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Studies on Ultrastructure and Antibacterial Activity of *Cantharellus cibarius* Fr

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Present study was undertaken to carry out taxonomic details, pure culture isolations and antimicrobial activity of a wild edible ectomycorrhizal mushroom *Cantharellus cibarius.* Macroscopic and microscopic characteristics were studied in the field and laboratory respectively. Antibacterial activities of methanolic and acetone extracts of *Cantharellus cibarius* were investigated *in-vitro* against two pathogenic bacteria *Listeria monocytogenes* and *Pseudomonas aeruginosa* following agar well diffusion method, using four different concentrations (25, 50, 75, 100%). The methanolic and acetone extracts of the mushroom showed significant reduction in the growth of the tested bacteria. The extracts showed maximum inhibitory growth against *Listeria monocytogenes* as compared to *Pseudomonas aeruginosa*. There is need of further studies to isolate and characterize the antibacterial moieties in this fungus for practical disease control measures.

Key words: *Cantharellus cibarius*, antibacterial, *in-vitro*, *Listeria monocytogenes*, *Pseudomonas aeruginosa.*

Mushrooms are the fleshy, spore-bearing fruiting bodies of some higher fungi. The use of mushrooms as human food and delicacy had been known to the mankind from the days of human history and civilization. More than 2000 species of mushrooms are edible throughout the world. Large number of naturally occurring edible fungi are eaten by tribal and hilly people after collection from wild habitats[1-3]. It is believed that mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Antimicrobial compounds could be isolated from many mushroom species and some proved to be of benefit for humans[4]. Several important compounds including bioactive polysaccharides (lentinan), dietary fibers, ergosterol, vitamins B_1, B_2, C and minerals have been isolated from the fruiting body, mycelia and culture medium of the mushrooms. Recent studies have shown their medicinal attributes including anti-tumor, antimicrobial and cholesterol lowering activities[5-7]. The rate of consumption of mushrooms in many countries including India has increased considerably in recent times and hence it has become imperative to explore new sources of edible mushrooms which people have been traditionally consuming in different parts of H.P. Present investigations were undertaken to study taxonomy and antimicrobial activity of *Cantharellus cibarius,* which is a wild edible mushroom quite popular among the tribals and locals in Shimla district and existing in abundance in the North Western Himalayan region.

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MATERIALS AND METHODS

Materials used in the present study were pure cultures and fruiting bodies of *Cantharellus cibarius*. Two species of bacterial pathogens (*Listeria monocytogenes* and *Pseudomonas aeruginosa)* were also used to check antibacterial activity of methanolic and acetone extract of *Cantharellus cibarius.*

Test bacteria

Pure cultures of two species of pathogenic bacteria (*L. monocytogenes* and *P. aeruginosa)* were maintained on nutrient medium.

Survey, collection and taxonomic studies

Fruiting bodies of *C. cibarius* were collected from Rampur (Nankhari) forest and Shimla (Summerhill) forest and its vicinity from July-September 2014. Collection sites were visited regularly usually after every spell of rain. The fruiting bodies were collected carefully with the help of forceps. Various characters which help in the identification of specimens e.g. shape, size and colour of the stipe and pileus were recorded by examining the specimens with naked eye. For microscopic studies both dried as well as wet preserved specimens were used. The dried parts were kept for few minutes in 95% ethyl alcohol (to expel out the air) and then in water. Such parts were revived by soaking in 3% KOH whereas wet preserved specimens were used directly after washing with water. Microscopic details of the specimens were worked out in laboratory with the help of research microscope. This included the study of mycelium and spores. For clarity the sections were mounted in 1% cotton blue and lactophenol. This wild edible ectomycorrhizal mushroom was identified a *Cantharellus cibarius* following Singer (1987).

Preparation of mushroom extract

Fruiting bodies of *C. cibarius* were dried under aseptic conditions. Dried mushrooms were pulverized in a blender and 50 g each of the powdered samples were soaked separately in 300 ml of methanol and 300 ml of acetone in an Erlenmayer flask. The flasks were covered with aluminium foil and allowed to stand for 7 days for extraction. These extracts were filtered through Whatman filter paper no.1 and were evaporated and dried using rotary evaporator at 40^0C[8]. Extracts were collected and stock solution of 40 mg/ml was prepared.

