

# Harvesting Bioactive Compounds with Broad Spectrum from Possible Poisonous *Clitocybe* spp.

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## Abstract

From ancient times mushrooms have been used by humans in different forms. It has found its way in different cuisines, medicines and other important aspects of our life. We have discovered and worked on different species of mushrooms, however many species of *Clitocybe* are still undiscovered. In this research we will be focusing benefits of the compounds extracted from species of *Clitocybe* (Undiscovered). The compounds present in *Clitocybe* have been confirmed to have a wide range of beneficial effects including antitumor, bacteriostatics, antifungal, antiviral, anti-inflammatory, antioxidant, anti-aging, antidiabetic, hypoglycemic, immune stimulant, anti-proliferative, neuro-protective, cardio-protective, and chemo-preventive properties. It has also found its way in food industry with no table characteristics as milk-coagulant, flavorings agent, organic food preservative, and yeast culture. Other known application as biological pest control has also been confirmed.

**Keywords:** *Clitocybe* • Milk coagulant • Immune stimulant • Bioactive compounds

## Introduction

Not from the modern era even from the beginning it become more difficult to find the solution for the treatment of various diseases, because of the development of bacterial resistance to antibiotics and antimicrobial agents lost their effectiveness [1]. Therefore it is more necessary to discover or explore new antimicrobial drugs [2]. Basidiomycetes are well known for their various medicinal properties as well as antifungal, antitumor, antiallergic, anti-inflammatory and also used in treatment of diabetes, antihepatotoxicity [3]. Prior to study the bioactivity of mushrooms and broad spectrum of their bioactive compounds, it's more necessary to determine their antimicrobial activity [4]. Most of the research is based on the isolation of fleshy fungi, extract and their antibacterial compounds [5]. Mushrooms also added the nutritional value as well as antimicrobial activity, feed and medicinal [6,7]. Thus, novel antimicrobial compounds have been investigated from mushroom. Mushrooms spp. are exceptionally rich natural resource of bioactive

compounds such as fatty acids, amino acids, tocopherol content, carotenoids ( $\beta$ -carotene, lycopene), flavonoids, ascorbic acid, and anthocyanidins [8-10].

Total mushroom spp. are 0.14 million. Out of these 14000 are known and 7000 are edible. 2,000 species are considered to be safe and 700 are reported with pharmacological properties [11]. But still there are some genres those who are not discovered at much extend due to their toxic nature. *Clitocybe* one of them, they contain about 0.1% to 0.33% of muscarine [12]. *Clitocybe* spp. mainly grow on dead decaying logs, grass and leaf litters [13].

There is name in all of this is *Clitocybe*. *Clitocybe* belongs to order Agaricales and family Tricholomataceae. (Phenotypic character). Small percentage of *Clitocybe* spp. is poisonous but most of the *Clitocybe* spp. is non-toxic and approximately 300 species of *Clitocybe* reported worldwide [14]. In totality about 22 species have been reported from India [15-17]. Reported first time *Clitocybe dilatata* and *C. hydrogramma* and *C. nebularis* from Poonch district of in Jammu And Kashmir State India. Still there are some spp. of *Clitocybe* are undiscovered with their bioactivity and medicinal value.

## Literature Review

In 1943 E.E. Morse first time introduces *Tricholoma sclerotoideum*, later on in 1945 placed in genus *Clitocybe* and knows as *Clitocybe sclerotoidea*. It is well known for mycoparasitic property [18].

Similarly *Clitocybe nuda* was earlier known as *Lepista nuda* or *Clitocybe subconnexa*. Found in Southeast Asia and the Middle East. Mainly used in different style of cuisine. Therefore they are studied for their nutritional value, detailed chemical composition and antimicrobial activity [19]. *C. geotropa* and *C. metachroa* reported from Kurdistan region in Iraq [20].

## Nutritional value

Manjunathan coworker in 2011 studied wild mushroom *Clitocybe* spp. from Tamil Nadu, in India for their nutritional value. They reported protein (24.8%), carbohydrates (42.0%), lipids/fats (1.24%), and fiber (13.04%). Mineral nutrients were determined such as calcium 208 mg/g, Iron 61.4 mg/g, manganese 120 mg/g, copper 9.0 mg/g, zinc 6.2 mg/g, sodium 85.84 mg/g, potassium 1369.1 mg/g, magnesium 120 mg/g (Figure 1).

