Dissertation Title	Commercialization of *Hericium* and Evaluation of Its Bioactive Compounds
Author	Didsanutda Gonkhom
Degree	Doctoral of Philosophy (Biological Science)
Advisor	Adjunct Professor Kevin David Hyde, Ph. D.
Co-Advisor	Naritsada Thongklang, Ph. D.

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