Screening of mushroom extracts for antibacterial activity

Screening of methanolic and acetone extracts of *C. cibarius* was done using agar well diffusion method. Nutrient Agar Medium (Beef extract 1g, Yeast extract 2g, Sodium chloride 1g, Peptone 5g, Agar 20g, Distilled water 1lt) was used throughout the investigation for the growth of microorganisms. The medium was autoclaved at 121.6^0C for 30 minutes. The plates were left over night at room temperature to check for any contamination to appear. Bacteria were grown in nutrient broth for 24 hours.100 µl of bacterial suspension were spread on nutrient agar plates. Agar wells of 8mm diameter were prepared with the help of sterilized stainless steel cork borer. Five wells were prepared in each agar plate. The wells in each plate were loaded with control, 25%, 50%, 75%, 100% conc. prepared separately by dissolving extract in methanol and acetone. The conc. labeled as control contained 0% conc. of mushroom extract. The plates containing bacterial colonies were incubated at 37^0C for 24 hours in incubation chamber. All the tests were repeated in triplicates. Diameter of bacterial colonies of treatment and control sets were measured in mutually perpendicular direction on second day. Percentage inhibition of bacteria against fungal extract was calculated after subtracting the value of control from the value of extracts using control as standard[9].

$$\text{Percentage of growth inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets[10].

RESULTS AND DISCCUSSION

Macroscopic and microscopic characteristics of *Cantharellus cibarius* (Plate 1 A)

Fruiting bodies of *C. cibarius* were analysed for macroscopic and microscopic characters. Pileus- 2 - 12 cm wide, convex at first with inrolled margin (edges) often becoming funnel shaped with a wavy margin. It can be quite irregularly shaped. The color ranges from egg yolk yellow to yellow orange and rarely has pink tones. Older specimens are more likely to be more orange especially after being rained on a few times. Gills- are actually ridges that are forked and usually with blunt edges that are of same color as the rest of the mushroom. They are often quite wavy and always run down the stem (decurrent). Stipe- central, solid, tapering towards the base and about the same color as the rest of the mushroom. Hyphae- 3µm-5.5 µm in diameter, thin walled with clamp connections

Table 1. Percentage inhibition of *L. monocytogenes* and *P. aeruginosa* against methanolic extract of *C. cibarius* at different concentrations

Concentration (%)	Percentage inhibition of growth of test bacteria (mm ± S.E.)	
	L. monocytogenes	*P. aeruginosa*
Control	0.00 ±0.00	0.00 ± 0.00
25	18.00 ±0.03	18.82 ± 0.03
50	20.00 ± 0.06	20.00 ± 0.06
75	24.23 ±0.09	21.52 ± 0.09
100	26.56 ± 0.12	23.52 ± 0.15

Each data represents mean of three replicates ± S.E.

Table 2. Percentage inhibition of *L. monocytogenes* and *P. aeruginosa* against acetone extract of *C. cibarius* at different concentrations

Concentration (%)	Percentage inhibition of growth of test bacteria (mm ± S.E.)	
	L. monocytogenes	*P. aeruginosa*
Control	0.00 ±0.00	0.00 ± 0.00
25	21.52 ±0.06	18.82 ± 0.12
50	24.70 ± 0.06	19.52 ± 0.03
75	27.75 ±0.13	20.35 ± 0.09
100	36.00 ± 0.35	23.05 ± 0.06

Each data represents mean of three replicates ± S.E.

and septate. Basidia- slender and club-shaped, usually 5 spored with sterigmata 5µm-6µm long and dimensions of 75-85µm by 6-9µm. Clamp connections present. Basidiospores- 7-11µm x 4.5-6 µm, smooth, thin walled, ellipsoid, inamyloid, hyaline (translucent). They sometimes have tiny oil droplets. (Plate 1 D)

Habit and Habitat

Fruiting bodies of *Cantharellus cibarius* were found in association with mycorrhizal host plants, either with conifers or hardwood trees. They are often found with oaks, birch and pines especially when the forest has plenty of moist, mossy undergrowth.

Collection examined

H.P. Shimla, Nankhari, July, 2014, HPUB 51326; H.P. Observatory Hill, July, 2014, HPUB 51357; H.P. Shimla, Glen, August, 2014, HPUB 51394.

