 ± 5.08^{b}	75.60 ± 2.74^{a}
	2	68.20 ± 4.55^{c}	69.60 ± 4.77^{b}	71.00 ± 4.47^{b}	69.60 ± 4.77^{b}
	3	78.00 ± 0.00^{a}	77.00 ± 2.24^{a}	78.00 ± 0.00^{a}	78.00 ± 0.00^{a}
Number of flushes	1	3	3	3	3
	2	3	3	3	3
	3	2	2	2	2
Total Yield (grams)	1	83.72^{b}	98.15^{b}	78.04^{b}	104.01^{b}
	2	123.7^{a}	102.86^{a}	85.79^{a}	109.24^{a}
	3	21.1^{c}	30.26^{c}	30.26^{c}	34.64^{c}
Biological Efficiency (%)	1	19.58 ± 9.80^{ab}	15.64 ± 9.24^{ab}	12.95 ± 4.32^{ab}	14.76 ± 8.30^{ab}
	2	27.19 ± 15.72^{a}	18.79 ± 13.68^{a}	16.11 ± 13.64^{a}	19.77 ± 10.74^{a}
	3	3.75 ± 4.02^{b}	4.17 ± 5.09^{b}	5.93 ± 6.46^{b}	6.27 ± 5.89^{b}

Note * Time taken for flush 1 (days): range of the time from the mycelium growth until mushroom fruiting bodies are produced on the substrate treatment bag.

The morphological characteristics of the fruiting bodies of *H. erinaceus* cultivated were branched. It produces a white to yellowish, slender, round, or uneven, with thick or dense hairs or thorns, an average size of 5–10 cm, a stem diameter of 1–3 cm, and long. The mature fruiting bodies grown on all growing substrates are shown in Figure 4.4. As a result of the growing substrate, no special or specific characters were detected.

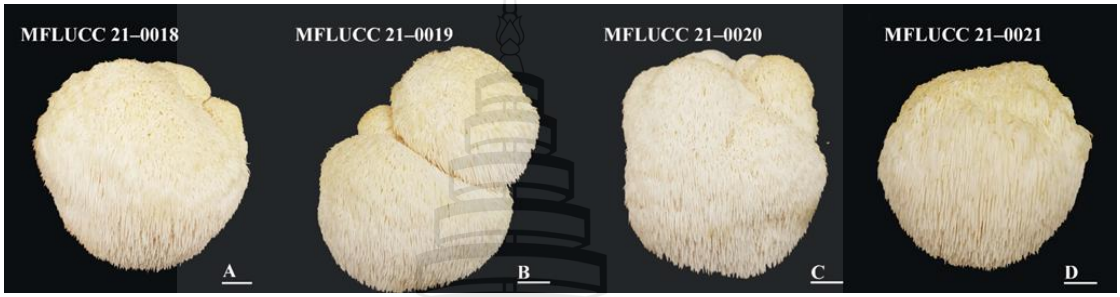


Figure 4.4 The Mature fruiting bodies of *H. erinaceus* strains MFLUCC 21-0018 (A), MFLUCC 21-0019 (B), MFLUCC 21-0020 (C), and MFLUCC 21-0021 (D) after 10 weeks. Scale bars = 1 cm.

6.3.4 Cultivation of *Hericium coralloides*

Hericium coralloides strain MFLUCC 21-0050 initiated pinhead growth within 2 weeks (14-18 days) after opening the substrate treatment bags at the time when the mycelial fully grew. The first flush of fruiting bodies occurs in the next 21-28 days, with individual fruiting bodies exhibiting a fresh weight ranging from 43 to 87 grams each. The fruiting bodies are produced at 18−24 °C and a relative humidity of 90−95%.

In the three different substrates, the mushroom products gave the highest fresh weight in the second substrate treatment. *Hericium coralloide* grown on the substrate treatments ranged from 43 to 87 g/600 g, with a B.E. of 18.43–19.00%. The average weight of the mushrooms, the total yield, and the B.E. of the *H. coralloide* were affected by growth on three different substrates. The total yield of *Hericium coralloides* strain MFLUCC 21-0050 on the three substrates varied between 138 and 142 g/kg of substrate, while B.E. varied between 18.43% and 19.00% (Table 4.13). *Hericium coralloides* strain MFLUCC 21-0050 shows more favorable growth under the second substrate treatment than under the first and third substrate treatments. The development of fruiting body growth of *H. coralloides* was shown in Figure 4.5.

Table 4.13 Comparison of the first flush yield of *Hericium coralloides* in various substrates

Parameters	Treatment number	MFLUCC 21–0050
Fresh weight of fruiting bodies of flush 1 (grams)	1	56.56 ± 11.67[a]
	2	55.29 ± 11.21[a]
	3	57.01± 17.95[a]
Time taken for flush 1 (days)*	1	38
	2	38
	3	40
Number of flushes	1	3
	2	3
	3	3
Total Yield (grams)	1	141.4
	2	138.2
	3	142.5
Biological Efficiency (%)	1	18.85 ± 3.9[a]
	2	18.43 ± 3.7[a]
	3	19.00 ± 5.9[a]

Note * Time taken for flush 1 (days): range of the time from the mycelium growth until mushroom fruiting bodies are produced (pinhead) on the substrate treatment bag. The different letters in the same column for each treatment indicate a significant difference ($p = 0.05$).

Figure 4.5 The development of fruiting body growth of *Hericium coralloides* strain MFLUCC 21-0050. a – c. *H. coralloides* growing on substrate treatment number 1, d – f *H. coralloides* growing on substrate treatment number 2, g – h *H. coralloides* growing on substrate treatment number 3. Scale bar = 1 cm.

The morphological characteristics of the fruiting bodies of *Hericium coralloides* cultivated were branched. It produces a white to yellowish, slender, round, or uneven, with thick or dense hairs or thorns, an average size of 4–11 cm, a stem diameter of 1 to 2 cm, and long. The mature fruiting bodies grown on all growing substrates are shown

in Figure 4.6. As a result of the growing substrate, no special or specific characters were detected.

Figure 4.6 The Mature fruiting bodies of *Hericium coralloides* strain MFLUCC 21-0050. a – c Mature fruiting bodies from substrate treatment number 1 – 3 respectively. Scale bar = 1 cm.

4.3.5 Nutritional Analysis of *Hericium erinaceus* and *H. corealloides*

The proximate compositions are presented in Table 4.14. The protein content was found between 15.30% and 19.56%, the crude fiber content was found between 10.89% and 11.68%, the fat content was found between 2.01% and 3.09%, the ash content was found between 8.84% and 9.49%, the carbohydrate content was found between 57.22% and 62.18%, the moisture content was found between 86.91% and 88.06%, and the dry matter was between 11.94% and 13.09%. While *H. coralloides*, the protein content was found 18.81%, the crude fiber content was found 4.61%, the fat content was found 6.43%, the ash content was found 13.88%, the carbohydrate content was found 49.98%, the moisture content was found 10.90%, and the energy was 333.03 kcal. The proximate compositions of *H. coralloides* are presented in Table 4.15.

Table 4.14 Proximate composition of four strains of *Hericium erinaceus* expressed as percentage (%)

Parameters	Strain			
	MFLUCC 21–0018	**MFLUCC 21–0019**	**MFLUCC 21–0020**	**MFLUCC 21–0021**
Ash	9.02 ± 0.10^{b}	8.95 ± 0.21^{b}	9.49 ± 0.10^{a}	8.84 ± 0.10^{b}
Carbohydrates	61.39 ± 1.61^{a}	60.90 ± 1.68^{a}	57.22 ± 0.43^{b}	62.18 ± 2.21^{a}
Crude fiber	10.96 ± 0.91^{a}	11.65 ± 0.17^{a}	10.89 ± 0.19^{a}	11.68 ± 0.14^{a}
Fat	3.02 ± 1.71^{a}	3.09 ± 1.44^{a}	2.85 ± 0.43^{a}	2.01 ± 1.96^{a}
Moisture	87.18 ± 4.31^{a}	86.92 ± 3.73^{a}	86.91 ± 2.56^{a}	88.06 ± 2.67^{a}
Protein	15.62 ± 0.51^{b}	15.36 ± 0.21^{b}	19.56 ± 0.12^{a}	15.30 ± 0.16^{b}

Table 4.15 Proximate composition in the mushroom fruiting bodies of *H. coralloides*

Parameters	Proximate composition
Ash	13.88 g/100g
Carbohydrate	49.98 g/100g
Crude fiber	4.61 g/100g
Energy	333.03 kcal/100g
Fat	6.43 g/100g
Moisture	10.90 g/100g
Protein	18.81 g/100g

4.4 Discussions

Commercial mushrooms such as *Auricularia*, *Flammulina*, and *Lentinula* have been remarkably popular in the world market (Chang & Wasser, 2018). In Thailand, these mushrooms are consumed, yet the price is not sufficiently high for export to the world market. *Hericium* has been consumed in a niche market and would likely reach a broader market if cultivation could be made more efficient.

Several studies have investigated the mycelial growth of *Hericium*, including *H. abietis*, *H. alpestre* (currently valid name: *H. flagellum*), *H. americanum*, *H. coralloides*, *H. erinaceus*, and *H. laciniatum* growth on PDA (Han et al., 2005). Figlas et al. (2007) suggested the growth of the mycelium of *H. erinaceus* on the MYPA medium at 25 °C. According to Julian et al. (2018), PDA was appropriate for *H. erinaceus* and SDA was suitable for *H. coralloides*. However, Bich et al. (2018) reported that PDA supplemented with fresh mushroom extract was the most suitable medium for mycelial growth of *H. erinaceus*. In this study, the mycelial cultures of the different strains of *Hericium* species were studied in various culture conditions. The results revealed that OMYA and CDA were suitable for *H. erinaceus*, while MYPA was suitable for the growth of *H. coralloides*. These data indicate that the optimal culture media and the nutrient requirements for mycelial growth differ, depending on the *Hericium* strain used.

Varying temperatures showed that the mycelium growth of *Hericium* was similar at 16–35 °C. This result was in agreement with the results reported by Han et al. (2005) and Imtiaj et al. (2008), which reported an extended range of temperature for the growth of *Hericum* mycelium at 20–30 °C. However, Bich et al. (2018) reported that the optimum temperature for vegetative growth of *Hericium* is 25 °C. This study recommends a temperature of 25 °C for *H. erinaceus,* while the growth of *H. coralloides* may occur at a variety of temperatures.

The pH values most suitable for the mycelial growth of *H. erinaceus* and *H. coralloides* were in the range of pH 4-5.5. This result was similar to the report by Boddy et al. (2011), which reported *H. cirrhatum*, *H. coralloides*, and *H. erinaceus* optimum growth at pH 5.5, and Imtiaj et al. (2008) presented the most favorable growth at pH 6.

Moreover, the pH range for mycelial growth of medicinal mushrooms such as *Phlebopus portentosus* included suitable growth at pH 4 (Thongklang et al., 2011), and Shim et al. (2005) revealed that pH 7 was the optimum for the growth of *Macrolepiota procera*.

The most suitable agricultural substrate and cereal grain for mycelium growth of *H. erinaceus,* which showed the best vegetative mycelium growth, are coir and wheat, respectively, while the substrates suitable for the mycelium growth of *H. coralloides* are wheat and rice. This result was similar to those of Siwulski and Sobieralski (2005), who reported the highest yields of *H. erinaceus* strains CS 91 and DSM 11325 on wheat bran. In addition, Hoa and Wang (2015) reported that brown rice was the most favorable to the mycelial growth of *Pleurotus ostreatus* and *P. cystidiosus*.

For the effect of carbon and nitrogen sources on mycelial growth, *H. erinaceus* and *H. coralloides* had the most favorable growth on molasses and yeast extract, respectively, which is in agreement with Hoa and Wang (2015), recording molasses as a good carbon source for *Pleurotus ostreatus* and *P. cystidiosus*. According to Shim et al. (2005), maltose was the best for mycelial growth. Moreover, Wiriya et al. (2014) reported that sucrose was the best carbon source for mycelial growth. So, both aforementioned studies showed that disaccharides were better than monosaccharides; however, molasses contains a surplus of 43% sugars (Jamir et al., 2021). Besides, Thai and Keawsompong (2019) showed that yeast extract was the most suitable for *Tricholoma crissum*, and Gbolagade et al. (2006) found that yeast extract enhanced the greatest mycelial growth of *Lentinus subnudus*. Wiriya et al. (2014) also reported that organic nitrogen sources were the best to promote mycelial growth. However, molasses and yeast extract were the complex media. Palmonari et al. (2020) reported that molasses is a by-product of sugar extract, and Ramadhani et al. (2022) said that sugar was widely known as a carbon source. In addition, yeast extract was estimated to contain 40% organic carbon (Holwerda et al., 2012), while Tomé (2021) reported yeast extract content of nitrogenous compounds at 45 to 70%, which included 80% of protein nitrogen and 10–12% of nucleic acid nitrogen. Additionally, molasses served as a carbon source, and yeast extract served as a nitrogen source. The ratio of media components of 10:1 was the best for the mycelial growth of *H. erinaceus* and *H.*

coralloides. This result was similar to that of et al. (2005), who reported for *Macrolepiota procera* an optimum carbon to nitrogen ratio ($NaNO_3$/D-glucose) of 10:1.

Hericium cultivation, the four strains observed of *Hericium erinaceus* are MFLUCC 21-0018, MFLUCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021 demonstrate the outcome of the sawdust substrate content augmented with cereal grains, including red sorghum (Treatment 2), receiving the high biological efficiency of fruiting bodies; similar results have been reported by Siwulski and Sobieralski (2005) that the cereal grain, including rye grain, was the best supplement for the cultivation of *H. erinaceus*.

All strains in this study produced high yields in the second substrate treatment, just as Siwulski and Sobieralski (2005) reported that when wheat bran was added to sawdust, high yields were produced. According to Figlas et al. (2007), who grew *Hericium* on a sawdust substrate supplemented with grain, including sunflower seed hulls, and Wang et al. (2010), who added the grain cottonseed hulls to the substrate, the addition of the grain resulted in an increase in mushroom yield. In this study, we assumed that the first and third substrate treatments had only carbon sources, whereas the second substrate treatment included a 2% addition of yeast powder as a nitrogen source. We agreed with Cheng et al. (2021) and Gonkhom et al. 2022, who reported that organic nitrogen sources were supplemented for mycelial growth. The mean number of days taken for pinhead formation was estimated to be 57−68 days, then the fruiting bodies were produced as a flush after 7−10 days. So, the mean number of days taken for flush 1 of four strains of *H. erinaceus* was 67−78 days, all were shorter than Bunroj et al. (2017), who reported the mean number of days taken for pinhead formation was 57−149 days.

The cultivation of *Hericium coralloides* strain MFLUCC 21-0050, the weight of the fruiting body as flush 1 of substate treatments 1, 2, and 3 shows 56.56, 55.29, and 57.01 grams/bag, with the yield as 141.4, 138.2, and 142.5 grams/ 600 grams, respectively. That shows the growth value of this mushroom on three different substrate treatments has no significant differences ($p = 0.05$). The total yield has reported similar results with Ko et al. (2005) and produced more weight than Atila et al. (2021), who reported 101 grams/ kg of *H. coralloides* fruiting body. As the ingredients for prepared substrate treatment base on para-rubber sawdust and additional ingredients, substrate

treatment number 1 added rice bran and sugar, substrate treatment number 2 added red sorghum, gypsum ($CaSO_4 \cdot 2H_2O$), lime (CaO), molasses, rice bran, and yeast powder, and substrate treatment number 3 added gypsum ($CaSO_4 \cdot 2H_2O$), lime (CaO), magnesium sulphate ($MgSO_4$), molasses, rice bran, rice powder, and sugar show the cost of ingredients on table 4.16. The cost of cultivation substrate was 16.37$, 96.17$, and 15.97$, respectively. The cost of substrate treatment number 1 was 16.37$ shows a lower price than 2 and 3.

Table 4.16 The price of additional ingredients for cultivation substrate treatments

Substrate	*Cost ($/kg)	Substrate Treatment (kg)		
		No. 1	No. 2	No. 3
Red sorghum	0.61	-	15	-
Gypsum ($CaSO_4 \cdot 2H_2O$)	3.00	-	1	0.4
Lime (CaO)	0.10	-	1	0.8
Magnesium sulfate ($MgSO_4$)	0.29	-	-	0.2
Molasses	0.25	-	1	-
Para rubber sawdust	0.15	95	77	88
Rice bran	0.04	3	3	9
Rice flour	0.35	-	-	0.8
Sugar	1.00	2	-	0.8
Yeast powder	36.00	-	2	-

Note No.1 = Substrate Treatment Number 1, No.2 = Substrate Treatment Number 2, No.3 = Substrate Treatment Number 3

* Cost of each of the additional ingredients from the Alibaba website.

Mushrooms have been considered as a good source of nutritional value. They have been well documented in terms of being an excellent source of protein, carbohydrates, minerals, and vitamins. However, the nutritional value of mushrooms varies depending on the species, substrate, and conditions in which the mushrooms were cultivated in Phonemany et al. (2021). In this chapter, *Hericium erinaceus* was closely examined in ash, lipid, carbohydrates, crude fiber, fat, elemental content, and protein (Atila et al., 2021; Gonkhom et al., 2024). The fruiting body of *H. coralloides* had the protein content 18.81%, and the ash content 13.88% higher than Atila et al. (2021), who reported 17.4% of protein content and 8.5% of ash content.

CHAPTER 5

IDENTIFICATION OF CHEMICAL CONSTITUENTS IN *HERICIUM*

5.1 Introduction of *Hericium* Metabolite

Hericium, a medicinal fungus, is well-known for its distinctive appearance as well as its health benefits (Kostanda et al., 2024). This edible fungus has gained interest due to its bioactive components, especially its metabolites, which promote its therapeutic characteristics (Wang et al., 2019; Kumar et al., 2021).

Numerous studies have identified various bioactive chemical constituents within *Hericium* (Jianzhao et al., 2024). *Hericium* is an abundant source of bioactive compounds that enhance its therapeutic properties (Kostanda et al., 2024). The small-molecular-weight and high-molecular-weight compounds have been isolated and identified from this mushroom (Thongbai et al., 2015; Szućko-Kociuba et al., 2023). Its main chemical components include polysaccharides (like β-glucans), terpenoids (erinacines and hericenones), amino acids (ergothioneine), fatty acids, phenolic compounds, sterols, and essential minerals (Banerjee et al., 2024; Singh et al., 2025).

The bioactive compounds of *Hericium* have been extensively studied for their neuroprotective effects (Tong et al., 2023). According to preclinical and clinical research, these metabolites may decrease the occurrence of neurodegenerative disorders, improve memory, and cognitive function (Saitsu et al., 2019; Priori et al., 2023; Roda et al., 2023). Furthermore, metabolites of *Hericium* have been demonstrated to have anti-inflammatory, antioxidant, and anti-cancer properties (Ray et al., 2024).

In this chapter, a liquid chromatography coupled to quadrupole–time-of-flight–mass spectrometry (LC–QTOF–MS) method for identification analysis compared with the Personal Compound Database and Library (PCDL) was used to rapidly identify the chemical components of *Hericium erinaceus* and *H. coralloides* extracts. In addition, two previously unreported isoindolinone-type meroterpenoids were identified from the dried fruiting bodies of *H. coralloides*.

5.2 Materials and Methods

5.2.1 Sample Preparation

The crude extract preparation of *H. erinaceus* (MFLUCC 21-0018−21-0021), *H. coralloides* (MFLUCC 21-0050), and one of the dried fruiting body samples of *H. coralloides* was obtained from KÄÄPÄ Biotech, Finland, where it had been cultivated under artificial conditions was following Section 3.6 of Chapter 3 (General Material and Methodology).

5.2.2. LC-ESI-QTOF-MS/MS Characterization

The characterization of *Hericium* compounds using LC-ESI-QTOF-MS/MS followed a modified version of the method by Ma et al. (2019). An Agilent 1200 series HPLC system (Agilent Technologies, CA, USA) coupled with an Agilent 6520 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, CA, USA) was employed for the analysis. Separation was achieved using a Synergi Hydro-RP reversed-phase column (250 × 4.6 mm i.d., 4 μm particle size) from Phenomenex (Lane Cove, NSW, Australia), paired with a Phenomenex C18 ODS guard column (4.0 × 2.0 mm i.d.). The column temperature was maintained at 25 °C, and a 5 μL sample injection volume was used. The mobile phase included two eluents: Eluent A (0.5% acetic acid in water, v/v) and Eluent B (acetonitrile/water/acetic acid in a 50:49.5:0.5 ratio, v/v/v). The gradient elution profile was as follows: 10–25% B (0–20 min), 25–35% B (20–30 min), 35–40% B (30–40 min), 40–55% B (40–70 min), 55–80% B (70–75 min), 80–90% B (75–77 min), 90–100% B (77–79 min), 100–10% B (79–82 min), and isocratic at 10% B (82–85 min). The flow rate was maintained at 0.8 mL/min. Nitrogen gas pressure was set to 45 psi with a flow rate of 5 L/min at 300 °C, while the sheath gas was supplied at 11 L/min at 250 °C. The capillary voltage was 3.5 kV, and the nozzle voltage was set at 500 V. A full mass scan was performed across an m/z range of 50–1300. MS/MS analysis operated in automatic mode with fragmentation energies of 10, 15, and 30 eV. Peak identification was conducted in both positive and negative ionization modes, and all instrument control, data acquisition, and processing were performed using MassHunter workstation software (Qualitative Analysis, version B.03.01) from Agilent Technologies (Santa Clara, CA, USA).

5.2.3 Instrumentation and Analytical Procedures

For the crude extract of the dried fruiting body samples of *H. coralloides* from Finland, HPLC-DAD/MS analyses were carried out using an amaZon speed ETD ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) operating in both positive and negative ionization modes. Chromatographic separation was performed with a C18 Acquity UPLC BEH column (Waters), using a solvent system composed of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B). The elution gradient began with 5% B for 0.5 minutes, ramped to 100% B over 20 minutes, and was held isocratically at 100% B for 10 minutes. The flow rate was 0.6 mL/min, with UV/Vis detection monitored at 190–600 nm and 210 nm.

High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was conducted on a MaXis ESI-TOF mass spectrometer (Bruker Daltonics), coupled with an Agilent 1260 series HPLC-UV system (Agilent Technologies, Santa Clara, CA, USA). The same type of C18 Acquity UPLC BEH column and solvent system was used. The gradient began at 5% B for 0.5 minutes, increased to 100% B over 19.5 minutes, and was maintained isocratically at 100% B for an additional 5 minutes. The analysis was performed at a flow rate of 0.6 mL/min and a column temperature of 40°C. UV/Vis detection was recorded from 200 to 600 nm. Molecular formulas were determined using Compass DataAnalysis 4.4 SR1 software and the Smart Formula algorithm (Bruker Daltonics).

NMR spectra were recorded on a Bruker Avance III 500 spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz) using DMSO-d_6 as the solvent, with chemical shifts reported in ppm and coupling constants in Hz. Optical rotation measurements were made in DMSO using an Anton Paar MCP-150 polarimeter (589 nm, 100 mm path length). UV spectra were obtained with a Shimadzu UV/Vis 2450 spectrophotometer (Kyoto, Japan), and electronic circular dichroism (ECD) spectra were recorded on a J-815 spectropolarimeter (Jasco, Pfungstadt, Germany) in DMSO.

All chemicals and solvents used (analytical and HPLC grade) were sourced from AppliChem GmbH (Darmstadt, Germany), Avantor Performance Materials (Deventer, Netherlands), Merck KGaA (Darmstadt, Germany), and Carl Roth GmbH & Co. KG (Karlsruhe, Germany) (Winnie et al., 2024).

5.3 Results

5.3.1 Total Ion Chromatogram

The total ion chromatogram of *Hericium erinaceus* strain MFLUCC 21-0018 is shown in Figure 5.1, *H. erinaceus* strain MFLUCC 21-0019 is shown in Figure 5.2, *H. erinaceus* strain MFLUCC 21-0020 is shown in Figure 5.3, *H. erinaceus* strain MFLUCC 21-0021 is shown in Figure 5.4, and *H. corealloides* strain MFLUCC 21-0050 is shown in Figure 5.5.

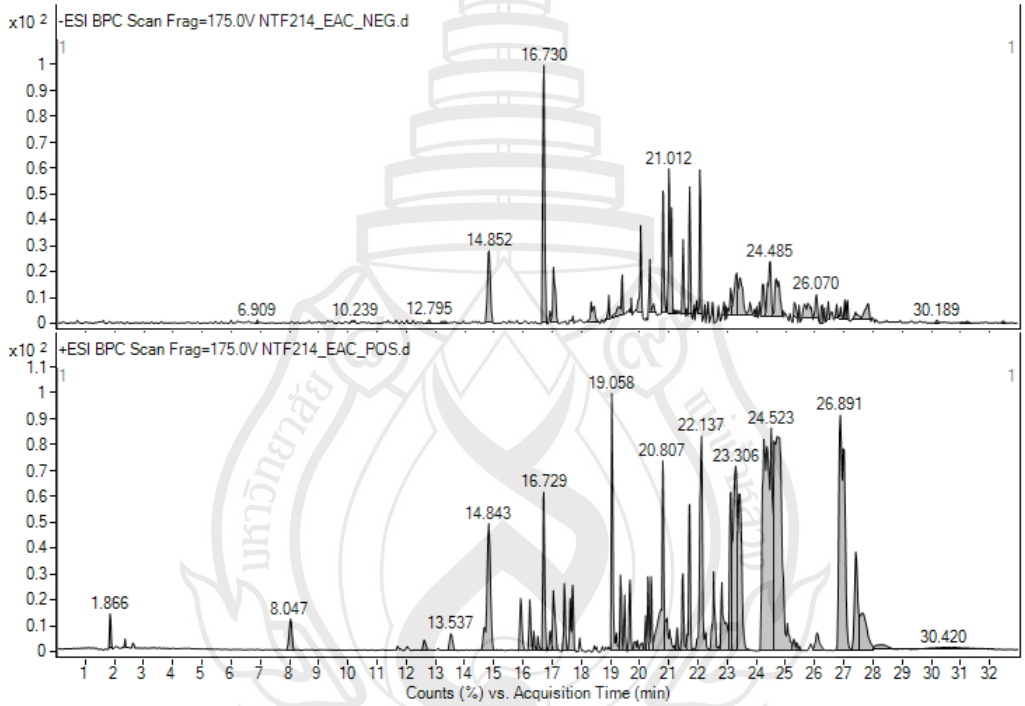


Figure 5.1 Total ion chromatogram of *Hericium erinaceus* strain MFLUCC 21-0018 in the positive ion mode (pos) and negative ion mode (neg)

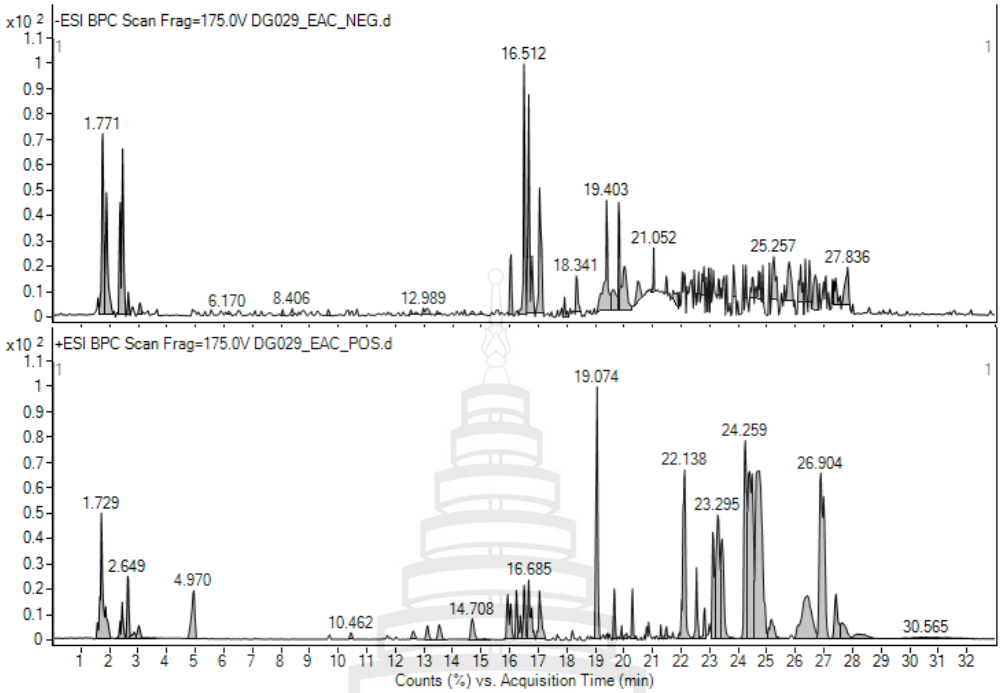


Figure 5.2 Total ion chromatogram of *H. erinaceus* strain MFLUCC 21-0019 in the positive ion mode (pos) and negative ion mode (neg)

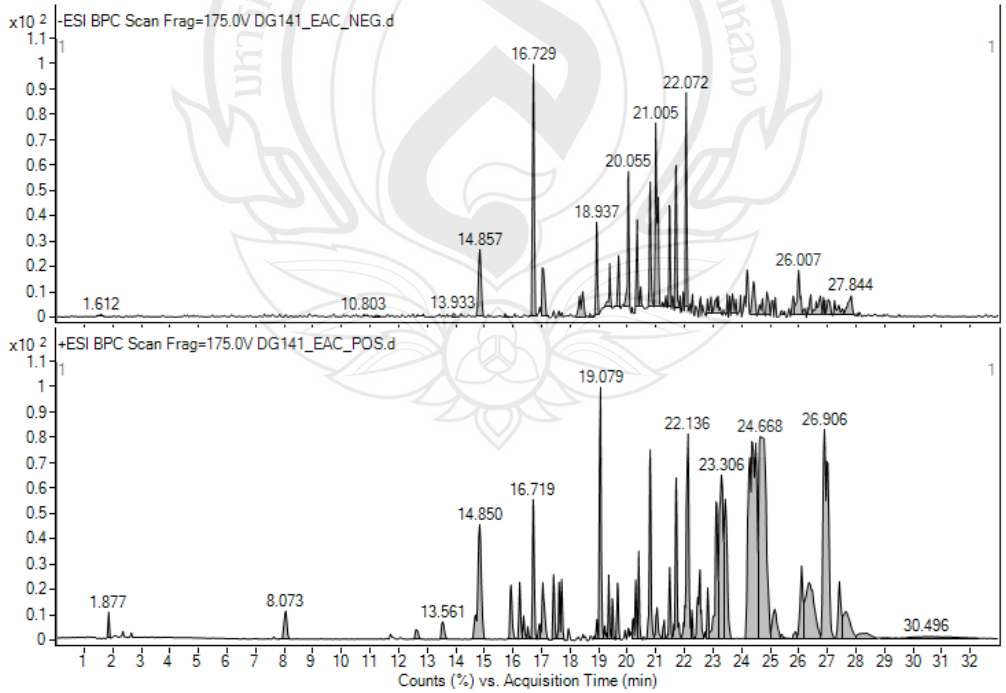


Figure 5.3 Total ion chromatogram of *H. erinaceus* strain MFLUCC 21-0020 in the positive ion mode (pos) and negative ion mode (neg)

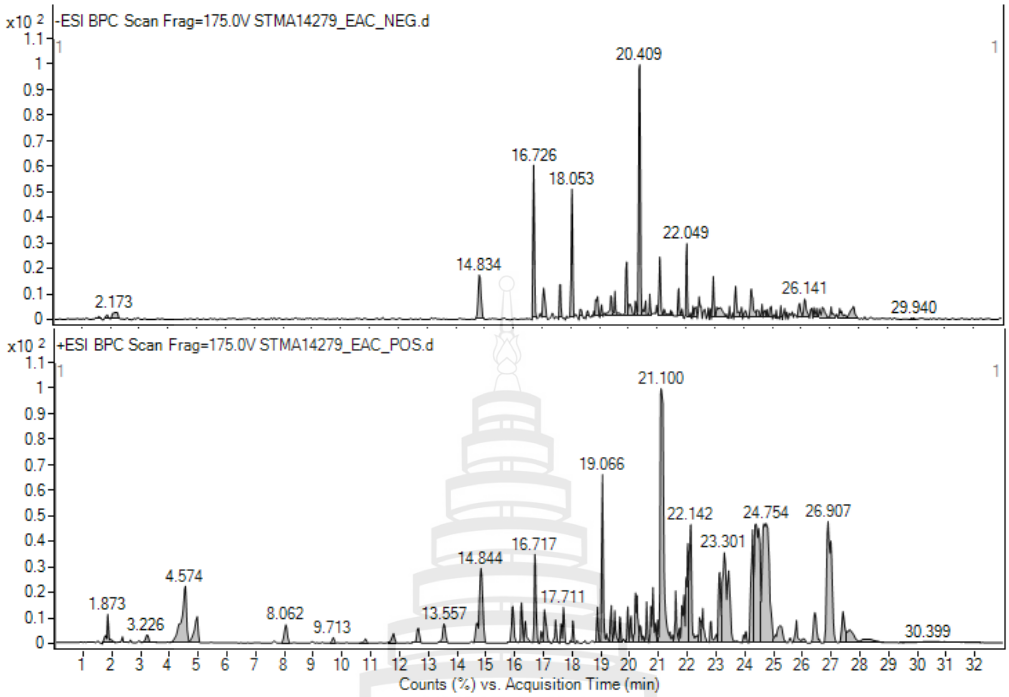


Figure 5.4 Total ion chromatogram of *H. erinaceus* strain MFLUCC 21-0021 in the positive ion mode (pos) and negative ion mode (neg)

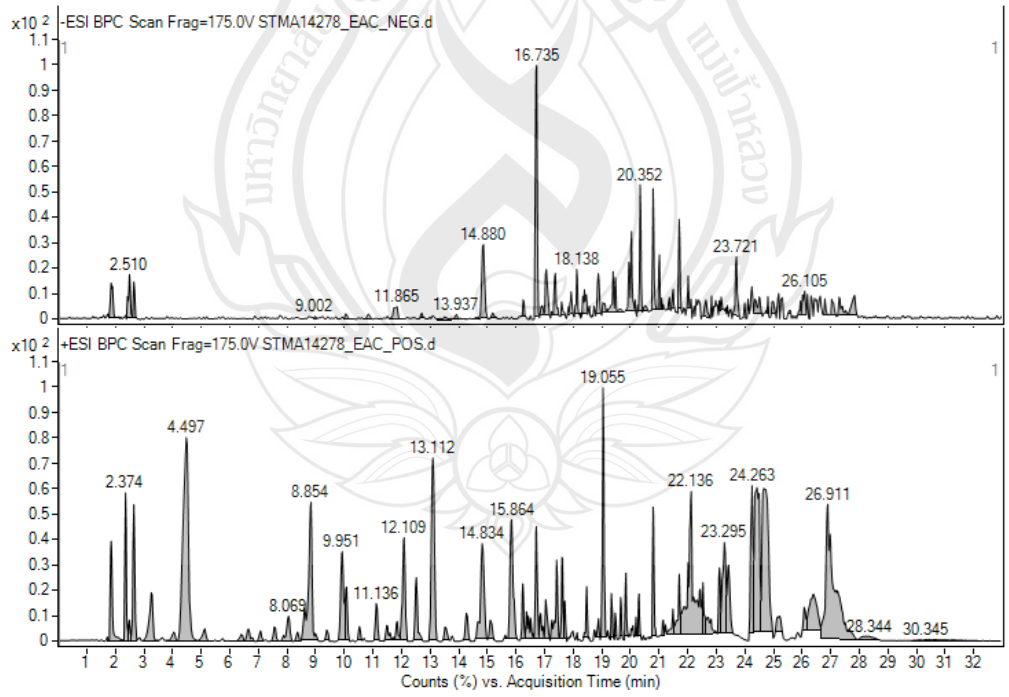


Figure 5.5 Total ion chromatogram of *H. coralloides* strain MFLUCC 21-0050 in the positive ion mode (pos) and negative ion mode (neg)

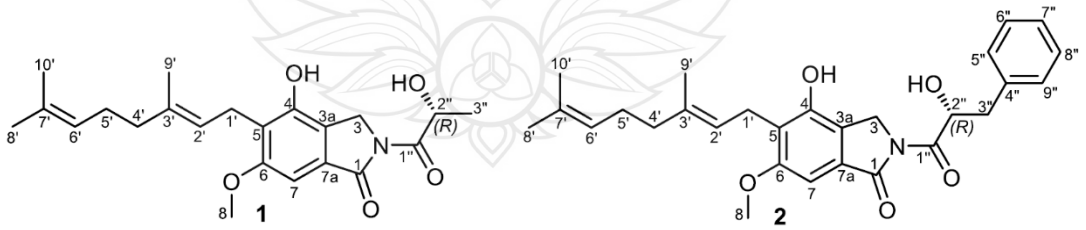
5.3.2 Compound Identification of *Hericium erinaceus* and *H. coralloides*

The compounds of *H. erinaceus* were analyzed by LC-MSQTOF; the identification of structure was based on the retention time, MS data, and MS2 data compared with the Personal Compound Database and Library (PCDL) with an online database of Kansas State University, USA. Compounds with library scores higher than 50% were further selected for *m*/*z* verification and MS/MS analysis.

