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Highlights on the Wood Blue-Leg Mushroom *Clitocybe Nuda* and Blue-Milk Mushroom *Lactarius Indigo* Ecology and Biological Activities

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Review Article

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Abstract

The need to improve the life quality together with the increase in the frequency of treating diseases attracted the attention of many researches to view food as a source of nutritional and therapeutical agents. Since earliest times, several mushrooms have been reported as a nutritious food with valuable medicinal properties. The genus *Clitocybe* and *Lactarius* belonging to Basidiomycota fungi, is a potential group of edible mushrooms that are distributed in Europe, North America, Asia, Australia and Mexico. The studies on *Clitocybe nuda* and *Lactarius indigo* species have revealed high nutritional and medicinal potentials. This review aims to present *Clitocybe* and *Lactarius genus* importance as both food and medicine, and which will offer a new vision to researchers to develop new drugs from natural sources.

Keywords: Medicinal Mushrooms; *Clitocybe nuda*; *Lactarius Indigo*; Biological Activities

Introduction

Mushrooms (Phylum Basidiomycota and Ascomycota) naturally produce numerous substances with bioactive properties such as antitumoral, antidiabetic, immunomodulatory, antioxidant, trypanocide, leishmanicide, anti-inflammatory, antiviral and antimicrobial [1-10]. The research on new substances have biological activities especially as antimicrobial is highly necessary, due to the emergence of resistant bacterial strains and new opportunistic species. Mushrooms produce a variety of bioactive compounds that are known to have a potential source of biological activities. Natural antioxidants can protect against free radicals without any side effects. For millennia, mushrooms have been valued by humankind as an edible and medical resource. The Basidiomycetes mushrooms are famous for their use as sources of therapeutic bioactive compounds, such as *Geastrum fimbriatum* and *Hydnellum peckii* which exhibit promising anticoagulant activity. *Handkea utriformis*, *Hericium erinaceus*, *Sparassis crispa*, *Agaricus blazei* and *Ganoderma oregonense* have wound-healing capabilities, *Trametes versicolor* and *Dictyophora indusiata* show promising antioxidant, antimicrobial, antihyperlipidemia, antitumor and immunity enhancement effects, *Fomes fomentarius* and *Polyporus squamosus* have significant importance as antifungal, antibacterial, anti-inflammatory, antioxidant, antitumor and antiviral agents [3,5,10-17].

Many mushroom genera are famous for their promising therapeutic capabilities and one of the mushrooms attracting attention is *Ganoderma lucidum* (Reishi), *Lentinus edodes*

(Shiitake), *Inonotus obliquus* (Chaga) and many others have been collected and used for hundreds of years in Korea, China, Japan, and eastern Russia. Those practices still form the basis of modern scientific studies of fungal medical activities, especially in the field of stomach, prostate, and lung cancers. All these vital activities have been reported from extracts of fruit bodies of these mushrooms or their biologically active isolated compounds [18-28]. Polysaccharides are the best known and most potent mushroom derived substances with antitumor and immunomodulating properties. Biologically active polysaccharides are widespread among higher Basidiomycetes mushrooms, and most of them have unique structures in different species. Moreover, different strains of one Basidiomycetes or Ascomycetes species can produce polysaccharides with different properties [29-39]. This review describes pharmacologically active compounds from mushrooms.

Clitocybe Nuda

Clitocybe nuda also known as *Lepista nuda*, and sometimes given the common name "blewit or blue-Leg mushroom. *Clitocybe nuda* belonging to; Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family Tricholomataceae. *Clitocybe nuda* is fairly easily recognized when fresh and young, but older specimens can be confused with many potential look-alikes. Gorgeous shades of lilac and lavender on the cap, gills, and stem fade quickly; the cap becomes brownish, and the gills and stem fade to buff. But this color transformation is one of the mushroom›s unique features, along with its pale pinkish spore print, its lack of a partial veil, and its tendency to grow in plenty on organic remains. *Clitocybe nuda* may look innocent enough, from a human perspective, *Clitocybe nuda* sends out tiny hyphae that penetrate bacteria colonies and kill them, sucking up their nutrients.