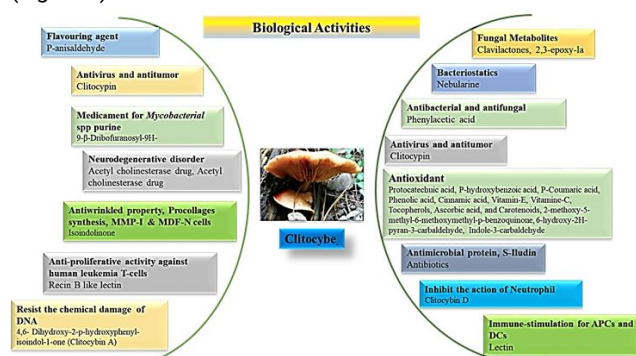


Figure 1. Various biological activities of the *Clitocybe*.

### **Clitocybe nebularis**

*Clitocybe nebularis* full package of bioactive compounds. But still there are some compounds present such as *Nebularine* and phenyl acetic acid. Subsequently they put forward the research of isolation of medicinal compounds such as *nebularine*, 9- $\beta$ -Dribofuranosyl-9H-purine which used as medicament for *Mycobacterial spp.* such as *Mycobacterium tuberculosis*, *M. phlei*, *M. avium* and *Brucella abortus*. Aspartic Proteases (APs) (EC 3.4.23) proteolytic enzymes has been isolated from *Clitocybe nebularis*. APs have been used in large amount in the dairy industry as milk coagulating enzymes in the manufacture of cheese as well as in food industry. It has been reported that worldwide turnover of industrial enzymes isolated from mushrooms is \$1 billion. Proteases are one of the largest groups of industrial enzymes and whose turnover is 60% all over

the world. Bioactive compounds have been reported from sporocarps of *C. nebularis*. *Nebularine* a purine riboside with bacteriostatic activity was the first biologically active compound isolated and identified from this mushroom. The study was led by *C. nebularis* were collected from Dukyu Mountain Korea and isolated various compound such as *nebularine* and *phenylacetic acid*, *purine*, *uracil*, *adenine*, *uridine*, *benzoic acid* and *mannitol*. However only two compounds *nebularine* and *phenylacetic acid* showed average results for against *Magnaphorthe grisea*, *Trichophyton mentagrophytes*, and *phenylacetic acid* but showed best potency against the growth of *Pythium ultium* (Table 1).

**Table 1.** Bioactive compounds isolated from *Clitocybe spp* and their various activities.

S.No.	<i>Clitocybe spp.</i>	Compound	Activity
1	<i>C. nebularis</i>	Nebularine	Bacteriostatics
		9- $\beta$ -Dribofuranosyl-9H-purine	medicament for <i>Mycobacterial spp</i>
		Phenylacetic acid	Antibacterial and antifungal
		Clitocybin	Antivirus and antitumor
		Lectin	Immune-stimulation for APCs and DCs
		Recin B like lectin	Anti-proliferative activity against human leukemia T-cells.
		Polysaccharids	Antioxidants
		Phenols	
		Ribonucleosides	
2	<i>Clitocybe clavipes</i>	Clavilactones	Fungal Metabolites
		2,3-epoxy-la.	Fungal Metabolites
3	<i>Clitocybe geotropa</i>	Acetyl cholinesterase drug	Neurodegenerative diorder
4	<i>Clitocybe nevulatus</i>	Acetyl cholinesterase drug	Neurodegenerative diorder
5	<i>Clitocybe aurantiaca</i>	4,6-Dihydroxy-2-p-hydroxyphenyl-isoindol-1-one (Clitocybin A)	Resist the chemical damage of DNA
		Clitocybin D	Inhibit the action of Neutrophil
		Isoindolinone	Antiwrinkled property procollages synthesis, MMP-I and MDF-N cells
6	<i>C. odora</i>	P-anisaldehyde	Flavouring agent and aromatic
		Protocatechuic acid	Antioxidant
		P-hydroxybenzoic acid	
		P-Coumaric acid	
		Phenolic acid	
		Cinnamic acid	
		Vitamin-E	
		Vitamine-C	

	<i>C. nuda</i>	Phenolics, Tocopherols, Ascorbic acid, and Carotenoids.	Antioxidant
	<i>C. nuda</i>	2-methoxy-5-methyl-6-methoxymethyl-p-benzoquinone, 6-hydroxy-2H-pyran-3-carbaldehyde, Indole-3-carbaldehyde	Antioxidant
7	<i>C. maxima C. geotropa</i>	Laccase enzyme	Inhibit Tumor cell HIV-reverse transcriptase
8	<i>C. sinopics</i>	Antimicrobial protien	Antibiotics
9	<i>C. illuden</i>	S-lludin, M-lludin	Antibiotics

A novel protein Clitocybin was reported from *C. nebularis*, used as cysteine protease inhibitor which has defense mechanism against pathogen and Mycoviruses as well as antitumor agents.