The preliminarily identified compounds of *Hericium erinaceus* strain MFLUCC 21-0018 were classified into 145 compounds when compared with PCDL., Detailed results are shown in Table 5.1. The discovered compounds of *H. erinaceus* strain MFLUCC 21-0019 were categorized into 168 compounds are presented in Table 5.2. The *H. erinaceus* strain MFLUCC 21-0020 was classified into 169 compounds are presented in Table 5.3, 170 compounds of *H. erinaceus* strain MFLUCC 21-0021 are shown in Table 5.4, and 176 compounds of *H. coralloides* strain MFLUCC 21-0050 are shown in Table 5.5.

5.3.3 Two Isoindolinone Derivatives Isolated from the Fruiting Bodies of *H. coralloides*

Two new isoindolinone derivatives, named corallocins D (1) and E (2), were isolated in low quantities from the ethyl acetate extract of *H. coralloides* fruiting bodies. Their structures were elucidated using HR-ESIMS, NMR spectroscopy, and ECD spectral analysis, revealing that both compounds share a geranyl side chain and an isoindolinone core, with corallocin D containing a 3-hydroxybutyryl moiety and corallocin E featuring a 3-hydroxy-4-phenylbutyryl group (Figure 5.6).



Source Winnie et al. (2024)

Figure 5.6 The Structures of corallocins D (1) and E (2)

Table 5.1 Characterization of Compounds in *Hericium erinaceus* strain MFLUCC 21-0018 by Using LC-QTOF

No.	RT	Formula	Mass	m/z	Base Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
1	18.956	$C_{14}H_{18}O_3$	234.1257	233.1184	277.1085	$[M-H]^-$	Erinachromane B	0.44	99.86
2	21.607	$C_{14}H_{28}O_3$	244.2039	243.1966	445.2236	$[M-H]^-$	(r)-3-Hydroxy myristic acid	0.14	99.81
3	20.042	$C_{25}H_{36}O_6$	432.2506	477.2492	477.2492	$[M-H]^-$	Erinacine A	-1.4	99.79
4	26.07	$C_{18}H_{34}O_2$	282.256	281.2488	381.1744	$[M-H]^-$	Ethyl palmitoleate	0.59	99.77
5	22.126	$C_{16}H_{32}O_3$	272.2349	271.2276	805.9871	$[M-H]^-$	16-Hydroxyhexadecanoic acid	-0.95	99.69
6	18.956	$C_{14}H_{16}O_3$	232.1102	277.1085	277.1085	$[M-H]^-$	coralcuparene	1.2	99.51
7	18.956	$C_{15}H_{18}O_5$	278.1157	277.1085	277.1085	$[M-H]^-$	hericioic acid E	1.01	99.51
8	24.251	$C_{37}H_{58}O_5$	582.4288	581.4216	555.4057	$[M-H]^-$	Hericene B	0.67	99.5
9	23.339	$C_{35}H_{56}O_5$	556.4133	555.4059	555.4059	$[M-H]^-$	Hericene A	0.86	99.45
10	19.473	$C_{11}H_{14}O_3$	194.0945	193.0872	325.185	$[M-H]^-$	4-Ethoxy ethylbenzoate	0.95	99.45
11	21.155	$C_{25}H_{38}O_6$	434.2656	479.2654	303.1607	$[M-H]^-$	Erinacine C	-2.75	99.43
12	21.965	$C_{25}H_{34}O_6$	430.2352	429.228	429.228	$[M-H]^-$	Erinacine S	-0.78	99.38
13	12.69	$C_8H_{15}NO_3$	173.1053	172.098	130.9663	$[M-H]^-$	N-Acetyl-l-leucine	0.69	99.32
14	21.474	$C_{12}H_{14}O_2$	190.0995	235.0977	445.2241	$[M+HCOOL]^-$	eulatachromene	0.8	99.28
15	21.474	$C_{13}H_{16}O_4$	236.105	235.0977	445.2241	$[M-H]^-$	Erinachromane A	0.53	99.28
16	20.758	$C_{25}H_{38}O_7$	450.2605	495.2595	477.2499	$[M-H]^-$	Erinacine T	-2.77	99.23

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
17	19.365	$C_{12}H_{16}O_3$	208.1098	207.1027	229.0874	$[M-H]^-$	B-Asarone	-0.46	99.17
18	24.251	$C_{18}H_{32}O_2$	280.2405	279.2331	555.4059	$[M-H]^-$	9(z),11(e)-Conjugated linoleic acid	0.9	99.11
19	19.504	$C_{10}H_{20}O_3$	188.1413	187.134	325.185	$[M-H]^-$	10-Hydroxydecanoic acid	0.34	99.07
20	20.733	$C_{21}H_{42}O_4$	358.3084	403.3066	477.2501	$[M+HCOOL]^-$	1-Stearoylglycerol	0.17	99.02
21	23.064	$C_{12}H_{26}O_4S$	266.1554	265.1481	555.4059	$[M-H]^-$	Dodecyl sulfate	0.83	98.93
22	27.405	$C_{18}H_{30}O_3S$	326.1918	325.1845	805.9874	$[M-H]^-$	4-Dodecylbenzenesulfonic acid	0.67	98.63
23	22.508	$C_{20}H_{28}O_2$	300.2088	299.2014	805.9872	$[M-H]^-$	Isotretinoin	-0.47	98.57
24	21.499	$C_{25}H_{34}O_7$	446.2313	445.224	295.2282	$[M-H]^-$	Hericinoid C	1.84	98.47
25	20.338	$C_{18}H_{34}O_4$	314.2462	313.2389	431.2442	$[M-H]^-$	(±)9,10-Dihydroxy-12z-octadecenoic acid	1.54	98.28
26	20.338	$C_{16}H_{30}O_2$	254.2251	313.2389	431.2442	$[M-H]^-$	Δ2-Trans-hexadecenoic acid	1.88	98.28
27	18.043	$C_{22}H_{27}NO_7$	417.1778	416.1721	966.0026	$[M-H]^-$	caputmedusins E	-2.23	98.25
28	21.703	$C_{28}H_{44}O_9$	524.2985	523.2909	479.2656	$[M-H]^-$	erinacine Q2	-0.04	98.1
29	20.175	$C_{25}H_{36}O_7$	448.2469	447.2395	339.2003	$[M-H]^-$	Hericinoid A	1.74	97.93
30	20.488	$C_{10}H_{18}O_4$	202.1211	201.1137	325.1855	$[M-H]^-$	3-Tert-butyladipic acid	2.68	97.86
31	21.703	$C_{26}H_{40}O_7$	464.2781	523.291	479.2657	$[M-H]^-$	Erinacine V	1.54	97.57
32	22.189	$C_{21}H_{38}O_4$	354.276	399.2744	805.9872	$[M-H]^-$	1-Linoleoyl glycerol	-2.95	97.57

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
33	19.673	$C_{22}H_{26}O_6$	386.1733	431.1712	325.1848	$[M + HCOOL]^-$	Bis(methylbenzylidene) sorbitol	0.92	97.55
34	20.086	$C_{24}H_{36}O_5$	404.256	449.2542	477.2495	$[M + HCOOL]^-$	Lovastatin	-0.78	97.46
35	18.457	$C_{25}H_{38}O_8$	466.2577	511.2559	329.2343	$[M - H]^-$	Erinacine J	2.14	97.34
36	18.457	$C_{18}H_{34}O_5$	330.2415	329.2343	329.2343	$[M - H]^-$	(15z)-9,12,13-Trihydroxy- 15-octadecenoic acid	2.6	97.33
37	16.534	$C_{13}H_{15}NO_4$	249.1003	248.0932	130.9663	$[M - H]^-$	Erinacerin M	0.58	97.32
38	16.771	$C_{19}H_{28}N_2O_6$	380.1952	379.1879	723.5048	$[M - H]^-$	Erinacerin N	1.3	97.31
39	22.274	$C_{27}H_{42}O_7$	478.2919	523.2901	805.9855	$[M + HCOOL]^-$	Erinacine D	-2.4	97.1
40	21.499	$C_{18}H_{30}O_2$	278.2245	277.2177	295.2283	$[M - H]^-$	Pinolenic acid	-0.14	97.09
41	1.897	$C_9H_{12}N_2O_6$	244.0688	243.0614	130.9657	$[M - H]^-$	Uridine	-3.18	96.92
42	17.956	$C_{15}H_{18}O_6$	294.1106	293.1032	805.9876	$[M - H]^-$	Erinaceolactone G	0.75	95.26
43	2.645	$C_5H_5N_5$	135.0536	134.0464	130.9656	$[M - H]^-$	Adenine	-6.48	92.17
44	20.607	$C_{11}H_{13}NO_3$	207.0897	266.103	311.1688	$[M - H]^-$	N-Acetyl-L-phenylalanine 1-(Carboxymethyl)	0.57	90.57
45	17.511	$C_{25}H_{36}O_8$	464.2428	463.2358	966.0021	$[M - H]^-$	Erinacine G	3.89	90.39
46	21.499	$C_{20}H_{30}O_3$	318.2177	363.216	445.224	$[M - H]^-$	11-α-hydroxy-17-methyltestosterone	-5.63	89.87

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
47	17.865	$C_{18}H_{21}NO_5$	331.1424	330.1348	966.0018	$[M-H]^-$	Erinacerin C	1.19	88.45
48	2.645	$C_{10}H_{13}N_5O_4$	267.0965	312.0946	130.9656	$[M-H]^-$	Adenosine	-0.79	88.27
49	23.152	$C_{37}H_{56}O_6$	596.4063	655.4197	555.4057	$[M-H]^-$	Hericenone I	-2.29	88.21
50	17.17	$C_9H_{16}O_4$	188.105	187.0977	341.115	$[M-H]^-$	Azelaic acid	0.6	87.3
51	18.432	$C_{10}H_{16}O$	152.1202	197.1185	329.2344	$[M-H]^-$	R-ipsdienol	0.32	87.28
52	20.359	$C_{10}H_{16}$	136.1254	195.1392	431.2445	$[M+HCOOL]^-$	Limonene	1.36	86.96
53	18.875	$C_{12}H_{22}O_4$	230.152	229.1446	805.987	$[M-H]^-$	Dodecanedioic acid	0.84	86.86
54	16.877	$C_9H_{10}O_3$	166.0628	165.0558	949.6718	$[M-H]^-$	Ethyl paraben	-1.29	86.83
55	20.378	$C_{12}H_{18}O_2$	194.1305	239.1289	431.2442	$[M-H]^-$	Sedanolide	-0.83	86.36
56	19.261	$C_{12}H_{16}O_2$	192.1151	237.1133	325.1851	$[M-H]^-$	Senkyunolide A	0.13	85.64
57	14.852	$C_8H_{14}O_4$	174.0891	173.0819	497.3356	$[M-H]^-$	Suberic acid	-0.42	85.42
58	11.747	$C_6H_{12}O_3$	132.0789	131.0717	966.002	$[M-H]^-$	2-Hydroxycaproic acid	1.92	82.54
59	18.75	$C_{18}H_{19}NO_6$	345.1213	344.114	186.104	$[M-H]^-$	Erinacerin S	0.15	82
60	26.421	$C_{19}H_{22}O_5$	330.1475	389.1618	805.9877	$[M+HCOOL]^-$	Hericenone A	2.23	81.7
61	20.462	$C_{20}H_{30}O_4$	334.2149	333.2079	325.1855	$[M-H]^-$	2-Hydroxy-4,5′,8a′-trimethyl-1′-oxo-4-vinyloctahydro-1′h-spiro[cyclopentane-1,2′-naphthalene]-5′-carboxylic acid	1.4	81.42

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
62	20.065	$C_{15}H_{18}O_3$	246.125	291.123	477.2496	$[M-H]^-$	Arglabin	-2.37	80.88
63	13.225	$C_{10}H_{10}O_4$	194.0587	193.0513	966.0026	$[M-H]^-$	Erinaceolactone B	3.92	80.73
64	20.042	$C_{27}H_{42}O_8$	494.2857	539.2856	477.2494	$[M+HCOOL]^-$	Erinacine K	-4.55	79
65	13.861	$C_{12}H_{12}N_2O_2$	216.0901	275.1038	130.9663	$[M+HCOOL]^-$	2,3,4,9-Tetrahydro-1h-β-carboline-3-carboxylic acid	1.06	78.83
66	2.44	$C_5H_7NO_3$	129.0428	128.0353	174.956	$[M-H]^-$	4-Oxoproline	1.56	78.68
67	26.042	$C_{20}H_{32}O_2$	304.2378	349.236	381.1744	$[M+HCOOL]^-$	Arachidonic acid	-7.85	78.44
68	23.616	$C_{24}H_{30}O_6$	414.2054	413.1986	555.4071	$[M-H]^-$	Bis(4-ethylbenzylidene) sorbitol	2.92	78.33
69	21.748	$C_{19}H_{24}O_4$	316.1683	361.167	479.2653	$[M+HCOOL]^-$	Hericenone J	2.56	77.92
70	17.72	$C_{26}H_{38}O_6$	446.2636	445.2565	445.2565	$[M-H]^-$	(-)-Erinacin A-d3	-7.26	77.04
71	20.914	$C_{12}H_{10}N_4O_2$	242.0815	301.0962	479.2647	$[M-H]^-$	Lumichrome	4.77	76.76
72	12.896	$C_9H_{14}O_4$	186.0883	185.0814	966.0013	$[M-H]^-$	cyclohexanecarboxylic acid	-4.86	76.41
73	29.264	$C_6H_{14}O_6$	182.0794	181.0723	174.9563	$[M-H]^-$	Galactitol	1.8	76.26
74	21.934	$C_{27}H_{38}O_9$	506.2526	505.2437	805.9868	$[M-H]^-$	Erinacine R	1.97	76.25
75	17.956	$C_{14}H_{14}O_6$	278.0791	277.0714	805.9879	$[M-H]^-$	Erinaceolactone H	0.27	75.99

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
76	21.237	$C_{19}H_{22}O_6$	346.1413	345.1331	311.1698	$[M-H]^-$	Corallocin A	-0.97	75.78
77	10.41	$C_7H_{12}O_4$	160.0733	159.0661	966.0013	$[M-H]^-$	3-Methyladipic acid	-1.76	75.73
78	5.68	$C_7H_{10}O_3$	142.062	187.0601	174.9555	$[M-H]^-$	Erinapyrone B	-6.73	72.82
79	27.173	$C_{37}H_{60}O_6$	600.4378	659.4515	805.9875	$[M-H]^-$	5′-hydroxyhericenes B	-1.95	72.6
80	18.479	$C_{15}H_{18}O_7$	310.1045	309.0966	723.5044	$[M-H]^-$	hericioic acid G	-2.38	72.48
81	24.51	$C_{28}H_{46}O$	398.3534	457.3674	581.421	$[M+HCOOL]^-$	ergosta-7, 22-dien-3β-ol	-3.65	71.69
82	20.949	$C_{18}H_{30}O_3$	294.2173	293.21	477.2499	$[M-H]^-$	9-Oxo-10(e),12(e)-octadecadienoic acid	-7.44	71.61
83	21.207	$C_{28}H_{31}NO_7$	493.2077	552.2216	311.1694	$[M+HCOOL]^-$	caputmedusins C	-4.71	71.57
84	20.758	$C_{22}H_{36}O_3$	348.268	347.2613	477.2499	$[M-H]^-$	3-Methyl-5-(5,5,8atrimethyl-2-methylene-7- oxodecahydro-1- naphthalenyl) pentyl acetate	4.44	70.7
85	23.094	$C_{35}H_{56}O_6$	572.4077	617.4039	555.4056	$[M-H]^-$	5′-hydroxyhericenes A	0.09	69.68
86	22.353	$C_{41}H_{77}NO_9$	727.5553	786.5734	805.9865	$[M-H]^-$	Cerebroside B	-6.19	68.79
87	22.987	$C_{11}H_{18}N_2O_2$	210.1357	269.1524	555.4059	$[M+HCOOL]^-$	Cyclo(leucylprolyl)	-5.14	68.35
88	22.126	$C_{20}H_{38}O_2$	310.2857	355.2829	287.1655	$[M+HCOOL]^-$	Ethyl oleate	-4.88	68.07
89	17.087	$C_{19}H_{23}NO_7$	377.1489	376.1408	341.115	$[M-H]^-$	caputmedusins G	3.75	67.83

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
90	19.504	$C_{21}H_{44}NO_7P$	453.2816	498.279	325.1851	$[M-H]^-$	Glycerophospho-n- palmitoyl ethanolamine	-8.74	66.78
91	26.07	$C_{43}H_{52}N_2O_{12}$	788.3479	787.3405	381.1744	$[M-H]^-$	caputmedusins B	-5.25	65.95
92	8.023	$C_8H_{10}O_4$	170.0576	169.0496	174.9561	$[M-H]^-$	Herierin IV	-1.61	65.77
93	26.07	$C_{46}H_{76}O_5$	708.5714	753.5706	805.9875	$[M-H]^-$	Erinarol F	3.07	64.85
94	24.485	$C_{19}H_{24}O_5$	332.1646	377.1631	581.4215	$[M-H]^-$	Erinacerin B	6.58	64.6
95	17.056	$C_{20}H_{24}O_7$	376.1539	435.1675	341.115	$[M-H]^-$	Erinaceolactone E	4.5	64.08
96	20.846	$C_{35}H_{54}O_7$	586.3843	645.398	477.2495	$[M-H]^-$	3-Hydroxyhericenone F	-4.53	60.48
97	16.771	$C_{12}H_{22}O_{12}$	358.1134	403.108	723.5047	$[M-H]^-$	Lactobionic acid	6.26	60.03
98	17.911	$C_{15}H_{16}O_7$	308.0868	353.0848	805.9879	$[M+HCOOL]^-$	hericioic acid F	-9.21	59.56
99	24.369	$C_{26}H_{36}O_6$	444.2536	443.2473	555.4057	$[M-H]^-$	Hericinoid B	5.38	59.49
100	20.488	$C_{27}H_{40}O_8$	492.2727	491.2658	325.1853	$[M-H]^-$	Erinacine P	0.87	58.47
101	16.485	$C_{55}H_{90}O_7$	862.6612	921.6758	130.9663	$[M+HCOOL]^-$	Hericene H	-8.61	58.42
102	20.822	$C_{27}H_{31}NO_4$	433.2273	493.2455	477.2497	$[M+HCOOL]^-$	Hericenone B	4.64	58.4
103	8.296	$C_9H_{13}N_3O_5$	243.0842	242.0801	966.0039	$[M-H]^-$	Cytarabine	-5.53	56.39
104	17.568	$C_{14}H_{17}NO_5$	279.1104	324.1067	966.002	$[M+HCOOL]^-$	hericioic acid A	-0.83	55.61
105	21.703	$C_{26}H_{41}O_7$	465.2815	524.2953	479.2657	$[M-H]^-$	Erinacine Z1	-8.08	55.16
106	23.188	$C_{37}H_{54}O_6$	594.3898	653.4022	555.4055	$[M-H]^-$	Hericenone H	-3.73	53.09

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
107	23.947	$C_{20}H_{39}NO_2$	325.2994	370.2958	555.406	$[M-H]^-$	Oleoyl ethanolamide	3.93	52.67
108	24.233	$C_{30}H_{46}O_4$	470.3386	469.3349	555.4058	$[M-H]^-$	18-β-glycyrrhetinic acid	-2.1	52.54
109	19.753	$C_{25}H_{39}O_7$	451.2658	496.2632	325.1848	$[M-H]^-$	Erinacine Z2	-8.31	52
110	18.835	$C_{24}H_{31}NO_7$	445.2136	444.2031	277.1085	$[M-H]^-$	Erinacerin W	7.96	51.78
111	24.687	$C_{19}H_{23}NO_5$	345.1555	344.1479	581.4223	$[M-H]^-$	hericioic acid D	-6.06	51.57
112	19.258	$C_{28}H_{40}O$	392.3079	393.3152	192.1409	$[M-H]^-$	ergosta-4, 6, 8(14), 22-tetraen-3-one	-0.14	96.66
113	17.959	$C_9H_8O_3$	164.0471	187.0362	185.0391	$[M+Na]^+$	2-Hydroxycinnamic acid	-1.46	96.4
114	26.866	$C_{20}H_{32}O$	288.2464	306.2802	284.2993	$[M+NH_4]^+$	Erinacol	3.88	94.06
115	19.41	$C_{15}H_{20}O_3$	248.1414	249.1487	288.2934	$[M+H]^+$	Atractylenolide III	0.63	86.33
116	3.268	$C_{10}H_8O_4$	192.0418	193.0493	116.9784	$[M+H]^+$	Erinaceolactone A	-2.14	83.09
117	18.98	$C_{14}H_{18}O_3$	234.1258	235.1332	274.2785	$[M+H]^+$	Erinachromane B	0.84	82.33
118	22.367	$C_{22}H_{32}O_4$	360.2311	383.2203	501.2878	$[M+H]^+$	Erinacine I	2.89	80.99
119	21.524	$C_{20}H_{32}O_3$	320.2377	321.2438	457.2609	$[M+H]^+$	(3 s)-3-Methyl-5- [(1 s,8ar)-2,5,5,8atetramethyl-4-oxo1,4,4a,5,6,7,8,8aoctahydro-1- naphthalenyl] pentanoic acid	8.12	79.34

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
120	21.092	$C_{18}H_{28}O_3$	292.205	293.2125	287.1679	$[M + H]^+$	9 s,13r-12- Oxophytodienoic acid	3.92	79.3
121	22.041	$C_{14}H_{26}O_4$	258.1849	281.1741	149.0261	$[M + Na]^+$	Tetradecanedioic acid	6.82	75.97
122	20.108	$C_{19}H_{24}O_5$	332.1643	333.1715	311.1893	$[M + H]^+$	Erinacerin B	5.66	74.37
123	15.002	$C_{18}H_{23}NO_6$	349.156	367.1898	453.3484	$[M + NH_4]^+$	Erinacerin I	9.85	71.22
124	12.626	$C_{28}H_{33}NO_5$	463.2329	481.2665	476.3118	$[M + NH_4]^+$	Corallocin E	-6.51	69.5
125	0.929	$C_6H_6O_6$	174.0152	197.0082	116.9783	$[M + H]^+$	Trans-aconitic acid	-7.07	69.42
126	22.16	$C_{18}H_{35}NO_2$	297.2682	320.2595	149.0262	$[M + H]^+$	2-Aminooctadec-4-yne- 1,3-diol	4.69	68.71
127	16.247	$C_{17}H_{21}NO_5$	319.1401	337.1742	652.4178	$[M + H]^+$	Erinacerin R	-5.8	67.25
128	20.424	$C_{11}H_{15}N_5O_3S$	297.0909	315.1245	189.0937	$[M + NH_4]^+$	5′-s-Methyl-5′-thioadenosine	4.62	66.77
129	23.962	$C_{27}H_{33}NO_3$	419.2501	442.2398	217.1076	$[M + H]^+$	Isohericerin	9.69	66.08
130	17.055	$C_{22}H_{26}N_2O_6$	414.1821	415.1887	435.1811	$[M + H]^+$	Erinacerin P	7.26	65.86
131	20.221	$C_{18}H_{37}NO_2$	299.2838	300.2914	316.3249	$[M + H]^+$	Palmitoyl ethanolamide	4.62	63.68
132	18.951	$C_{15}H_{18}O_4$	262.1207	263.1289	274.279	$[M + H]^+$	spirobenzofuran	0.84	62.61
133	27.587	$C_{19}H_{21}NO_7$	375.1314	393.1666	310.3144	$[M + NH_4]^+$	caputmedusins J	-1.02	59.89
134	17.167	$C_{11}H_{12}N_2O_2$	204.0908	227.0826	435.181	$[M + Na]^+$	L-Tryptophan	4.3	58.44
135	10.894	$C_7H_6O_5$	170.0216	171.029	116.9783	$[M + H]^+$	Gallic acid	0.54	57.93
136	20.198	$C_{16}H_{19}NO_5$	305.1253	323.158	415.2166	$[M + NH_4]^+$	Erinacerin G	-3.36	57.32
137	18.858	$C_{19}H_{21}NO_7$	375.1345	393.167	274.2785	$[M + NH_4]^+$	caputmedusins J	7.13	57.12

Table 5.1 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
120	21.092	$C_{18}H_{28}O_3$	292.205	293.2125	287.1679	$[M + H]^+$	9 s,13r-12- Oxophytodienoic acid	3.92	79.3
138	17.599	$C_{15}H_{10}O_8$	318.039	319.0438	401.2693	$[M + H]^+$	Avenacein Y	4.36	56.21
139	26.063	$C_{37}H_{60}O_5$	584.4408	603.4904	675.43	$[M + NH_4]^+$	Hericene C	-5.59	56.08
140	20.108	$C_{21}H_{28}NO_5$	374.1955	375.2071	149.0258	$[M + H]^+$	Erinaceolactam D	-3.35	54.56
141	21.729	$C_{29}H_{34}N_2O_3$	458.2601	459.2676	457.2617	$[M + H]^+$	Corallocin C	6.95	53.75
142	20.053	$C_{23}H_{31}N_2O_5$	415.222	433.2622	316.3248	$[M + NH_4]^+$	Erinaceolactam E	-3.19	52.51
143	25.874	$C_{43}H_{81}NO_9$	755.5869	773.6282	691.518	$[M + H]^+$	Cerebroside D	-5.59	52.28
144	21.853	$C_{46}H_{76}O_5$	708.5725	733.5735	317.2127	$[M + Na]^+$	Erinarol F	4.55	52.08
145	19.784	$C_{17}H_{27}N_3O_{17}P_2$	607.0838	608.0844	260.1678	$[M + H]^+$	Udp-n-acetylglucosamine	3.66	50.08

Table 5.2 Characterization of Compounds in *Hericium erinaceus* strain MFLUCC 21-0019 by Using LC-QTOF

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
1	20.037	$C_{25}H_{36}O_6$	432.251	477.2494	293.1798	$[M-H]^-$	Erinacine A	-0.52	99.74
2	21.606	$C_{14}H_{28}O_3$	244.2036	243.1963	805.9864	$[M-H]^-$	(r)-3-Hydroxy myristic acid	-0.93	99.65
3	24.28	$C_{18}H_{32}O_2$	280.2404	279.2331	805.9867	$[M-H]^-$	9(z),11(e)-Conjugated linoleic acid	0.53	99.52
4	19.737	$C_{12}H_{14}O_2$	190.0995	189.0922	325.1851	$[M-H]^-$	eulatachromene	0.63	99.49
5	22.05	$C_{27}H_{33}NO_3$	419.2464	418.2392	805.9867	$[M-H]^-$	Isohericerin	0.85	99.46
6	22.152	$C_{16}H_{32}O_3$	272.2349	271.2277	805.9862	$[M-H]^-$	16-Hydroxyhexadecanoic acid	-0.96	99.33
7	19.513	$C_{10}H_{20}O_3$	188.1415	187.1343	325.1853	$[M-H]^-$	10-Hydroxydecanoic acid	1.2	99.22
8	20.882	$C_{25}H_{38}O_6$	434.2675	479.2657	311.1697	$[M-H]^-$	Erinacine C	1.5	98.96
9	12.716	$C_8H_{15}NO_3$	173.1048	172.0975	966.0029	$[M-H]^-$	N-Acetyl-l-leucine	-2.16	98.92
10	2.521	$C_9H_{12}N_2O_6$	244.07	243.0627	128.0356	$[M-H]^-$	Uridine	1.87	98.88
11	26.119	$C_{18}H_{34}O_2$	282.2562	281.2489	805.9868	$[M-H]^-$	Ethyl palmitoleate	1.03	98.86
12	16.898	$C_{14}H_{17}NO_5$	279.1108	278.1035	130.9662	$[M-H]^-$	hericioic acid A	0.62	98.84
13	19.485	$C_{11}H_{14}O_3$	194.0946	193.0874	325.1853	$[M-H]^-$	4-Ethoxy ethylbenzoate	1.78	98.79
14	21.516	$C_{28}H_{33}NO_5$	463.2358	462.2285	295.2284	$[M-H]^-$	Corallocin E	-0.1	98.69
15	18.462	$C_{18}H_{34}O_5$	330.2412	329.2339	329.2339	$[M-H]^-$	(15z)-9,12,13-Trihydroxy- 15-octadecenoic acid	1.89	98.61

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
16	18.92	$C_{24}H_{31}NO_7$	445.2109	444.2033	325.1847	$[M-H]^-$	Erinacerin W	1.86	98.56
17	2.471	$C_5H_7NO_3$	129.043	128.0356	290.0885	$[M-H]^-$	4-Oxoproline	2.85	98.51
18	27.417	$C_{18}H_{30}O_3S$	326.1918	325.1845	805.9872	$[M-H]^-$	4-Dodecylbenzenesulfonic acid	0.75	98.4
19	2.667	$C_5H_5N_5$	135.0549	134.0476	134.0476	$[M-H]^-$	Adenine	2.88	98.35
20	23.181	$C_{12}H_{26}O_4S$	266.1555	265.1483	805.9871	$[M-H]^-$	Dodecyl sulfate	1.33	98.29
21	18.976	$C_{14}H_{16}O_3$	232.1103	277.1085	325.1848	$[M+HCOOL]^-$	coralcuparene	1.74	98.27
22	18.976	$C_{15}H_{18}O_5$	278.1157	277.1085	325.1848	$[M-H]^-$	hericioic acid E	1.01	98.27
23	19.38	$C_{12}H_{16}O_3$	208.1102	207.103	229.0876	$[M-H]^-$	B-Asarone	1.32	97.73
24	1.712	$C_6H_{14}O_6$	182.0792	181.072	341.1097	$[M-H]^-$	Galactitol	0.7	97.5
25	18.976	$C_{14}H_{18}O_3$	234.1252	233.1184	325.1848	$[M-H]^-$	Erinachromane B	-1.62	97.27
26	16.795	$C_{19}H_{28}N_2O_6$	380.1953	379.1879	949.6727	$[M-H]^-$	Erinacerin N	1.6	97.08
27	13.946	$C_{12}H_{12}N_2O_2$	216.0904	275.1042	130.9663	$[M+HCOOL]^-$	2,3,4,9-Tetrahydro-1h-β-carboline-3-carboxylic acid	2.35	96.99
28	18.92	$C_{25}H_{38}O_7$	450.2624	495.2603	325.1848	$[M+HCOOL]^-$	Erinacine T	1.33	96.96
29	15.336	$C_{11}H_{13}NO_3$	207.0896	206.0823	130.9661	$[M-H]^-$	N-Acetyl-L-phenylalanine 1-(Carboxymethyl)	0.49	96.64
30	18.073	$C_{25}H_{38}O_8$	466.2564	511.2551	805.9887	$[M+HCOOL]^-$	Erinacine J	-0.5	96.46
31	2.732	$C_9H_{11}NO_3$	181.0737	180.0663	243.062	$[M-H]^-$	L-Tyrosine	-1.12	96.26

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
32	19.673	$C_{22}H_{26}O_6$	386.1733	431.1714	325.1853	$[M + HCOOL]^-$	Bis(methylbenzylidene) sorbitol	0.83	96.07
33	16.898	$C_{19}H_{21}NO_7$	375.1322	420.1309	1062.7568	$[M + HCOOL]^-$	caputmedusins J	0.96	93.99
34	22.05	$C_{29}H_{34}N_2O_3$	458.2564	503.2548	805.9867	$[M + HCOOL]^-$	Corallocin C	-1.13	90.71
35	20.336	$C_{18}H_{34}O_4$	314.2463	313.2388	339.2004	$[M - H]^-$	(±)9,10-Dihydroxy-12z-octadecenoic acid	1.93	90.57
36	20.336	$C_{16}H_{30}O_2$	254.2251	313.2388	339.2004	$[M + HCOOL]^-$	Δ2-Trans-hexadecenoic acid	2.1	90.57
37	3.077	$C_{19}H_{22}O_6$	346.1411	345.1339	130.0871	$[M - H]^-$	Corallocin A	-1.66	90.49
38	23.476	$C_{30}H_{46}O_4$	470.3394	515.3379	805.9872	$[M + HCOOL]^-$	18-β-glycyrrhetinic acid	-0.52	90.36
39	17.292	$C_{25}H_{36}O_7$	448.2459	493.2452	966.0011	$[M + HCOOL]^-$	Hericinoid A	-0.36	89.72
40	23.142	$C_{37}H_{56}O_6$	596.408	655.4214	805.9873	$[M + HCOOL]^-$	Hericenone I	0.48	88.9
41	1.735	$C_5H_{12}O_5$	152.0686	151.0612	341.1096	$[M - H]^-$	Ribitol	0.61	88.41
42	1.735	$C_{12}H_{22}O_{12}$	358.112	357.1045	341.1096	$[M - H]^-$	Lactobionic acid	2.31	88.35
43	1.904	$C_4H_4O_4$	116.0106	115.0036	290.0882	$[M - H]^-$	Fumaric acid	-3.11	87.6
44	19.431	$C_{10}H_{16}$	136.1252	181.1235	325.1851	$[M + HCOOL]^-$	Limonene	0.29	87.43
45	20.037	$C_{19}H_{24}O_4$	316.1681	361.1662	293.1798	$[M + HCOOL]^-$	Hericenone J	2.01	87.08
46	17.878	$C_{10}H_{18}O_4$	202.1207	201.1134	966.0026	$[M - H]^-$	3-Tert-butyladipic acid	0.83	86.88
47	17.176	$C_9H_{16}O_4$	188.1052	187.0978	341.1152	$[M - H]^-$	Azelaic acid	1.58	86.41

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
48	19.02	$C_{14}H_{26}O_4$	258.1828	257.176	325.185	$[M-H]^-$	Tetradecanedioic acid	-1.32	86.4
49	24.28	$C_{43}H_{81}NO_9$	755.5979	814.6061	805.986	$[M+HCOOL]^-$	Cerebroside D	8.92	86.33
50	20.365	$C_{12}H_{18}O_2$	194.1306	239.1289	325.1845	$[M+HCOOL]^-$	Sedanolide	-0.66	86.12
51	18.871	$C_{12}H_{22}O_4$	230.152	229.1447	325.1848	$[M-H]^-$	Dodecanedioic acid	0.87	85.51
52	19.313	$C_{20}H_{30}O_4$	334.2152	333.2073	325.1854	$[M-H]^-$	2-Hydroxy-4,5′,8a′-trimethyl-1′-oxo-4-vinyloctahydro-1′h-spiro[cyclopentane-1,2′-naphthalene]-5′-carboxylic acid	2.29	85.21
53	17.413	$C_8H_{14}O_4$	174.0896	173.0823	966.0016	$[M-H]^-$	Suberic acid	2.32	84.39
54	19.02	$C_{12}H_{16}O_2$	192.1159	237.1128	421.2276	$[M+HCOOL]^-$	Senkyunolide A	4.39	83.69
55	20.997	$C_{15}H_{20}O_3$	248.1402	293.1388	311.1695	$[M+HCOOL]^-$	Atractylenolide III	-4.05	83.35
56	3.077	$C_{17}H_{21}NO_5$	319.1398	378.1541	130.0871	$[M+HCOOL]^-$	Erinacerin R	-6.89	83.21
57	22.118	$C_{20}H_{38}O_2$	310.2859	355.2844	805.9862	$[M+HCOOL]^-$	Ethyl oleate	-4.09	81.84
58	17.935	$C_{15}H_{18}O_6$	294.1106	293.1031	805.9883	$[M-H]^-$	Erinaceolactone G	0.83	81.28
59	17.974	$C_{14}H_{14}O_6$	278.0796	277.0724	966.003	$[M-H]^-$	Erinaceolactone H	2.12	80.9
60	19.454	$C_{10}H_{10}O_4$	194.0588	239.0571	325.1851	$[M+HCOOL]^-$	Erinaceolactone B	4.69	80.6
61	18.227	$C_{27}H_{29}NO_8$	495.1902	494.1826	966.0025	$[M-H]^-$	caputmedusins D	1.74	80.59
62	21.318	$C_{25}H_{34}O_6$	430.2349	429.2287	311.1694	$[M-H]^-$	Erinacine S	-1.41	80.22

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
63	21.637	$C_7H_{12}O_4$	160.0732	159.067	311.1689	$[M-H]^-$	3-Methyladipic acid	-2.23	79.62
64	24.28	$C_{22}H_{36}O_3$	348.2669	393.2655	805.9867	$[M+HCOOL]^-$	3-Methyl-5-(5,5,8atrimethyl-2-methylene-7- oxodecahydro-1-naphthalenyl) pentyl acetate	1.28	79.2
65	17.778	$C_{18}H_{21}NO_5$	331.1422	390.1559	966.002	$[M+HCOOL]^-$	Erinacerin C	0.64	77.76
66	24.497	$C_{35}H_{56}O_6$	572.4073	571.4013	132.9236	$[M-H]^-$	5′-hydroxyhericenes A	-0.74	77.64
67	9.751	$C_7H_{11}NO_5$	189.0623	188.055	966.0026	$[M-H]^-$	N-acetyl-dl-glutamic acid	-7.57	77.54
68	20.013	$C_{27}H_{33}NO_4$	435.2423	434.2343	293.1798	$[M-H]^-$	Corallocin B	3.01	76.08
69	2.776	$C_{26}H_{29}NO_5$	435.2022	480.1998	130.0866	$[M+HCOOL]^-$	Erinacerin Q	-5.46	74.71
70	20.555	$C_{21}H_{27}NO_5$	373.1886	372.1817	325.1852	$[M-H]^-$	Erinaceolactam B	-0.88	74.33
71	22.278	$C_{27}H_{42}O_7$	478.2924	523.2919	805.9863	$[M+HCOOL]^-$	Erinacine D	-1.41	73.68
72	22.278	$C_{26}H_{40}O_7$	464.2775	523.2919	805.9863	$[M+HCOOL]^-$	Erinacine V	0.28	73.68
73	22.278	$C_{28}H_{44}O_9$	524.2988	523.2919	805.9863	$[M-H]^-$	erinacine Q2	0.56	73.68
74	1.688	$C_{17}H_{27}N_3O_{17}P_2$	607.0829	606.0754	341.1099	$[M-H]^-$	Udp-n-acetylglucosamine	2.18	73.6
75	20.495	$C_{27}H_{40}O_8$	492.2751	491.2665	325.1849	$[M-H]^-$	Erinacine P	5.7	72.95
76	24.28	$C_{41}H_{77}NO_9$	727.56	772.5572	132.9236	$[M+HCOOL]^-$	Cerebroside B	0.24	70.47
77	21.195	$C_{25}H_{34}O_7$	446.2267	445.2217	311.1686	$[M-H]^-$	Hericinoid C	-8.43	69.68
78	17.688	$C_{28}H_{31}NO_7$	493.2092	538.2046	966.0019	$[M+HCOOL]^-$	caputmedusins C	-1.63	68.14