Antimicrobial screening of *Cantharellus cibarius*

Antibacterial activity of methanolic extract of *C. cibarius*

Methanolic extract of *C. cibarius* was seen to show gradual inhibition in the growth of *Listeria monocytogenes* and *Pseudomonas aeruginosa*. This inhibition increased with the increase in concentration of the extract and reached at its maximum at 100% concentration of the mushroom extract. Control wells showed no inhibition against test bacteria. Stock solution of concentration 40 mg/ml was considered as 100% conc. and other concentrations were prepared by serial dilution of stock solution. It is clear from Table 1. and Histogram 1 that methanolic extract of *C. cibarius* gave percent inhibition of around 18% at 25% conc., 20% at 50% conc., 24.23% at 75% conc. and 26.56% at100% conc. in case of *L. monocytogenes* (Plate 1 F). Similar trend of increase in percent inhibition of growth was noticed in case

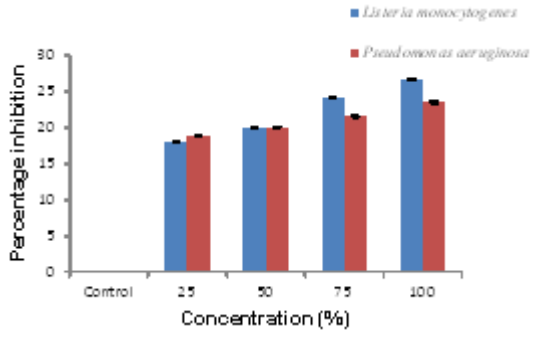

Fig. 1. Percentage inhibition of growth of *L. monocytogenes* and *P. aeruginosa* at different concentrations of methanolic extract of *C. cibarius*

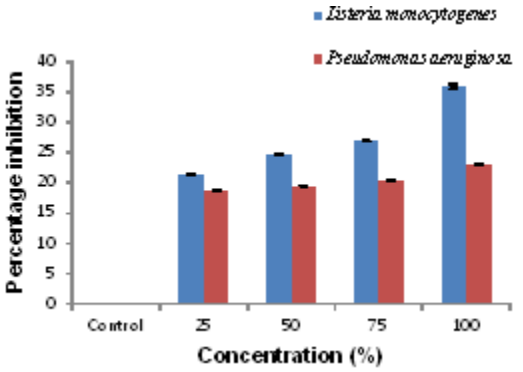

Fig. 2. Percentage inhibition of growth of *L. monocytogenes* and *P. aeruginosa* at different concentrations of acetone extract of *C. cibarius*

of *P. aeruginosa* (Plate 1 H) when the concentration of methanolic extract of *C. cibarius* was gradually increased (i.e. 18.82% at 25% conc., 20% at 50% conc., 21.52% at 75% conc. and 23.52% at 100% conc.).

Antibacterial activity of acetone extract of *C. cibarius*

Simliar trends of gradual growth inhibition were also observed when acetone extract of *C. cibarius* was used against test bacteria. This inhibition increased with the increase in concentration of the extract and reached at its maximum at 100% concentration of the mushroom extract. Control wells showed no inhibition against test bacteria. Stock solution of concentration 40 mg/ml was considered as 100% conc. and other concentrations were prepared by serial dilution of stock solution. It is clear from Table 2 and Histogram 2 that acetone extract of *C. cibarius* showed percent inhibition of around 21.52% at 25% conc., 24.70% at 50% conc., 27.75% at 75% conc. and 36% at 100% conc. in case of *Listeria monocytogenes.* (Plate 1 G) Similar trend of increase in growth inhibition was observed in case of