The novel lectin isolated from the basidiomycetes mushroom *Clitocybe nebularis*. The important function of lectin in immunology showed the immune-stimulation for antigen-presenting cells and the Dendritic Cells (DCs). Prior to the study another protein ricin B-likelectin isolated and purified from *Clitocybe nebularis* which possesses anti-proliferative activity against human leukaemic T cells. *C. nebularis* with lectin show rich versatile positive biological activity in human and veterinary medicine. CNL therefore can be regarded as a promising candidate for different applications in medicine. *Clavilactones* (3a,b-quinones and 3c-hydroquinone) are fungal metabolites and a 2,3-epoxy-lactone). They were isolated from the culture medium of the non-toxicogenic basidiomycetae *Clitocybe clavipes* and have tendency to inhibitors of protein tyrosine kinases.

*C. nebularis* showed good biological activity due to the presence polysaccharides, phenols, ribonucleosides, proteins as antioxidant. However the presence of lectines and isolectins in the extract of *C. nebularis* has contradictory due to their versatile biological activities. As well as in agriculture, for plant protection, including insecticidal and anti-nutritional effects. Coworker in 2008 isolated and studied the structure elucidation of compound 4,6-dihydroxy-2-*p*-hydroxyphenyl-isoindol-1-one known as clitocybin A from *Clitocybe aurantiaca* by NMR and MS spectroscopy analysis. They reported the good antioxidant activity of clitocybin-A against any chemical damage of DNA and apoptosis due to accumulation of Oxygen reactive species (ROS) and also showed antioxidant activity against DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid) and Superoxide. Similarly Clitocybins a novel isoindolinone free radical

Later on in 2009, A nobel compound 4-(4,6-dihydroxy-3-methoxy-3H-isoindol-1-yl)-benzoic acid was purified from *Clitocybe aurantiaca* by NMR and MS spectroscopy analysis. The main function of this compound is to inhibit the overactive action of neutrophil elactase which is also known as clitocybin D. and to protect the humans from inflammatory related diseases.

In recent study of 2017 a nobel compound isoindolinone (Clitocybin-A) has been isolated from *Clitocybe aurantiaca*. The isoindolinone was investigated for skin related properties such as antiwrinkled properties, procollagen synthesis, and matrix metalloproteinase-1 (MMP-1) expression, in human primary dermal fibroblast-neonatal (HDF-N) cells.

## Discussion

*C. odora*: Another name is associated with good quality is *C. odora*. *C. odora* species also added as a value of source of aromatic compound such as *p*-anisaldehyde (4-methoxybenzaldehyde, methyl-anisate, Benzaldehyde and Benzyl alcohol as flavouring agent in food industry. Investigated the *p*-Anisaldehyde, an aromatic compound from sporocarp of *C. odora*. By using the method hydro-distillation and solvent extraction through GC/MS technique. About 81.4% *p*-Anisaldehyde identified in hydro-distillation and by Solvent extraction 66.8%.

Distinct odor can also use also for taxonomic purpose for mushroom identification. *C. odora* had reported with best antioxidant properties such as presence protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, phenolic acids, cinnamic acid and vitamin E and C. In modern days it's more effective and safe to use natural resources to use as medicine and antimicrobials.

## *Clitocybe nuda*

A study was reported in *Clitocybe nuda*, where tendency of *Clitocybe Nuda* Extract (CNE) was studied to control the non-communicable diseases, diabetes and hyperlipidemia. Extract of *Clitocybe Nuda* (CNE) was given orally to the mice with different doses/kg/day such as 0.2 gm/kg, 0.5 gm/kg, and 1.0 gm/kg. The mice were pretreated with high fat diet to see the significant changes in their blood glucose level, experiment resulted with Gain in body weight but white adipose tissue and hepatic triacylglycerol content reduce. The protein content of GLUT4 (glucose transporter 4) increase which helps to improve insulin stimulation, glucose disposal and glycogen storage in muscle cells. CNE further switching on the AMPK also resulted in increase the glucose uptake primarily in muscle cells simultaneously decreased hepatic glucose production enhance the lipid metabolism.