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
79	23.181	$C_{37}H_{54}O_6$	594.3932	653.4072	265.1483	$[M + HCOOL]^-$	Hericenone H	1.93	67.29
80	24.519	$C_{40}H_{75}NO_9$	713.5454	772.5575	805.9867	$[M + HCOOL]^-$	Cerebroside E	1.73	66.56
81	22.05	$C_{22}H_{32}O_4$	360.2284	419.2423	805.9867	$[M + HCOOL]^-$	Erinacine I	-4.74	64.58
82	18.699	$C_{15}H_{18}O_3$	246.1233	305.139	966.0024	$[M + HCOOL]^-$	Arglabin	-9.3	64.16
83	20.807	$C_{22}H_{29}NO_5$	387.2044	386.1986	311.1698	$[M - H]^-$	Corallocin D	-0.49	64.15
84	2.387	$C_{20}H_{25}NO_6$	375.1706	420.1696	128.0356	$[M + HCOOL]^-$	Erinacerin D	6.45	61.96
85	24.28	$C_{37}H_{60}O_5$	584.446	645.4696	805.9864	$[M + HCOOL]^-$	Hericene C	3.34	60.78
86	18.592	$C_{23}H_{29}NO_7$	431.193	430.1868	325.1846	$[M - H]^-$	caputmedusins F	-3.29	60.61
87	27.581	$C_{51}H_{86}O_7$	810.6372	870.6489	116.9287	$[M + HCOOL]^-$	Hericene E	-0.2	58.9
88	2.497	$C_8H_{10}O_5$	186.0524	245.0675	128.0354	$[M + HCOOL]^-$	Erinapyrone C	-2.4	58.24
89	19.797	$C_{15}H_{20}O_4$	264.1342	263.1276	325.1851	$[M - H]^-$	hydrospirobenzofuran	-7.56	56.57
90	16.707	$C_{15}H_{17}NO_5$	291.1106	336.1073	836.5882	$[M + HCOOL]^-$	hericioic acid B	-0.12	56.33
91	1.688	$C_9H_{13}N_3O_5$	243.0879	302.1014	341.1098	$[M + HCOOL]^-$	Cytarabine	9.94	56.02
92	19.485	$C_{21}H_{44}NO_7P$	453.2832	452.2766	325.1855	$[M - H]^-$	Glycerophospho-n- palmitoyl ethanolamine	-5.17	55.77
93	24.804	$C_{46}H_{76}O_4$	692.5727	691.5626	805.987	$[M - H]^-$	Erinarol A	-2.43	54.24
94	18.029	$C_{22}H_{27}NO_7$	417.1813	416.1728	261.0774	$[M - H]^-$	caputmedusins E	6.13	53.8
95	2.471	$C_{46}H_{74}O_4$	690.559	735.5652	128.0356	$[M + HCOOL]^-$	Erinarol C	0.45	50.61

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
96	27.543	$C_{53}H_{86}O_7$	834.6319	880.6488	311.1698	$[M + HCOOL]^-$	Hericene F	-6.51	50.22
97	1.753	$C_6H_{12}O_6$	180.0645	239.0773	341.1097	$[M - H]^-$	L-Sorbose	6.08	50.09
98	24.259	$C_{16}H_{33}NO$	255.2565	256.2638	282.2805	$[M + H]^+$	Hexadecanamide	1.14	99.63
99	13.101	$C_{11}H_{18}N_2O_2$	210.1368	211.1441	453.3446	$[M + H]^+$	Cyclo(leucylprolyl)	-0.07	99.62
100	24.414	$C_{10}H_{16}O$	152.1201	170.1539	282.2803	$[M + NH_4]^+$	R-ipsdienol	-0.2	99.52
101	4.668	$C_{15}H_{18}O_7$	310.105	328.1388	120.0804	$[M + NH_4]^+$	hericioic acid G	-0.86	99.49
102	3.042	$C_{10}H_{13}N_5O_5$	283.0916	284.0988	152.0567	$[M + H]^+$	Guanosine	-0.31	99.27
103	4.031	$C_{11}H_{15}N_5O_4$	281.1122	282.1195	116.9761	$[M + H]^+$	2′-o-Methyladenosine	-0.58	99.25
104	25.505	$C_{19}H_{34}O$	278.2612	296.295	217.1051	$[M + NH_4]^+$	9, 12-octadecadienoic acid methyl ester	0.98	99.12
105	3.042	$C_5H_5N_5O$	151.0494	152.0567	152.0567	$[M + H]^+$	Guanine	0.15	99.09
106	20.8	$C_{20}H_{28}O_2$	300.2085	301.2158	301.2158	$[M + H]^+$	Isotretinoin	-1.36	99.01
107	14.966	$C_{19}H_{23}NO_5$	345.1575	346.1647	116.9762	$[M + H]^+$	hericioic acid D	-0.42	98.84
108	2.649	$C_{10}H_{13}N_5O_4$	267.0971	268.1043	268.1043	$[M + H]^+$	Adenosine	1.12	98.83
109	20.518	$C_{24}H_{30}O_6$	414.2041	415.2114	147.0904	$[M + H]^+$	Bis(4-ethylbenzylidene) sorbitol	-0.25	98.7
110	22.68	$C_{28}H_{40}O$	392.3085	393.3158	425.2157	$[M + H]^+$	ergosta-4, 6, 8(14), 22-tetraen-3-one	1.56	98.62
111	20.765	$C_{21}H_{42}O_4$	358.3085	359.3156	147.0916	$[M + H]^+$	1-Stearoylglycerol	0.52	98.38

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
112	2.387	$C_{10}H_{13}N_5O_3$	251.102	274.0912	124.0383	$[M + Na]^+$	2′-Deoxyadenosine	0.67	98.35
113	21.959	$C_{24}H_{36}O_5$	404.2557	405.263	420.2552	$[M + H]^+$	Lovastatin	-1.33	98.26
114	24.754	$C_{18}H_{35}NO$	281.2726	282.2799	282.2799	$[M + H]^+$	Oleamide	2.7	97.62
115	2.565	$C_{15}H_{16}O_7$	308.0889	326.1228	268.1034	$[M + NH_4]^+$	hericioic acid F	-2.24	97.45
116	20.876	$C_9H_{10}O_3$	166.0637	167.0709	167.0709	$[M + H]^+$	Ethyl paraben	4.31	96.02
117	20.607	$C_{19}H_{25}NO_3$	315.1839	316.1908	316.1908	$[M + H]^+$	5-(2E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-hydroxy-6-methoxy-1-isoindoline (isoindolinone derivative)	1.36	95.71
118	26.462	$C_{18}H_{37}NO$	283.2884	284.2957	553.3922	$[M + H]^+$	Stearamide	3.21	94.69
119	10.388	$C_{11}H_9NO_2$	187.0632	188.0705	340.2601	$[M + H]^+$	Indole-3-acrylic acid	-0.54	94.19
120	2.734	$C_7H_{10}O_3$	142.0637	165.0529	268.1025	$[M + Na]^+$	Erinapyrone B	4.96	93.02
121	3.042	$C_4H_6O_5$	134.0228	152.0567	152.0567	$[M + NH_4]^+$	Dl-Malic acid	9.53	90.1
122	16.246	$C_6H_{12}O_3$	132.0782	133.0857	652.4128	$[M + H]^+$	2-Hydroxycaproic acid	-3.32	89
123	4.534	$C_7H_{10}O_7$	206.0436	224.0773	116.9764	$[M + NH_4]^+$	3-Hydroxy-3-(methoxycarbonyl) pentanedioic acid	4.37	88.36
124	23.656	$C_{18}H_{37}NO_2$	299.2824	300.2902	685.4368	$[M + H]^+$	Palmitoyl ethanolamide	0	87.03
125	20.8	$C_{18}H_{30}O_2$	278.2266	301.2158	301.2158	$[M + Na]^+$	Pinolenic acid	7.16	87

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
126	21.612	$C_9H_8O_3$	164.0477	165.0547	675.5179	$[M + H]^+$	2-Hydroxycinnamic acid	2.21	86.86
127	5.079	$C_{20}H_{25}NO_7$	391.1623	409.1967	120.0804	$[M + NH_4]^+$	caputmedusins I	-2.06	86.59
128	2.649	$C_4H_6O_4$	118.0275	136.0619	268.104	$[M + NH_4]^+$	Succinic acid	7.22	86.09
129	23.918	$C_{21}H_{38}O_4$	354.2751	355.2825	217.1048	$[M + H]^+$	1-Linoleoyl glycerol	-5.49	85.16
130	21.77	$C_{18}H_{30}O_3$	294.2189	295.2263	675.5182	$[M + H]^+$	9-Oxo-10(e),12(e)-octadecadienoic acid	-1.86	84.71
131	22.192	$C_{20}H_{30}O_3$	318.2202	319.2268	149.0257	$[M + H]^+$	11-α-hydroxy-17-methyltestosterone	2.08	84.35
132	20.765	$C_{20}H_{32}O_2$	304.2404	327.2299	301.2163	$[M + Na]^+$	Arachidonic acid	0.6	83.87
133	23.041	$C_{19}H_{32}O_2$	292.2407	293.2479	280.2645	$[M + H]^+$	9(z),11(e),13(e)- Octadecatrienoic acid methyl ester	1.75	83.58
134	22.262	$C_{18}H_{35}NO_2$	297.2682	298.2762	149.0259	$[M + H]^+$	2-Aminooctadec-4-yne- 1,3-diol	4.78	83.49
135	26.904	$C_{20}H_{32}O$	288.2431	306.2768	284.2958	$[M + NH_4]^+$	Erinacol	-7.84	83.48
136	25.864	$C_{25}H_{36}O_8$	464.2403	487.2299	559.5169	$[M + Na]^+$	Erinacine G	-1.51	82.77
137	20.112	$C_{13}H_{16}O_4$	236.1043	237.1117	311.1853	$[M + H]^+$	Erinachromane A	-2.44	82.21
138	22.299	$C_{18}H_{28}O_3$	292.2053	293.211	149.0254	$[M + H]^+$	9 s,13r-12- Oxophytodienoic acid	5.03	80.8
139	1.713	$C_7H_{15}NO_3$	161.1044	162.1116	118.0858	$[M + H]^+$	L(-)-Carnitine	-4.71	80.67
140	2.329	$C_6H_6O_6$	174.016	197.0053	118.0861	$[M + Na]^+$	Trans-aconitic acid	-2.79	80.65

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
141	20.112	$C_{19}H_{24}O_5$	332.1638	333.1691	311.1873	$[M + H]^+$	Erinacerin B	4.17	79.43
142	22.472	$C_{10}H_8O_4$	192.0438	193.0511	512.5072	$[M + H]^+$	Erinaceolactone A	8.14	79.38
143	1.815	$C_{12}H_{10}N_4O_2$	242.0796	260.1138	118.0859	$[M + NH_4]^+$	Lumichrome	-3.16	77.26
144	22.744	$C_{20}H_{34}O_2$	306.2553	307.262	254.2476	$[M + H]^+$	Linolenic acid ethyl ester	-2.03	77.13
145	22.359	$C_{20}H_{32}O_3$	320.2357	321.2424	149.0233	$[M + H]^+$	(3 s)-3-Methyl-5- [(1 s,8ar)-2,5,5,8atetramethyl-4-oxo1,4,4a,5,6,7,8,8aoctahydro-1-naphthalenyl] pentanoic acid	1.73	76.92
146	22.33	$C_{21}H_{32}O_4$	348.2294	349.2356	149.0255	$[M + H]^+$	Herialpin B	-1.77	76.52
147	17.056	$C_{22}H_{25}NO_8$	431.1601	454.1497	435.1753	$[M + Na]^+$	Pseurotin A	4.86	73.09
148	22.619	$C_{27}H_{42}O_8$	494.2923	495.2997	425.2148	$[M + H]^+$	Erinacine K	8.79	71.54
149	20.8	$C_{25}H_{27}NO_7$	453.1803	471.2142	301.2158	$[M + NH_4]^+$	Erinacerin K	3.4	71.52
150	1.696	$C_{19}H_{22}O_7$	362.1388	385.1279	118.085	$[M + H]^+$	Erinaceolactone D	6.33	70.67
151	2.387	$C_{16}H_{19}NO_5$	305.1281	306.1344	124.0377	$[M + H]^+$	Erinacerin G	5.72	69.02
152	2.387	$C_{15}H_{18}O_4$	262.1227	263.1301	124.0377	$[M + H]^+$	spirobenzofuran	8.27	68.43
153	23.918	$C_{20}H_{39}NO_2$	325.2988	326.3064	217.1049	$[M + H]^+$	Oleoyl ethanolamide	2.3	67.98
154	16.402	$C_{19}H_{23}NO_7$	377.1498	378.1567	679.512	$[M + H]^+$	caputmedusins G	6.19	67.95
155	20.823	$C_{11}H_{15}N_5O_3S$	297.0886	315.1222	167.0704	$[M + H]^+$	5′-s-Methyl-5′-thioadenosine	-3.22	67.22

Table 5.2 (continue)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
156	21.862	$C_{46}H_{76}O_5$	708.5716	731.564	365.1365	$[M + Na]^+$	Erinarol F	3.26	63.8
157	20.518	$C_{23}H_{31}N_2O_5$	415.2237	433.2551	147.091	$[M + NH_4]^+$	Erinaceolactam E	1.07	62.1
158	12.045	$C_{12}H_{13}ClO_5$	272.0434	290.0771	169.0758	$[M + NH_4]^+$	Erinaceolactone C	-6.3	59.41
159	21.329	$C_{18}H_{23}NO_6$	349.1556	367.1895	279.2324	$[M + NH_4]^+$	Erinacerin I	8.67	59.15
160	20.141	$C_6H_8O_7$	192.0279	211.0638	311.1856	$[M + NH_4]^+$	Citric acid	4.91	58.98
161	23.361	$C_{37}H_{60}O_6$	600.4394	623.4264	280.2646	$[M + Na]^+$	5′-hydroxyhericenes B	0.73	57.73
162	21.743	$C_{27}H_{31}NO_4$	433.2244	434.2321	457.2567	$[M + H]^+$	Hericenone B	-2.14	57.1
163	22.894	$C_5H_8O_5$	148.0363	149.044	254.2485	$[M + H]^+$	D-α-hydroxyglutaric acid	-5.84	56.87
164	21.743	$C_{25}H_{39}O_7$	451.2689	469.2973	457.2567	$[M + NH_4]^+$	Erinacine Z2	-1.49	53.91
165	2.387	$C_{24}H_{27}NO_7$	441.18	442.1913	124.0383	$[M + H]^+$	Erinacerin U	2.85	52.75
166	17.54	$C_{15}H_{14}O_6$	290.0796	308.1121	116.9758	$[M + NH_4]^+$	Hericiofuranoic Acid	1.9	51.88
167	24.156	$C_{19}H_{24}O_6$	348.1573	367.1937	256.2641	$[M + NH_4]^+$	Hericenone K	-0.06	51.04
168	19.288	$C_{16}H_{12}O_6$	300.0663	301.0739	192.1371	$[M + H]^+$	Diosmetin	9.84	50.51

Table 5.3 Characterization of Compounds in *Hericium erinaceus* strain MFLUCC 21-0020 by Using LC-QTOF

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
1	22.134	$C_{16}H_{32}O_3$	271.2278	272.2351	805.987	$[M-H]^-$	16-Hydroxyhexadecanoic acid	-0.21	99.91
2	21.553	$C_{14}H_{28}O_3$	243.1965	244.2038	445.2235	$[M-H]^-$	(r)-3-Hydroxy myristic acid	-0.32	99.86
3	21.15	$C_{25}H_{38}O_6$	479.2653	434.2669	303.1606	$[M+HCOOL]^-$	Erinacine C	0.2	99.79
4	12.677	$C_8H_{15}NO_3$	172.0981	173.1053	805.9877	$[M-H]^-$	N-Acetyl-l-leucine	0.89	99.74
5	19.081	$C_{10}H_{20}O_3$	187.1342	188.1415	421.2276	$[M-H]^-$	10-Hydroxydecanoic acid	1.27	99.63
6	18.436	$C_{18}H_{34}O_5$	329.2335	330.2408	329.2335	$[M-H]^-$	(15z)-9,12,13-Trihydroxy- 15-octadecenoic acid	0.45	99.58
7	26.007	$C_{18}H_{34}O_2$	281.2489	282.2562	381.1743	$[M-H]^-$	Ethyl palmitoleate	1.13	99.55
8	18.937	$C_{14}H_{16}O_3$	277.1085	232.1103	277.1085	$[M+HCOOL]^-$	coralcuparene	1.54	99.33
9	18.937	$C_{15}H_{18}O_5$	277.1085	278.1158	277.1085	$[M-H]^-$	hericioic acid E	1.28	99.33
10	21.967	$C_{25}H_{34}O_6$	429.2286	430.236	429.2286	$[M-H]^-$	Erinacine S	1	99.27
11	18.937	$C_{14}H_{18}O_3$	233.1188	234.126	277.1086	$[M-H]^-$	Erinachromane B	1.7	99.04
12	23.136	$C_{12}H_{26}O_4S$	265.1482	266.1555	805.9871	$[M-H]^-$	Dodecyl sulfate	1.24	98.88
13	1.935	$C_9H_{12}N_2O_6$	243.0625	244.0699	174.9562	$[M-H]^-$	Uridine	1.59	98.86
14	19.461	$C_{11}H_{14}O_3$	193.0874	194.0946	325.185	$[M-H]^-$	4-Ethoxy ethylbenzoate	1.71	98.85
15	19.708	$C_{12}H_{14}O_2$	189.0925	190.0998	491.2294	$[M-H]^-$	eulatachromene	2.03	98.82

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
16	20.322	$C_{25}H_{38}O_7$	495.2605	450.2623	431.2448	$[M + HCOOL]^-$	Erinacine T	1.11	98.57
17	20.185	$C_{25}H_{36}O_7$	447.2396	448.2468	339.2005	$[M - H]^-$	Hericinoid A	1.57	98.53
18	20.483	$C_{18}H_{34}O_4$	313.2387	314.246	313.2387	$[M - H]^-$	(±)9,10-Dihydroxy-12z-octadecenoic acid	1.05	98.31
19	20.483	$C_{16}H_{30}O_2$	313.2387	254.2249	313.2387	$[M + CH_3COOL]^-$	Δ2-Trans-hexadecenoic acid	1.27	98.31
20	19.313	$C_{18}H_{30}O_3S$	325.1849	326.1921	277.1085	$[M - H]^-$	4-Dodecylbenzenesulfonic acid	1.58	98.21
21	22.171	$C_{21}H_{38}O_4$	399.2749	354.2764	287.1658	$[M + HCOOL]^-$	1-Linoleoyl glycerol	-1.64	98.13
22	22.267	$C_{27}H_{42}O_7$	523.292	478.2938	805.9872	$[M + HCOOL]^-$	Erinacine D	1.48	98.08
23	22.267	$C_{26}H_{40}O_7$	523.292	464.278	805.9872	$[M + CH_3COOL]^-$	Erinacine V	1.36	98.08
24	22.267	$C_{28}H_{44}O_9$	523.292	524.2993	805.9872	$[M - H]^-$	erinacine Q2	1.55	98.08
25	16.55	$C_{13}H_{15}NO_4$	248.0932	249.1005	966.0018	$[M - H]^-$	Erinacerin M	1.5	97.97
26	24.66	$C_{37}H_{58}O_5$	581.421	582.428	805.987	$[M - H]^-$	Hericene B	-0.71	97.89
27	21.198	$C_{25}H_{34}O_7$	445.2233	446.2305	311.1694	$[M - H]^-$	Hericinoid C	0.21	97.83
28	18.914	$C_{24}H_{31}NO_7$	444.2027	445.2109	277.1084	$[M - H]^-$	Erinacerin W	1.82	97.71
29	19.375	$C_{12}H_{16}O_3$	207.1029	208.1101	229.0875	$[M - H]^-$	B-Asarone	0.67	97.57

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
30	17.698	$C_{15}H_{18}O_6$	293.1032	294.1106	445.2564	$[M-H]^-$	Erinaceolactone G	0.77	96.86
31	23.435	$C_{35}H_{56}O_5$	555.4051	556.4145	805.9868	$[M-H]^-$	Hericene A	3.12	96.34
32	16.75	$C_{19}H_{28}N_2O_6$	379.1876	380.1946	723.5044	$[M-H]^-$	Erinacerin N	-0.27	96.28
33	19.252	$C_{26}H_{29}NO_5$	434.1978	435.204	325.1851	$[M-H]^-$	Erinacerin Q	-1.42	95.68
34	25.84	$C_{37}H_{58}O_6$	597.4147	598.4237	355.1587	$[M-H]^-$	5′-hydroxyhericenes C	0.65	95.22
35	24.202	$C_{43}H_{81}NO_9$	814.6042	755.5902	379.1584	$[M+CH_3COOL]^-$	Cerebroside D	-1.24	94.78
36	17.881	$C_{18}H_{21}NO_5$	330.1355	331.1428	805.9883	$[M-H]^-$	Erinacerin C	2.36	94.64
37	17.15	$C_9H_{16}O_4$	187.0979	188.1053	341.1152	$[M-H]^-$	Azelaic acid	2.53	94.53
38	16.75	$C_{10}H_{18}O_4$	261.1346	202.1203	723.5048	$[M+CH_3COOL]^-$	3-Tert-butyladipic acid	-0.82	93.64
39	17.109	$C_{19}H_{23}NO_7$	376.1404	377.1488	341.1151	$[M-H]^-$	caputmedusins G	3.59	92.43
40	20.611	$C_{19}H_{25}NO_3$	314.1763	315.1839	325.1851	$[M-H]^-$	5-(2E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-hydroxy-6-methoxy-1-isoindoline (isoindolinone derivative)	1.52	92.36
41	22.504	$C_{20}H_{28}O_2$	299.2016	300.2091	805.9871	$[M-H]^-$	Isotretinoin	0.41	92.1
42	16.708	$C_{15}H_{17}NO_5$	290.1035	291.1105	723.5044	$[M-H]^-$	hericioic acid B	-0.76	90.7
43	23.136	$C_{37}H_{56}O_6$	655.4233	596.4096	805.9884	$[M+CH_3COOL]^-$	Hericenone I	3.2	88.95

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
44	18.573	$C_{23}H_{29}NO_7$	430.1866	431.1946	966.0011	$[M-H]^-$	caputmedusins F	0.45	88.73
45	21.483	$C_{20}H_{30}O_3$	363.2159	318.2175	445.224	$[M+HCOOL]^-$	11-α-hydroxy-17-methyltestosterone	-6.28	87.71
46	20.611	$C_{11}H_{13}NO_3$	266.1037	207.0904	311.1697	$[M+CH_3COOL]^-$	N-Acetyl-L-phenylalanine 1-(Carboxymethyl)	3.99	87.48
47	18.103	$C_{19}H_{23}NO_5$	404.1721	345.1582	966.0023	$[M+CH_3COOL]^-$	hericioic acid D	1.78	87.28
48	20.037	$C_{27}H_{42}O_8$	539.2865	494.2871	477.2502	$[M+HCOOL]^-$	Erinacine K	-1.82	86.99
49	20.361	$C_{12}H_{18}O_2$	239.129	194.1306	431.2448	$[M+HCOOL]^-$	Sedanolide	-0.63	86.66
50	18.874	$C_{12}H_{22}O_4$	229.1445	230.152	277.1084	$[M-H]^-$	Dodecanedioic acid	0.66	86.62
51	19.84	$C_{14}H_{26}O_4$	257.176	258.1832	325.1852	$[M-H]^-$	Tetradecanedioic acid	0.41	86.18
52	20.055	$C_{15}H_{18}O_3$	291.1238	246.1255	477.2499	$[M+HCOOL]^-$	Arglabin	-0.36	85.93
53	12.834	$C_6H_{14}O_6$	181.0713	182.0786	966.0022	$[M-H]^-$	Galactitol	-2.21	85.19
54	20.769	$C_{20}H_{32}O_3$	319.2283	320.2354	477.2506	$[M-H]^-$	(3 s)-3-Methyl-5- [(1 s,8ar)-2,5,5,8atetramethyl-4-oxo1,4,4a,5,6,7,8,8aoctahydro-1- naphthalenyl] pentanoic acid	0.74	84.54

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
55	14.827	$C_8H_{14}O_4$	173.0821	174.0892	497.3356	$[M-H]^-$	Suberic acid	0.22	84.51
56	19.313	$C_{20}H_{30}O_4$	333.207	334.2142	325.1851	$[M-H]^-$	2-Hydroxy-4,5′,8a′-trimethyl-1′-oxo-4-vinyloctahydro-1′h-spiro[cyclopentane-1,2′-naphthalene]-5′-carboxylic acid	-0.53	83.63
57	17.824	$C_{20}H_{25}NO_6$	374.1608	375.168	805.9872	$[M-H]^-$	Erinacerin D	-0.42	83.5
58	21.483	$C_{28}H_{33}NO_5$	462.2288	463.2361	445.224	$[M-H]^-$	Corallocin E	0.45	83.25
59	20.541	$C_{19}H_{24}O_4$	361.1663	316.1689	477.2505	$[M+HCOOL]^-$	Hericenone J	4.55	83.14
60	22.106	$C_{20}H_{38}O_2$	355.2857	310.2878	287.1663	$[M+HCOOL]^-$	Ethyl oleate	2.02	83.05
61	20.982	$C_{15}H_{20}O_3$	293.1402	248.1415	477.2504	$[M+HCOOL]^-$	Atractylenolide III	0.95	82.19
62	21.005	$C_{22}H_{32}O_4$	359.2232	360.2323	477.2501	$[M-H]^-$	Erinacine I	6.12	82.13
63	11.663	$C_6H_{12}O_3$	131.0713	132.0787	966.0021	$[M-H]^-$	2-Hydroxycaproic acid	0.41	80.96
64	17.729	$C_{26}H_{38}O_6$	445.2568	446.2639	445.2568	$[M-H]^-$	(-)-Erinacin A-d3	-6.63	80.67
65	26.388	$C_{19}H_{22}O_5$	389.1617	330.1476	805.9877	$[M+CH_3COOL]^-$	Hericenone A	2.53	79.88
66	18.739	$C_{18}H_{19}NO_6$	344.1135	345.121	186.1038	$[M-H]^-$	Erinacerin S	-0.76	79.17

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
67	24.182	$C_{40}H_{75}NO_9$	772.556	713.5432	379.1584	$[M + CH_3COOL]^-$	Cerebroside E	-1.31	77.66
68	5.664	$C_7H_{10}O_3$	187.0603	142.0622	174.9557	$[M + HCOOL]^-$	Erinapyrone B	-5.26	77.39
69	24.182	$C_{35}H_{56}O_6$	571.4006	572.4072	379.1582	$[M - H]^-$	5′-hydroxyhericenes A	-0.94	77.35
70	2.442	$C_5H_7NO_3$	128.0346	129.0418	966.0007	$[M - H]^-$	4-Oxoproline	-5.9	76.91
71	13.873	$C_{12}H_{12}N_2O_2$	275.1032	216.0898	965.9999	$[M + CH_3COOL]^-$	2,3,4,9-Tetrahydro-1h-β-carboline-3-carboxylic acid	-0.44	75.33
72	20.409	$C_{28}H_{40}O$	391.3012	392.3041	431.2442	$[M - H]^-$	ergosta-4, 6, 8(14), 22-tetraen-3-one	-9.65	75.15
73	17.939	$C_{14}H_{14}O_6$	277.0702	278.0774	966.0025	$[M - H]^-$	Erinaceolactone H	-5.76	74.64
74	24.182	$C_{41}H_{77}NO_9$	772.5547	727.5538	379.1584	$[M + HCOOL]^-$	Cerebroside B	-8.25	72.59
75	20.433	$C_{21}H_{32}O_4$	393.2257	348.2306	477.2501	$[M + HCOOL]^-$	Herialpin B	1.44	71.92
76	19.517	$C_{21}H_{44}NO_7P$	498.2793	453.2816	325.1851	$[M + HCOOL]^-$	Glycerophospho-n-palmitoyl ethanolamine	-8.74	71.46
77	17.042	$C_{20}H_{24}O_7$	435.1676	376.1525	341.1151	$[M + CH_3COOL]^-$	Erinaceolactone E	0.86	71.42
78	24.477	$C_{28}H_{44}O_3$	487.3423	428.3279	461.2009	$[M + CH_3COOL]^-$	ergosterol peroxide	-2.68	70.81
79	20.361	$C_{35}H_{54}O_7$	631.3868	586.3926	431.2448	$[M + HCOOL]^-$	3-Hydroxyhericenone F	9.67	68.44
80	27.295	$C_{37}H_{60}O_6$	659.4512	600.4375	311.1692	$[M + CH_3COOL]^-$	5′-hydroxyhericenes B	-2.44	68.06

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
81	23.308	$C_{30}H_{46}O_4$	515.3347	470.3376	805.9869	$[M + HCOOL]^-$	18-β-glycyrrhetinic acid	-4.38	67.98
82	18.459	$C_{15}H_{18}O_7$	309.096	310.1035	329.234	$[M - H]^-$	hericioic acid G	-5.71	67.51
83	18.937	$C_{15}H_{14}O_6$	289.0703	290.0771	277.1085	$[M - H]^-$	Hericiofuranoic Acid	-6.71	64.95
84	17.546	$C_{14}H_{17}NO_5$	324.1068	279.1095	966.002	$[M + HCOOL]^-$	hericioic acid A	-4.11	64.67
85	19.903	$C_{19}H_{24}O_6$	347.1475	348.1549	477.2502	$[M - H]^-$	Hericenone K	-6.98	63.66
86	17.075	$C_7H_{12}O_4$	159.0651	160.0731	341.115	$[M - H]^-$	3-Methyladipic acid	-3.13	63.52
87	20.817	$C_{29}H_{50}O$	459.3806	414.3873	477.2505	$[M + HCOOL]^-$	β-sitosterol	2.7	61.49
88	16.496	$C_{55}H_{90}O_7$	921.6747	862.6607	966.0018	$[M + CH_3COOL]^-$	Hericene H	-9.2	60.23
89	17.66	$C_{28}H_{31}NO_7$	538.2054	493.2087	966.0023	$[M + HCOOL]^-$	caputmedusins C	-2.82	59.41
90	24.202	$C_{35}H_{54}O_6$	569.3844	570.3908	379.1582	$[M - H]^-$	Hericenone C	-2.11	58.83
91	16.805	$C_{12}H_{22}O_{12}$	357.1095	358.1116	723.5047	$[M - H]^-$	Lactobionic acid	1.19	55.71
92	20.957	$C_{27}H_{29}NO_8$	541.1898	495.1891	477.2504	$[M + HCOOL]^-$	caputmedusins D	-0.39	52.94
93	22.106	$C_{28}H_{39}NO_4$	498.2826	453.2876	287.1655	$[M + HCOOL]^-$	Sambutoxin	-0.76	51.59
94	24.451	$C_{46}H_{76}O_4$	691.5665	692.5683	132.9236	$[M - H]^-$	Erinarol A	-8.73	50.19
95	20.1	$C_{17}H_{21}NO_5$	379.1558	319.141	477.25	$[M + CH_3COOL]^-$	Erinacerin R	-3.13	50.03
96	24.297	$C_{16}H_{33}NO$	255.2564	256.2637	282.2804	$[M + H]^+$	Hexadecanamide	0.64	99.86

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
96	24.297	$C_{16}H_{33}NO$	255.2564	256.2637	282.2804	$[M + H]^+$	Hexadecanamide	0.64	99.86
97	25.511	$C_{19}H_{34}O$	278.2607	296.2945	217.1045	$[M + NH_4]^+$	9, 12-octadecadienoic acid methyl ester	-0.99	99.76
98	21.035	$C_{18}H_{32}O_2$	280.2401	281.2473	339.2516	$[M + H]^+$	9(z),11(e)-Conjugated linoleic acid	-0.55	99.75
99	18.436	$C_{25}H_{38}O_8$	466.2566	467.264	225.1015	$[M + H]^+$	Erinacine J	-0.07	99.71
100	22.733	$C_{20}H_{34}O_2$	306.2559	324.2898	324.2898	$[M + NH_4]^+$	Linolenic acid ethyl ester	0	99.68
101	26.906	$C_{18}H_{37}NO$	283.2878	284.2951	310.3116	$[M + H]^+$	Stearamide	0.99	99.49
102	4.063	$C_{11}H_{15}N_5O_4$	281.1122	282.1196	116.9761	$[M + H]^+$	2′-o-Methyladenosine	-0.7	99.39
103	25.297	$C_{18}H_{35}NO$	281.2721	282.2793	338.3422	$[M + H]^+$	Oleamide	0.66	99.38
104	20.814	$C_{25}H_{36}O_6$	432.2515	455.2405	301.2158	$[M + Na]^+$	Erinacine A	0.62	99.27
105	23.306	$C_{10}H_{16}O$	152.1205	170.1543	280.2647	$[M + NH_4]^+$	R-ipsdienol	2.29	99.15
106	20.347	$C_{18}H_{30}O_2$	278.2249	279.232	415.2124	$[M + H]^+$	Pinolenic acid	1.09	99.07
107	2.671	$C_{10}H_{13}N_5O_4$	267.0971	268.1044	268.1044	$[M + H]^+$	Adenosine	1.44	98.81
108	23.591	$C_{22}H_{36}O_3$	348.2663	366.3001	280.2637	$[M + NH_4]^+$	3-Methyl-5-(5,5,8atrimethyl-2-methylene-7- oxodecahydro-1-naphthalenyl) pentyl acetate	-0.55	98.75
109	21.467	$C_{13}H_{16}O_4$	236.1046	237.1118	279.2317	$[M + H]^+$	Erinachromane A	-0.93	98.63

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
110	23.945	$C_{20}H_{39}NO_2$	325.298	326.3053	217.1046	$[M + H]^+$	Oleoyl ethanolamide	-0.09	98.62
111	12.095	$C_{11}H_{18}N_2O_2$	210.1365	211.1437	116.9761	$[M + H]^+$	Cyclo(leucylprolyl)	-1.71	98.44
112	2.671	$C_5H_5N_5$	135.0545	136.0618	268.1044	$[M + H]^+$	Adenine	0.37	98.39
113	20.882	$C_{21}H_{42}O_4$	358.308	359.3155	301.2172	$[M + H]^+$	1-Stearoylglycerol	-0.74	98.31
114	18.793	$C_{24}H_{36}O_5$	404.2556	422.2896	163.0382	$[M + NH_4]^+$	Lovastatin	-1.6	98.18
115	23.99	$C_{27}H_{33}NO_3$	419.246	442.2352	217.1046	$[M + Na]^+$	Isohericerin	-0.08	98.12
116	20.882	$C_9H_{10}O_3$	166.0635	167.0707	167.0707	$[M + H]^+$	Ethyl paraben	2.93	98.05
117	20.31	$C_{24}H_{30}O_6$	414.2054	437.1943	415.2128	$[M + H]^+$	Bis(4-ethylbenzylidene) sorbitol	2.73	98.02
118	16.719	$C_{12}H_{16}O_2$	192.1148	210.1487	679.5128	$[M + H]^+$	Senkyunolide A	-1.05	97.69
119	18.95	$C_9H_8O_3$	164.0479	165.0552	165.0552	$[M + H]^+$	2-Hydroxycinnamic acid	3.6	97.68
120	18.436	$C_{18}H_{30}O_3$	294.2184	295.2259	225.1015	$[M + H]^+$	9-Oxo-10(e),12(e)-octadecadienoic acid	-3.6	97.23
121	22.023	$C_{27}H_{33}NO_3$	419.2474	420.2545	420.2545	$[M + H]^+$	Isohericerin	3.13	96.06
122	17.514	$C_{25}H_{36}O_8$	464.2412	465.2484	296.1493	$[M + H]^+$	Erinacine G	0.29	95.92
123	23.691	$C_{18}H_{37}NO_2$	299.2818	300.2894	280.2633	$[M + H]^+$	Palmitoyl ethanolamide	-2.12	93.25
124	22.247	$C_{18}H_{35}NO_2$	297.2644	298.2753	501.2857	$[M + H]^+$	2-Aminooctadec-4-yne- 1,3-diol	-7.89	91.95