Clitocybe nuda ecology: Saprobic; growing alone, scattered, or in clusters in organic debris in woods or in urban locations; late summer and fall; widely distributed in North America. Cap: 4-20 cm; convex with an enrolled margin when young, becoming broadly convex to nearly flat or with an uplifted, wavy margin in age; surface smooth, slightly sticky when moist; sometimes finely cracked over the center; usually dull purple, or purplish with brown shades when fresh, fading to brownish, flesh colored, tan, or lighter. Gills: Attached to the stem sometimes by a notch or beginning to run down it; close or crowded; pale lavender to lilac, fading to buff, pinkish buff, or brownish. Stem: 3-10 cm long; 1-3 cm thick at apex; equal, or enlarged at the base; dry; finely hairy, and/or mealy near the apex; pale purple or colored like the gills; becoming brownish in age; base often covered with lilac to buff mycelium. Flesh: Thick; soft; purplish to lilac-buff or whitish. Odor and Taste: Taste not distinctive, pleasant, or slightly bitter; odor fragrant. Spore Print: Pinkish. Microscopic Details: Spores 5.5-8 x 3.5-5 µ; ellipsoid; roughened or sometimes smooth; in amyloid. Cystidia absent Pileipellis a cutis of hyphae 1-4 µ wide. Clamp connections present (Figures 1, 2) [40-43].

Figure 1: *Clitocybe nuda,* (Photo was taken by: Damon Tighe. Locality: United States, California, Knowland Park, Oakland (Cited in: *https://mycoportal.org***)**.

Figure 2: *Clitocybe nuda,* (Photo was taken by Evan Casey. Locality: United States, California, San Bernardino, Yucaipa (Cited in: *https://mycoportal.org***)**.

Clitocybe nuda biological activities

Clitocybe nuda (Fr.) (*Lepista nuda,* commonly known as wood blewit or blue stalk mushroom) is an edible woodland mushroom found in Europe, North America, Asia, and Australia [44]. Due to its special cologne and delicate texture, it has been cultivated in France, Holland, Britain, and Taiwan. Several bioactive extracts from *Clitocybe nuda* have been found to exhibit antioxidant and antimicrobial properties [45]. Furthermore to its edible properties, many researchers have conducted biological activity studies on *Clitocybe nuda*. Many studies have shown that *Clitocybe nuda* mushroom has, antimicrobial, cytotoxic, enzymatic, antiviral and antiproliferative activities [46]. As a result, *Clitocybe nuda*, which is an edible mushroom, has important medicinal properties in addition to its nutritional properties [47]. *Clitocybe nuda* is reported to exhibit many biological

activities. *Clitocybe nuda* extract was studied its action on HL60 (leukemia) and MCF7 (breast cancer) cancer cell lines by Özmen and Değirmenci [48]. The extract showed fairly significant (IC50 ~15 mg/mL) of biologic activity compared with drugs protocatechuic acid, paclitaxel and doxorubicin against HL-60 cell line with regard to both proliferation and apoptotic effects (>%75).

Clitocybe nuda was extracted, with 95% ethanol and the extract showed good antibacterial activity against four pathogenic foodborne bacteria: *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* and *Staphylococcus aureus* [49,50]. Secondary metabolites of *Clitocybe nuda* displayed antimicrobial activity against *Phytophthora capsici*. The culture filtrate of *Clitocybe nuda* was extracted with ethanol and chromatographically separated on a Sephadex LH-20 column and fractionated on a silica gel column to give eight fractions. These fractions were tested for the ability to inhibit zoospore germination of *Phytophthora capsici*. The most active fraction was further purified by silica gel column chromatography to yield three compounds: 2-methoxy-5-methyl-6-methoxymethyl-p-benzoquinone, 6-hydroxy-2H-pyran-3-carbaldehyde, and indole-3-carbaldehyde. At a concentration of 500 mg/L, indole-3-carbaldehyde showed complete inhibition of zoospore germination, while 2-methoxy-5-methyl-6-methoxymethyl-p-benzoquinone and 6-hydroxy-2H-pyran-3-carbaldehyde, showed inhibition rates of 97 and 86%, respectively.

The culture filtrates of *Clitocybe nuda* was studied by Chen and Huang, and reported that *Clitocybe nuda* culture filtrates was able to completely inhibit spore germination of *Colletotrichum higginsianum* [51]. The culture filtrate of *Clitocybe nuda* contained substances that had the capacity to completely inhibit spore germination of *Alternaria brassicicola. Clitocybe nuda* culture filtrate showed complete suppression of spore germination of *Phytophthora capsici* and moderately inhibited spore germination of *Fusarium oxysporum*. The culture filtrates of *Clitocybe nuda* and *Coprinus comatus* effectively reduced the disease severity of Phytophthora blight of pepper caused by *Phytophthora capsici*. All these results suggest that substances from edible mushrooms have the potential to be developed into biocontrol agents for the control of plant diseases [51].