*C. nuda* is well known for their medicinal properties and cure metabolic disorder and reported antioxidant activity due to the presence of phenolics, tocopherols, ascorbic acid, and carotenoids. The three compounds 2-methoxy-5-methyl-6-methoxymethyl-*p*-benzoquinone, 6-hydroxy-2H-pyran-3-carbaldehyde, and indole-3-carbaldehyde of *Clitocybe nuda* showed inhibitory results for *Phytophthora capsici*.

The study reported the antibacterial activity against *Listeria monocytogenes* and *Salmonella typhimurium* is very stable at a wide pH range of 5-10. Therefore, the mushroom extract can be applied in foods with different pH levels to control microbial growth improving the safety. *Clitocybe nuda* was extracted with 95% ethanol. The extract had better antibacterial activity against *Listeria monocytogene* and *Salmonella typhimurium* than against *E. coli* and *Staphylococcus spp.* Now these days in food industries, a commercial world, and food preservative play important role to increase the shelf life of product. So once the food went through series of trial such as from production to sales market and reached to consumer. The food needs good preservative they may maintain the better quality of food and inhibit the spoilage. But prolong use of artificial food preservatives have bad effects on health. The free radicals cause various health issues. Therefore the use of organic preservative from mushroom act as antioxidant which can protect from oxidative stress release from reactive oxygen species. Mushroom exhibit antioxidant activity due to present of secondary metabolites vitamins A and C, beta-phenolic compounds, steroids and terpenes. *Clitocybe nuda* play important role in immune modulator. They have the tendency enhance the immune response of DNA Vaccine by activating the antigen presenting cells such as dendritic cells and exhibit the property of anti-hyperlipidemic and anti-hyperglycemic effects.

### C. Alexandri

*Clitocybe alexandri*, the whole fruiting body from Portugal in 2008 was subjected to investigate their antioxidant activity as well as antitumor activity in the ethanolic phenolic extract. Extract was tested *in vitro* for cancer cell growth inhibition against the four human tumor cell lines such as Breast (MCF-7), Lungs (NCI-H460), Colon (HCT-15), Gastric cancer (AGS).

*Clitocybe alexandri* investigated for antimicrobial activity by using different solvents, ethylacetate and methanolic extract. Both the extracts ethylacetate extract and methanolic extract showed good results however ethylacetate extract showed better result for yeast culture, *Candida albicans* and *Saccharomyces cerevisiae* whereas methanolic extract showed best activity against bacterial culture gram+ve, gram-ve.

*Clitocybe maxima* protein extract was tested against the three cases of abnormalities such as Human liver cancer cell lines (HepG2) and human breast cancer cell lines (MCF-7). Reported under microscope best results in change in cell death morphology. Laccase enzyme was isolated from *Clitocybe maxima*. Laccase enzyme has the tendency to inhibit and manifest the tumor cell and HIV -I reverse transcriptase.

Similarly *Clitocybe geotropa* studied with volatile chloroform and ethanolic extracts. Both the solvent extract showed significant capacity against gram negative, *Bacillus cereus* and *Proteus vulgaris*. Therefore volatile solvents are also play important role to release the essential components from fruiting body extract of fungus.

Antimicrobial protein is now becoming a great interest because of their tendency to resist the diseases not only in animals but also against the plant pathogens. Now in phylum basidiomycetes known as macrofungus has also reported an AMP (Antimicrobial protein). Zheng coworker isolated the novel antimicrobial protein from the extract of *Clitocybe sinopics*.

First time in 1947 Mattick and Hirsch isolated the two proteins Nisin, a lantibiotic (AMP) from *Lactococcus lactis*. and from other bacteria. And various AMP from animals. Sea animals (*Aplysia dactylomela*). Cecropin protein from Silkworm; *Bombyx mori*.

### Clitocybe inversa

In 1952 first time reported the antibiotics illudin S and illudin M from *Clitocybe illuden*. From basidiomycetes. However *Clitocybe illudens* is toxic but still these similar two compounds were isolated from different fungus *Granulobasidium vellereum* were reported with cytotoxic effect in tumor cells. The illudin-M and illudin-S compounds belong to family of sesquiterpenes. Therefore there is literature found which showed the results of terpenoids, affective for sporozoan such as *Plasmodium gallinaceum*.