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
125	2.671	$C_4H_6O_4$	118.0277	136.0616	116.9763	$[M + NH_4]^+$	Succinic acid	9.59	91.65
126	18.057	$C_{22}H_{27}NO_7$	417.1766	418.1852	543.3172	$[M + H]^+$	caputmedusins E	-5.25	91.33
127	19.671	$C_{22}H_{26}O_6$	386.1752	387.1823	387.1823	$[M + H]^+$	Bis(methylbenzylidene) sorbitol	5.78	88.82
128	20.065	$C_{10}H_{16}$	136.1258	137.1325	316.3216	$[M + H]^+$	Limonene	4.35	87.84
129	21.639	$C_{18}H_{30}O_2$	278.2241	279.2318	457.2565	$[M + H]^+$	Pinolenic acid	-1.81	85.38
130	1.31	$C_6H_6O_6$	174.0161	197.0057	116.9766	$[M + Na]^+$	Trans-aconitic acid	-1.96	84.75
131	26.906	$C_{20}H_{32}O$	288.2432	306.2769	284.2958	$[M + NH_4]^+$	Erinacol	-7.42	84.67
132	23.013	$C_{19}H_{32}O_2$	292.2402	293.2473	254.248	$[M + H]^+$	9(z),11(e),13(e)- Octadecatrienoic acid methyl ester	-0.07	84.63
133	20.113	$C_{19}H_{24}O_5$	332.1621	333.1694	299.2023	$[M + H]^+$	Erinacerin B	-0.79	83.93
134	23.097	$C_{20}H_{32}O_2$	304.2412	305.2474	280.2647	$[M + H]^+$	Arachidonic acid	3.22	83.33
135	20.957	$C_{10}H_{10}O_4$	194.0581	195.0652	397.2375	$[M + H]^+$	Erinaceolactone B	0.77	83.22
136	20.985	$C_{27}H_{40}O_8$	492.2729	515.2624	339.2517	$[M + Na]^+$	Erinacine P	1.21	82.7
137	21.148	$C_{15}H_{18}O_4$	262.1184	263.1285	199.1697	$[M + H]^+$	spirobenzofuran	-7.86	82.23
138	9.393	$C_8H_{10}O_4$	170.0572	171.0643	116.9761	$[M + H]^+$	Herierin IV	-4.37	81.72
139	21.306	$C_{18}H_{28}O_3$	292.2054	293.2102	200.2007	$[M + H]^+$	9 s,13r-12- Oxophytodienoic acid	5.39	81.67

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
140	17.861	$C_{15}H_{16}O_7$	308.0895	309.0969	185.0358	$[M + H]^+$	hericioic acid F	-0.22	79.06
141	21.874	$C_{25}H_{36}O_6$	432.2537	455.2416	317.2099	$[M + Na]^+$	Erinacine A	5.73	78.99
142	2.377	$C_5H_5N_5O$	151.0507	152.0582	202.1811	$[M + H]^+$	Guanine	8.57	77.85
143	18.556	$C_{15}H_{20}O_4$	264.137	287.127	260.1064	$[M + Na]^+$	hydrospirobenzofuran	3.15	77.23
144	22.449	$C_{10}H_8O_4$	192.042	193.0495	512.505	$[M + H]^+$	Erinaceolactone A	-1.48	74.37
145	17.034	$C_{12}H_{10}N_4O_2$	242.0794	265.0682	435.1765	$[M + H]^+$	Lumichrome	-3.88	74.27
146	20.411	$C_7H_6O_5$	170.0228	171.0293	189.0903	$[M + H]^+$	Gallic acid	7.56	74.1
147	20.814	$C_{25}H_{27}NO_7$	453.1807	471.2145	301.2164	$[M + NH_4]^+$	Erinacerin K	4.35	73.18
148	21.724	$C_{29}H_{34}N_2O_3$	458.2562	459.2635	457.2575	$[M + H]^+$	Corallocin C	-1.68	71.27
149	17.342	$C_{27}H_{38}O_9$	506.249	507.2554	116.9733	$[M + H]^+$	Erinacine R	-5.03	70.92
150	18.591	$C_{21}H_{27}NO_5$	373.1915	396.1799	225.1011	$[M + Na]^+$	Erinaceolactam B	6.79	70.72
151	23.908	$C_{28}H_{44}O$	396.3431	397.3449	256.2641	$[M + H]^+$	ergosterol	9.68	68.29
152	20.43	$C_{25}H_{39}O_7$	451.2684	475.2613	189.0905	$[M + Na]^+$	Erinacine Z2	-2.58	68.2
153	20.045	$C_{23}H_{31}N_2O_5$	415.225	433.2598	299.2024	$[M + NH_4]^+$	Erinaceolactam E	4.13	67.85
154	17.059	$C_{22}H_{25}NO_8$	431.1612	454.1507	435.1764	$[M + Na]^+$	Pseurotin A	7.35	65.94
155	21.639	$C_{27}H_{33}NO_4$	435.2408	453.2745	279.232	$[M + H]^+$	Corallocin B	-0.42	64.71

Table 5.3 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
156	2.849	$C_5H_{12}O_5$	152.0671	175.0566	116.9763	$[M + Na]^+$	Ribitol	-8.88	64.4
157	22.559	$C_{22}H_{29}NO_5$	387.2069	405.2411	425.2158	$[M + NH_4]^+$	Corallocin D	5.96	63.42
158	20.882	$C_{11}H_{15}N_5O_3S$	297.0874	315.1211	301.2164	$[M + NH_4]^+$	5′-s-Methyl-5′-thioadenosine	-7.29	61.44
159	22.023	$C_{24}H_{27}NO_7$	441.179	459.2142	420.2545	$[M + NH_4]^+$	Erinacerin U	0.49	60.97
160	26.047	$C_{18}H_{23}NO_6$	349.1536	367.187	553.3916	$[M + NH_4]^+$	Erinacerin I	3.05	60.54
161	20.142	$C_6H_8O_7$	192.0281	211.0641	316.3209	$[M + NH_4]^+$	Citric acid	5.67	59.36
162	25.137	$C_{22}H_{26}N_2O_6$	414.1812	415.1893	338.3421	$[M + H]^+$	Erinacerin P	5.17	58.45
163	20.411	$C_8H_{14}O_7$	222.0724	245.0624	189.0903	$[M + Na]^+$	Ethylβ-d-glucuronide	-6.99	57.52
164	21.827	$C_{46}H_{76}O_5$	708.5714	731.5588	381.262	$[M + Na]^+$	Erinarol F	2.95	57.08
165	25.854	$C_{37}H_{60}O_5$	584.4454	587.4643	691.5113	$[M + H]^+$	Hericene C	2.24	56.06
166	26.047	$C_{16}H_{19}NO_5$	305.1258	323.1599	553.3919	$[M + NH_4]^+$	Erinacerin G	-1.83	53.55
167	27.456	$C_7H_{10}O_7$	206.0442	229.0336	310.3109	$[M + Na]^+$	3-Hydroxy-3-(methoxycarbonyl) pentanedioic acid	7.27	51.57
168	16.62	$C_{10}H_{13}N_5O_5$	283.093	301.126	317.15	$[M + NH_4]^+$	Guanosine	4.88	51.13
169	19.539	$C_{17}H_{27}N_3O_{17}P_2$	607.0774	625.1159	288.2889	$[M + NH_4]^+$	Udp-n-acetylglucosamine	-6.82	51.05

Table 5.4 Characterization of Compounds in *Hericium erinaceus* strain MFLUCC 21-0021 by Using LC-QTOF

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
1	19.488	$C_{10}H_{20}O_3$	188.1413	187.134	325.1851	$[M-H]^-$	10-Hydroxydecanoic acid	0.15	99.81
2	11.485	$C_{10}H_{10}O_4$	194.0579	193.0507	193.0507	$[M-H]^-$	Erinaceolactone B	0.18	99.75
3	22.321	$C_{14}H_{28}O_3$	244.2037	243.1964	243.1964	$[M-H]^-$	(r)-3-Hydroxy myristic acid	-0.73	99.74
4	10.464	$C_6H_6O_3$	126.0317	125.0244	130.9662	$[M-H]^-$	Pyrogallol	0.26	99.73
5	23.117	$C_{19}H_{24}O_6$	348.1575	347.1503	265.1482	$[M-H]^-$	Hericenone K	0.48	99.71
6	22.38	$C_{19}H_{24}O_5$	332.1622	331.155	331.155	$[M-H]^-$	Erinacerin B	-0.51	99.59
7	24.292	$C_{18}H_{32}O_2$	280.2405	279.2332	379.1586	$[M-H]^-$	9(z),11(e)-Conjugated linoleic acid	1.02	99.58
8	21.513	$C_{28}H_{33}NO_5$	463.2363	462.2292	311.1695	$[M-H]^-$	Corallocin E	0.94	99.5
9	20.539	$C_{21}H_{27}NO_5$	373.1897	372.1819	325.1849	$[M-H]^-$	Erinaceolactam B	2.01	99.47
10	1.82	$C_6H_{14}O_6$	182.0789	181.0717	126.9047	$[M-H]^-$	Galactitol	-0.77	99.37
11	20.617	$C_{19}H_{25}NO_3$	315.1839	314.1766	314.1766	$[M-H]^-$	5-(2E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-hydroxy-6-methoxy-1-isoindoline (isoindolinone derivative)	1.38	99.23
12	18.43	$C_{18}H_{34}O_5$	330.2409	329.2336	275.0566	$[M-H]^-$	(15z)-9,12,13-Trihydroxy- 15-octadecenoic acid	0.92	99.19
13	19.74	$C_{12}H_{14}O_2$	190.0996	189.0923	325.1851	$[M-H]^-$	eulatachromene	1.18	99.1

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
14	19.378	$C_{12}H_{16}O_3$	208.1099	207.1027	229.0875	$[M-H]^-$	B-Asarone	-0.15	98.97
15	18.946	$C_{14}H_{18}O_3$	234.126	233.1187	277.1088	$[M-H]^-$	Erinachromane B	1.58	98.96
16	26.141	$C_{18}H_{34}O_2$	282.2563	281.2491	631.3289	$[M-H]^-$	Ethyl palmitoleate	1.51	98.95
17	18.266	$C_{15}H_{18}O_6$	294.1108	293.1035	355.1405	$[M-H]^-$	Erinaceolactone G	1.55	98.92
18	22.149	$C_{16}H_{32}O_3$	272.2347	271.2274	805.9862	$[M-H]^-$	16-Hydroxyhexadecanoic acid	-1.78	98.92
19	16.791	$C_{15}H_{16}O_7$	308.0904	307.0829	723.5044	$[M-H]^-$	hericioic acid F	2.54	98.7
20	25.749	$C_{30}H_{46}O_4$	470.3391	529.3538	805.987	$[M+CH_3COOL]^-$	18-β-glycyrrhetinic acid	-0.99	98.69
21	20.12	$C_9H_{16}O_4$	188.1052	187.0979	293.1802	$[M-H]^-$	Azelaic acid	1.83	98.62
22	23.218	$C_{12}H_{26}O_4S$	266.1554	265.1482	265.1482	$[M-H]^-$	Dodecyl sulfate	0.99	98.61
23	2.366	$C_4H_6O_5$	134.0211	133.0138	133.0138	$[M-H]^-$	Dl-Malic acid	-3.18	98.54
24	19.488	$C_{11}H_{14}O_3$	194.0947	193.0875	325.1853	$[M-H]^-$	4-Ethoxy ethylbenzoate	1.94	98.54
25	16.768	$C_{19}H_{28}N_2O_6$	380.1949	379.1878	723.5043	$[M-H]^-$	Erinacerin N	0.34	98.47
26	18.946	$C_{14}H_{16}O_3$	232.1106	277.1088	277.1088	$[M+HCOOL]^-$	coralcuparene	2.75	98.29
27	20.539	$C_{22}H_{29}NO_5$	387.2049	386.1975	325.185	$[M-H]^-$	Corallocin D	0.82	98.15
28	16.726	$C_{15}H_{17}NO_5$	291.1112	290.1039	723.505	$[M-H]^-$	hericioic acid B	1.66	98.11

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
29	27.693	$C_{18}H_{30}O_3S$	326.192	325.1847	311.1694	$[M-H]^-$	4-Dodecylbenzenesulfonic acid	1.41	98.11
30	20.176	$C_{23}H_{29}NO_7$	431.1946	430.1873	293.1799	$[M-H]^-$	caputmedusins F	0.49	97.91
31	17.108	$C_{18}H_{21}NO_5$	331.1428	376.1411	341.1152	$[M+HCOOL]^-$	Erinacerin C	2.37	97.87
32	5.7	$C_8H_{10}O_4$	170.0578	169.0505	130.9661	$[M-H]^-$	Herierin IV	-0.9	97.44
33	19.548	$C_{19}H_{22}O_6$	346.1425	345.1352	345.1352	$[M-H]^-$	Corallocin A	2.51	96.98
34	22.049	$C_{27}H_{33}NO_3$	419.2471	418.2397	418.2397	$[M-H]^-$	Isohericerin	2.43	96.93
35	1.856	$C_5H_{12}O_5$	152.0683	151.0609	126.9047	$[M-H]^-$	Ribitol	-1.07	96.55
36	21.751	$C_{26}H_{36}O_6$	444.2514	503.2656	315.1607	$[M+CH_3COOL]^-$	Hericinoid B	0.53	96.34
37	1.856	$C_8H_{14}O_7$	222.0737	267.0719	126.9046	$[M+HCOOL]^-$	Ethylβ-d-glucuronide	-1.04	96.04
38	20.884	$C_{25}H_{38}O_6$	434.2666	479.2649	311.1694	$[M+HCOOL]^-$	Erinacine C	-0.58	95.94
39	2.539	$C_9H_{12}N_2O_6$	244.0691	243.0618	174.9558	$[M-H]^-$	Uridine	-1.79	95.6
40	13.071	$C_9H_8O_3$	164.0474	209.0457	130.9664	$[M+HCOOL]^-$	2-Hydroxycinnamic acid	0.49	95.3
41	18.603	$C_{15}H_{18}O_4$	262.1201	261.1134	431.1721	$[M-H]^-$	spirobenzofuran	-1.73	95.19
42	4.825	$C_7H_{10}O_3$	142.0632	187.0612	966.0018	$[M+HCOOL]^-$	Erinapyrone B	1.13	94.89
43	22.049	$C_{29}H_{34}N_2O_3$	458.2582	503.2552	837.4848	$[M+HCOOL]^-$	Corallocin C	2.78	94.72
44	20.31	$C_{25}H_{38}O_7$	450.2617	495.2599	415.1768	$[M+HCOOL]^-$	Erinacine T	-0.23	93.95

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
45	10.464	$C_7H_6O_5$	170.0212	169.0143	130.9663	$[M-H]^-$	Gallic acid	-1.92	93.28
46	23.456	$C_{20}H_{32}O$	288.2428	287.2381	265.1482	$[M-H]^-$	Erinacol	-8.59	92.4
47	10.464	$C_7H_{12}O_4$	160.0735	159.066	130.9662	$[M-H]^-$	3-Methyladipic acid	-0.46	91.14
48	15.347	$C_{11}H_{13}NO_3$	207.0893	206.082	966.0005	$[M-H]^-$	N-Acetyl-L-phenylalanine 1-(Carboxymethyl)	-1.41	89.37
49	2.366	$C_4H_4O_4$	116.0108	115.0035	133.0138	$[M-H]^-$	Fumaric acid	-1.16	87.59
50	20.365	$C_{10}H_{16}$	136.1251	195.139	415.1782	$[M+CH_3COOL]^-$	Limonene	-0.47	87.49
51	25.525	$C_{21}H_{38}O_4$	354.2787	399.277	805.9876	$[M+HCOOL]^-$	1-Linoleoyl glycerol	4.77	87.45
52	18.877	$C_{12}H_{22}O_4$	230.152	229.1447	277.1087	$[M-H]^-$	Dodecanedioic acid	0.82	86.76
53	11.434	$C_6H_{12}O_3$	132.0791	131.0718	130.9663	$[M-H]^-$	2-Hydroxycaproic acid	3.1	86.73
54	14.953	$C_8H_{14}O_4$	174.0894	173.0822	497.3353	$[M-H]^-$	Suberic acid	0.98	86.65
55	18.535	$C_{12}H_{18}O_2$	194.1304	253.1444	431.1721	$[M+CH_3COOL]^-$	Sedanolide	-1.28	86.61
56	17.868	$C_{10}H_{18}O_4$	202.1209	201.1136	966.002	$[M-H]^-$	3-Tert-butyladipic acid	2.05	86.49
57	19.826	$C_{14}H_{26}O_4$	258.1832	257.1759	325.185	$[M-H]^-$	Tetradecanedioic acid	0.25	86.2
58	17.374	$C_{15}H_{18}O_7$	310.1055	309.098	215.0123	$[M-H]^-$	hericioic acid G	0.95	85.58
59	20.096	$C_{18}H_{34}O_4$	314.2457	313.2386	293.18	$[M-H]^-$	(±)9,10-Dihydroxy-12z-octadecenoic acid	0.05	85.23

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
60	20.096	$C_{16}H_{30}O_2$	254.2248	299.2228	293.1802	$[M + HCOOL]^-$	Δ2-Trans-hexadecenoic acid	0.85	85.2
61	17.4	$C_{15}H_{14}O_6$	290.0781	289.0714	215.0123	$[M - H]^-$	Hericiofuranoic Acid	-3.33	84.61
62	20.508	$C_{27}H_{31}NO_4$	433.2245	478.2232	325.1848	$[M + HCOOL]^-$	Hericenone B	-1.88	84.6
63	9.415	$C_4H_6O_4$	118.0264	117.0191	966.0017	$[M - H]^-$	Succinic acid	-1.62	84.53
64	17.71	$C_{15}H_{20}O_4$	264.1363	323.1505	155.1081	$[M + CH_3COOL]^-$	hydrospirobenzofuran	0.38	84.38
65	20.508	$C_{21}H_{32}O_4$	348.23	393.2282	325.1848	$[M + HCOOL]^-$	Herialpin B	-0.2	83.36
66	17.948	$C_{14}H_{14}O_6$	278.0784	277.0711	291.0884	$[M - H]^-$	Erinaceolactone H	-2.43	83.21
67	23.645	$C_{28}H_{44}O_3$	428.3261	473.3251	805.9867	$[M + HCOOL]^-$	ergosterol peroxide	-6.99	82.85
68	17.677	$C_{13}H_{16}O_4$	236.1043	281.1021	155.1081	$[M + HCOOL]^-$	Erinachromane A	-2.51	82.74
69	21.014	$C_{25}H_{36}O_7$	448.2467	447.2393	412.2133	$[M - H]^-$	Hericinoid A	1.27	82.53
70	19.253	$C_{15}H_{20}O_3$	248.1411	293.1401	325.185	$[M + HCOOL]^-$	Atractylenolide III	-0.65	81.68
71	26.141	$C_{20}H_{32}O_2$	304.2378	349.2361	631.3287	$[M + HCOOL]^-$	Arachidonic acid	-8.1	80.9
72	18.633	$C_{25}H_{34}O_6$	430.2352	475.2339	433.1876	$[M + HCOOL]^-$	Erinacine S	-0.8	80.26
73	17.71	$C_{26}H_{38}O_6$	446.2638	445.2567	155.1083	$[M - H]^-$	(-)-Erinacin A-d3	-6.84	79.33
74	22.293	$C_{26}H_{40}O_7$	464.2778	523.2914	805.9866	$[M + CH_3COOL]^-$	Erinacine V	0.78	79.03
75	22.293	$C_{28}H_{44}O_9$	524.2986	523.2914	805.9866	$[M - H]^-$	erinacine Q2	0.05	79.03

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
76	1.856	$C_6H_{10}O_6$	178.0471	237.0613	126.9047	$[M + CH_3COOL]^-$	Δ-Gluconic acid δ-lactone	-3.77	78.47
77	20.482	$C_{27}H_{40}O_8$	492.2732	491.2666	325.1849	$[M - H]^-$	Erinacine P	1.73	78.32
78	20.232	$C_{20}H_{30}O_4$	334.2142	393.2297	331.1555	$[M + CH_3COOL]^-$	2-Hydroxy-4,5′,8a′-trimethyl-1′-oxo-4-vinyloctahydro-1′h-spiro[cyclopentane-1,2′-naphthalene]-5′-carboxylic acid	-0.51	78.32
79	17.006	$C_{25}H_{38}O_8$	466.2568	511.2552	341.115	$[M + HCOOL]^-$	Erinacine J	0.28	78.31
80	18.782	$C_{19}H_{22}O_7$	362.1345	361.1284	277.1087	$[M - H]^-$	Erinaceolactone D	-5.63	78.3
81	11.897	$C_8H_{15}NO_3$	173.1041	172.0977	130.9662	$[M - H]^-$	N-Acetyl-l-leucine	-6.33	77.41
82	1.856	$C_6H_{12}O_6$	180.0635	179.0558	126.9047	$[M - H]^-$	L-Sorbose	0.42	77.13
83	21.874	$C_{20}H_{32}O_3$	320.233	365.2313	837.4859	$[M + HCOOL]^-$	(3 s)-3-Methyl-5- [(1 s,8ar)-2,5,5,8atetramethyl-4-oxo1,4,4a,5,6,7,8,8aoctahydro-1- naphthalenyl] pentanoic acid	-6.64	76.83
84	4.825	$C_6H_{14}O_6$	182.0792	181.0717	130.9663	$[M - H]^-$	Galactitol	0.63	76.68
85	16.553	$C_{13}H_{15}NO_4$	249.0999	248.0932	307.0827	$[M - H]^-$	Erinacerin M	-0.69	76.52
86	17.71	$C_{15}H_{18}O_3$	246.1261	305.1403	155.1081	$[M + CH_3COOL]^-$	Arglabin	1.95	76.37

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
87	18.669	$C_{27}H_{38}O_9$	506.2501	565.266	431.1725	$[M + CH_3COOL]^-$	Erinacine R	-2.88	73.43
88	19.113	$C_{21}H_{44}NO_7P$	453.2814	498.2798	331.1195	$[M + HCOOL]^-$	Glycerophospho-n- palmitoyl ethanolamine	-9.03	70.15
89	22.049	$C_{22}H_{32}O_4$	360.2296	419.2436	837.4866	$[M + CH_3COOL]^-$	Erinacine I	-1.25	69.76
90	16.479	$C_{55}H_{90}O_7$	862.6627	921.677	307.0827	$[M + CH_3COOL]^-$	Hericene H	-6.94	66.69
91	25.917	$C_{35}H5_4O_6$	570.3909	629.4039	355.1588	$[M + CH_3COOL]^-$	Hericenone C	-2.01	66.63
92	1.856	$C_7H_{10}O_7$	206.0423	205.0334	126.9047	$[M - H]^-$	3-Hydroxy-3-(methoxycarbonyl) pentanedioic acid	-1.93	63.78
93	21.513	$C_{26}H_{40}O_7$	464.2812	523.2943	311.1695	$[M + CH_3COOL]^-$	Erinacine V	8.08	63.33
94	12.342	$C_{11}H_9NO_2$	187.0632	246.0774	130.9662	$[M + CH_3COOL]^-$	Indole-3-acrylic acid	-0.9	62.25
95	16.9	$C_{19}H_{21}NO_7$	375.1343	420.1301	949.671	$[M + HCOOL]^-$	caputmedusins J	6.76	61.68
96	18.974	$C_{11}H_{18}N_2O_2$	210.135	269.1483	277.1087	$[M + CH_3COOL]^-$	Cyclo(leucylprolyl)	-8.47	59.46
97	16.38	$C_{16}H_{19}NO_5$	305.1279	350.1253	723.5056	$[M + HCOOL]^-$	Erinacerin G	5.16	58.5
98	17.178	$C_{20}H_{24}O_7$	376.1519	421.152	341.1151	$[M + HCOOL]^-$	Erinaceolactone E	-0.91	58.07
99	22.472	$C_{46}H_{80}O_4$	696.6079	695.5975	315.1605	$[M - H]^-$	Erinarol E	3.24	57.98
100	20.845	$C_{19}H_{24}NO_4$	330.1712	375.1772	311.1694	$[M + HCOOL]^-$	Erinaceolactam A	2.02	55.92

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
101	24.31	$C_{35}H_{54}O_7$	586.3855	645.3977	631.3279	$[M + CH_3COOL]^-$	3-Hydroxyhericenone F	-2.42	54.38
102	18.205	$C_{15}H_{10}O_8$	318.0391	317.0308	291.0883	$[M - H]^-$	Avenacein Y	4.89	53.37
103	23.145	$C_{22}H_{36}O_3$	348.2636	407.2826	257.2121	$[M + CH_3COOL]^-$	3-Methyl-5-(5,5,8atrimethyl-2-methylene-7-oxodecahydro-1-naphthalenyl) pentyl acetate	-8.11	52.69
104	18.177	$C_{19}H_{23}NO_5$	345.1593	404.1717	291.0884	$[M + CH_3COOL]^-$	hericioic acid D	4.94	51.18
105	24.863	$C_{46}H_{74}O_4$	690.5639	689.5423	805.9863	$[M - H]^-$	Erinarol C	7.5	50.19
106	24.507	$C_{41}H_{77}NO_9$	727.5587	772.5559	379.1584	$[M + HCOOL]^-$	Cerebroside B	-1.63	50.11
107	22.123	$C_{19}H_{24}NO_4$	330.1718	377.1611	805.9844	$[M + HCOOL]^-$	Erinaceolactam A	3.79	50.09
108	19.458	$C_{25}H_{39}O_7$	451.2695	496.264	325.1853	$[M + HCOOL]^-$	Erinacine Z2	-0.17	50.05
109	22.438	$C_{19}H_{24}O_4$	316.1676	317.1749	317.1749	$[M + H]^+$	Hericenone J	0.52	99.69
110	22.438	$C_{10}H_8O_4$	192.0423	193.0496	317.1749	$[M + H]^+$	Erinaceolactone A	0.02	99.64
111	20.868	$C_9H_{10}O_3$	166.063	167.0702	333.1701	$[M + H]^+$	Ethyl paraben	-0.22	99.55
112	25.53	$C_{19}H_{34}O$	278.2611	296.2949	431.2778	$[M + NH_4]^+$	9, 12-octadecadienoic acid methyl ester	0.5	99.52

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
113	20.304	$C_{24}H_{30}O_6$	414.2035	437.1933	315.158	$[M + H]^+$	Bis(4-ethylbenzylidene) sorbitol	-1.83	99.26
114	26.907	$C_{18}H_{37}NO$	283.2872	284.2945	310.3108	$[M + H]^+$	Stearamide	-1.05	99.26
115	21.165	$C_{27}H_{33}NO_4$	435.2412	436.2485	284.334	$[M + H]^+$	Corallocin B	0.47	99.24
116	20.04	$C_{27}H_{42}O_8$	494.2879	517.2773	315.1586	$[M + Na]^+$	Erinacine K	-0.06	99.24
117	2.667	$C_{10}H_{13}N_5O_4$	267.0971	268.1044	268.1044	$[M + H]^+$	Adenosine	1.38	99.16
118	23.301	$C_{10}H_{16}O$	152.1201	170.1539	280.2639	$[M + NH_4]^+$	R-ipsdienol	0.11	99.14
119	24.754	$C_{18}H_{35}NO$	281.2723	282.2796	338.3422	$[M + H]^+$	Oleamide	1.69	99.08
120	17.098	$C_{19}H_{23}NO_7$	377.147	378.1543	435.1762	$[M + H]^+$	caputmedusins G	-1.24	98.91
121	17.436	$C_{15}H_{18}O_5$	278.1149	296.1487	296.1487	$[M + NH_4]^+$	hericioic acid E	-1.8	98.75
122	22.273	$C_{27}H_{42}O_7$	478.2931	501.2826	707.4553	$[M + Na]^+$	Erinacine D	0.19	98.47
123	12.671	$C_{14}H_{17}NO_5$	279.111	280.1181	476.3073	$[M + H]^+$	hericioic acid A	1	98.31
124	20.982	$C_{26}H_{29}NO_5$	435.2038	436.2111	414.2289	$[M + H]^+$	Erinacerin Q	-1.71	98.17
125	24.286	$C_{16}H_{33}NO$	255.257	256.2643	282.2808	$[M + H]^+$	Hexadecanamide	3.13	97.14
126	16.717	$C_{12}H_{16}O_2$	192.1143	210.1482	340.2597	$[M + NH_4]^+$	Senkyunolide A	-3.59	96.37
127	5.066	$C_{11}H_{12}O_5$	224.0682	242.1023	141.0548	$[M + NH_4]^+$	Erinaceolactone F	-1.25	96.27

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
128	20.804	$C_{20}H_{28}O_2$	300.2081	301.2152	331.1532	$[M + H]^+$	Isotretinoin	-2.82	95.86
129	21.593	$C_{16}H_{12}O_6$	300.0642	301.0713	312.3636	$[M + H]^+$	Diosmetin	2.81	95.56
130	21.894	$C_{37}H_{56}O_6$	596.4076	614.4411	596.4308	$[M + NH_4]^+$	Hericenone I	-0.19	94.08
131	21.96	$C_{24}H_{36}O_5$	404.2557	405.2625	598.4492	$[M + H]^+$	Lovastatin	-1.46	93.03
132	21.867	$C_{25}H_{36}O_6$	432.2532	455.2423	596.4332	$[M + Na]^+$	Erinacine A	4.73	92.57
133	19.668	$C_{22}H_{26}O_6$	386.1749	387.1821	387.1821	$[M + H]^+$	Bis(methylbenzylidene) sorbitol	5.13	91.25
134	21.867	$C_{23}H_{31}N_2O_5$	415.2257	433.2594	596.4326	$[M + NH_4]^+$	Erinaceolactam E	5.72	86.51
135	2.667	$C_5H_5N_5$	135.0547	136.0619	268.1044	$[M + H]^+$	Adenine	1.64	86.32
136	1.897	$C_9H_{11}NO_3$	181.0743	182.0816	202.181	$[M + H]^+$	L-Tyrosine	2.06	85.47
137	19.542	$C_{19}H_{22}O_5$	330.146	353.135	288.2886	$[M + H]^+$	Hericenone A	-2.3	85.2
138	21.552	$C_{18}H_{30}O_2$	278.2246	279.2324	284.3315	$[M + H]^+$	Pinolenic acid	0.09	84.76
139	21.236	$C_{16}H_{17}NO_6$	319.1077	337.1416	284.3314	$[M + NH_4]^+$	Erinacerin T	6.76	84.64
140	21.983	$C_{18}H_{30}O_3$	294.2205	295.2274	598.449	$[M + H]^+$	9-Oxo-10(e),12(e)-octadecadienoic acid	3.45	84.41
141	19.846	$C_9H_{14}O_4$	186.0894	209.0786	333.1675	$[M + Na]^+$	cyclohexanecarboxylic acid	0.89	83.96

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
142	25.198	$C_{19}H_{32}O_2$	292.2427	293.2471	312.3267	$[M + Na]^+$	9(z),11(e),13(e)-Octadecatrienoic acid methyl ester	8.58	83.38
143	16.209	$C_{18}H_{19}NO_6$	345.1214	346.1287	652.4125	$[M + H]^+$	Erinacerin S	0.52	83.22
144	20.131	$C_{24}H_{31}NO_7$	445.2103	446.2173	193.0485	$[M + H]^+$	Erinacerin W	0.64	82.79
145	22.25	$C_{18}H_{35}NO_2$	297.267	298.2745	149.0243	$[M + H]^+$	2-Aminooctadec-4-yne- 1,3-diol	0.8	82.52
146	4.077	$C_{11}H_{15}N_5O_4$	281.1131	282.1203	116.9764	$[M + H]^+$	2′-o-Methyladenosine	2.51	82.34
147	22.322	$C_{20}H_{30}O_3$	318.2181	341.209	663.4334	$[M + H]^+$	11-α-hydroxy-17-methyltestosterone	-4.35	82.18
148	32.803	$C_6H_6O_6$	174.0163	197.0061	116.9767	$[M + Na]^+$	Trans-aconitic acid	-0.62	80.92
149	20.868	$C_{21}H_{42}O_4$	358.3081	359.3153	284.3394	$[M + H]^+$	1-Stearoylglycerol	-0.69	80.81
150	26.084	$C_{25}H_{36}O_8$	464.2402	487.2301	559.5164	$[M + Na]^+$	Erinacine G	-1.66	80.55
151	16.62	$C_8H_{10}O_5$	186.0526	209.0422	317.1474	$[M + Na]^+$	Erinapyrone C	-1.26	78.75
152	19.907	$C_{20}H_{34}O_2$	306.253	324.2868	333.167	$[M + NH_4]^+$	Linolenic acid ethyl ester	-9.39	77.28
153	17.051	$C_{12}H_{10}N_4O_2$	242.0786	243.0861	435.1756	$[M + H]^+$	Lumichrome	-7.48	76.87
154	21.309	$C_{18}H_{28}O_3$	292.2047	293.2094	284.3314	$[M + Na]^+$	9 s,13r-12- Oxophytodienoic acid	3.09	76.8
155	16.556	$C_{15}H_{10}O_5$	270.0518	288.0859	679.5116	$[M + NH_4]^+$	Apigenin	-3.83	76.12

Table 5.4 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
156	20.912	$C_{20}H_{38}O_2$	310.2864	328.3207	333.1701	$[M + NH_4]^+$	Ethyl oleate	-2.65	75.55
157	17.051	$C_{22}H_{25}NO_8$	431.1605	454.1499	435.1757	$[M + Na]^+$	Pseurotin A	5.85	74.52
158	21.236	$C_{28}H_{46}O$	398.3537	399.361	284.332	$[M + H]^+$	ergosta-7, 22-dien-3β-ol	-2.97	74.44
159	20.304	$C_{28}H_{40}O$	392.3075	410.3426	415.2111	$[M + H]^+$	ergosta-4, 6, 8(14), 22-tetraen-3-one	-0.93	71.74
160	21.593	$C_{37}H_{54}O_6$	594.3924	612.4262	312.3629	$[M + NH_4]^+$	Hericenone H	0.63	71.21
161	19.066	$C_{11}H_{15}N_5O_3S$	297.0896	315.1231	274.275	$[M + NH_4]^+$	5′-s-Methyl-5′-thioadenosine	0.01	66.24
162	21.575	$C_{37}H_{58}O_6$	598.4221	616.4552	312.3631	$[M + NH_4]^+$	5′-hydroxyhericenes C	-2.11	66.04
163	11.143	$C_{16}H_{12}O_5$	284.0676	307.0573	116.9752	$[M + Na]^+$	Acacetin	-3.04	65.77
164	21.481	$C_{37}H_{58}O_5$	582.4335	600.4667	596.4315	$[M + NH_4]^+$	Hericene B	8.69	65.58
165	21.481	$C_{37}H_{60}O_5$	584.4419	602.4759	312.3628	$[M + NH_4]^+$	Hericene C	-3.76	65.32
166	22.036	$C_{24}H_{27}NO_7$	441.1789	459.2139	420.2555	$[M + NH_4]^+$	Erinacerin U	0.44	63.64
167	20.157	$C_{43}H_{52}N_2O_{12}$	788.3538	806.3865	317.1742	$[M + NH_4]^+$	caputmedusins B	2.3	63.5
168	20.547	$C_{21}H_{28}NO_5$	374.1948	375.2007	316.1907	$[M + H]^+$	Erinaceolactam D	-5.26	59.41
169	2.948	$C_{12}H_{13}ClO_5$	272.0448	273.0522	116.9761	$[M + H]^+$	Erinaceolactone C	-1.22	58.94
170	20.25	$C_6H_8O_7$	192.0268	211.0631	193.0488	$[M + NH_4]^+$	Citric acid	-1.04	52.01

Table 5.5 Characterization of Compounds in *Hericium coralloides* strain MFLUCC 21-0050 by Using LC-QTOF

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
1	21.41	$C_{14}H_{28}O_3$	244.2037	243.1965	311.1694	$[M-H]^-$	(r)-3-Hydroxy myristic acid	-0.68	99.82
2	24.487	$C_{35}H_{56}O_6$	572.4079	571.4006	805.9863	$[M-H]^-$	5′-hydroxyhericenes A	0.33	99.81
3	18.449	$C_{18}H_{34}O_5$	330.2408	329.2335	251.093	$[M-H]^-$	(15z)-9,12,13-Trihydroxy-15-octadecenoic acid	0.48	99.75
4	1.72	$C_6H_{14}O_6$	182.0789	181.0716	341.1085	$[M-H]^-$	Galactitol	-0.91	99.72
5	21.714	$C_{25}H_{38}O_6$	434.2667	479.2649	479.2649	[M + HCOOL] −	Erinacine C	-0.41	99.71
6	26.077	$C_{18}H_{34}O_2$	282.2561	281.2488	381.1744	$[M-H]^-$	Ethyl palmitoleate	0.7	99.62
7	22.133	$C_{16}H_{32}O_3$	272.2348	271.2276	805.9863	$[M-H]^-$	16-Hydroxyhexadecanoic acid	-1.44	99.6
8	2.51	$C_9H_{12}N_2O_6$	244.0698	243.0626	243.0626	$[M-H]^-$	Uridine	1.27	99.53
9	26.105	$C_{37}H_{58}O_6$	598.4237	597.4165	805.9872	$[M-H]^-$	5′-hydroxyhericenes C	0.67	99.48
10	24.487	$C_{35}H_{56}O_5$	556.4125	555.4056	805.9865	$[M-H]^-$	Hericene A	-0.53	99.44
11	19.507	$C_{10}H_{20}O_3$	188.1414	187.1342	329.2339	$[M-H]^-$	10-Hydroxydecanoic acid	0.74	99.44
12	12.728	$C_8H_{15}NO_3$	173.1055	172.0982	172.0982	$[M-H]^-$	N-Acetyl-l-leucine	1.61	99.33
13	16.381	$C_{15}H_{17}NO_5$	291.1109	350.1248	130.9663	$[M + CH_3COOL]^-$	hericioic acid B	0.62	99.32
14	16.381	$C_{16}H_{19}NO_5$	305.1264	350.1248	130.9663	[M + HCOOL] −	Erinacerin G	0.31	99.32
15	22.214	$C_{25}H_{34}O_6$	430.2358	475.2337	805.9885	$[M-H]^-$	Erinacine S	0.54	99.3

