

Study on Ultrastructure and Antibacterial Activity of *Lactarius sanguifluus* (Paulet) Fr

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The aim of the present investigation was to study ultrastructure (mycelium and spores) of *Lactarius sanguifluus* (Paulet) Fr. under Scanning Electron Microscope (SEM). Antibacterial activity of methanolic extract of *L. sanguifluus* was investigated in-vitro against two pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* following agar well diffusion method, using four different concentrations (25, 50, 75, 100%). The methanolic extract of the mushroom showed significant reduction in the growth of the tested bacteria. The extract showed maximum inhibitory growth against *E. coli* as compared to *S. aureus*. There is need of further studies to isolate and characterize the antibacterial moieties in this mushroom for practical disease control measure.

Key words: *Lactarius sanguifluus*, SEM, antibacterial, In-vitro, *S. aureus*, *E. coli*.

Mushrooms have become attractive as a functional food and as source for development of drugs and nutraceuticals, namely for antioxidants and antimicrobial compounds. In addition to dried mushrooms, alternative or substitute mushroom products (mycelium) could also be used as food and food flavoring material, or in the formulation of nutraceuticals and functional foods. The nutritional value and taste components of some mushroom mycelia have also been studied¹. 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 of them have proved to be of benefit for humans². Several important compounds including bioactive polysaccharides (lentinan), dietary fiber, ergosterol, vitamins B1, B2, 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 activities³⁻⁵. Although there is tremendous progress in human medicine; bacterial, fungal and viral diseases are still threatening the public health especially in the developing countries. Therefore further research about investigation of new antimicrobial substances should be conducted⁶. Present investigations were undertaken to study ultrastructure and antibacterial activity of *L. sanguifluus*, which is a wild edible mushroom quite popular among the tribals and existing in abundance in the North Western Himalayan region.

MATERIALS AND METHODS

Materials used in the present study were: Fruiting bodies and pure cultures of *L. sanguifluus*. Two species of bacterial pathogens (*Staphylococcus aureus* and *Escherichia coli*) were also used to check antibacterial activity of methanolic extract of *L. sanguifluus*.

Procurement of test bacteria

Pure cultures of two species of pathogenic bacteria (*S. aureus* and *E. coli*) were

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procured from IGMCI, Department of Microbiology, Shimla. Cultures were maintained on nutrient medium.

Methodology

Survey, collection and taxonomic studies: Fruiting bodies of *L. sanguifluus* were collected from Rampur (Nankhari) forest and its vicinity from July-September 2012. 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. The specimens were identified by following^{7,8} (Singer and Lincoff). For microscopic studies both dried as well as wet preserved specimens were used. The dried parts were kept for few minute 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. The anatomical details of the specimens were worked out by cutting free hand sections of the materials. 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.

SEM studies

Surface of mycelium and spores were imaged with the help of Scanning Electron Microscope (SEM). For mounting spores, spore print was taken on glass slide and spores were transferred from glass slide to carbon tape. Mycelial samples were mounted on carbon tape and were placed on the stub then stub was placed in Environmental Scanning Electron Microscope Mode (ESEM MODE) under vacuum and desired pressure, the images of the samples were obtained on screen.

Preparation of mushroom extract

Fruiting bodies of *L. sanguifluus* were dried under aseptic conditions. Dried mushrooms were pulverized in a blender and 50g each of the powdered samples were soaked separately in 300ml of methanol in an Erlenmeyer 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°C. The extracts were collected and stock solution of conc. 50 mg/ml was prepared.

Screening of mushroom extract for antibacterial activity

Screening of methanolic extract of *L. sanguifluus* 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 121.60°C 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. One well was prepared in each agar plate. The well in each plate was loaded with 25%, 50%, 75%, 100% conc. prepared separately by dissolving extract in methanol. The plates containing bacterial colonies were incubated at 37°C 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 standard¹⁰.

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

Control = Average diameter of bacterial colony in control.

Test = Average diameter of bacterial colony in treatment sets¹¹.