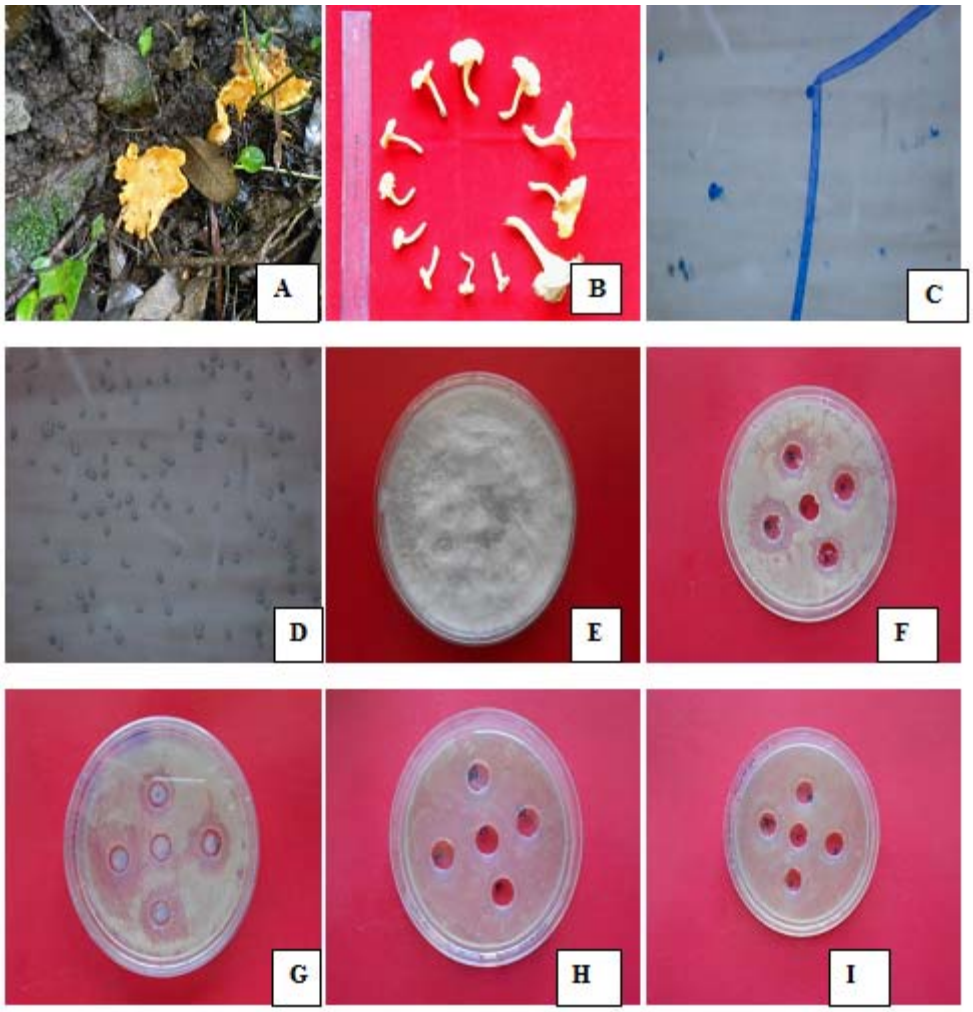

Plate 1: A *Cantharellus cibarius* in its natural habitat.**B** Collection of specimen in laboratory. **C-D** Photograph of mycelium and spores. **E** Petriplate containing pure culture of *Cantharellus cibarius.* **F** Inhibition of growth of *Listeria monocytogenes* at different conc. of methanolic extract of *Cantharellus cibarius.* **G** Inhibition of growth of *Listeria monocytogenes* at different conc. of acetone extract of *Cantharellus cibarius.* **H** Inhibition of growth of *Pseudomonas aeruginosa* at different conc. of methanolic extract of *Cantharellus cibarius.***I** Inhibition of growth of *Pseudomons aeruginosa* at different conc. of acetone extract of *Cantharellus cibarius.*

Pseudomonas aeruginosa (Plate 1 I) when the concentration of acetone extract of *C. cibarius* was increased (i.e. 18.82% at 25% conc., 19.52% at 50% conc., 20.35% at 75% conc. and 23.05% at 100% conc.) Bacterium *Listeria monocytogenes* is known to cause Listriosis, severe cases of which may lead to meningitis. Thus *C. cibarius* may be used as an agent for the treatment of such severe disorders.

The results of the present investigations are in agreement with the information compiled by[11] Turkoglu *et al*. on the antimicrobial activity of *Laetiporus sulphureus* that showed that Gram-negative bacteria were less susceptible than Gram-positive strains. In this study also acetone extract of *C. cibarius* showed more inhibition against *L. monocytogenes* as compared to *P. aeruginosa*. It is inferred from this study that the mushroom under investigation must be containing certain antibacterial moieties which need further study for its isolation and characterization[4].

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