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
16	11.749	$C_6H_{12}O_3$	132.079	131.0717	121.03	$[M-H]^-$	2-Hydroxycaproic acid	2.39	99.21
17	1.899	$C_{10}H_{13}N_5O_5$	283.0916	282.0843	243.0623	$[M-H]^-$	Guanosine	-0.39	99.17
18	20.547	$C_{21}H_{27}NO_5$	373.1893	372.182	325.1851	$[M-H]^-$	Erinaceolactam B	1.09	99.16
19	18.428	$C_{24}H_{36}O_5$	404.2566	449.2548	248.1294	$[M+HCOOL]-$	Lovastatin	0.71	99.14
20	1.72	$C_5H_{12}O_5$	152.0682	151.0609	134.0472	$[M-H]^-$	Ribitol	-2.09	99.09
21	19.319	$C_{22}H_{36}O_3$	348.2664	407.2801	325.1851	$[M+CH_3COOL]^-$	3-Methyl-5-(5,5,8atrimethyl-2-methylene-7-oxodecahydro-1-naphthalenyl) pentyl acetate	-0.03	98.97
22	20.031	$C_{27}H_{42}O_8$	494.2883	539.2868	477.2498	$[M+HCOOL]-$	Erinacine K	0.69	98.96
23	15.291	$C_{11}H_{13}NO_3$	207.0899	206.0826	966.0025	$[M-H]^-$	N-Acetyl-L-phenylalanine 1-(Carboxymethyl)	1.77	98.96
24	24.256	$C_{18}H_{32}O_2$	280.2405	279.2332	379.1587	$[M-H]^-$	9(z),11(e)-Conjugated linoleic acid	0.98	98.96
25	26.565	$C_{21}H_{38}O_4$	354.277	353.2696	805.9873	$[M-H]^-$	1-Linoleoyl glycerol	0	98.95
26	2.489	$C_5H_7NO_3$	129.043	128.0357	243.0625	$[M-H]^-$	4-Oxoproline	2.9	98.9
27	20.547	$C_{14}H_{16}O_3$	232.1095	231.1024	325.1852	$[M-H]^-$	coralcuparene	-1.87	98.84

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
28	19.459	$C_{11}H_{14}O_3$	194.0946	193.0874	329.2338	$[M-H]^-$	4-Ethoxy ethylbenzoate	1.55	98.84
29	18.428	$C_{25}H_{38}O_7$	450.262	449.2548	248.1295	$[M-H]^-$	Erinacine T	0.51	98.83
30	10.672	$C_{12}H_{12}N_2O_2$	216.0904	275.104	130.9663	$[M+CH_3COOL]^-$	2,3,4,9-Tetrahydro-1h-β-carboline-3-carboxylic acid	2.38	98.78
31	19.38	$C_{12}H_{16}O_3$	208.1098	207.1028	229.0874	$[M-H]^-$	B-Asarone	-0.93	98.76
32	21.507	$C_{28}H_{33}NO_5$	463.2366	462.2294	619.3033	$[M-H]^-$	Corallocin E	1.53	98.64
33	2.664	$C_5H_5N_5$	135.0547	134.0474	134.0474	$[M-H]^-$	Adenine	1.7	98.6
34	23.235	$C_{12}H_{26}O_4S$	266.1555	265.1483	805.9868	$[M-H]^-$	Dodecyl sulfate	1.28	98.51
35	19.255	$C_{26}H_{29}NO_5$	435.2051	434.1978	421.2272	$[M-H]^-$	Erinacerin Q	1.11	98.49
36	17.85	$C_{10}H_{18}O_4$	202.1208	201.1135	234.114	$[M-H]^-$	3-Tert-butyladipic acid	1.62	98.33
37	17.686	$C_{25}H_{36}O_8$	464.244	463.2345	463.2345	$[M-H]^-$	Erinacine G	6.46	98.3
38	17.286	$C_{25}H_{36}O_7$	448.2462	493.2447	395.0756	$[M+HCOOL]-$	Hericinoid A	0.28	98.13
39	17.92	$C_{17}H_{21}NO_5$	319.1428	378.1566	378.1566	$[M+CH_3COOL]^-$	Erinacerin R	2.47	98.08
40	27.721	$C_{18}H_{30}O_3S$	326.192	325.1848	116.9287	$[M-H]^-$	4-Dodecylbenzenesulfonic acid	1.43	98.08
41	21.507	$C_{25}H_{34}O_7$	446.2313	445.2241	619.3034	$[M-H]^-$	Hericinoid C	1.97	98.05
42	20.177	$C_{27}H_{33}NO_4$	435.2413	434.2336	293.1797	$[M-H]^-$	Corallocin B	0.8	97.94

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
43	21.761	$C_{19}H_{24}O_4$	316.1679	315.1607	479.2657	$[M-H]^-$	Hericenone J	1.5	97.47
44	22.271	$C_{27}H_{42}O_7$	478.2934	523.2914	805.987	[M + HCOOL] −	Erinacine D	0.75	97.42
45	22.271	$C_{26}H_{40}O_7$	464.2782	523.2914	805.987	$[M+CH_3COOL]^-$	Erinacine V	1.7	97.42
46	22.271	$C_{28}H_{44}O_9$	524.2987	523.2914	805.987	$[M-H]^-$	erinacine Q2	0.38	97.42
47	18.799	$C_{42}H_{50}N_2O_{12}$	774.3368	773.3294	478.1878	$[M-H]^-$	caputmedusins A	0.55	97.31
48	15.588	$C_7H_{10}O_3$	142.0631	141.0557	130.9663	$[M-H]^-$	Erinapyrone B	0.9	97.18
49	22.033	$C_{27}H_{33}NO_3$	419.2451	418.2378	418.2378	$[M-H]^-$	Isohericerin	-2.27	97.06
50	22.807	$C_{35}H_{54}O_6$	570.3919	569.385	805.9872	$[M-H]^-$	Hericenone C	-0.22	96.71
51	24.435	$C_{40}H_{75}NO_9$	713.5447	772.5573	805.9869	$[M+CH_3COOL]^-$	Cerebroside E	0.68	95.74
52	26.105	$C_{37}H_{58}O_5$	582.4299	581.4213	805.9874	$[M-H]^-$	Hericene B	2.48	95.43
53	18.891	$C_{24}H_{31}NO_7$	445.2108	444.2032	444.2032	$[M-H]^-$	Erinacerin W	1.74	95.36
54	16.566	$C_{13}H_{15}NO_4$	249.1005	248.0931	966.0016	$[M-H]^-$	Erinacerin M	1.57	94.75
55	18.249	$C_{27}H_{29}NO_8$	495.1904	494.1831	355.1408	$[M-H]^-$	caputmedusins D	2.21	94.65
56	18.554	$C_{23}H_{29}NO_7$	431.1957	430.1883	248.1299	$[M-H]^-$	caputmedusins F	2.96	94.05
57	16.989	$C_{22}H_{26}N_2O_6$	414.1795	413.1723	385.2352	$[M-H]^-$	Erinacerin P	0.97	93.64
58	21.53	$C_{18}H_{30}O_2$	278.2243	277.2174	619.3034	$[M-H]^-$	Pinolenic acid	-0.93	93.45
59	24.46	$C_{41}H_{77}NO_9$	727.5584	772.5571	805.9866	[M + HCOOL] −	Cerebroside B	-1.91	93.39

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
60	11.556	$C_{27}H_{40}O_8$	492.2741	551.2879	130.9661	$[M + CH_3COOL]^-$	Erinacine P	3.58	92.39
61	16.913	$C_{14}H_{17}NO_5$	279.111	278.1041	949.6725	$[M - H]^-$	hericioic acid A	1.25	92.34
62	16.269	$C_{14}H_{26}O_4$	258.1832	317.1974	166.0514	$[M + CH_3COOL]^-$	Tetradecanedioic acid	0.52	91.97
63	21.714	$C_{29}H_{34}N_2O_3$	458.2585	457.251	479.2658	$[M - H]^-$	Corallocin C	3.29	90.66
64	18.58	$C_{20}H_{30}O_4$	334.2155	379.2134	248.13	[M + HCOOL] −	2-Hydroxy-4,5′,8a′-trimethyl-1′-oxo-4-vinyloctahydro-1′h-spiro[cyclopentane-1,2′-naphthalene]-5′-carboxylic acid	3.28	88.96
65	20.373	$C_{35}H_{54}O_7$	586.387	631.3855	431.2448	[M + HCOOL] −	3-Hydroxyhericenone F	0.09	88.39
66	19.673	$C_{27}H_{31}NO_4$	433.2223	478.2249	325.1851	[M + HCOOL] −	Hericenone B	-6.9	87.6
67	17.186	$C_9H_{16}O_4$	188.1048	187.0975	215.0119	$[M - H]^-$	Azelaic acid	-0.08	87.02
68	14.88	$C_8H_{14}O_4$	174.0892	173.0819	497.3354	$[M - H]^-$	Suberic acid	-0.09	86.91
69	19.43	$C_{10}H_{16}$	136.125	181.1233	325.1849	[M + HCOOL] −	Limonene	-1.18	86.36
70	16.913	$C_9H_8O_3$	164.0474	163.0402	949.6725	$[M - H]^-$	2-Hydroxycinnamic acid	0.6	86.35
71	23.143	$C_{37}H_{56}O_6$	596.4085	595.4	805.9867	$[M - H]^-$	Hericenone I	1.37	86.32
72	18.055	$C_{22}H_{27}NO_7$	417.1797	416.1722	279.0882	$[M - H]^-$	caputmedusins E	2.18	85.97
73	3.085	$C_5H_5N_5O$	151.0491	150.0415	966.0013	$[M - H]^-$	Guanine	-2.14	85.9

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
74	19.557	$C_{12}H_{14}O_2$	190.0996	249.1136	329.2338	$[M + CH_3COOL]^-$	eulatachromene	1.26	85.66
75	20.914	$C_{15}H_{18}O_4$	262.1194	261.1128	311.169	$[M - H]^-$	spirobenzofuran	-4.4	85.38
76	20.325	$C_{16}H_{30}O_2$	254.2248	313.2388	431.2449	$[M + CH_3COOL]^-$	Δ2-Trans-hexadecenoic acid	0.74	85.2
77	20.099	$C_{18}H_{34}O_4$	314.2444	313.2384	477.2497	$[M - H]^-$	(±)9,10-Dihydroxy-12z-octadecenoic acid	-4.33	85.16
78	17.873	$C_{18}H_{21}NO_5$	331.1427	330.1351	388.141	$[M - H]^-$	Erinacerin C	2.17	84.96
79	20.268	$C_{12}H_{18}O_2$	194.1308	239.1291	431.2449	[M + HCOOL] −	Sedanolide	0.41	84.95
80	13.23	$C_{10}H_{10}O_4$	194.0577	193.0505	130.9664	$[M - H]^-$	Erinaceolactone B	-0.94	84.54
81	1.72	$C_8H_{14}O_7$	222.0732	221.0664	134.0472	$[M - H]^-$	Ethylβ-d-glucuronide	-3.27	84.41
82	18.864	$C_{12}H_{22}O_4$	230.1521	229.1447	444.2036	$[M - H]^-$	Dodecanedioic acid	1.42	84.25
83	20.373	$C_{15}H_{20}O_3$	248.1408	307.1545	431.2447	$[M + CH_3COOL]^-$	Atractylenolide III	-1.59	82.7
84	20.979	$C_{14}H_{18}O_3$	234.1244	293.1389	477.2496	$[M + CH_3COOL]^-$	Erinachromane B	-5.07	81.87
85	18.709	$C_{15}H_{18}O_5$	278.1155	277.1087	282.1142	$[M - H]^-$	hericioic acid E	0.39	81.33
86	2.51	$C_8H_{10}O_5$	186.052	245.0655	243.06	$[M + CH_3COOL]^-$	Erinapyrone C	-4.69	80.15
87	19.507	$C_{21}H_{44}NO_7P$	453.2825	498.2802	329.2341	[M + HCOOL] −	Glycerophospho-n- palmitoyl ethanolamine	-6.69	78.98
88	18.554	$C_{19}H_{23}NO_5$	345.1596	344.1511	248.1299	$[M - H]^-$	hericioic acid D	5.71	78.42

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
89	21.881	$C_{20}H_{32}O_3$	320.2325	319.2266	297.2436	[M + HCOOL] −	(3 s)-3-Methyl-5- [(1 s,8ar)-2,5,5,8atetramethyl-4-oxo1,4,4a,5,6,7,8,8aoctahydro-1-naphthalenyl] pentanoic acid	-8.34	77.78
90	19.619	$C_{19}H_{24}O_6$	348.1576	347.1508	325.1852	$[M - H]^-$	Hericenone K	0.96	76.93
91	17.686	$C_5H_8O_5$	148.0371	207.0505	966.0011	$[M + CH_3COOL]^-$	D-α-hydroxyglutaric acid	-0.52	75.65
92	16.477	$C_{55}H_{90}O_7$	862.6636	921.6771	966.002	$[M + CH_3COOL]^-$	Hericene H	-5.83	74.99
93	18.983	$C_{19}H_{23}NO_7$	377.1457	376.1384	495.2602	$[M - H]^-$	caputmedusins G	-4.56	74.79
94	17.716	$C_{26}H_{38}O_6$	446.2632	445.2561	463.2345	$[M - H]^-$	(-)-Erinacin A-d3	-8.16	73.82
95	24.167	$C_{19}H_{32}O_2$	292.2398	351.2541	805.9875	$[M + CH_3COOL]^-$	9(z),11(e),13(e)-Octadecatrienoic acid methyl ester	-1.38	73.52
96	13.23	$C_{11}H_{12}O_5$	224.0698	283.083	130.9665	$[M - H]^-$	Erinaceolactone F	5.97	70.61
97	22.773	$C_{20}H_{38}O_2$	310.286	369.2984	805.988	$[M + CH_3COOL]^-$	Ethyl oleate	-3.69	68.38
98	1.72	$C_{12}H_{22}O_{12}$	358.1098	357.1018	341.1085	$[M - H]^-$	Lactobionic acid	-3.77	65.62
99	24.487	$C_{37}H_{60}O_5$	584.4414	583.434	805.9863	$[M - H]^-$	Hericene C	-4.64	63.01
100	18.679	$C_{15}H_{18}O_3$	246.1255	305.1399	444.2036	$[M + CH_3COOL]^-$	Arglabin	-0.45	61.41

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
101	26.077	$C_{43}H_{52}N_2O_{12}$	788.3499	787.3399	381.1744	$[M-H]^-$	caputmedusins B	-2.74	57.71
102	1.757	$C_6H_{12}O_6$	180.0635	179.0556	243.0621	$[M-H]^-$	L-Sorbose	0.36	53.49
103	18.428	$C_{37}H_{60}O_6$	600.4412	645.4448	248.1293	[M + HCOOL] −	5′-hydroxyhericenes B	3.68	52.41
104	19.589	$C_{28}H_{31}NO_7$	493.2126	492.2035	325.1852	$[M-H]^-$	caputmedusins C	5.07	52.12
105	17.824	$C_{20}H_{25}NO_6$	375.1693	374.163	388.1411	$[M-H]^-$	Erinacerin D	2.96	50.68
106	16.763	$C_{19}H_{28}N_2O_6$	380.1946	381.2019	679.5124	$[M+H]^+$	Erinacerin N	-0.32	99.88
107	14.99	$C_{18}H_{23}NO_6$	349.1527	367.1866	453.3442	$[M+NH_4]^+$	Erinacerin I	0.57	99.85
108	27.357	$C_{18}H_{37}NO$	283.2877	284.295	537.3959	$[M+H]^+$	Stearamide	0.53	99.77
109	12.533	$C_{11}H_{18}N_2O_2$	210.1369	211.1441	211.1441	$[M+H]^+$	Cyclo(leucylprolyl)	0.13	99.52
110	20.804	$C_{25}H_{36}O_6$	432.2514	455.2406	301.2161	$[M+Na]^+$	Erinacine A	0.48	99.25
111	25.304	$C_{18}H_{35}NO$	281.2719	282.2791	312.326	$[M+H]^+$	Oleamide	0.03	99.08
112	2.665	$C_{10}H_{13}N_5O_4$	267.0971	268.1043	268.1043	$[M+H]^+$	Adenosine	1.23	99.02
113	16.728	$C_{12}H_{16}O_2$	192.115	210.1489	679.5127	$[M+NH_4]^+$	Senkyunolide A	-0.27	98.99
114	24.263	$C_{16}H_{33}NO$	255.2566	256.2638	256.2638	$[M+H]^+$	Hexadecanamide	1.44	98.91
115	25.491	$C_{19}H_{34}O$	278.2608	296.2945	559.5176	$[M+NH_4]^+$	9, 12-octadecadienoic acid methyl ester	-0.69	98.57
116	20.788	$C_{22}H_{29}NO_5$	387.2054	388.2118	301.2167	$[M+H]^+$	Corallocin D	2.04	98.51

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
117	18.442	$C_{25}H_{38}O_8$	466.2569	467.2646	568.3135	$[M + H]^+$	Erinacine J	0.59	98.4
118	4.064	$C_{11}H_{15}N_5O_4$	281.113	282.1203	282.1203	$[M + H]^+$	2′-o-Methyladenosine	2.18	98.4
119	19.714	$C_{20}H_{28}O_2$	300.2089	301.2161	387.1805	$[M + H]^+$	Isotretinoin	-0.09	98.29
120	22.68	$C_{28}H_{40}O$	392.3087	393.3158	537.3962	$[M + H]^+$	ergosta-4, 6, 8(14), 22-tetraen-3-one	1.98	98.25
121	20.312	$C_{24}H_{30}O_6$	414.2055	437.1942	415.2133	$[M + H]^+$	Bis(4-ethylbenzylidene) sorbitol	2.93	98.25
122	9.065	$C_{11}H_9NO_2$	187.0627	205.0965	261.1231	$[M + NH_4]^+$	Indole-3-acrylic acid	-3.3	97.95
123	9.065	$C_{11}H_{12}N_2O_2$	204.0893	205.0965	261.1231	$[M + H]^+$	L-Tryptophan	-3.02	97.95
124	20.888	$C_9H_{10}O_3$	166.0624	167.0697	301.216	$[M + H]^+$	Ethyl paraben	-3.32	97.77
125	20.587	$C_{19}H_{25}NO_3$	315.1836	316.1907	316.1907	$[M + H]^+$	5-(2E)-3′,7′-dimethyl-2′,6′-octadienyl]-4-hydroxy-6-methoxy-1-isoindoline (isoindolinone derivative)	0.65	97.32
126	24.67	$C_{10}H_{16}O$	152.12	170.1539	282.2801	$[M + NH_4]^+$	R-ipsdienol	-0.96	97.32
127	25.831	$C_{37}H_{54}O_6$	594.391	595.3983	559.519	$[M + H]^+$	Hericenone H	-1.72	96.87
128	6.673	$C_6H_6O_3$	126.031	127.0382	127.0382	$[M + H]^+$	Pyrogallol	-5.66	96.68
129	21.236	$C_{18}H_{37}NO_2$	299.2819	300.2893	537.3957	$[M + H]^+$	Palmitoyl ethanolamide	-1.86	96.61
130	3.88	$C_9H_{11}NO_3$	181.0741	199.1077	116.9764	$[M + NH_4]^+$	L-Tyrosine	1.13	95.92

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
131	9.359	$C_8H_{10}O_4$	170.0587	171.066	227.1401	$[M + H]^+$	Herierin IV	4.94	95.31
132	16.216	$C_{18}H_{19}NO_6$	345.1209	368.11	652.4122	$[M + H]^+$	Erinacerin S	-0.87	94.23
133	20.735	$C_{21}H_{42}O_4$	358.3089	359.3158	301.217	$[M + H]^+$	1-Stearoylglycerol	1.72	93.2
134	15.64	$C_9H_{14}O_4$	186.0897	204.1234	336.1664	$[M + NH_4]^+$	cyclohexanecarboxylic acid	2.78	93.03
135	19.483	$C_{18}H_{30}O_3$	294.2211	295.2278	288.2908	$[M + H]^+$	9-Oxo-10(e),12(e)-octadecadienoic acid	5.43	92.78
136	13.112	$C_{19}H_{22}O_5$	330.148	353.1375	211.1435	$[M + Na]^+$	Hericenone A	3.88	92.75
137	7.534	$C_{16}H_{12}O_5$	284.0684	285.0757	138.055	$[M + H]^+$	Acacetin	-0.24	91.74
138	22.235	$C_{18}H_{35}NO_2$	297.2668	298.2746	537.397	$[M + H]^+$	2-Aminooctadec-4-yne- 1,3-diol	0.09	91.23
139	22.453	$C_{10}H_8O_4$	192.0432	193.0504	512.5064	$[M + H]^+$	Erinaceolactone A	5.04	90.91
140	17.896	$C_{19}H_{22}O_7$	362.1355	380.1688	380.1688	$[M + NH_4]^+$	Erinaceolactone D	-2.99	90.21
141	3.045	$C_4H_6O_5$	134.0226	152.0563	155.0811	$[M + NH_4]^+$	Dl-Malic acid	7.68	89.82
142	19.674	$C_{22}H_{26}O_6$	386.175	387.1823	387.1823	$[M + H]^+$	Bis(methylbenzylidene) sorbitol	5.33	89.24
143	18.926	$C_{23}H_{31}N_2O_5$	415.2253	433.2589	446.218	$[M + NH_4]^+$	Erinaceolactam E	4.82	87.9
144	9.951	$C_{19}H_{22}O_6$	346.1437	369.1331	227.1388	$[M + Na]^+$	Corallocin A	5.96	87.5
145	2.665	$C_4H_6O_4$	118.0275	136.0619	268.1043	$[M + NH_4]^+$	Succinic acid	7.27	87.07

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
146	16.452	$C_{19}H_{24}O_5$	332.1632	355.1529	213.1582	$[M + Na]^+$	Erinacerin B	2.52	86.71
147	21.479	$C_{13}H_{16}O_4$	236.105	237.1122	621.3181	$[M + H]^+$	Erinachromane A	0.4	86.5
148	20.961	$C_{20}H_{30}O_3$	318.2202	319.2266	537.3963	$[M + H]^+$	11-α-hydroxy-17-methyltestosterone	2.31	85.25
149	17.028	$C_{12}H_{10}N_4O_2$	242.0798	243.087	435.1763	$[M + H]^+$	Lumichrome	-2.35	84.48
150	20.037	$C_{20}H_{32}O_2$	304.2411	305.2476	517.2789	$[M + H]^+$	Arachidonic acid	2.92	84.26
151	19.88	$C_{20}H_{34}O_2$	306.2535	324.2875	248.1046	$[M + NH_4]^+$	Linolenic acid ethyl ester	-7.85	83.7
152	13.536	$C_7H_{15}NO_3$	161.1051	184.0945	520.3338	$[M + Na]^+$	L(-)-Carnitine	-0.76	82.09
153	20.529	$C_{21}H_{28}NO_5$	374.1957	375.2029	515.4138	$[M + H]^+$	Erinaceolactam D	-2.83	80.52
154	26.911	$C_{20}H_{32}O$	288.2431	306.277	284.2956	$[M + NH_4]^+$	Erinacol	-7.79	79.79
155	18.57	$C_{20}H_{24}O_7$	376.1528	377.1611	250.1396	$[M + H]^+$	Erinaceolactone E	1.68	78.01
156	3.37	$C_6H_6O_6$	174.016	197.0057	100.0761	$[M + Na]^+$	Trans-aconitic acid	-2.68	77.83
157	20.804	$C_{25}H_{27}NO_7$	453.1805	471.2148	301.2164	$[M + NH_4]^+$	Erinacerin K	3.96	76.76
158	21.338	$C_{22}H_{32}O_4$	360.2293	361.2352	537.3957	$[M + H]^+$	Erinacine I	-1.98	70.46
159	19.027	$C_{24}H_{27}NO_7$	441.1803	442.187	274.2764	$[M + H]^+$	Erinacerin U	3.5	70.32
160	21.338	$C_{18}H_{23}NO_6$	349.1542	367.188	537.3954	$[M + NH_4]^+$	Erinacerin I	4.7	69.56
161	20.356	$C_{18}H_{28}O_3$	292.2016	293.2091	301.217	$[M + H]^+$	9 s,13r-12- Oxophytodienoic acid	-7.57	69.47

Table 5.5 (continued)

No.	RT	Formula	Mass	m/z	Peak	Adduct ions	Compound name	Error (ppm)	Score (Tgt)
162	17.053	$C_{22}H_{25}NO_8$	431.1611	454.1505	435.1763	$[M + Na]^+$	Pseurotin A	7.12	68.72
163	21.205	$C_{21}H_{32}O_4$	348.2328	349.2357	537.3964	$[M + H]^+$	Herialpin B	7.78	68.3
164	18.798	$C_{25}H_{39}O_7$	451.2714	469.3067	480.2028	$[M + NH_4]^+$	Erinacine Z2	4.01	63.37
165	1.873	$C_{10}H_{13}N_5O_3$	251.1019	252.11	136.0624	$[M + H]^+$	2′-Deoxyadenosine	0.15	60.47
166	26.024	$C_{46}H_{76}O_4$	692.5679	710.6065	553.3907	$[M + NH_4]^+$	Erinarol A	-9.29	60.38
167	18.57	$C_{15}H_{10}O_5$	270.0551	288.0874	250.1396	$[M + NH_4]^+$	Apigenin	8.27	60.17
168	6.673	$C_{13}H_8NO_7$	290.0289	313.0196	127.0376	$[M + Na]^+$	Herialpin A	-4.2	57.61
169	19.244	$C_{15}H_{14}O_6$	290.0814	313.0703	288.2899	$[M + Na]^+$	Hericiofuranoic Acid	8.06	57.36
170	25.831	$C_{46}H_{76}O_4$	692.5711	710.6082	559.519	$[M + Na]^+$	Erinarol A	-4.65	57.28
171	17.948	$C_{20}H_{25}NO_7$	391.1633	414.1536	295.1067	$[M + Na]^+$	caputmedusins I	0.38	56.08
172	2.464	$C_{15}H_{10}O_8$	318.04	336.0757	124.042	$[M + NH_4]^+$	Avenacein Y	7.76	54.99
173	20.441	$C_{11}H_{15}N_5O_3S$	297.0882	315.1217	609.3472	$[M + NH_4]^+$	5′-s-Methyl-5′-thioadenosine	-4.62	52.52
174	3.274	$C_{11}H_{13}ClO_2$	212.0588	235.0485	155.081	$[M + Na]^+$	1-(5-chloro-2-hydroxyphenyl)-3-methylbutan-1-one	-7.54	52.02
175	20.498	$C_{20}H_{39}NO_2$	325.2982	326.3078	515.4136	$[M + H]^+$	Oleoyl ethanolamide	0.26	51.65
176	21.756	$C_{29}H_{50}O$	414.3889	415.3964	457.2555	$[M + H]^+$	β-sitosterol	6.59	50.61

Discussions

Two novel isoindolinone derivatives, named corallocins D (1) and E (2), were isolated from the fruiting bodies of the basidiomycete *Hericium coralloides* (Winnie et al., 2024). Isoindolinone-type metabolites are well-documented as major secondary metabolites in both basidiomes and cultured samples of *Hericium* species (Wang et al., 2015; Wittstein et al., 2016; Ryu et al., 2021; Sum et al., 2023).

The study successfully identified a wide range of chemical constituents in *Hericium erinaceus* and *H. coralloides* using LC-ESI-QTOF-MS/MS. The results provided comprehensive insight into the bioactive compounds present in these mushrooms, contributing to the growing body of research on their medicinal potential.

One of the key findings was the identification of various bioactive metabolites, including polysaccharides, terpenoids (erinacines and hericenones), fatty acids, sterols, and phenolic compounds. These compounds have been linked to significant pharmacological activities such as neuroprotection, antioxidation, and anti-inflammatory properties. Previous studies, such as those by Kostanda et al. (2024) and Banerjee et al. (2024), have also reported similar findings, reinforcing the therapeutic importance of *Hericium* species.

Among the identified compounds, erinacines and hericenones stand out due to their potential to stimulate nerve growth factor (NGF) production, which plays a crucial role in cognitive function and neurodegenerative disease management (Tong et al., 2023). Additionally, the presence of phenolic compounds such as gallic acid, quercetin derivatives, and catechins highlights the strong antioxidant properties of these mushrooms (Saitsu et al., 2019).

Furthermore, the study found significant variations in the chemical profiles between different *Hericium* strains, with some strains containing a higher concentration of specific bioactive compounds. For instance, *H. coralloides* exhibited a richer profile of erinacines compared to *H. erinaceus*, which may suggest a variance in their neuroprotective capabilities.

The use of LC-ESI-QTOF-MS/MS proved to be an efficient analytical method for the rapid identification of these chemical constituents. The high accuracy and

sensitivity of this technique enabled the detection of even trace amounts of bioactive molecules, ensuring a robust characterization of *Hericium* metabolites. Comparisons with the Personal Compound Database and Library (PCDL) further validated the identified compounds, making the findings reliable and reproducible.

Overall, this study contributes valuable knowledge to the field of mycochemistry, demonstrating the vast potential of *Hericium* species as sources of medicinally important bioactive compounds. Future research could focus on isolating and testing specific compounds for their pharmacological efficacy in clinical settings, further validating their therapeutic applications.

CHAPTER 6

SCREENING OF BIOLOGICAL ACTIVITY OF *HERICIUM*

6.1 Introduction to the Bioactivity of *Hericium*

Hericium are part of the Hericiaceae family and are known for their distinctive, spiny, white fruiting bodies that resemble a lion's mane or coral formations (Arora, 1986). The two species most frequently researched are *Hericium erinaceus* and *H. coralloides* (Atila, 2019). *Hericium* was an edible mushroom that was then developed to be used medicinally (Thongbai et al., 2015). This mushroom is widely recognized as a highly nutritious food and is used for medicine (Jayachandran et al., 2017), although it is common in Asia, Europe, and North America (Boddy, 2016; Ouali et al., 2020).

Numerous studies have examined the antimicrobial, antioxidant, and cytotoxic effects of these mushrooms, revealing that they contain several bioactive compounds with potential therapeutic benefits (Suleiman et al., 2022; Han et al., 2013). *Hericium* species have demonstrated significant antimicrobial activity against a range of bacterial and fungal pathogens (Song et al., 2020). The compounds in these mushrooms, including polysaccharides, terpenoids, and phenolic acids, may weaken the cell wall structure of microbes, preventing their growth (Łysakowska et al., 2023; Suleria, 2024). However, the compounds of *Hericium* have powerful antioxidant effects, cytotoxic effects (Wong et al., 2009; Hetland et al., 2020), and anticancer effects (Zhou et al., 2014; Ryu et al., 2018).

In this chapter, we determined the antimicrobial activity, antioxidant activity, and cytotoxic activity of *H. erinaceus* and *H. corlloides* extracts. Also, determine the capacity of fruiting body extract of the selected strain of *H. coralloides* to cancer cell lines.

6.2 Materials and Methods

6.2.1 Preparation of the Crude Extract

The crude extract of *H. erinaceus* and *H. coralloides* was prepared following Section 3.7.1 of Chapter 3 (General Material and Methodology).

6.2.2 Selected Compounds Preparation of *H. coralloides*

6.2.2.1 Sample Collection

The selected strain of *Hericium* was used in this study. *Hericium coralloides* strain MFLUCC 21-0050 was characterized morphologically as described in Section 3.1 of Chapter 3.

6.2.2.2 Preparation of extracts

The 100 g of dried mushroom powders of *Hericium coralloides* were refluxed with 95% acetone (1000 mL), and placed on a shaker at 120 rpm at room temperature (25±2 °C) for 24 hr. After filtering the sample, 1000 mL of acetone was used twice to eliminate the residue. The collected filtrate was subsequently extracted using ethyl acetate according to the previously mentioned method (Wittstein et al., 2016) and dried under reduced pressure using a rotary evaporator. The following produced 15 grams of crude ethyl acetate extract.

6.2.2.3 Chromatographic separation

Thin-layer chromatography (TLC) was utilized after silica gel column chromatography to separate the dried extracts. For silica gel column chromatography, the dried extract was dissolved in 2 combinations of solvents. The ratios of two combinations of the solvents were EtOAc: hexane = 20: 80 in order for the polarity to shift monotonically. The carrier of the silica gel column was Kieselgel 60. Following the loading of the samples onto the silica gel column, the solvent was used to elute the column. A schematic diagram of the purification and isolation of compounds from *H. coralloides* is shown in Figure 6.1.

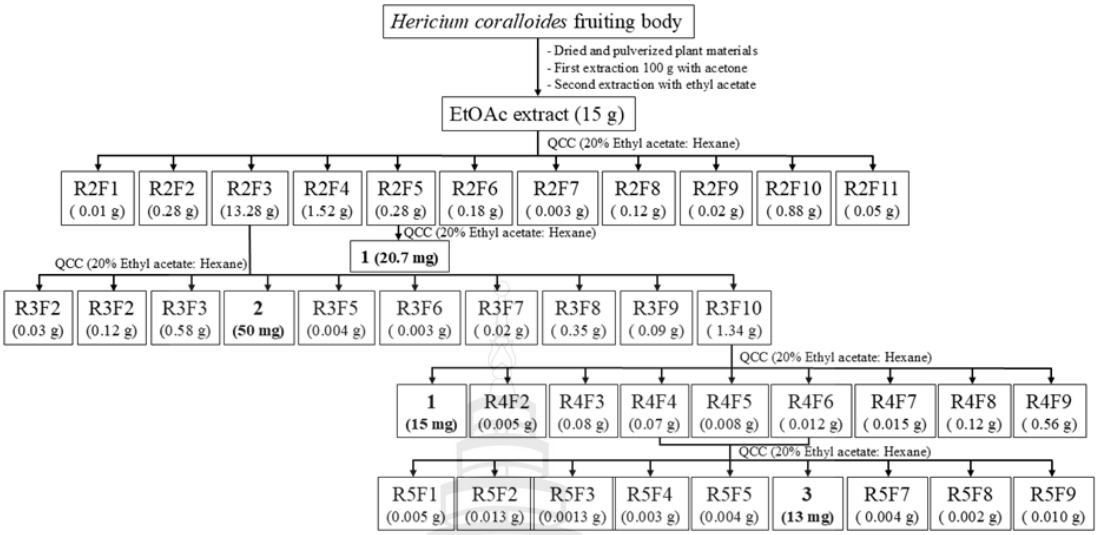


Figure 6.1 Schematic diagram of the extraction and isolation of compounds 1–3 from fruiting body of *Hericium coralloides*

For an additional four products of secondary metabolites, the ethyl acetate extract was purified by chromatography on a silica gel column, using a n-hexane gradient system: ethyl acetate (20:80) (R2), resulting in eleventh fractions, and fraction 5 (compound 1) was collected for NMR analysis. After that, R2F3 (20% ethyl acetate: n-hexane) (R3) was further separated according to the previously mentioned method, resulting in ten fractions, and fraction 4 (compound 2) was collected. And fraction 10 was used in the silica column with a solvent of 20% acetone: n-hexane (R4), resulting in nine fractions, collected fraction 1 (compound 1). Fraction 4 was combined with fraction 6 (20% ethyl acetate: n-hexane) (R3) and was further separated according to the previously mentioned method, resulting in ten fractions, and collected fraction 6 (compound 3). All fractions were collected from the solvent. The structure of the compounds, compound 1 (R2F6 and R4F1), compound 2 (R3F4), and compound 3 (R5F6), was identified by correlating the experimental NMR data with values published in previous studies.

6.2.2.4 Compounds characterizations

The structures of the isolated compounds were determined using spectroscopic techniques, with ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data compared with previously published physical properties and NMR spectra.

6.2.3 Screening and Investigation of the Secondary Metabolite Production of *Hericium*

6.2.3.1 Anti-microbial assay

Crude extract samplers of *H. erinaceus* and *H. coralloides* were used for the anti-microbial assay. The methods were following Section 3.7.2 of Chapter 3 (General Material and Methodology).

6.2.3.2 Anti-oxidants Assay

Crude extract samplers of *H. erinaceus* and *H. coralloides* were used for the antioxidant assay. The methods were following Section 3.7.3 of Chapter 3 (General Material and Methodology).

6.2.3.3 Cytotoxicity assay

Crude extract samples of *H. erinaceus* and *H. coralloides* were used for the cytotoxicity assay. The methods were following Section 3.7.4 of Chapter 3 (General Material and Methodology).

6.2.3.4 Anti-cancer Assay

The selected compounds of *H. coralloides* were used for the anticancer activity. Prepared cell cultures and anti-cancer assay as described in Section 3.7.5 of Chapter 3.

6.3 Results

6.3.1 Selected Compound from *H. coralloides*

Four compounds have been identified from the fruiting bodies of *Hericium coralloides*, which belong to five metabolites along with sterol, unsaturated fatty acid ester, diterpenoid, unsaturated fatty alcohol, and triterpene. The ^{1}H, and ^{13}C NMR spectral data of compounds 1-3 revealed the presence of various functional groups (Figure 6.2).