RESULTS AND DISCUSSION

Macroscopic and microscopic characteristics of *L. sanguifluus*: Fruiting bodies of *L. sanguifluus* were analysed for macroscopic and microscopic characters. Pileus- 6-15 cm in diameter, cap is carrot coloured, depression in the centre, paler zones alternating and margins enrolled when young becoming decurved to plane with age. Stipe- 5-8cm long, 0.8-1.5cm diameter, usually paler and dull then pileus, solid when young, hollowing

with age. Lamellae- subdecurrent to decurrent, dull purplish red, easily separable from flesh often spotted greenish with age. Latex- deep blood red to purplish red which becomes muddy orange red in mature specimens (Plate 1a). Spore print- pale yellowish. Basidia- $35-55 \times 8-10.5 \mu\text{m}$, clavate, thin walled and tetrasporic. The mycelium and spores were observed under Scanning Electron Microscope at different magnification at pressure 2.98e-1 Torr. The mycelial hyphae ranges in diameter from 1.8- $4.8 \mu\text{m}$, branched and septate. Basidiospores- $6-8 \mu\text{m} \times 6-7.2 \mu\text{m}$, broadly ellipsoid to subglobose, widely spaced irregular bands with both heavy and light cross connections to produce a broken or nearly complete reticulum, surface

ornamentations were clearly visible (Plate 1 b&c). Basidiospores of *L. strigosipes*, *L. aerolatus* and *L. minusculus* were observed under Scanning Electron Microscope and report similar sizes and ornamentations¹².

Antibacterial activity

Methanolic extract of *L. sanguifluus* was seen to show gradual inhibition in the growth of *S. aureus* and *E. coli*. There was a regular increase in inhibition of *S. aureus* and *E. coli* when subjected to increase in concentration of the extract and reached at its maximum at 100% conc. of the mushroom extract. Stock solution of conc. 50mg/ml was considered as 100% conc. and other concentrations were prepared by serial dilution of stock solution. It is evident from Table 1. and Histogram 1 that methanolic extract of *L. sanguifluus* against *S. aureus* and *E. coli* failed to produce any inhibition in petriplates kept as control but showed 20.59% inhibition at 25% conc., 24.12% at 50% conc., 26.82% at 75% conc. and 30% at 100% concentration in case of *S. aureus* (Plate 1 i-m) and 21.42% at 25% conc., 24.94% at 50% conc., 28.24% at 75% conc. and 30.94% at 100% conc. of methanolic extract in case of *E. coli* (Plate 1 d-h). Some earlier workers¹³⁻¹⁵ has reported strong antibacterial activities of *Lactarius* species against different pathogenic bacteria. The result of the present study are in agreement with the work of I6 (Ramesh and Patter) who reported strong

Table 1. Percentage inhibition of *S. aureus* and *E. coli* against methanolic extract of *L. sanguifluus* at different concentrations

Concen. (In %)	Percentage inhibition of growth of test bacteria(mm $\pm$ S.E)	
	<i>S. aureus</i>	<i>E. coli</i>
Control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
25%	20.59 $\pm$ 0.50	21.42 $\pm$ 0.73
50%	24.12 $\pm$ 0.29	24.94 $\pm$ 0.44
75%	26.82 $\pm$ 0.60	28.24 $\pm$ 0.76
100%	30.00 $\pm$ 3.01	30.94 $\pm$ 2.49

Each data represent mean of three replicates $\pm$ S.E.

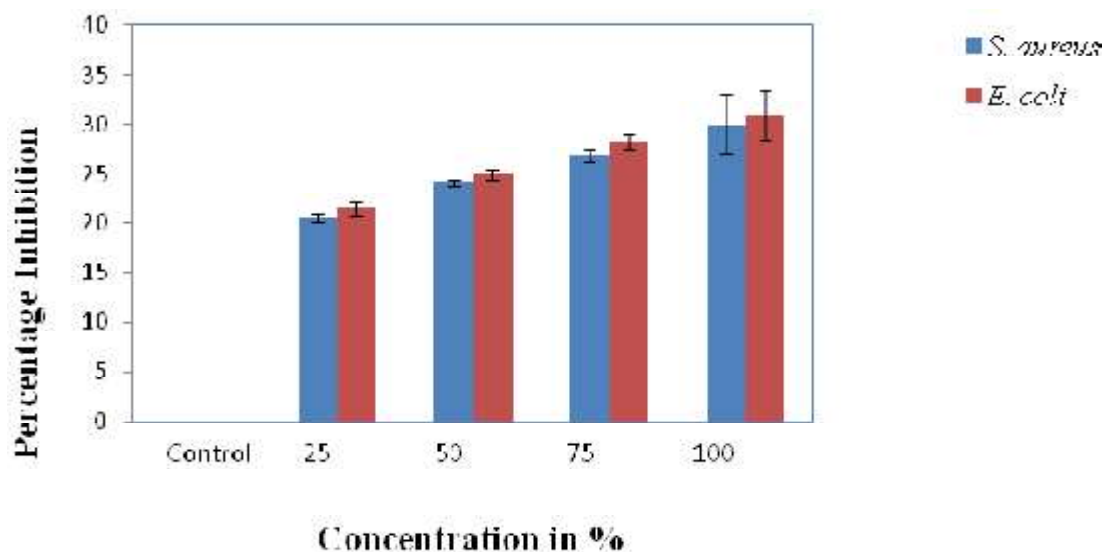


Fig. 1. Percentage inhibition of growth of *S. aureus* and *E. coli* against methanolic extract of *L. sanguifluus* at different concentrations.