### Biopest control

*Clitocybe spp.* are not well known to overcome the medical conditions but also well known for biological pest control. The clitocine was the compound isolated from *Clitocybe inversa* field insects known as bollworm *Pectinophora gossypiella*. *Clitocybe nebularis* showed the antagonistic relationship between soil bacteria (*Bacillus spp.*) and fungus (*Phytophthora Spp.*), that leads the best example to study the tendency of *Clitocybe nebularis* to inhibit soil bacteria and fungal infection to the plants by *Phytophthora Spp.* And control the pathogens in plants or forest in large scale by using the natural source instead of using the chemicals. The lectin is best known protein present in the fruiting body of most of the mushroom among of which *Clitocybe nebularis* is one of them. Lectin protects the fruiting bodies against from insects, nematodes and amoebae.

### Poisonous muscarine

*Clitocybe spp.* is equally important medically as well as few of the species are deadly due presence of poisonous compound such as muscarin cause muscarinic syndrome. *Clitocybe spp.* that contains higher amounts of muscarine includes *Clitocybe cerussata*, *Clitocybe dealbata*, *Clitocybe*

*dilatata*, *Clitocybe rivulosa*. Intake of these mushrooms cause serious health problem within 15 minutes to 2 hours such as gastrointestinal problem, excessive constriction of the pupil of the eye, hyper-secretion and cause bradycardia which can be treated with atropine. And mainly one component which lead the poisoning is Tetrahydro-4-hydroxy-N,N,N-5-tetramethyl-2-furanmethanaminium and 2-methyl-3-hydroxy-5-(N,N,N-trimethylammonium) methylente-trahydrofuran chloride, which was reported in *Amanita muscaria* and 2-methyl-3-hydroxy-5-(N,N,N-trimethylammonium) methylente-trahydrofuran chloride (13.1.14), which was reported in *Amanita muscaria* and in larger amounts in *Clitocybe serussata*, *C. dealbata*, *C. phyllophilla* and *C. rivulosa*. Muscarine structure is very similar to acetylcholine and bind to the same receptors. It is not hydrolyzed by cholinesterase causing a par sympathomimetic symptomatology.

Still there are few species of *Clitocybe*, *Clitocybe acromelalga* and *Clitocybe rivulosa* which cause syndrome Delayed rhabdomyolysis and Abdominal pain, diarrhea, and intense sweating but the causal components not yet discovered. The responsible toxin acromelic acids found *C. amoenolens* and *Clitocybe acromelalga* cause erythromelalgia syndrome has been described in late 19<sup>th</sup> century in Japan and South Korea and in 1996 in France and then Italy.

There is some species whose purified components are known for medicinal properties on the other hand they also cause serious health damage such as *Clitocybe nebulari*. *C. nebulari* cause acute gastroenteritis without liver failure due to GI toxic production. But the mortality rate in this case is rare. In *Clitocybe dealbata* and *C. illudens muscarin* was reported which act upon neurotransmitter acetylcholine and leads to various complications such as sweating, diarrhea, brochial secretion, increased saliva, cardiac arrest, abdominal pain.

### Conclusion

*Clitocybe* has a lot of potential in the field of medicine and healthcare. The novel compounds found from species of *Clitocybe* would help cater against the bacterial resistance developed for antibiotics and antimicrobial drugs. Few species of *Clitocybe* have been reported to be poisonous but on extracting the compounds many were found to have a lot of benefits. Our study would help in extracting compounds having lot of benefits.

The compounds present in *Clitocybe* have been confirmed to have a wide range of beneficial effects including antitumor, bacteriostat, antifungal, antiviral, anti-inflammatory, antioxidant, anti-aging, antidiabetic, hypoglycemic, immune stimulant, anti-proliferative, Neuro-protective, cardio-protective, and chemopreventive properties. It has also found its way in food industry with no table characteristics as milk-coagulant, flavorings agent, organic food preservative, yeast culture. Other known application as Biological pest control has also been confirmed.

As mentioned in the research, many species are still undiscovered, after we complete our research we expect to find novel compounds from natural resource. This would be very useful in a time when artificially acquired compounds are notorious for their side effects.

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