Compound 1 was isolated as a white solid. Its molecular formula was determined as $C_{28}H_{44}O$ based on the HR-ESI-MS, revealing a protonated molecular ion and a sodium adduct at *m/z* 397.348 $[M + H]^+$ and 419.3202 $[M + Na]^+$, respectively. The ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.57 (dd, J = 5.7, 2.5 Hz, 1H), 5.38 (dt, J = 5.5, 2.7

Hz, 1H), 5.25–5.14 (m, 2H), 3.64 (tt, J = 11.3, 4.3 Hz, 1H), 2.47 (ddd, J = 14.3, 4.8, 2.4 Hz, 1H), 2.28 (ddq, J = 14.0, 11.5, 2.3 Hz, 1H), 2.10–1.93 (m, 3H), 1.93–1.82 (m, 4H), 1.60 (s, 5H), 1.54–1.43 (m, 3H), 1.41–1.22 (m, 6H), 1.03 (d, J = 6.6 Hz, 3H), 0.94 (s, 3H), 0.92 (d, J = 6.8 Hz, 3H), 0.85–0.80 (m, 6H), 0.63 (s, 3H). The ^{13}C NMR (125 MHz, $CDCl_3$): δ 141.52, 139.93, 135.72, 132.12, 119.74, 116.43, 70.62, 55.88, 54.71, 46.39, 42.98, 42.97, 40.94, 40.57, 39.23, 38.52, 37.18, 33.24, 32.14, 28.44, 23.14, 21.25, 20.10, 17.75, 16.43, 12.20. Based on these ^{1}H and ^{13}C NMR spectral data, compound 1 was identified as (22E)-ergosta-7,9,22-trien-3*β*-ol compared to previously reported data (Chen et al., 2017).

Compound 2 was isolated as a green-brown oil. Its molecular formula was established as $C_{35}H_{56}O_5$ determined based on the HR-ESI-MS *m/z* 555.4064 $[M + H]^-$, indicating eight degrees of unsaturation. The ^{1}H NMR (500 MHz, $CDCl_3$): δ 12.36 (d, J = 4.1 Hz, 1H), 10.11 (d, J = 4.2 Hz, 1H), 6.53 (d, J = 4.2 Hz, 1H), 5.41–5.28 (m, 5H), 5.17 (t, J = 7.0 Hz, 1H), 5.09–5.03 (m, 1H), 3.92 (d, J = 3.8 Hz, 4H), 3.34 (d, J = 7.0 Hz, 2H), 2.77 (t, J = 6.5 Hz, 1H), 2.34 (t, J = 7.5 Hz, 2H), 2.04 (ddt, J = 18.0, 13.0, 6.8 Hz, 5H), 1.99–1.90 (m, 2H), 1.77 (d, J = 4.4 Hz, 3H), 1.71–1.54 (m, 14H), 1.38–1.20 (m, 31H), 0.92–0.80 (m, 5H), 0.01 (s, 1H). The ^{13}C NMR (125 MHz, $CDCl_3$): δ 193.49, 173.63, 173.58, 163.88, 163.29, 138.81, 138.80, 136.16, 131.63, 130.63, 130.42, 130.11, 128.47, 128.30, 124.75, 121.60, 118.48, 113.28, 106.01, 63.38, 56.30, 40.17, 34.64, 34.62, 32.33, 32.30, 31.92, 30.17, 30.10, 30.06, 30.04, 29.97, 29.84, 29.76, 29.75, 29.72, 29.62, 29.53, 29.51, 29.48, 27.62, 27.60, 27.57, 27.55, 27.15, 27.08, 26.06, 26.03, 25.28, 25.25, 23.09, 22.97, 21.76, 18.05, 16.50, 14.52, 14.47. Based on these ^{1}H and ^{13}C NMR spectral data, compound 2 was identified as hericene A compared to previously reported data (Chen et al., 2017).

Compound 3 was isolated as a white solid. Its molecular formula as $C_{28}H_{44}O$ was determined based on the HR-ESI-MS unravelling a protonated molecular ion and a sodium adduct at *m/z* 397.348 $[M + H]^+$ and 419.3202 $[M + Na]^+$, respectively, indicating seven degrees of unsaturation. The ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.50 (d, J = 8.5 Hz, 1H), 6.29 (d, J = 8.5 Hz, 0H), 6.24 (d, J = 8.5 Hz, 1H), 5.44–5.10 (m, 3H), 4.03–3.90 (m, 1H), 3.86 (s, 0H), 2.17 (s, 18H), 2.15–2.06 (m, 1H), 2.05–1.80 (m, 7H), 1.79–1.66 (m, 2H), 1.66–1.38 (m, 14H), 1.38–1.14 (m, 7H), 1.09 (s, 0H), 1.00 (dd, J = 6.6, 2.2 Hz, 3H), 0.94–0.85 (m, 7H), 0.85–0.77 (m, 10H), 0.74 (s, 0H), -0.00 (s, 2H).

The ^{13}C NMR (125 MHz, $CDCl_3$): δ 207.17, 135.55, 135.34, 132.45, 130.89, 82.30, 79.57, 77.41, 77.16, 76.91, 66.61, 56.34, 51.82, 51.22, 44.70, 42.91, 39.88, 39.48, 37.11, 37.07, 34.83, 33.21, 31.08, 30.26, 28.79, 23.54, 21.02, 20.77, 20.09, 19.78, 18.32, 17.70, 13.01. Based on these ^{1}H and ^{13}C NMR spectral data, compound 3 was identified as ergosterol, which is consistent with previously published data for ergosterol (Chen et al., 2020; Kang et al., 2003).

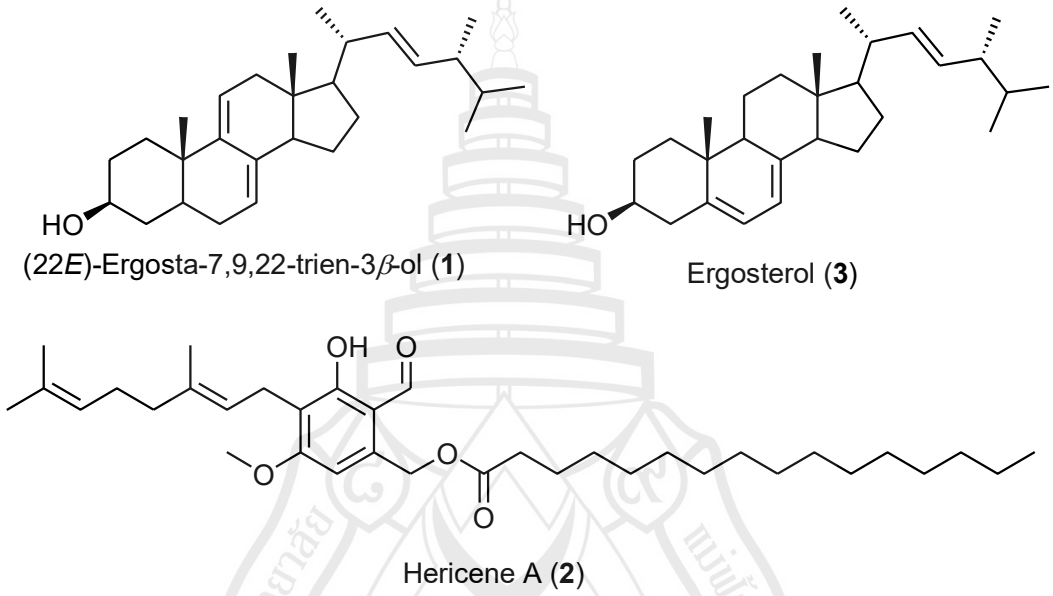


Figure 6.2 Structure of (22E)-ergosta-7,9,22-trien-3b-ol (1), hericene A (2), and ergosterol (3).

6.3.2 Screening of the Biological Activity of the *Hericium erinaceus* and *H. corealloides*

6.3.2.1 Antimicrobial activity

6.3.2.1.1 Agar disc diffusion

The antimicrobial activities of four *Hericium erinaceus* and *H. coralloides* extracts were determined by the agar disc diffusion method against 20 isolates that included 19 bacteria and one yeast. Disk diffusion tests, extracts showed determined inhibition effects against *A. baumannii*, *B. cereus*, *B. subtilis*, *E. faecalis*, *E. faecium*, *M. luteus*, *P. acnes*, *P. mirabilis*, *S. typhimurium*, *S. flexneri*, *S. aureus*, *S. epidermidis*, *S. mutans*, *S. pyogenes*, and *V. cholera* and inhibited yeast growth. The crude extract of *H. erinaceus* (MFLUCC 21–0019) showed a larger diameter of the

inhibition zone with *S. mutans* (31 mm), *P. acnes* (30 mm), *S. pyogenes* (29 mm), and *S. aureus* (25 mm), respectively. While other crude extracts gave an inhibition zone of less than 20 mm. Furthermore, all extracts did not have an effect on *K. pneumoniae*, *P. aeruginosa*, and *S. typhi* (Table 6.1).

6.3.2.1.2 Minimum inhibitory concentration (MIC), and Minimal bactericidal concentration (MBC) or minimum fungicidal concentration (MFC)

The water extracts, ethyl acetate extract, and methyl alcohol showed stronger antimicrobial activity, respectively. In particular, the MIC values between 10 and 0.625 mg/mL against *A. baumannii*, *B. cereus*, *B. subtilis*, *E. faecium*, *E. coli*, *M. luteus*, *S. aureus*, *S. epidermidis*, *S. flexneri*, *S. mutans*, *S. pyogenes*, *S. typhimurium*, *V. cholera*, and *C. albicans*. Although the MBC or MFB values range from 10 to 1.25 mg/mL against *A. baumannii*, *B. cereus*, *B. subtilis*, *E. coli*, *M. luteus*, *S. aureus*, *S. epidermidis*, *S. flexneri*, *S. mutans*, *S. pyogenes*, *S. typhimurium*, *V. cholera,* and *C. albicans* (Table 6.2). However, *E. faecalis*, *P. acnes,* and *P. mirabilis* did not have an effect on the MIC and MBC test. However, the MBC values of all crude extracts did not have an effect on *S. epidermidis*. (Table 6.3).

Table 6.1 Antimicrobial activity of *Hericium* in different solvents

Test Organism	Diameter of the inhibition zone (mm)																	
																Positive control		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**a**	**b**	**c**
A. baumannii	-	-	7	-	-	15	6	-	-	-	-		-	-	-	46	34	27
B. cereus	15	11	11	12	11	18	11	-	-	9	-	-	11	-	-	19	38	22
B. subtilis	7	7	16	7	-	19	9	-	9	-	-	-	8	-	-	41	36	29
E. coli	-	-	-	-	-	-	-	-	-	-	-	11	-	-	-	29	27	14
E. faecalis	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	43	30	26
E. faecium	-	-	-	-	-	11	8	7	8	7	-	-	-	-	7	41	29	26
K. pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	28	11
M. luteus	8	-	-	-	-	-	8	-	-	8	7	-	11	-	-	56	49	41
P. acnes	-	-	-	-	-	30	-	-	-	-	-	7	-	-	-	75	48	50
P. aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19	34	14
P. mirabilis	-	-	-	-	-	8	-	-	-	-	-	-	-	8	-	33	33	7
S. aureus	7	-	21	7	7	25	10	-	10	-	-	-	7	-	-	46	33	23
S. epidermidis	-	-	-	-	-	9	7	-	-	-	-	11	-	-	-	31	41	26
S. flexneri	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	8	29	18
S. mutans	-	-	17	-	-	31	11	-	-	-	-	-	-	-	-	64	38	35
S. pyogenes	10	-	26	-	9	29	8	7	18	-	-	-	-	-	-	58	39	35
S. typhi	-	-	-	-	-	-	-	-	-	-	-	19	-	-	-	11	33	11
S. typhimurium	-	-	-	-	-	11	7	7	7	7	7	-	-	-	-	38	38	16
V. cholera	-	-	-	-	-	6	-	-	-	9	-	-	-	-	7	19	25	9
C. albicans	8	-	15	7	-	16	8	-	9	8	-	-	9	-	-	34	41	27

Note (-) = No inhibition, 1 = MFLUCC 21–0018 EAEs, 2 = MFLUCC 21–0018 MeOHEs,3 = MFLUCC 21–0018 Wes, 4 = MFLUCC 21–0019 EAEs, 5 = MFLUCC 21–0019 MeOHEs, 6 = MFLUCC 21–0019 Wes, 7 = MFLUCC 21–0020 EAEs, 8 = MFLUCC 21–0020 MeOHEs, 9 = MFLUCC 21–0020 Wes, 10 = MFLUCC 21–0021 EAEs, 11= MFLUCC 21–0021 MeOHEs, 12 = MFLUCC 21–0021 WEs, 13 = MFLUCC 21–0050 EAEs, 14= MFLUCC 21–0050 MeOHEs, 15 = MFLUCC 21–0050 WEs, a = Ampicillin, b = Gentamycin, c = Vancomycin.

Table 6.2 The minimum inhibitory concentration (MIC) of the *Hericium* extracts

	MIC (µg/mL)														
Test Organism	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
A. baumannii	-	-	1280	-	-	1280	160	-	-	-	-	-	-	-	-
B. cereus	-	-	-	-	-	-	160	-	-	1280	-	-	160	-	-
B. subtilis	160	-	-	-	-	-	640	-	-	-	-	-	320	-	-
E. faecium	-	-	-	-	-	-	1280	-	-	640	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1280
M. luteus	320	-	-	1280	1280	-	320	-	-	640	-	-	320	-	-
S. aureus	160	-	-	1280	1280	-	80	-	-	-	-	-	320	-	-
S. epidermidis	-	-	-	-	-	-	1280	-	-	-	-	-	-	-	-
S. flexneri	-	-	-	-	-	-	-	-	-	160	-	-	-	-	-
S. mutans	-	-	1280	-	-	1280	640	-	-	-	-	-	-	-	-
S. pyogenes	80	-	-	-	320	1280	320	1280	-	-	-	1280	-	-	-
S. typhimurium	-	-	-	-	-	1280	-	-	1280	1280	-	-	-	-	-
V. cholera	-	-	-	-	-	-	-	-	-	1280	-	-	-	-	1280
C. albicans	80	-	1280	-	-	1280	160	-	1280	1280	-	160	1280	-	-

Note (-) = No inhibition, 1 = MFLUCC 21–0018 EAEs, 2 = MFLUCC 21–0018 MeOHEs,3 = MFLUCC 21–0018 Wes, 4 = MFLUCC 21–0019 EAEs, 5 = MFLUCC 21–0019 MeOHEs, 6 = MFLUCC 21–0019 Wes, 7 = MFLUCC 21–0020 EAEs, 8 = MFLUCC 21–0020 MeOHEs, 9 = MFLUCC 21–0020 Wes, 10 = MFLUCC 21–0021 EAEs, 11= MFLUCC 21–0021 MeOHEs, 12 = MFLUCC 21–0021 WEs, 13 = MFLUCC 21–0050 EAEs, 14= MFLUCC 21–0050 MeOHEs, 15 = MFLUCC 21–0050 WEs.

Table 6.3 The minimal bactericidal concentration (MBC) of the *Hericium* extracts

Test Organism	MBC (µg/mL)														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
A. baumannii	-	-	1280	-	-	1280	160	-	-	-	-	-	-	-	-
B. cereus	-	-	-	-	-	-	160	-	-	1280	-	-	160	-	-
B. subtilis	320	-	-	-	-	-	1280	-	-	-	-	-	320	-	-
E. faecium	-	-	-	-	-	-	>1280	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	>1280
M. luteus	640	-	-	-	1280	-	640	-	-	640	-	-	1280	-	-
S. aureus	>1280	-	-	-	-	-	-	-	-	-	-	-	320	-	-
S. flexneri	-	-	-	-	-	-	-	-	-	160	-	-	-	-	-
S. mutans	-	-	1280	-	-	1280	640	-	-	-	-	-	-	-	-
S. pyogenes	80	-	-	-	640	-	320	1280	-	-	-	1280	-	-	-
S. typhimurium	-	-	-	-	-	1280	-	-	1280	1280	-	-	-	-	-
V. cholera	-	-	-	-	-	-	-	-	-	1280	-	-	-	-	1280
C. albicans	320	-	-	-	-	1280	160	-	1280	1280	-	160	>1280	-	-

Note (-) = No inhibition, 1 = MFLUCC 21–0018 EAEs, 2 = MFLUCC 21–0018 MeOHEs,3 = MFLUCC 21–0018 Wes, 4 = MFLUCC 21–0019 EAEs, 5 = MFLUCC 21–0019 MeOHEs, 6 = MFLUCC 21–0019 Wes, 7 = MFLUCC 21–0020 EAEs, 8 = MFLUCC 21–0020 MeOHEs, 9 = MFLUCC 21–0020 Wes, 10 = MFLUCC 21–0021 EAEs, 11= MFLUCC 21–0021 MeOHEs, 12 = MFLUCC 21–0021 WEs, 13 = MFLUCC 21–0050 EAEs, 14= MFLUCC 21–0050 MeOHEs, 15 = MFLUCC 21–0050 WEs.

6.3.2.2 Antioxidant activity

Antioxidant activity of *Hericium erinaceus* and *H. coralloides* extracts obtained from mycelium. All crude extracts of *Hericium* strains were observed at a concentration of 1 mg/mL. MFLUCC 21–0018 (MeOHEs) showed a higher potential to eliminate free radicals. The ability of *Hericium* to scavenge DPPH radicals is shown in Table 6.4.

Table 6.4 Antioxidant activity of *Hericium* extracts

Isolate	**DPPH activity (IC_{50}, mg/mL)**
MFLUCC 21–0018 (EAEs)	175.08 ± 1.9
MFLUCC 21–0018 (MeOHEs)	003.48 ± 0.4
MFLUCC 21–0018 (EWEs)	159.06 ± 3.9
MFLUCC 21–0019 (EAEs)	067.13 ± 3.5
MFLUCC 21–0019 (MeOHEs)	045.19 ± 4.3
MFLUCC 21–0019 (EWEs)	074.99 ± 4.2
MFLUCC 21–0020 (EAEs)	182.07 ± 4.0
MFLUCC 21–0020 (MeOHEs)	018.41 ± 5.2
MFLUCC 21–0020 (EWEs)	166.12 ± 1.8
MFLUCC 21–0021 (EAEs)	046.63 ± 3.8
MFLUCC 21–0021 (MeOHEs)	207.07 ± 14.3
MFLUCC 21–0021 (EWEs)	390.41 ± 5.3
MFLUCC 21–0050 (EAEs)	460.86 ± 4.3
MFLUCC 21–0050 (MeOHEs)	354.11 ± 13.0
MFLUCC 21–0050(EWEs)	37.75 ± 1.6
Standard: Ascorbic acid	8.67 ± 0.3

6.3.2.3 Cytotoxicity test

The toxicity of the crude extract of *H. erinaceus* and *H. coralloides* in L929 (Mouse fibroblast cell line) and KB 3–1 (Human cancer cell lines). The crude extracts had detectable cytotoxic effects in two cell lines (Table 6.5).

Table 6.5 The cytotoxic activity of *Hericium* extracts

Compound crude extract	**IC50 µg/mL**	
	KB 3–1	**L929**
MFLUCC 21–0018 (EAEs)	-	-
MFLUCC 21–0018 (MeOHEs)	-	-
MFLUCC 21–0018 (EWEs)	-	-
MFLUCC 21–0019 (EAEs)	-	-
MFLUCC 21–0019 (MeOHEs)	-	-
MFLUCC 21–0019 (EWEs)	-	-
MFLUCC 21–0020 (EAEs)	-	-
MFLUCC 21–0020 (MeOHEs)	-	-
MFLUCC 21–0020 (EWEs)	-	-
MFLUCC 21–0021 (EAEs)	-	-
MFLUCC 21–0021 (MeOHEs)	-	-
MFLUCC 21–0021 (EWEs)	-	-
MFLUCC 21–0050 (EAEs)	-	-
MFLUCC 21–0050 (MeOHEs)	-	-
MFLUCC 21–0050 (EWEs)	-	-
Positive control	0.000070	0.00070

6.3.2.4 Anticancer test

6.3.2.4.1 Exhibits cytotoxic effect on cancer cells

All compounds (1-3) were initially investigated for their cytotoxicity against three cancer cell lines: A549, Huh-7, and SW-480. These cells were treated with various concentrations of compounds 1-3 for 72 h, and cell viability was then assessed using the MTT assay. The results showed that compounds 1-3 exhibited varying levels of cytotoxicity on cancer cells (Figure 6.3). The IC_{50} values are summarized in Table 6.6.

Among these compounds, ergosterol (3) had the strongest cytotoxic activity against A549 (IC_{50} = 4.61 ± 0.04 µg/mL), Huh-7 (IC_{50} = 4.267 ± 0.04 µg/mL), and SW-480 (IC_{50} = 5.209 ± 0.05 µg/mL). Therefore, ergosterol (3) is the best for exhibiting cytotoxic effect and is selected for further investigation into its effect on other malignant properties.

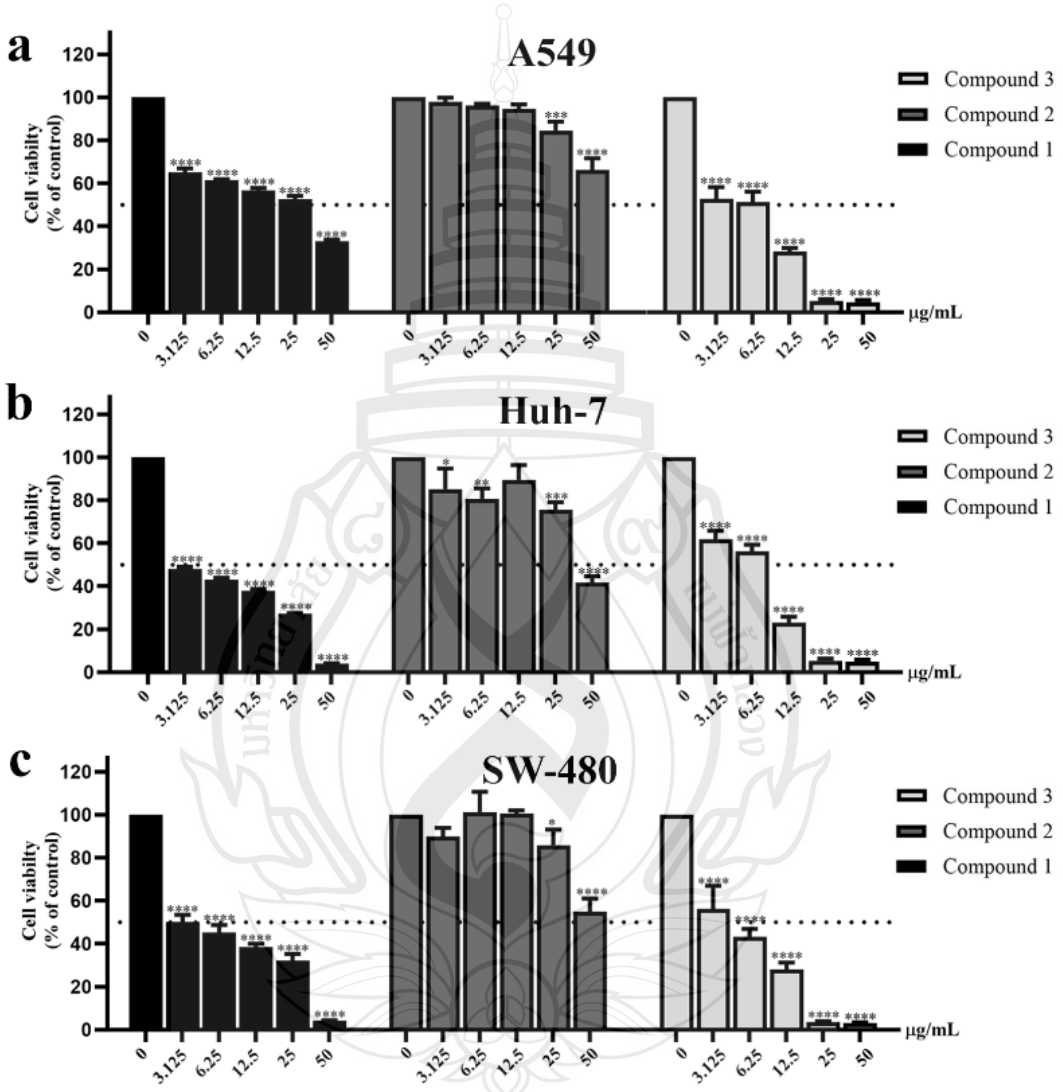


Figure 6.3 Cytotoxicity of compounds isolated from *H. coralloides* against (a) A549, (b) Huh-7, and (c) SW-480 cells. Bar graphs represent the mean ± SD (n=3). * $p < 0.03$, ** $p < 0.02$, *** $p < 0.002$, **** $p < 0.0001$ vs. untreated control (0 µg/mL)

Table 6.6 IC_{50} of compound 1-3 of *Hericium coralloides* on various cancer cell lines

	IC_{50} value ± SD		
Cell lines	**A549**	**Huh-7**	**SW-480**
(22*E*)-ergosta-7,9,22-trien-3*β*-ol (**1**)	16.39 ± 0.06	26.54 ± 0.17	23.87 ± 0.22
Hericene A (**2**)	16.27 ± 0.06	8.14 ± 0.03	17.74 ± 0.08
Ergosterol (**3**)	4.61 ± 0.04	5.209 ± 0.05	4.267 ± 0.04

6.3.2.4.2 Effects of ergosterol (3) on colony formation of cancer cells

From a primary tumor, when a cell gets disconnected, it usually adheres to a distant part to grow and metastasize. The clonogenic formation assay is one of the standard protocols to study adhesion (Thakor et al., 2017). Compounds 4 induced a significant decrease in colony formation of A549, Huh-7, and SW-480 cell lines in concentrations of 0–10 μg/ml. As depicted in Figure 6.4, the compound 3 displayed the lowest colony formation units, further substantiating the antiproliferative effect of compound 3 of *H. coralloides*.

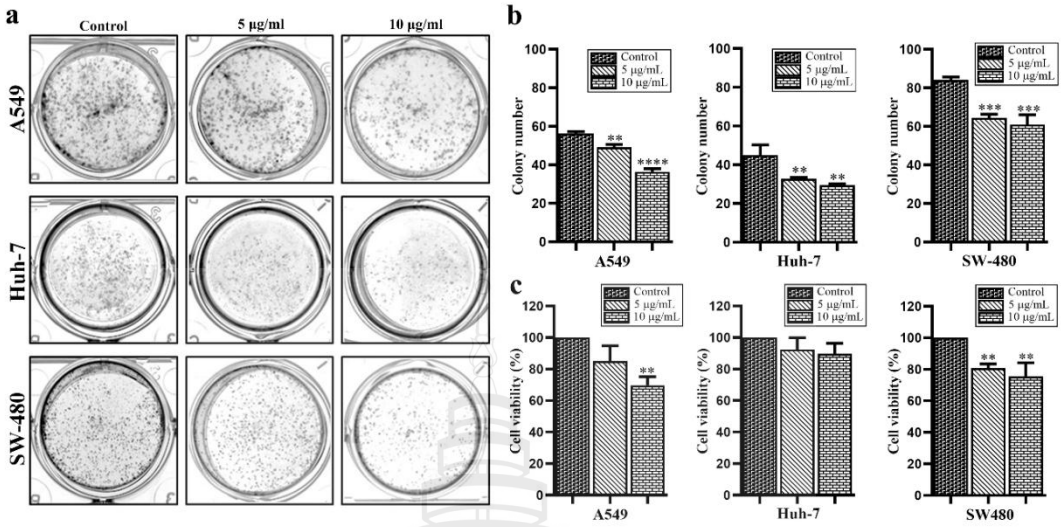


Figure 6.4 Effects of ergosterol (3) on colony formation of cancer cells. (a) Representative images of the formed colonies of A549, Huh7, and SW-480 cells after treatment with varying concentrations of ergosterol (3). (b) Number of the formed colonies and (c) cell viability of A549, Huh7, and SW-480 cells after a 7-day culture period. Bar graphs represent the mean ± SD (n=3). * $p < 0.03$, ** $p < 0.02$, *** $p < 0.002$, **** $p < 0.0001$ vs. untreated control (0 µg/mL)

6.3.2.4.3 Effect of ergosterol (3) on the migration of cancer cells

The effects of ergosterol (3) on the migration ability of cancer cell lines were evaluated using a wound healing-scratch assay. The results demonstrated that ergosterol (3) significantly inhibited the migration of A549 and Huh-7 cells. However, no significant effect was observed for SW-480 cells (Figure 6.5). These findings suggest that ergosterol (3) selectively suppresses the migratory potential of certain cancer cell lines.

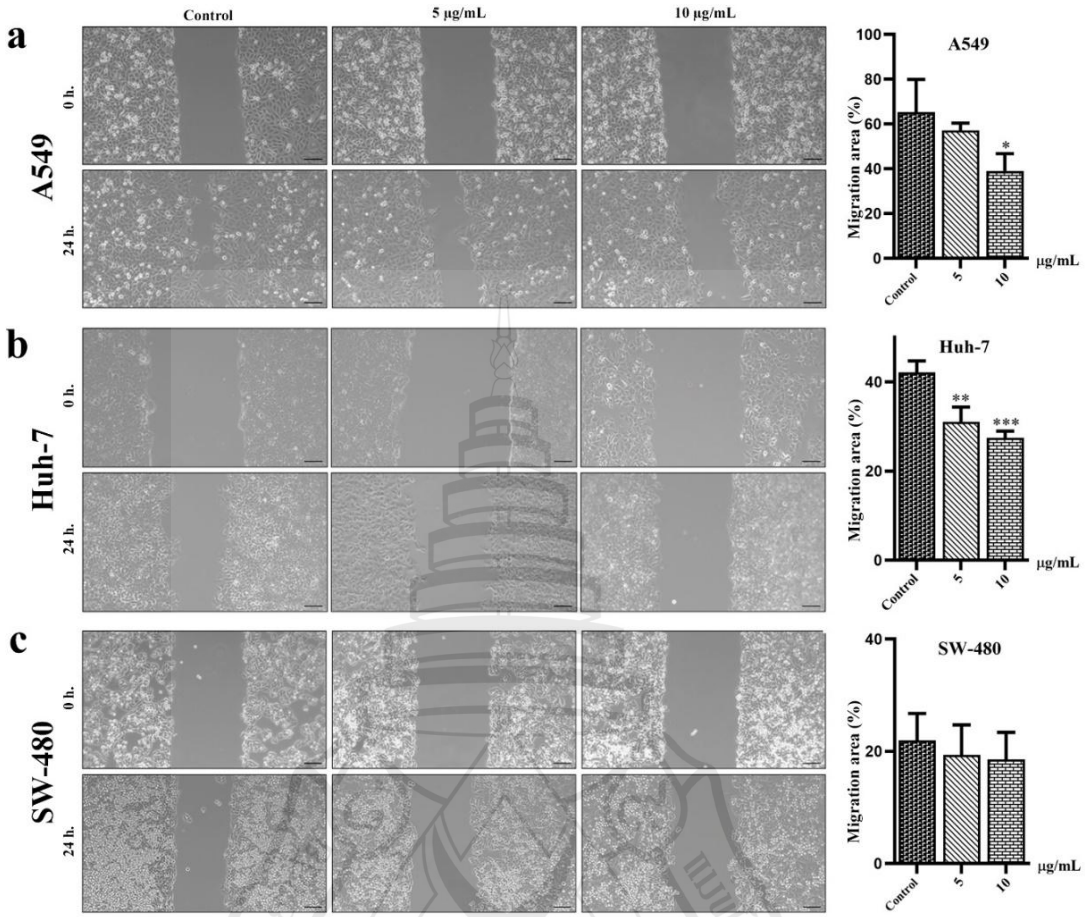


Figure 6.5 Effect of ergosterol (3) on the migration of cancer cells. Representative images of the wound area at 0 hours and 24 hours post-scratching, along with the percentage of migration area for (a) A549, (b) Huh-7, and (c) SW-480 cells after treatment with varying concentrations of ergosterol (4). Scale bar = 100 µm. Bar graphs represent the mean ± SD (n=3). * $p < 0.03$, ** $p < 0.02$, *** $p < 0.002$, **** $p < 0.0001$ vs. untreated control (0 µg/mL)

6.4 Discussions

The crude extracts of three solvents showed high inhibition for the water extract, the ethyl acetate extract, and the methyl alcohol extract, respectively, which is consistent with Khan et al. (2013), who reported that crude water provides high components of arabinose, glucose, and rhamnose. According to Younis et al. (2015), *P. ostreatus* water extracts contain antimicrobial compounds that are effective against a

wide spectrum of bacteria and fungi. Furthermore, the agar disc diffusion test showed a determined inhibition effect against bacteria with a range of 7–30 mm, which is closely/ aligned with Kwak et al. (2015), who presented the antibacterial activity of different extracts of *H. erinaceus,* 2.4–26.5 mm. Agar disc diffusion showed a determined inhibition effect against with a range 7-11 mm, were closely/agrees with Kwak (2018), who presented the antibacterial activity of different extracts *H. coralloides* with 11 mm. However, the MIC and MBC/MFC showed antibacterial activity of four strains of *H. erinaceus* inhibitory against gram-positive bacteria and yeast that were similar to Chanthaphon et al. (2008) and Kim et al. (2012).

According to Kim et al. (2019), the antioxidant activity of the extract of *H. erinaceus* was higher than the standard test, except that the crude MeOH extract of MFLUCC 21–018 showed an antioxidant value of 3.48 ± 0.4 mg / mL and showed stronger antioxidant activity and greater capacity to remove free radicals. According to Hou et al. (2015), the IC_{50} value of *Hericium erinaceus* oligosaccharide (HEO-A) for the removal of DPPH radicals was 12.5 mg / mL, indicating that HEO-A had a significant effect on the removal of DPPH radicals, especially when used in large amounts. The inhibitory ability of four strains of *H. erinaceus* isolated from ethyl acetate and water was found to be higher than that of HEO-A in this study. The results of antioxidant activity were consistent with Gąsecka et al. (2020), who found a significant reduction in the capacity of *H. erinaceus* to scavenge DPPH radicals.

For cytotoxic activity, all crude *Hericium erinaceus* and *Hericium coralloides* extracts have no activity on KB 3–1 and L929. A previous study did not show detectable cytotoxic effects in RAW 264.7 cells from the extract of *H. erinaceus* (Kim et al., 2012). Lee et al. (2015) reported that cerebroside E from *H. erinaceus* had no cytotoxic effect on human umbilical vein vascular endothelial cells, and 11 known compounds isolated from the fruiting body of *H. erinaceus* presented weak cytotoxicity against A549 and HeLa cell lines (Wang et al., 2015).

However, *Hericium coralloides* produces several phytochemical groups such as polysaccharides, triterpenoids, phenolic compounds, sterols, fatty acids, amino acids, and other compounds (Jianzhao et al., 2024). Previous phytochemical investigations on *H. coralloides* have revealed the presence of alkaloids such as corallocin B-D (Wittstein et al., 2016), benzofurans such as corallocin A, hydrospirobenzofuran, spirobenzofuran,

sesquibenzopyran (Wittstein et al., 2016; Kim et al., 2018), phenols such as coralcuparene (Kim et al., 2018), sterols such as ergosterol (Lazur et al., 2024), terpenoids such as erinacine (Koga et al., 2024), and polysaccharides (Zhang et al., 2024). This study identified three known compounds along with (22E)-ergosta-7,9,22-trien-3b-ol (1), hericene A (2), and ergosterol (3) (Figure 4). The ^{1}H, and ^{13}C NMR spectral data of the compound revealed the presence of various functional groups.

In addition, cancer is a worldwide public health problem and is the second leading cause of death (Cao et al., 2021). Approximately 1.8 million deaths were associated with lung cancer (18.7%), followed by colorectal (9.3%) and liver (7.8%) cancer deaths in 2022 (Bray et al., 2024). Several natural substances from both terrestrial and marine sources have been found to have significant anticancer properties. Rangsinth et al. (2023) reported that the pharmacological properties of ergosterol included anticancer properties as well. The potential anticancer properties of ergosterol have been investigated (Table 6.7).