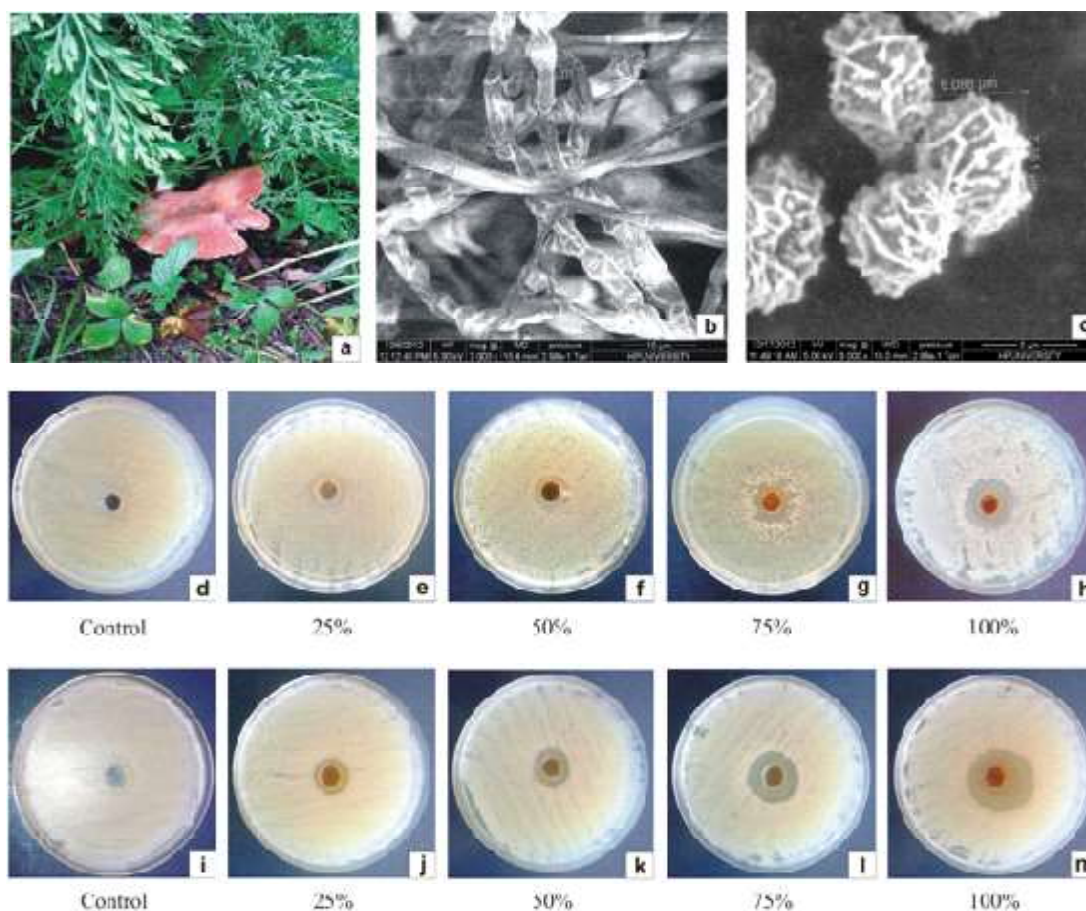


Fig. 2. (a) *Lactarius sanguifluus* in its natural habitat. (b-c) Scanning electron microscopic images of mycelium and spores of *Lactarius sanguifluus*. (d-h) inhibition of growth of *E. coli* at different concentration of methanolic extract of *Lactarius sanguifluus*. (i-m) inhibition of growth of *S. aureus* at different concentration of methanolic extract of *Lactarius sanguifluus*

antibacterial activity of extract of *Clavaria vermiculris* and *Marasmius oreades* against Gram-negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria and comparatively less activity against Gram-positive (*Bacillus subtilis* and *S. aureus*) bacteria. As per our study also *L. sanguifluus* showed more inhibition against *E. coli* as compared to *S. aureus*. 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.

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