Lactarius indigo

Lactarius indigo belonging to; Basidiomycota; Class: Agaricomycetes; Order: Russulales; Family Russulaceae. *Lactarius indigo* it is a truly beautiful, blue species that exudes dark blue milk when damaged with a knife point. Only *Lactarius indigo, Lactarius chelidonium* and *Lactarius paradoxus* come close in appearance and they only do so when very young, before they have begun to develop their brown and yellow shades (they also have yellowish and brown milk, respectively). *Lactarius indigo* description; small cap (3-5 cm across), flesh that turns green within a few minutes of exposure, and a pure white spore print. *Lactarius indigo* was found in many diverse ecosystems from oak-hickory forests to ponderosa pine zones in the southwestern United States to cloud forests in Mexico. Ecology: Mycorrhizal with oaks and with pines; growing alone, scattered, or grouped; summer and fall; fairly widely distributed in North America from the northeast to the southwestern United States, Texas, and Mexico [52-54]. Cap: 5-15 cm; convex becoming flat or vase-shaped; the margin at first inrolled; deep to medium blue when fresh; grayish or silvery blue when faded; sometimes developing brownish areas when old; with concentric zones of color, or sometimes evenly colored; sticky or slimy when fresh; bruising and discoloring deep green, especially with age. Gills: Attached to the stem or beginning to run down it; close; colored like the cap or a little paler; becoming nearly yellowish at maturity; staining green. Stem: 2-8 cm long; 1-2.5 cm thick; equal or tapering to base; sometimes a little off-centre; slimy at first but soon dry; hard; hollowing; usually with potholes on the surface. Flesh: Whitish, turning indigo blue when cut; staining slowly greenish. Milk: Deep indigo blue; becoming dark green on exposure. Odor and Taste: Odor not distinctive; taste mild to (sometimes) slowly, slightly acrid. Spore Print: Cream. Microscopic Features: Spores 7-10 x 5.5-7.5 µ; broadly ellipsoid to subglobose; ornamentation about 0.5 µ high, as amyloid warts and connecting lines that sometimes form partial reticula. Pleuromacrocystidia cylindric-ventricose; inconspicuous; to about 60 x 8 µ. Cheilocystidia inconspicuous; clavate to subcylindric; to about 30 x 6 µ. Pileipellis an ixocutis (Figures 3, 4) [54, 55].

Figure 3: *Lactarius indigo,* (Photo was taken by: Patricia R. Miller. Locality: USA, Mississippi, Grenada, Grenada Dam, (Cited in: *https://mycoportal.org*).

Figure 4: *Lactarius indigo,* (Photo was taken by Patricia R. Miller. Locality: USA, Mississippi, Lafayette, Oxford, Washington Ave. (Cited in: *https://mycoportal.org*).

Lactarius indigo biological activities

The major role of *Lactarius indigo* in the world is in cooking. It is known for its fascinating color and the cultural significance it has in countries such as Mexico [56]. However, research has indicated the mushroom has antibacterial and cytotoxic properties [57]. Bioassays and cytotoxic assays were created to compare the inhibition of strains with only hexane and methanol versus with the *Lactarius indigo*. When tested against different bacteria, such as diarrheagenic *Escherichia coli* strains, the *Lactarius indigo* inhibited proliferation of certain pathogenic bacteria, the inhibitory effect depended on the bacteria it was tested against and the dosage of *Lactarius indigo*. Overall, the study indicated possible medicinal properties in *L. indigo* [57]. Both aqueous and organic extracts of *Lactarius indigo* basidiocarp have pharmacological activity, Ochoa-Zarzosa, et al., show that the basidiocarp of the edible *Lactarius indigo* is a source of pharmacological substances having varied therapeutic applications, which makes it necessary to perform further studies in that regard by isolating and characterizing the molecules responsible for the observed activities [58].

Conclusion

Basidiomycetous mushrooms represented by *Clitocybe nuda* and *Lactarius indigo* have a rich history of use as a food source and well-claimed medicinal properties. This review summarises a number of sources with details of nutritional content and beneficial compounds (Antimicrobial properties to antitumor, health-promoting nutrients and others). Despite these advances, there is much we have yet to understand and these hypogeal fruiting Basidiomycetes prove to be a fruitful source of novel medicinal compounds.

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