Table 6.7 The potential anticancer properties of ergosterol

Cancer	**Cell lines**	**Dose/ Concentration**	**Activity and Mechanism**	**Reference**
Bladder	N-butyl-N-(4-hydroxybutyl) nitrosamine	15 µg/kg/day for 3 weeks	Regulate inflammation-related signaling and suppress androgen signaling pathways.	Ikarashi et al. (2020)
		A diet that contains ergosterol 0.01–0.1% for 25 weeks	Inhibiting androgen signaling.	Yazawa et al. (2020)
Breast	normal breast cell lines, MCF10A, MCF12A	1–50 µM	Inhibit carcinogen-induced ROS, ERK activation, DNA oxidation, and DNA damage.	Pluchino et al. (2015)

Table 6.7 (continued)

Cancer	Cell lines	Dose/ Concentration	Activity and Mechanism	Reference
Breast	MCF7	IC_{50} = 112.65 µM	Trigger S-phase cell cycle arrest and promote apoptosis.	Subbiah and Abplanalp (2003), Hao et al. (2017)
	MDA-MB-231	IC_{50} = 20.3 µM	Inhibited the proliferation	Chen et al. (2017)
Esophageal	Eca-109	IC_{50} = 74.50 µM	Inhibited the proliferation	Lin et al. (2022)
Gastric	SGC-7901	IC_{50} = 41.60 µM	Inhibited the proliferation and induced apoptosis	Lin et al. (2022)
Liver	Hep3B and HepJ5	IC_{50} of Hep3B and HepJ5 cells from 14.54–6.66 µM and 18.65–4.07 µM, respectively, when combined with amphotericin B (5–25 µM)	Elevate ROS and LC3-II levels.	Lin et al. (2017)
	HepG2	IC_{50} = 22.1 µM	Inhibited the proliferation	Chen et al. (2017)
	Bel-7402	IC_{50} = 69.55 µM	Inhibited the proliferation	Lin et al. (2022)
Lung	A549	IC_{50} = 42.22 µM	Inhibited the proliferation	Lin et al. (2022)

Table 6.7 (continued)

Cancer	Cell lines	Dose/ Concentration	Activity and Mechanism	Reference
Prostrate	LNCaP human prostate adenocarcinoma cell	IC_{50} = 14.68 ± 1.01 μM	Inhibit the androgen receptor	Muñoz-Fonseca et al. (2021)
Sarcoma	Sarcoma 180-bearing mice	400 and 800 mg/kg for 20 days	N/A	Takaku et al. (2001)
Tumor	Matrigel-induced neovascularization in C57BL/6 mice	5, 10 and 20 mg/kg for 5 days	Inhibit angiogenesis	Takaku et al. (2001)

However, the effects of bioactive compounds in *H. coralloides* on cancer cells remain under investigation. Wittstein et al. (2016) reported that corallocin A-C, a class of terpenoids featuring an isoindolinone structure, has varying levels of antiproliferative activity. Among them, only corallocin B exhibited antiproliferative activity against MCF-7 (breast cancer), KB-3-1 (cervical cancer), and HUVEC (umbilical vein endothelial) cell lines. In contrast, Sum et al. (2024) found that corallocin D-E showed no significant effect on the proliferation of A431 (epidermoid carcinoma), SKOV-3 (ovarian cancer), PC3 (prostate cancer), and A549 (lung cancer) cell lines.

In this study, hericene A (2) and ergosterol (3) from *H. coralloides* demonstrated stronger antiproliferative activity against A549 cells compared to those reported by Sum et al. (2024), as well as Huh-7 (liver cancer) and SW-480 (colorectal cancer) cells. In addition, ergosterol (3) showed potent antiproliferative activity against A549 cells, with IC_{50} value of 4.6 μg/mL (11.60 μM), which is more effective than the findings of Lin et al. (2022), who reported an IC_{50} of 42.22 μM. Notably, ergosterol (3) also reduced colony formation and inhibited migration of A549, Huh-7, and SW-480 cells, suggesting the therapeutic potential of *H. coralloides* and its bioactive compounds, particularly ergosterol (3), in cancer treatment.

In addition, all three compounds exhibit minimal toxicity toward normal mammalian cell lines or immune cells at typical concentrations. A previous study showed that ergosterol exhibited $IC_{50} > 100$ μM in normal cell lines, indicating low cytotoxicity at doses effective in cancer models (Rangsinth et al., 2023). Ergosterol and (22E)-ergosta-7,9,22-trien-3*β*-ol exhibit low to no cytotoxicity in human normal cell lines at concentrations effective in cancer cells (Huang et al., 2021); quantitative toxicity data for hericene A are currently limited. In comparison, the doxorubicin had IC_{50} values that varied with assay conditions, around 2.2 μM at 12 h exposure (Maryam et al., 2017), and around 70 μM (0.07 mM) after 72 h in A549 lung cancer cells (Kashkin et al., 2010). This places doxorubicin's potency than of ergosterol (3). Although IC_{50} values for doxorubicin in SW-480 colorectal cancer are not directly reported, they are generally within the micromolar range.

CHAPTER 7

TAXONOMY AND PHYLOGENY OF *SCLERODERMA*

7.1 Introduction to *Scleroderma*

Ectomycorrhizal fungi are fungi that have a symbiotic relationship between the fungus and the feeder roots of many tree species in forests that benefit both parties (Charya & Garg, 2019), worldwide distribution in temperature and tropical regions (Corrales et al., 2018). The ectomycorrhizal fungi include the genera of *Scleroderma* (Ouatiki et al., 2022). Persoon (1801) created the genus *Scleroderma*, which was later updated by Guzmán (1970), who proposed infrageneric classifications such as sections *Sclerangium*, *Scleroderma*, and *Macrospora* based on morphology. Modern phylogenetic studies have further refined these groupings (de Menezes Filho et al., 2022; Wu et al., 2023). From ancient morphological-based classifications through more recent genetic investigations that have improved our understanding of species connections (Guzmán, 1970; Persoon, 1801; Wu et al., 2023), the history of *Scleroderma* demonstrates the evolving dynamics of fungal taxonomy. The evolution from *Lycoperdon verrucosum* to *S. verrucosum* is an illustration of the proof that scientific classification continues to evolve (Persoon, 1801).

There are 206 *Scleroderma* species records in the Index Fungorum (https://www.indexfungorum.org/Names/Names.asp, accessed on March 24, 2025), 76 species in Species Fungorum (https://www.speciesfungorum.org/Names/Names.asp, accessed on March 24, 2025). The genus belongs to the family Sclerodermataceae, order Boletales of the class Agaricomycetes (Binder & Hibbett, 2006).

Scleroderma species have traditionally been segregated according to the morphology of the basidiomata and the surface of the peridium, the type of dehiscence of the peridium, the color of the gleba, and the ornamentation of their basidiospores (de Menezes Filho et al., 2022). Moreover, the thickness and scaliness of the peridium, the

presence of stalks of the basidiome, and the form of the stipes form have been occasionally used to distinguish between species in the genus (Raut et al., 2020).

Although regions like Europe and America are relatively well studied for the taxonomy of *Scleroderma*, data are lacking particularly for tropical Africa (Sanon et al., 1997), and Asia (Farmer & Sylvia, 1998; Sims et al., 1999). In Thailand, 11 species have been reported namely, *S. areolatum*, *S. aurantium*, *S. bovista*, *S. cepa*, *S. citrinum*, *S. dictyosporum*, *S. flavidum*, *S. lycoperdoides*, *S. polyrhizum*, *S. sinnamariense*, *S. verrucosum* (Chandrasrikul, 2011) and *S. suthepense* (Kumla et al., 2013). *Scleroderma* species have been used to stimulate tree seeding growth as in nurseries and in the field (Ouatiki et al., 2022). Based on the physical traits of their basidiomes and basidiospores, several species of *Scleroderma* have been proposed (Guzmán, 1970; Guzmán et al., 2004). Recent studies (Ranjith et al., 2021; Raut et al., 2020) examined inter- and intraspecific variation among *Scleroderma* species using molecular analysis.

In this study, we aimed to describe the three new species and a new record of Scleroderma from Northern Thailand, based on macro- and microscopic characteristics and molecular phylogenetic methods.

7.2 Materials and Methods

7.2.1 Sample Collection and Morphological Identification

Fresh basidiome of *Scleroderma* were collected during the rainy season. The basidiomes were collecting in the top soil, near the tree. They were collected from May to June 2019 in Chiang Mai and Chiang Rai province. The specimens were characterized morphologically as described in the Section 3.1.3 of Chapter 3.

7.2.2 Phylogenetic Analysis

DNA extraction, primers used for PCR conditions and sequencing were in Table 3.2 in Section 3.2 of Chapter 3.

Phylogenetic analysis and sequence divergence were used to determine the recently discovered taxon related to other *Scleroderma* species. In the phylogenetic analysis, *Scleroderma* species from broader geographic regions were taken into

consideration to compare with our taxon. Bioedit Sequence Alignment Editor version 7.0.9.0 was used to verify ITS, LSU, RPB2, and TEF1-α sequences, and SeqMan was used to assemble the sequence (DNAstar, Madison, WI, USA). The National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/genbank/) was blasted against each sequence using the Basic Local Alignment Search Tool (BLAST) to ensure that it was from the correct genus and not contaminated, and to identify the closest matches.

A GenBank BLAST search was performed to check for similarity between the newly created sequences, 82 sequences of several *Scleroderma* species were obtained from various regions, which include our group (Table 7.1), *Pisolithus aurantioscabrosus* is closely related to *Scleroderma* (Martin et al., 2002; Wilson et al., 2012), was chosen as an outgroup. Then aligned using the MAFFT v. 7.11 online tool (https://mafft.cbrc.jp/alignment/software/ (accessed on 23 December 2023), all sequence alignments were trimmed separately using TrimAl to eliminate ambiguously aligned positions (Capella-Gutiérrez et al., 2009).

Each character set's length were 78 collections and 676 characters (including gaps) from ITS, 20 collections and 1402 characters from LSU, 20 collections and 1120 characters from RPB2, and 20 collections and 1035 characters from TEF1-α. The final dataset comprised 82 collections and 4233 characters from ITS+LSU+RPB2+TEF1-α. After checking for supported conflicts (BS < 70%) between single-gene maximum likelihood (ML) phylogenies, a concatenated four-locus data set was assembled.

Phylogenetic analysis using Maximum Likelihood (ML) was performed, followed by manual adjustments in raxmlGUI 2.0.13, along with Bayesian analysis, all conducted on the CIPRES Science Gateway version 3.3 web server (Miller et al., 2010), available at https://www.phylo.org/. A mixed-model (partitioned) scheme was employed for both Maximum Likelihood and Bayesian analyses, with the alignment split into four characters sets: ITS1+ITS2, LSU+5.8S, RPB2, and TEF1-α. The best-fit substitution models from jModelTest2 version 2.1.6 (Darriba et al., 2012) on XSEDE were chosen for Bayesian analysis. The models according to the best data were HKY+G for ITS, GTR+I+G for nrLSU, HKY+G for RPB2, and SYM+I+G for TEF1-α. Four independent runs, each with four chains, were performed for 1,000,000 generations,

with sampling occurring every 100 generations. The average standard deviation of the split frequencies at the end of the runs was 0.015009. The burn-in phase (25%) was determined by assessing stationarity in the generation-likelihood plot using Tracer version 1.7.1 (Rambaut et al., 2018). The resulting phylogenetic tree was visualised in Treeview 32 and further edited using Adobe Illustrator CS6.0.

Table 7.1 Phylogenetic analysis list of species, herbarium number, place of origin, and GenBank accession number

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
Pisolithus aurantioscabrosus	AWW297	Malaysia	EU718112	EU718146	FJ536648	FJ536681	Wilson et al. (2011)
Scleroderma areolatum	AWW211	USA	EU718115	EU718149	FJ536651	FJ536683	Wilson et al. (2011)
S. areolatum	PBM2208	Australia	N/A	EU718150	FJ536652	FJ536684	Wilson et al. (2011)
S. areolatum	TNS: F-82295	Japan	OQ025272	OQ025269	N/A	N/A	Kasuya et al. (2023)
S. areolatum	Kasuya-B4422	Japan	OQ025273	OQ025270	N/A	N/A	Kasuya et al. (2023)
S. areolatum	O3C_4	USA	JX030282	N/A	N/A	N/A	Bzdyk et al. (2018)
S. areolatum	23	Spain	MN684210	N/A	N/A	N/A	-
S. areolatum	Db-K	-	MH040288	N/A	N/A	N/A	Bzdyk et al. (2018)

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. areolatum	Bk-N	-	MH040301	N/A	N/A	N/A	Bzdyk et al. (2018)
S. *bermudense*	BZ3961	Belize	EU718118	DQ644137	FJ536654	FJ536686	Wilson et al. (2011)
S. bermudense	EUA09	-	OQ351725	N/A	N/A	N/A	Bullaín-Galardis et al. (2024)
S. bermudense	SUA03	-	OQ351729	N/A	N/A	N/A	Wilson et al. (2011)
S. bovista	MCA242	USA	EU718117	DQ644138	FJ536653	FJ536685	Wilson et al. (2011)
S. citrinum	AWW212	USA	EU718119	EU718151	FJ536655	FJ536687	Wilson et al. (2011)
S. citrinum	F-PRL5772	USA	GQ166907	N/A	N/A	N/A	Zhang et al. (2013)

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. citrinum	K (M) 17485	England	EU784413	N/A	N/A	N/A	Zhang et al. (2013)
S. citrinum	CITSCL1	USA	FM213344	N/A	N/A	N/A	Zhang et al. (2013)
S. citrinum	K (M) 53906	England	EU784414	N/A	N/A	N/A	Zhang et al. (2013)
S. columnare	CUB: Microbiology KHS3	Thailand	AB459512	N/A	N/A	N/A	Ruankaew Disyatat et al. (2016)
S. columnare	Scl1	Thailand	AB854700	N/A	N/A	N/A	Kaewgrajang et al. (2023)
S. columnare	CUB: Microbiology KHS10	Thailand	AB459519	N/A	N/A	N/A	Ruankaew Disyatat et al. (2016)
S. columnare	MFLU25-0110 (DG150)	Thailand	N/A	N/A	PX137624	PX126632	This study

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. columnare	MFLU25-0111 (DG153)	Thailand	PV444716	N/A	PX137625	PX126633	This study
S. dictyosporum	IR250	Burkina Faso	FJ840444	N/A	N/A	N/A	Sanon et al. (2009)
S. dictyosporum	IR408	Burkina Faso	FJ840445	N/A	N/A	N/A	Sanon et al. (2009)
S. meridionale	AWW218	USA	EU718121	EU718152	FJ536656	FJ536688	Wilson et al. (2011)
S. mcalpinei	OSC 24605	-	EU718122	DQ682999	FJ536657	N/A	Wilson et al. (2011)
S. nitidum	UFRN: Fungos 2034	Brazil	KU759904	KU759903	N/A	N/A	Raut et al. (2020)
S. nitidum	UFRN: Fungos 2219	Brazil	KU759908	N/A	N/A	N/A	Raut et al. (2020)
S. polyrhizum	AWW216	USA	EU718123	EU718153	FJ536658	FJ536689	Wilson et al. (2011)

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. polyrhizum	MA: Fungi-39352	Spain	MT270662	N/A	N/A	N/A	Ortiz-Rivero et al. (2021)
S. separatum	Ge5394	China	OQ554975	N/A	N/A	N/A	Wu et al. (2023)
S. separatum	ZLR31	China	OQ554974	N/A	N/A	N/A	Wu et al. (2023)
S. separatum	Ge4148	China	OQ554973	N/A	N/A	N/A	Wu et al. (2023)
S. separatum	MFLU 19-1347 (NTF066)	Thailand	PV444715	PV446742	N/A	N/A	This study
S. sinnamariense	SINSCL3 (SCLN)	Thailand	FM213358	N/A	N/A	N/A	Phosri et al. (2009)
S. sinnamariense	150728-29	China	MH513635	N/A	N/A	N/A	Zhang et al. (2020)
S. sinnamariense	SINSCL1 (SCLK4)	Thailand	FM213356	N/A	N/A	N/A	Phosri et al. (2009)

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. sinnamariense	SINSCL6 (SCLD1)	Thailand	FM213361	N/A	N/A	N/A	Phosri et al. (2009)
S. sinnamariense	SINSCL4 (SCLY5)	Thailand	FM213359	N/A	N/A	N/A	Phosri et al. (2009)
S. sinnamariense	CMU53:210-2	Thailand	HQ687222	N/A	N/A	N/A	Kumla et al. (2014)
S. sinnamariense	rpr-355	-	MW374160	N/A	N/A	N/A	Wang et al. (2022)
S. sinnamariense	HKAS122471	China: Yunnan	ON794312	N/A	N/A	N/A	Wang et al. (2022)
S. sinnamariense	SINSCL5 (SC1)	Thailand	FM213360	N/A	N/A	N/A	Phosri et al. (2009)
S. sinnamariense	DX2021-8-2	-	OL351633	N/A	N/A	N/A	-
S. sinnamariense	MFLU25-0112 (DG157)	Thailand	N/A	N/A	PX137633	PX207694	This study

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. sinnamariense	MFLU25-0113 (DG158)	Thailand	PV444717	N/A	PX137632	PX207695	This study
S. sinnamariense	MFLU25-0114 (DG159)	Thailand	PV444718	N/A	PX137631	PX137634	This study
S. sinnamariense	MFLU25-0115 (DG160)	Thailand	PV444719	N/A	PX137630	PX126634	This study
S. sinnamariense	MFLU 19-1647 (MO-DG020)	Thailand	PV444720	N/A	PX137626	PX126635	This study
S. sinnamariense	MFLU 19-1648 (MO-DG021)	Thailand	PV444721	N/A	N/A	N/A	This study
S. sinnamariense	MFLU 19-1649 (MO-DG022)	Thailand	PV444722	PV446743	PX137627	PX126636	This study
S. sinnamariense	MFLU 19-1650 (MO-DG023)	Thailand	PV444723	N/A	PX137628	N/A	This study
S. sinnamariense	MFLU 19-1652 (MO-DG034)	Thailand	PV444724	N/A	N/A	N/A	This study

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. sinnamariense	MFLU 19-1653 (MO-DG035)	Thailand	PV444725	N/A	PX137629	N/A	This study
S. sinnamariense	MFLU 19-1341 (NTF012)	Thailand	PV444726	N/A	N/A	N/A	This study
Scleroderma sp.	AWW260	Malaysia	EU718124	EU718155	FJ536660	FJ536691	Wilson et al. (2011)
Scleroderma sp.	AB96	Cameroon	KR819100	N/A	N/A	N/A	Michaëlla Ebenye et al. (2017)
Scleroderma sp.	YAAS-L5455	-	MT876542	N/A	N/A	N/A	-
Scleroderma sp.	YAAS-L5449	-	MT876541	N/A	N/A	N/A	-
Scleroderma sp.	SL2085	Singapore	OR354966	N/A	N/A	N/A	-
Scleroderma sp.	LH35	Malaysia	GQ268582	N/A	N/A	N/A	Peay et al. (2010)

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
Scleroderma sp.	ECM26-SERS	-	DQ146385	N/A	N/A	N/A	Yuwa-Amornpitak et al. (2006)
S. suthepense	AWW311	Malaysia	EU718125	EU718156	FJ536661	FJ536692	Wilson et al. (2011)
S. suthepense	CMU:55-SC2	Thailand	NR_132871	N/A	N/A	N/A	Kumla et al. (2013)
S. suthepense	JH-2016-0727-052	China	MH513626	N/A	N/A	N/A	Zhang et al. (2020)
S. suthepense	180508-08	China	MH513625	N/A	N/A	N/A	Zhang et al. (2020)
S. suthepense	MFLU25-0109 (DG146)	Thailand	N/A	N/A	N/A	PX126630	This study
S. suthepense	MFLU 19-1344 (NTF053)	Thailand	PV444727	N/A	N/A	N/A	This study

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. xanthochroum	AWW254	Malaysia	EU718126	EU718154	N/A	N/A	Wilson et al. (2011)
S. yunnanense	HKAS80386	-	MW493647	MW493703	N/A	N/A	Kasuya et al. (2023)
S. yunnanense	PERTH-7604645	China	MT270651	N/A	N/A	N/A	Ortiz-Rivero et al. (2021)
S. yunnanense	TNS: F-82294	Japan	OQ025271	OQ025268	N/A	N/A	Kasuya et al. (2023)
S. yunnanense	MFLU 19-1348 (NTF090)	Thailand	PV444728	N/A	N/A	N/A	This study
S. yunnanense	MFLU 19-1517 (DMSL-DG005)	Thailand	PV444729	N/A	N/A	PV749898	This study
uncultured fungus	ASV_419	-	LR993736	N/A	N/A	N/A	-
uncultured fungus	ASV_1014	-	LR994331	N/A	N/A	N/A	-

Table 7.1 (continued)

Species	Voucher no.	Country	GenBank accession no.				Reference
			ITS	LSU	RPB2	TEF1-α	
S. longistipes	MFLU 19-1655 (DG109)	Thailand	PV444712	PV446740	PX126607	PX121227	This study
S. longistipes	MFLU 19-1656 (DG110)	Thailand	PV444713	PV446741	PX121228	N/A	This study
S. magnisporum	MFLU 19-1345 (NTF062)	Thailand	PV444714	N/A	N/A	N/A	This study
S. microcarpum	MFLU 19-1349 (DG002)	Thailand	PV436898	N/A	N/A	N/A	This study

Note N/A: not available; new species is described in bold black

7.3 Results & Discussion

Based on *Scleroderma* species, they were formerly separated by morphology with basidiome size, shape change depending on soil and environment, and basidiospore morphology (Gonkhom et al., 2025; Kumla et al., 2013; Sanon et al., 2009; Watling, 2006). In this study, *Scleroderma* sp. nov. drawing on macromorphological and micromorphological traits, together with the phylogenetic analysis of the ITS, LSU, RPB2 and TEF1-α genes, three new species of *Scleroderma longistipes*, *Scleroderma magnisporum*, and *Scleroderma microcarpum* from northern Thailand were described; it enhanced our understanding of the diversityvariety of species of *Scleroderma*. Macroscopically, the size, color, and type of dehiscence of the basidiome, the color, and thickness of the peridium are crucial traits for identifying Scleroderma species. Microscopically, the size, shape, and ornamentation of the basidiospores are employed to differentiate species of this genus. Its basidiome are similar in character and/or in size, also basidiospores are similar in size to those of *S. seperatum*, *S. dictyosporum*, and *S. hypogaeum* (Cortez et al., 2011; Guzmán et al., 2013; Sims et al., 1995). Our ITS, LSU, RPB2, and TEF1-α sequence analysis clearly separates the species from the other reticulate spored Scleroderma species in the section.

7.3.1 Phylogenetic Analysis

The combined dataset of four genes was composed of 4233 bp (including the gaps), 676 bp for ITS, 1402 bp for nrLSU, 1120 bp for RPB2, 1035 bp for TEF1-α. The best RaxML phylogram, with a final likelihood value of -22414.937168, is presented. The matrix had 1563 distinct alignment patterns with 68.28% undetermined characters or gaps. The estimated base frequencies were as follows: A = 0.233328, C = 0.255317, G = 0.274489, and T = 0.236866; substitution rates, AC = 1.330307, AG = 4.855565, AT = 1.388503, CG = 1.501262, CT = 8.964382, and GT = 1.000000; gamma distribution shape parameter, α = 0.929050. The phylogram topology derived from the Bayesian analysis was similar to that derived from the ML analysis. Bootstrap values of ML ≥ 70% and bootstrap values of BI ≥ 0.90 are indicated in Figure 7.1.

Phylogenetic trees inferred from ML and MrBayes analyzes resulted in similar tree topologies; therefore, only the ML tree ITS + LSU + RPB2 + TEF1-α is shown with both the maximum likelihood bootstrap (BS) values and the Bayesian posterior probabilities (PP). In the phylogram, *Scleroderma longistipes* (MFLU 19-1655 and MFLU 19-1655) was closely related to *Scleroderma* sp. (Uncultured ectomycorrhiza ECM26 SERS, LH35 from Malaysia, and SL2085 from Singapore) with high statistical support as 100% BS/1.00 PP, 81% BS/0.93PP, and 90% BS/1.00 PP respectively. *Scleroderma microcarpum* (MFLU 19-1347) was closely related to the uncultured fungus ASV 1014 and ASV 419 with statistical support as 70% BS/0.94 PP. *Scleroderma magnisporum* MFLU 19-1345) was closely related to Scleroderma sp. YAAS L5449 and YAAS L5455 with statistical support as 74% BS/0.97 PP.

However, the species most closely related in the phylogenic tree of *Scleroderma microcarpum* (MFLU 19-1347) to *S. dictyosporum* (Voucher IR250) showed that the genetic distance between the ITS sequence of *Scleroderma microcarpum* (MFLU 19-1347) and *S. dictyosporum* was 11.81% (65/570). While *Scleroderma magnisporum* (MFLU 19-1345) and *Scleroderma microcarpum* (MFLU 19-1347) were 21.34% (95/445), supporting the distinction of the species. This result was similar to previous molecular phylogenetic studies that strongly support the distinction of *Scleroderma* species as genetically discrete lineages (Nouhra et al., 2012; Phosri et al., 2009; Wu et al., 2023). However, *Scleroderma* species are found in the temperate zone, tropical and subtropical regions, which may be related to the higher diversity of Scleroderma or ectomycorrhizal fungi in these climatic zones (Brundrett et al., 2005; Jeffries, 1999; Pradhan et al., 2011; Wu et al., 2023).

In addition, eleven species of *Scleroderma* have been recorded according to morphology (*S. areolatum*, *S. bovista*, *S. cepa*, *S. citrinum*, *S. dictyosporum*, *S. flavidum*, *S. lycoperdoides*, *S. polyrhizum*, *S. sinnamariense*, *S. verrucosum*, and *S. suthepense*) have been recorded in Thailand based on morphology (Chandrasrikul, 2011; Kumla et al., 2013; Gonkhom et al., 2025). Phylogenetic analysis confirmed the placement of *Scleroderma separatum* based on the ITS and LSU regions (Figure 7.1). The species of *S. separatum* exhibit similar shapes and sizes of their basidiome and basidiospores (Wu et al., 2023). Phylogenetic analysis based on ITS and LSU sequences

facilitated the confirmation of the species we analyzed, which has been officially recorded in Thailand.

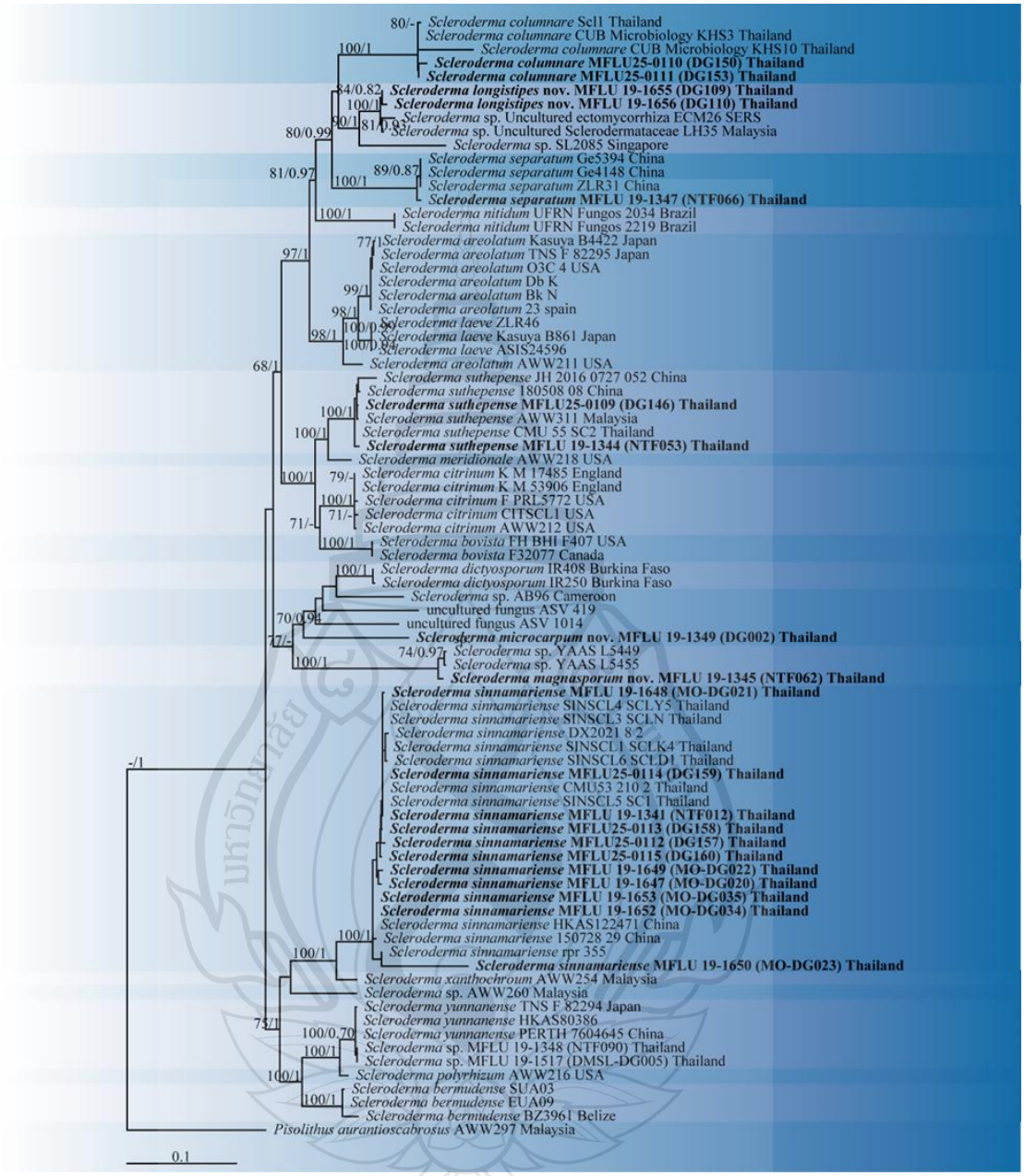


Figure 7.1 Phylogenetic tree obtained from the maximum likelihood analysis of Scleroderma species. Maximum Likelihood tree obtained from the alignment of ITS, LSU, RPB2, and TEF1-α sequence. The Bootstrap consensus tree was inferred from 1000 replicates. *Pisolithus aurantioscabrosus* was included as an outgroup

7.3.2 Taxonomy

7.3.2.1 *Scleroderma longistipes* Gonkhom, Sysouph. & Thongkl. sp. nov.

Index Fungorum number: IF903880

(Figure 7.2)

Diagnosis: Epigeous brown to burnt umber basidiomata with long stipe, rubbery pale brown peridium, hyaline to yellow brown hyphae in exoperidium, hyaline hyphae in eendoperidium, globose dark brown basidiospores with echinulate or spinose ornamentation.

Holotype: THAILAND. Chiang Rai Province, Mueang Chiang Rai District, Mae Fah Luang University campus, 04 June 2019, collected by Didsanutda Gonkhom, DG109 (MFLU 19-1655).

Etymology: The species name (l*ongistipes*) refers to the long stipe of the basidiomata.

Description: Basidiomata epigeous, 28–35 mm in diam., 42–60 mm in high, club shaped, with globular peridial head; with cracked to squamulose surface, brown (6E5) background when young, with fawn (7E4) to brown (6E5) or burnt umber (6F6) squamular cracks upon luteous background, hard skin, tough when mature. Stipe sub-cylindric, fat, with small irregular cracks at the top of strip, 35–45 × 10–13 mm, white background, covered with brown (6E5) fibrillose squamules. Rhizomorphs more aggregated at the base, white, branched and narrowing towards the base. Context white in peridium and stipe, turned dull red to greyish red 98B4-5) when cut. Peridium up to 5 mm wide when fresh, rubbery in consistence, pale brown (6D5).

Peridium layer formed by simple-septate hyphae. Exoperdium slightly thickened walls, composed of interwoven to ramified and superimposed hyphae, hyaline to yellow brown, 2.9–3.8 µm diam. Endoperdium thick, composed of interwoven hyphae, hyaline, 4.3–7.2 µm diam. Clamp connections present on endoperidium hyphae. Gleba brownish grey (9E2), greyish brown (9E3), or oxblood red (9E7) to dark brown (9F4-7), compact, and powdery when mature. Basidiospores (n= 50) globose, echinulate, dark brown in KOH, (13.4–)14.5–17.2(19.5) µm in diam., with brown spinose ornamentation (2.6–5.9 µm high). Basidia not seen

Habitat and distribution: Caespitose or fasciculated on soil, epigeous, in northern Thailand.

Additional specimens examined: THAILAND, Chiang Rai Province, Mueang Chiang Rai District, Mae Fah Luang University campus, 04 June 2019, collected by Didsanutda Gonkhom, DG110 (MFLU 19-1656).

Notes: *Scleroderma longistipes* is characterized by a larger brown basidiomata with a longer stipe, turned from dull red to greyish red when touched. The basidiospores are globose with longer brown spines. According to the phylogenetic analysis (Figure 7.1), Thai specimens of *Scleroderma longistipes* is identical an unknown species from Malaysia (LH35) (Peay et al., 2010), and an unknown species from Thailand (ECM26-SERS) (Yuwa-Amornpitak et al., 2006). However, these two specimens taxa were only identified as Sclerodermataceae species. *Scleroderma separatum* Z.W. Ge, R. Wu & L.R. Zhou, a species originally described from Yunnan, southwestern China, is a species to relate with *S. longistipes* by having stipe. However, *Scleroderma longistipes* appears closely related to *S. seperatum*, has smaller basidiomata, greenish yellow background, slender stipe (5–30 × 3–5 mm), smaller basidiospores (4.5–8.5 µm), and shorter basidiospore spines (1.2–2.5 µm) (Wu et al., 2023). Furthermore, *S. separatum* is related to *S. longistipes* by phylogenetic analysis with low bootstrap support (BS) (Figure 7.1).

Scleroderma longistipes is also similar to *S. columnare* Berk. & Broome. However, *S. columnare* has stellate dehiscence at upper part of basidiomata in old specimens (London, 1911), and *S. columnare* is also related to *S. longistipes* with low BS (Figure 7.1). Additionally, *S. nitidum* Berk. is morphologically similar to *S. longistipes*, shares the stipitate morphology but differs in having a glossy peridium, smaller basidiospores (5-7 µm) with denser, shorter spines, and no color change when bruised (Guzmán, 1970).

Figure 7.2 *Scleroderma longistipes* (MFLU 19-1655, holotype). A, B. basidiomata. C. scale on peridium surface. D. cut side of peridium of MFLU 19-1655. E, F. basidiomata. G. scale on peridium surface. H. cut side of peridium of Scleroderma longistipes (MFLU 19-1656). I. exoperidial hyphae. J. endoperidial hyphae. K. clamped hyphae of endoperidium. L–Q. Basidiospore. Scale bars: A, B, E, F = 10 mm, C, D, G, H = 5 mm, I, J = 50 µm, K = 20 µm, L–Q = 10 µm.

7.3.2.2 *Scleroderma microcarpum* Gonkhom, Sysouph. & Thongkl. sp. nov.

Index Fungorum number: IF903881

(Figure 7.3)

Diagnosis: Different from the similar species *S. dictyosporum* in having smaller basidiomata and larger basidiospores.

Holotype: THAILAND, Chiang Mai Province, Mae On District, 9 October 2019, collected by Didsanutda Gonkhom, DG002 (MFLU 19-1347).

Etymology: The species name "*microcarpum*" refers to the small size of the basidiomata.

Description: Basidiomata epigeous, 20–21 mm in diam. 10–20 mm in high, circular when young, depressed at maturity; surface smooth and with small scales when young stage, light yellow (5A4-5), covered with brown (7E6-8) squamules when mature. Stipe sessile or short pseudostipitate (less than 4 mm long). Rhizomorphs at the base, white, branched, narrowing towards base. Context up to 2 mm thick, light yellow (5A4-5).

Peridium layer formed by simple hyphae septate, hyaline to yellow brown. Exoperdium 6.9–9.3 µm in diam., with clamp connections. Endoperidium 4.2–8.2 µm in diam., with or without with clamp connections. Gleba white – yellowish white (3A1-2), compact, and powdery when mature. Basidiospores (n= 50) globose to subglobose, echinulate, grayish brown in KOH, (3.97–)6.07–6.51(–8.15) × (8.24–)10.77–11.61(–13.33) µm in diam. Basidia not observed.

Habitat and distribution: Caespitose or fasciculated on soil, epigeous, in northern Thailand.

Known distribution: Northern Thailand.

Note: *Scleroderma microcarpum* is characterized by small basidiomata with a smooth and small scale on the surface and larger basidiospores and globose echinulate. *Scleroderma microcarpum* is phylo-genetically related to *S. dictyosporum* Pat. With low BS (Figures 7.1). Both species have echinulate basidiospores. However, *S. dictyosporum* has a larger basidiomata (24–28 mm in diam) (Sanon et al., 2009), and has smaller basidiospores (7–9 µm wide) (Patouillard, 1896; Sanon et al., 2009).

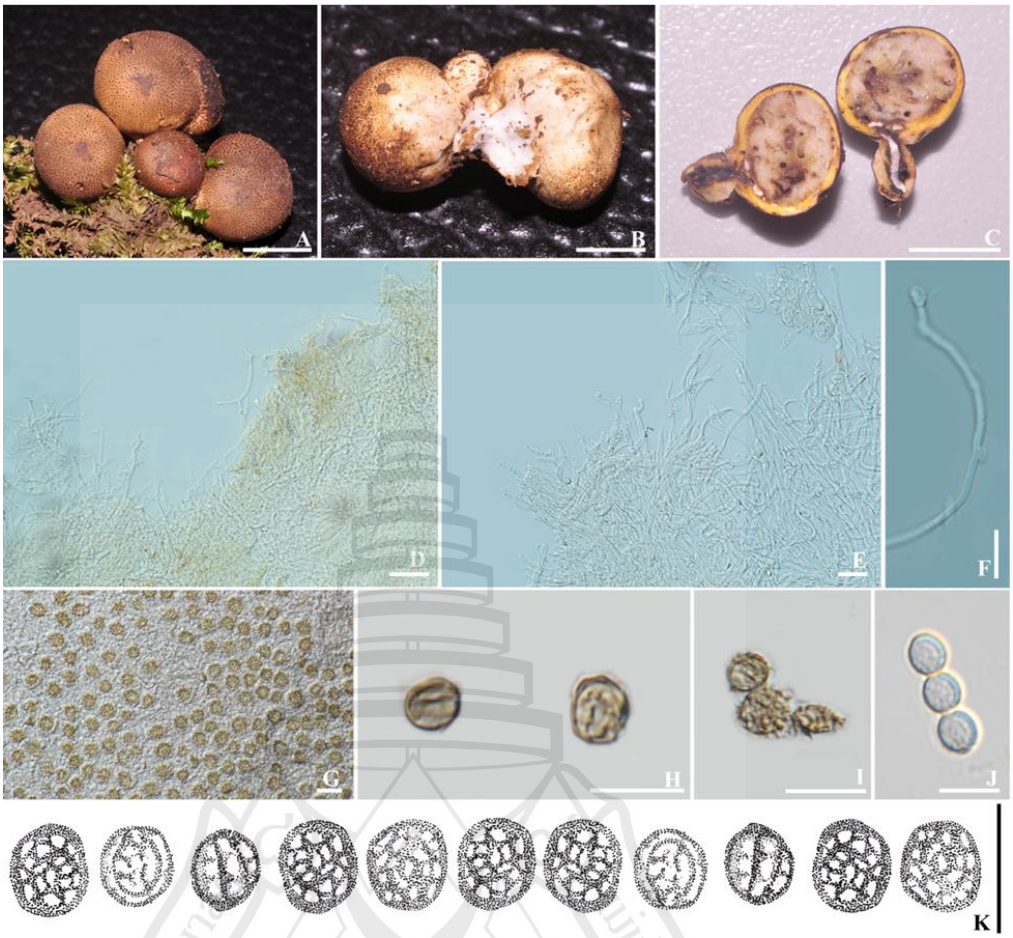


Figure 7.3 *Scleroderma microcarpum* (MFLU 19-1347, holotype). A, B. basidiomata. C. context of peridium. D. exoperidial hyphae. E. endoperidial hyphae. F. clamped hyphae of endoperidium. G–K. Basidiospore. Scale bars: A–C = 10 mm, D. = 20 µm, E = 50 µm, F = 20 µm, G–K = 10 µm.

7.3.2.3 *Scleroderma magnisporum* Gonkhom, Sysouph. & Thongkl. sp. nov.

Index Fungorum number: IF903882

(Figures 7.4)

Diagnosis: Epigeous basidiomata with irregular club shape, smooth to slightly cracked to squamulose peridial head, brown to burnt umber, sessile or short pseudostipitate stipe, white pale brown context, with hyphae simple-septate in both nndoperidium and exoperidium, dark brown globose to subglobose basidiospores with crowded spines.

Holotype: THAILAND, Chiang Rai Province, Mueang Chiang Rai District, 16 July 2010, collected by Naritsada Thongklang, NTF062 (MFLU 19-1345).

Etymology: The species name "*magnisporum*" refers to its larger basidiospores.

Description: Basidiomata epigeous, 35 mm in high, 22–34 mm in diam., club shaped, with an irregularly globular peridial head; surface smooth, slightly cracked to squamulose, brown (6E5) to burnt umber (6F6), on pale orange (5A3) background. Sessile or short pseudostipitate (10 mm high), brown (6E5). Context thick, up to 5 mm wide. Exoperidium composed of hyphae simple-septate, interwoven, hyaline to yellow, 4.1–5.8 µm in diam. Endoperidium layer formed by hyphae simple-septate, with slightly thickened walls, interwoven, hyaline, 5.8–9.6 µm in diam., and hyphae from the endoperidium toward the gleba pale yellow (4A3), and black in the mature gleba. Clamp connections present on endoperidium hyphae. Basidiospores (n=50) globose to subglobose, echinulate with crowded curved spines, dark brown in KOH, (7.67–)12.42–8.42(–13.46) × (10.33–)14.53–11.42(–15.50) µm including ornamentation. Basidia not seen.

Habitat and distribution: Solitary on soil, epigeous, in northern Thailand.

Known distribution: Northern Thailand.Note: *Scleroderma magnisporum* is characterized by a smooth, slightly cracked surface and larger basidiospores. The microcharacter of *S. magnisporum* is similar to that of *S. hypogaeum* Zeller. However, *S. hypogaeum*, originally described from Oregon, has a smooth, slightly cracked, or subscaly basidiomebasiodiome, with larger basidiospores up to 22–30 µm diam. (Zeller, 1922; Guzmán, 2013). *S. magnisporum* is phylo-genetically close to *Scleroderma microcarpum* (MFLU 19-1347) in this study (Figure 7.1). Both species have clearly different in their basidiomata size and shapes, the basidiomata of *S. microcarpum* is much smaller than of *S. magnisporum* (7.6–15.5 µm diam.). While *S. yunnanense* shares with *S. magnisporum* a smooth to faintly cracked peridium and large basidiospores (15-20 µm), pale yellow to ochre peridium (Guzmán, 1970; Zhang et al., 2013).

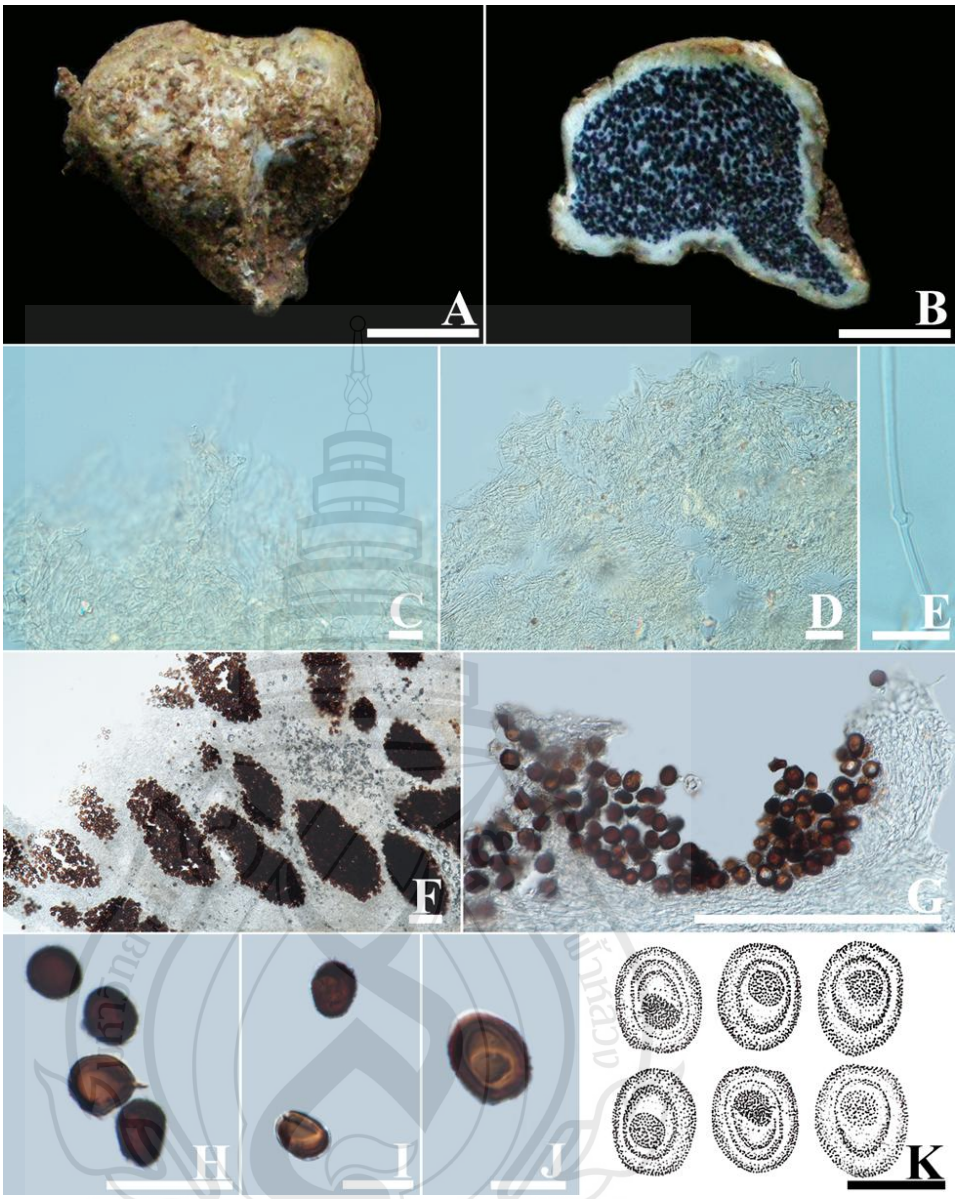


Figure 7.4 *Scleroderma magnisporum* (MFLU 19-1345, holotype). A. basidiomata. B. cut side of peridium. C. exoperidial hyphae. D. endoperidial hyphae. E. clamped hyphae of endoperidium. F−K. Basidiospore. Scale bars: A, B = 10 mm. C−E =20 µm, F−G = 50 µm., H = 20 µm., I−K = 10 µm.

7.3.2.3 *Scleroderma separatum* Z.W. Ge, R. Wu & L.R. Zhou.

IndexFungorium number: 847687

(Figure 7.5)

Description: Basidiomata are epigeous, 12–28 mm in diam., 17–45 mm in height, globose, subglobose to irregularly oblate, tan (3D3) to ochraceous–brown (5E2). Peridium is leathery, thin, 0.5–1.0 mm thick when fresh and was becoming much thinner when dry, hay (5C4) to greenish-yellow (3B4) background. Peridium layer formed by hyphae simple-septate, with slightly thickened walls, interwoven, hyaline, exoperidium 2.4−4.4 µm in diam, and endoperidium 4.1–6.0 µm in diam. with clamp connections. Gleba grey (8F1) –dark brown (8F5), compact, and powdery when mature. Stipe is subcylindric, 20–50 mm in length and 5–10 mm in diam., with numerous white rhizomorphs at the base. Basidiospores globose, occasionally subglobose, dark brown in KOH, (12.31–)13.23–14.35 (–16.49) × (11.53–)13.78–14.29(–16.30) µm in diam., including ornamentation (spinose up to 1.7–3.9 µm high), n = 50, coated by crowded curved spines. Basidia not seen.

Habitat and distribution: Caespitose or fasciculated on soil, epigeous, in tropical and temperate regions of China; Thailand.

Specimens examined: THAILAND, Chiang Mai Province, Mae Rim District, Mae Sa, 28 July 2010, MFLU 19-1347 (NTF066).

Note: This based on a single Thai specimen. Thai specimens are considered to be similar with specimens of *S. separatum* from Southwestern China, by having epigeous basidiomata. The molecular analysis also supports the identification (Figure 7.1). The species grows under forest dominated by *Pinus yunnanensis* (Wu et al., 2023). Nonetheless, the samples from Thailand were frequently taken from mixed forests or the litter of *Pinus* trees. These results suggest that this species is found on both *Quercus* and *Pinus*. Since the initial description, this is the second record of the species.

Figure 7.5 *Scleroderma separatum* (MFLU 19-1347) A. basidioma. B. cut side of peridium. C. exoperidial hyphae. D. endoperidial hyphae. E. clamped hyphae of endoperidium. F-L. Basidiospore. Scale bars: A, B = 10 mm. C = 50 µm, D = 20 µm, E = 10 µm, F-L = 20 µm.

Among 82 accessions, including newly described species, the length of the entire ITS, LSU, RPBS, and TEF1-α comprised 4,233 base. The species of *Scleroderma* known from this study include *Scleroderma columnare*, *S. sinnamariense*, and *S. suthepense* (Figure 7.6).

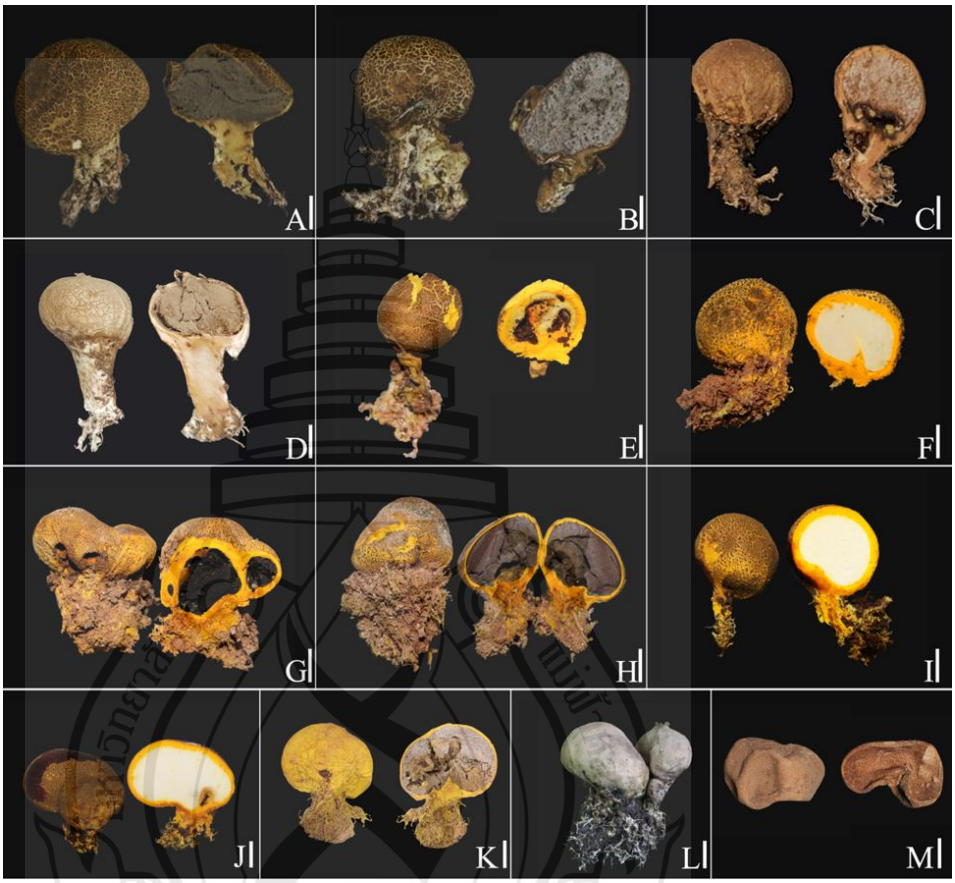


Figure 7.6 The mature basidiomata of *Scleroderma* spp. in this study. A −B. S. columnare. C−K. S. sinnamariense. L−M. S. suthepense. Scale bars = 10 mm

Key to *Scleroderma* species in Thailand

1a. Basidiome sessile or with a short pseudostipe.....	2
1b. Basidiome with a well-developed pseudostipe or stalk-like base.	10
2a. Basidiome globose to subglobose, peridium thick (1-2 mm) ..…	3
2b. Basidiome irregularly shaped, peridium thin (<1 mm) …….....	5
3a. Peridium yellowish to orangish-yellow, smooth to cracked	*S. cepa*

3b. Peridium brown, with distinct warts or scales	4
4a. Peridium covered with tough raised warts, yellow-brown	*S. citrinum*
4b. Peridium cracked, scaly, roughened, brown	*S. bovista*
5a. Peridium thin, leathery, yellowish white	6
5b. Peridium thick, with distinct cracks or subscaly	8
6a. Basidiospores 7-8.5 µm, dark brown, reticulate	*S. dictyosporum*
6b. Basidiospores larger than 8.5 µm, spiny	7
7a. Basidiospores 8-12 µm, round, net-like ridges	*S. verrucosum*
7b. Basidiospores 10-15 µm, slightly roughened texture	*S. columnare*
8a. Peridium smooth, brown, tough, thick (up to 5 mm)	*S. magnisporum*
8b. Peridium cracked or scaly, background yellowish	9
9a. Basidiospores 8.24-13.33 µm, rhizomorphs pale brown	*S. microcarpum*
9b. Basidiospores 12.31-16.49 µm, spines up to 3.9 µm	*S. separatum*
10a. Stipe well-developed, more than 3 cm long	11
10b. Stipe short, less than 3 cm, or absent	13
11a. Stipe sub-cylindric, cracked at the top, basidiospores 13.4-19.5 µm	*S. longistipes*
11b. Stipe short or irregular, basidiospores smaller (<14 µm)	12
12a. Basidiospores 7-12 µm, peridium star-shaped when split	*S. polyrhizum*
12b. Basidiospores 8-13 µm, peridium smooth to scaly	*S. suthepense*
13a. Peridium golden yellow, apex rupturing at maturity	*S. flavidum*
13b. Peridium brown to ochraceous, scaly or roughened	14
14a. Peridium leathery, verrucose, yellowish to lemon-yellow	*S. sinnamariense*
14b. Peridium brownish, smooth to scaly, spore mass dark brown	15
15a. Basidiome surface smooth, spore mass clearly olive	*S. lycoperdoides*
15b. Basidiome rough or scaly, spore mass brown to dark brown	*S. areolatum*

Overall, this study significantly advances our understanding of the diversity and taxonomy of *Scleroderma* species in northern Thailand, describing three new species included *Scleroderma longistipes*, *S. microcarpum*, and *S. magnisporum*. Also reporting a new record, *S. separatum* form Thailand. The research integrates

comprehensive morphological analyses with molecular phylogenetic methods, utilizing sequences from four loci (ITS, LSU, RPB2, and TEF1-α), to confirm the distinctiveness of these taxa. The findings contribute to our understanding of *Scleroderma* and establish the basis for future ecological and evolutionary research on ectomycorrhizal fungi.

CHAPTER 8

CONCLUSIONS

Basidiomycota, with a focus on the Agaricomycetes class, particularly the genera *Hericium* and *Scleroderma.* It highlighted the ecological, economic, and medicinal significance of these fungi, emphasizing their roles as decomposers, symbionts, and sources of bioactive compounds. The research objectives were clearly outlined, aiming to explore cultivation methods, nutritional and medicinal properties, and phylogenetic relationships of selected strains.

The literature review delved into the cultivation methods, health benefits, and economic importance of *Hericium*, showcasing their potential in neuroprotection, anti-inflammatory, and anti-cancer applications. For *Scleroderma*, the review discussed its ecological roles, inedibility or toxicity, and bioactive compounds, while also addressing gaps in taxonomy and cultivation research in Thailand. Synthesized existing knowledge, identifying areas for further investigation and underscoring the need for more studies on *Scleroderma*'s bioactive potential and cultivation techniques.

The methodologies for sample collection, morphological identification, DNA extraction, phylogenetic analysis, and cultivation optimization for *Hericium* and *Scleroderma*. It also outlined protocols for nutritional analysis, secondary metabolite screening, and biological activity assays. The rigorous and systematic approach described here ensures reproducibility and reliability of the research findings. The methodologies provide a clear roadmap for achieving the study's objectives, from laboratory experiments to statistical analyses.

8.1 Genus *Hericium*

This study provides practical guidelines for cultivating *Hericium* in Thailand, highlighting its nutritional value and potential for commercial production.

8.1.1 *Hericium erinaceus*

In this study, the optimal growth conditions of *Hericium erinaceus* strains MFLUCC 21-0018, MFLUCC 21-0019, and MFLUCC 21-0020 showed the most favorable growth on OMYA at a pH range of 4–4.5 at 25 °C. For spawn tests, coir was demonstrated to be optimal. *H. erinaceus* strain MFLUCC 21-0021 had the most favorable growth on CDA and a pH range of 4–5.5 at 25 °C. Coir grains were similarly optimal for the spawn test.

The cultivation of *Hericium erinaceus* provides a high yield of the fruiting body on the main substrate of para-rubber sawdust and cereal grains (red sorghum) with the addition of rice bran, yeast powder, lime (CaO), gypsum ($CaSO_4 \cdot 2H_2O$), and molasses. The short time taken to produce fruiting bodies. High in protein and crude fiber, all *H. erinaceus* strains can be consumed in a healthy diet. The presence of all these results increases interest in researching these mushrooms for potential applications in food and health-related disciplines, such as increasing the mushroom product's use as a meal replacement or medicine.

8.1.2 *Hericium coralloides*

In this study, the optimal growth conditions of *H. coralloides* strain MFLUCC 21-0050 showed favorable growth on MYPA for pH 5.5 at 30 °C and wheat grain for spawn tests. For the carbon and nitrogen sources, the best growth rates for all five strains were obtained using molasses and yeast extract, respectively, and the ratio of media components was 10:1 for the best mycelial growth.

The first successful cultivation of *H. coralloides* in Thailand achieved mature fruiting bodies across three substrate treatments with no statistically significant differences in yield. While all treatments supported growth effectively, substrate treatment 1 comprising para-rubber sawdust supplemented with rice bran and sugar proved to be the most cost-effective option. Therefore, it is recommended as the preferred substrate for the cultivation of *H. coralloides* under similar conditions. Nutritional analysis confirmed *H. coralloides* as a rich source of carbohydrates, protein, and crude fiber.

In addition, the study successfully identified a diverse array of bioactive compounds in *Hericium* species, including *H. erinaceus* and *H. coralloides,* using LC-QTOF-MS/MS analysis. A total of 145 compounds were characterized in *H. erinaceus*

strain MFLUCC 21-0018, 168 in MFLUCC 21-0019, 169 in MFLUCC 21-0020, 170 in MFLUCC 21-0021, and 176 in *H. coralloides* strain MFLUCC 21-0050. These compounds included terpenoids (e.g., erinacines, hericenones), phenolic acids, fatty acids, sterols, and polysaccharides, which are known for their neuroprotective, anti-inflammatory, antioxidant, and anti-cancer properties. The findings highlight the rich chemical diversity of *Hericium* and its potential therapeutic applications. The identified metabolites, such as erinacine A, hericenone B, and corallocin E, underscore the mushroom's significance in promoting cognitive health and combating neurodegenerative diseases. In addition, two novel isoindolinone derivatives, named corallocins D (1) and E (2), were isolated from the fruiting bodies of the basidiomycete *Hericium coralloides* (Winnie et al., 2024).

The bioactive potential of *Hericium erinaceus* and *H. coralloides*, focusing on their antimicrobial, antioxidant, cytotoxic, and anticancer properties. The study identified four bioactive compounds from *H. coralloides*, including ergosterol, which exhibited significant anticancer activity against lung (A549), liver (Huh-7), and colorectal (SW-480) cancer cell lines. The extracts demonstrated notable antimicrobial effects against gram-positive bacteria and yeast, with water and ethyl acetate extracts showing the highest efficacy. Antioxidant activity was also observed, particularly in methanol extracts. While the crude extracts showed no cytotoxicity to normal cells, ergosterol stood out for its potent antiproliferative, anti-migratory, and colony formation inhibitory effects on cancer cells. These findings highlight the therapeutic potential of *Hericium*, particularly ergosterol, as a natural source for developing anticancer and antimicrobial agents. Further research is needed to elucidate the mechanisms of action and optimize extraction methods for clinical applications.

8.2 Genus *Scleroderma*

The taxonomy and phylogeny of *Scleroderma* species, with a focus on specimens collected from Northern Thailand. Through morphological and molecular analyses (ITS, LSU, RPB2, and TEF1-α sequences), three new species, including *Scleroderma longistipes*, *S. microcarpum*, and *S. magnisporum* were identified and characterized. The study also documented the first record of *S. separatum* in Thailand. Phylogenetic analyses provided robust support for the distinctiveness of these species, revealing their evolutionary relationships with other *Scleroderma* taxa.

8.3 Research Advantages

8.3.1 The results of the optimal conditions of media, temperature, pH, types of spawn, carbon and nitrogen source, and carbon and nitrogen ratio for mycelium growth of *Hericium erinaceus* and *H. coralloides* are useful for further study.

8.3.2 This study provided the methodology to cultivate of *Hericium erinaceus* and *H. coralloides* in Thailand.

8.3.3 This study highlights the identification of bioactive compounds from *Hericium erinaceus* and *H. coralloides* through LC-QTOF-MS/MS analysis, offering valuable insight into their chemical profiles.

8.3.4 The results of the screening of the biological activity of *Hericium erinaceus* and *H. coralloide* show the effect on antimicrobial, antioxidant, and cytotoxic activities. The selected bioactive compound shows strong anticancer activity against lung (A549), liver (Huh-7), and colorectal (SW-480) cancer cell lines.

8.3.5 This study provided a checklist of *Scleroderma* in Thailand based on both morphology and phylogeny.

8.4 Future Work

Further experiments with diverse strains of *Hericium* species need to be conducted to improve productivity and biological efficiency. In addition, all of these experiments can be used to develop suitable methods for inducing their mushroom product formation on artificial media composed of agricultural by-products. However, these improved techniques can be used to enhance the mycelial production of *Hericium*, and the fungi may eventually be used for processing food products with high efficacy and compounds that are useful against nervous system diseases. One of the next goals of our studies is to investigate how effectively production of basidiomes can be accomplished. Given the fact that these fungi originate from temperate climate zones and fruit in nature in autumn, it may be necessary to start production at higher altitudes where the temperatures are not so high.

This research provides a foundation for further exploration of *Hericium* in pharmacology and functional foods, emphasizing their role as a valuable natural resource for health benefits. Future studies could focus on isolating these compounds to evaluate their bioactivity and mechanisms of action in detail.

Scleroderma, the findings contribute to the understanding of *Scleroderma* diversity in tropical regions and underscore the importance of integrating morphological and molecular data for accurate species identification. This research expands the known distribution of *Scleroderma* species and provides a foundation for future studies on their ecological roles and potential applications in forestry and medicine. Further investigations should explore the ecological interactions and biotechnological potential of the newly described species.

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APPENDIX A

CHEMICAL REAGENTS

Chemical Reagents used for this study

1) Potassium Hydroxide (KOH): 3-5% aqueous solution (Lagent et al., 1977)

- 3 (-5) g of potassium hydroxide
- 97 (-95) ml of water
- Place the material to be studied in a drop of potassium hydroxide on a glass slide; add Congo Red if desired

Use: 3-5% KOH is the reagent used to revive the hyphae of dried basidioms.

2) Congo Red: 1% aqueous solution (Lagent et al., 1977).

- 1 g of congo Red
- 99 ml of water

Use: Usually used in combination with water for fresh material or potassium hydroxide for dried material. Congo Red will stain the wall of hyphae to red, and this is used to observe walls hyphae and spores for drawing.

3) Distilled water (H_2O) (Lagent et al., 1977).

Use: The distilled water used to revive the hyphae of dried specimens of some species have thin hyphae, and will see the real colour.

4) Phosphate-Buffered Saline (PBS) (Franken et al., 2006)

Use: PBS a commonly used isotonic buffer to maintain pH and osmotic balance in biological staining protocols and washes in clonogenic assays, though they primarily mention crystal violet.

5) Cresyl violet (Franken et al., 2006)

- 0.1–0.2% cresyl violet in PBS or water

Use: Cresyl violet is used to stain viable cell colonies that have formed after the assay.

6) MTT (Tetrazolium salt) (Mosmann 1983)

Use: MTT, or 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, is a tetrazolium salt used in cell viability assays. The reagent is a yellow, water-soluble

dye taken up by metabolically active cells. The reagent reduced by dehydrogenases to formazan, a purple, water-insoluble crystal.

7) Dimethyl sulfoxide (DMSO) (Mosmann 1983)

Use: DMSO is commonly used in MTT Assay to dissolves insoluble formazan crystals for absorbance measurement.

APPENDIX B

ABSTRACT OF PUBLICATIONS

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DGfM

ORIGINAL ARTICLE

Optimal conditions for mycelial growth of medicinal mushrooms belonging to the genus *Hericium*

Didsanutda Gonkhom [1,2] · Thatsanee Luangharn [1] · Kevin D. Hyde [1,2] · Marc Stadler [3] · Naritsada Thongklang [1,2]

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Abstract
Hericium is a well-known genus that comprises edible and medicinal mushrooms with fleshy, distinctive white spines that hang from a tough, unbranched clump, and grows on dying or dead wood. In preparation for the artificial cultivation of these mushrooms in Thailand, an optimization of mycelial growth on different agar culture media, for various conditions (including temperature, pH, cereal grains, and agricultural waste, carbon sources, nitrogen sources, and the ratio of media components) was carried out. For this study, three strains of *H. erinaceus* (MFLUCC 21-0018, MFLUCC 21-0019, and MFLUCC 21-0020) were favorably grown on OMYA medium, at 25 °C and at a pH of 4–4.5, while one strain of *H. erinaceus* (MFLUCC 21-0021) grew favorably on CDA medium, at 25 °C and pH 5.5. The favorable condition for *H. coralloides* (MFLUCC 21-0050) growth was MYPA medium, at 30 °C and pH 5.5. All five strains presented higher mycelial growth on wheat grain. Carbon and nitrogen sources promoted higher rates using molasses and yeast extract respectively, and a ratio of these media components of 10:1 resulted in higher growth rates. The data presented provide growth requirements that will be useful in the future development of the cultivation of *Hericium* mushrooms.

Keywords Grain spawn · Media components · Medicinal mushroom · pH · Temperature

Introduction

Hericium Pers. is a well-known genus of medicinal mushrooms that belongs to *Hericiaceae*. They are known by different names including bear's head, bearded tooth, hog's head fungus, hóutóugū, lion's mane, monkey's head, old man's beard, pom pom, white beard, and yamabushitake (Thongbai et al. 2015; Sangtitanu et al. 2020). There are 66 records in the Index Fungorum (http://www.indexfungorum.org/Names/NAMES.ASP) and 48 records of taxa in MycoBank (https://www.mycobank.org/) and He et al. (2019) record 23 species of *Hericium*. The genus is cosmopolitan but its species occur in higher altitudes in warmer climates, mostly found in North and South America, Europe, Australia, China, Japan, and Asia (Ginns 1985; Boddy et al. 2011; Grumezescu and Holban 2018). In addition, one of the species known from Africa was actually collected around the equator in a Cameroonian mountain range (Jumbam et al. 2019). *Hericium* mushrooms have a serrated basidiome, with members that are classified as white rotters (Hallenberg et al. 2013). The basidiomes of these saprotrophic fungi generally grow with short stalks on a wide range of hardwood, in particular on old or dead broadleaved trees (Mizuno 1999).

Generally, *Hericium erinaceus* has been characterized to have a branched or unbranched hymenophore with structures supporting thorns of various lengths and growing in single or multiple clumps. They have been described as hanging, meaty, at first white, yet becoming yellowish (Ginns 1985), with fragile ice-like spines hanging from scaffolds, and

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Research Article

Cultivation and Nutrient Compositions of Medicinal Mushroom, *Hericium erinaceus* in Thailand

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Abstract

Hericium erinaceus, commonly known as "Lion's Mane," is widely edible, used for medicinal purposes, and has a long history of cultivation in China and Japan since 1990s. In this study, four strains of *Hericium erinaceus* (MFLUCC 21-0018, MFLCC 21-0019, MFLUCC 21-0020, and MFLUCC 21-0021) were grown for cultivation and proximate analysis. The mushroom was cultivated on three different substrate treatments, designed using a completely randomized design (CRD), harvested as fresh fruiting bodies, dried at 40–45 °C, and the total yield calculated. We pulverized the dried fruiting body for proximate composition and analyzed it according to standard procedures. The result showed that all *H. erinaceus* strains in three different substrate treatments produced mature fresh fruiting bodies when the temperature was 18–24 °C, while the second substrate treatment under conditions of the sawdust bag content 77% of para rubber sawdust, 15% of red sorghum, 3% of rice bran, 2% of yeast powder, 1% of lime (CaO), 1% of gypsum ($CaSO_4 \cdot 2H_2O$), and 1% of molasses produced a high yield of 85.79–123.7 grams/bag. Proximate analysis of the dried mushroom powder showed high levels of protein content between 15.30% and 19.56%. The cultivation of *H. erinaceus* in Thailand is a significant achievement, as this type of mushroom is generally valued for its nutritional and therapeutic properties.

Keywords: farming, lion's mane, nutritional contents

1. Introduction

Hericium Pers. (Basidiomycota) species are saprotrophs that grow on the wood of angiosperm trees, especially trees are belong to family Fagaceae [1]. *Hericium* belongs to the family Hericiaceae, order Russulales, and 71 records were reported in the Index Fungorum (www.indexfungorum.org). The characteristics of *Hericium* mushrooms were distinctive by shaggy spines with a furry appearance form; some were round balloons when young to mature stage, but they can age to a yellow or tan hue [2].

Hericium erinaceus is generally known as "Lion's Mane" and is widely distributed in deciduous forests [3]. It has a long tradition as an edible mushroom, but it was later developed to be used medicinally [4]. This mushroom is widely recognized as a

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Article
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Hericium: A review of the cultivation, health-enhancing applications, economic importance, industrial, and pharmaceutical applications

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Abstract

Hericium is a genus of edible mushroom with proven medicinal efficacy. The mycelium and basidiomata contain many nutrients and bioactive compounds with therapeutic uses. Recent and emerging evidence has shown that *Hericium* is helpful to various diseases with medicinal properties, such as anti-oxidant, anti-cancer, anti-diabetic, anti-hyperglycemic, hypolipidemic properties, anti-inflammatory, anti-microbial, anti-viral, and hepatoprotective. Over the past decade, many studies have been done on *Hericium* cultivation to produce enough basidiomata for culinary and medical purposes, due to its rarity in natural habitat. The purpose of this review is to provide the cultivation methods including indoor-outdoor cultivations and submerged culture methods, health-enhancing applications, economic importance, and industrial applications of *Hericium* mushroom.

Keywords – cultivation – economical – industrial applications – medicinal mushroom

Introduction

Species of *Hericium* Pers. (1794), are commonly considered as traditional food and traditional folk medicines in China (Shao et al. 2019). They are native to North America and found in the wild in East Asia countries and India (Das et al. 2011). They are rarely found in European countries but are common in Japan and North America. *Hericium* species have a variety of common names for e.g., lion's mane mushroom, hóutóugū, yamabushitake, monkey's head, Pom Pom, Bear's head, Hog's head fungus, Whitebeard, Old-man's beard, and Bearded tooth (Thongbai et al. 2015, Sangtitanu et al. 2020).

Hericium is a genus of edible and medicinal mushrooms that belongs to the family *Hericiaceae*, order *Russulales*, and class Agaricomycetes (Kirk et al. 2008, He et al. 2019). *Hericium* comprises 34 species with 66 taxon names listed in Index Fungorum (Index Fungorum 2021) and 23 species records in Notes of Genera in *Basidiomycota* (He et al. 2019). The basidiomata in this genus are white and fleshy, growing on dead trees or dried woods, the basidiomata are similar to fragile iced thorns which either hang from a branch, supporting the framework or as a tough unbranched cushion of tissue (Volk & Westmoreland 2009, Hallenberg et al. 2012, Kuo 2014). The dangling spines easily identify a mature specimen (Ouali et al. 2020). The

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Scleroderma: A review of the known species in Thailand

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Abstract

Scleroderma species belong to Gasteromycetes (Basidiomycota), characterised by their basidiospores maturing within the basidioma without forcible discharge from the basidia. Commonly known as earth balls, *Scleroderma* has been identified as suitable for human consumption. Additionally, these fungi exhibit medicinal properties through the production of bioactive compounds. However, there have been some concerns about the edibility and potential toxicity of this mushroom. In Asia, twenty-five species of *Scleroderma* have been documented, with eleven species identified in Thailand based on morphological evidence. This review aims to provide insights into the taxonomy, distribution, life cycle, and cultivation of *Scleroderma* species found in Thailand. Furthermore, we report the bioactive compounds produced by this genus and their economic significance.

Keywords – edible mushroom – Gasteromycetes – poisonous – puffball – taxonomy

Introduction

Scleroderma Pers. belongs to Sclerodermataceae with *Scleroderma verrucosum* (Bull.) Pers. as the type species (He et al. 2019). This genus is distributed worldwide in temperate, subtropical, and tropical regions (Sims et al. 1997, He et al. 2019). Several morphological and molecular studies have confirmed the systematic position of *Scleroderma*, placing it in the suborder Sclerodermatineae within Boletales (Binder & Bresinsky 2002, Hughey et al. 2000, Louzan et al. 2007). Most lineages within this suborder are recognised as ectomycorrhizal taxa (Binder & Hibbett 2006, Watling 2006). The taxonomy of *Scleroderma* is understudied particularly in tropical Africa (Sanon et al. 1997) and in Asia (Farmer & Sylvia 1998, Sims et al. 1999). There are 202 records with 121 species of *Scleroderma* in Index Fungorum 2025 (www.indexfungorum.org). However, only 46 species have been accepted by He et al. (2019).

A revised key of the genus *Scleroderma* was considered for the identification of 25 species in Asia (Sim et al. 1995, Sanon et al. 2009). In Thailand, only eleven species of *Scleroderma* based on morphology have been reported, namely *S. areolatum* Ehrenb., *S. bovista* Fr., *S. cepa* Pers., *S. citrinum* Pers., *S. dictyosporum* Pat., *S. flavidum* Ellis & Everh., *S. lycoperdoides* Schwein., *S. polyrhizum* (J.F. Gmel.) Pers., *S. sinnamariense* Mont., *S. verrucosum* (Bull.) Pers. (Chandrasrikul et al. 2011) and *S. suthepense* Kumla, Suwannar. & Lumyong (Kumla et al. 2013).

Scleroderma species are good candidates as symbionts for inoculation in afforestation initiatives involving pine and eucalyptus trees (Dell et al. 2002, Chen 2006). Typically, the well-

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5. Certificate of Training Program on Chemical and Laboratory Waste Management for Faculty Member, Researchers, and Relevant Personnel, 2024” December 16th, 2024.

6. Certificate of the online course on Standard Laboratory Safety, National Research Council of Thailand, 2025
7. Certificate of the Center of Excellence in Fungal Research Annual Conference 2025 (CEFR AC 2025). Presentation on the Ergosterol isolated from the medicinal mushroom Hericium coralloides as anticancer activity. 2nd May 2025.

PUBLICATION

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