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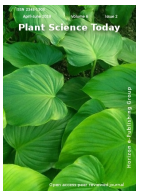

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**Research Article**

# Antimicrobial potential of some wild Macromycetes collected from Kashmir Himalayas

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**Abstract**

Alarming increase in microbial resistance to existing synthetic commercial antibiotics forced scientists to search for new antimicrobials from various alternative sources. The present study carried out during the year 2014-2015, presents the antimicrobial potential of some mushroom extracts against some commonly found pathogenic bacterial and fungal microbes. During the study four mushroom species, viz. *Lentinus tigrinus* (Bull.) Fr., *Fomitopsis pinicola* (Sw.) P.Karst, *Inonotus hispidus* (Bull.) P.Karst and *Ramaria formosa* (Pers.) Quel. were evaluated for their antimicrobial activity against both gram positive (*Bacillus subtilis* and *Staphylococcus aureus*), gram negative (*Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and fungi (*Saccharomyces cerevisiae Saccharomyces cerevisiae*, *Candida albicans*, *Penicillium chrysogenum* and *Aspergillus fumigates*). The results revealed that ethyl acetate and methanolic extract of all the mushroom extracts showed significant antimicrobial activity against most of the bacterial and fungal microbes. However, the aqueous extract of these mushrooms was found either lacking or conferring insignificant antimicrobial activity. The ethyl acetate extracts of *Ramaria formosa* and *Lentinus tigrinus* produced more promising results against the bacterial microbes than fungal counterparts. Both ethyl acetate and methanolic extracts of *Fomitopsis pinicola* and *Inonotus hispidus* exhibited strong antimicrobial activity against the selected set of microbes. The antibacterial and antifungal activity exhibited by *Fomitopsis pinicola* at the concentration 150mg/ml was almost parallel to 10µg gentamycin and 50µg nystatin respectively. Therefore, *Fomitopsis pinicola* signifies as one of the promising mushroom species possessing strong antimicrobial activity against broad spectrum of microbes.

**Keywords:** mushroom extracts; pathogenic microbes; antimicrobial activity; Kashmir

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## Introduction

Mushrooms have been appreciated by man since the times immemorial not only for their flavor, deliciousness and nutritive excellence but for their medicinal attributes (1). They are now the subject of interest for many ethnobotanists and medical researchers. Of the 14,000- 15,000 known species of

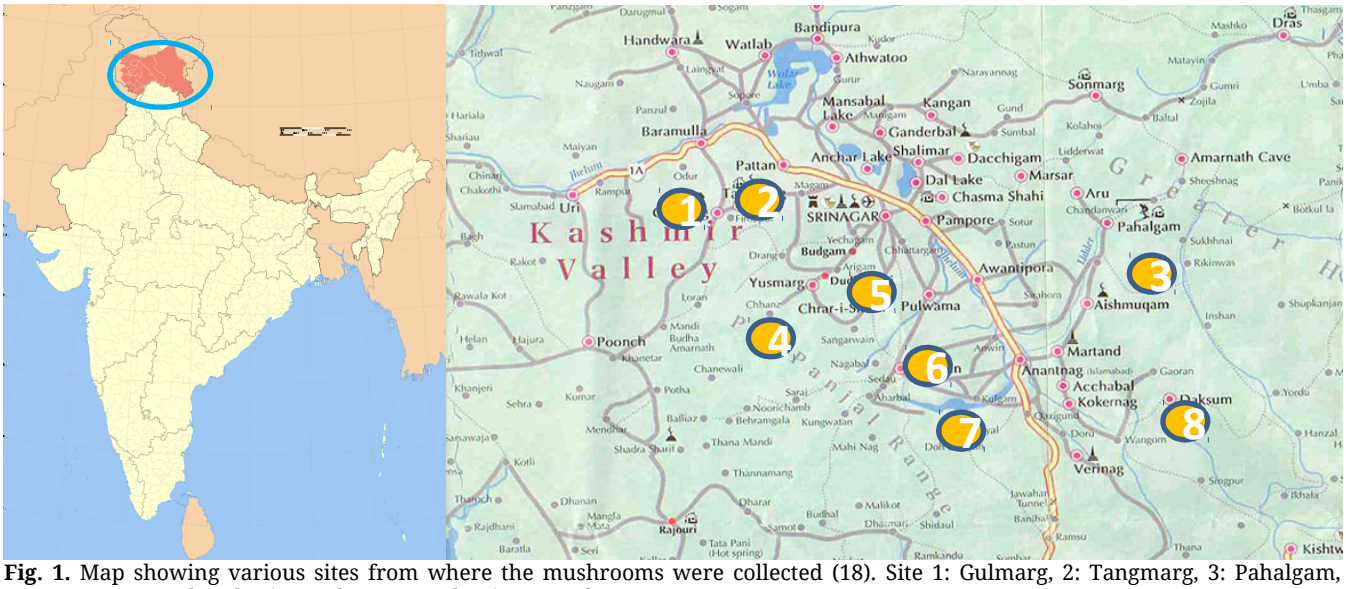


**Fig. 1.** Map showing various sites from where the mushrooms were collected (18). Site 1: Gulmarg, 2: Tangmarg, 3: Pahalgam, 4: Yusmarg, 5: Duthpatheri, 6: Pulwama, 7: Shopian, 8: Daksum.

mushrooms, there are some 1,800 species possessing medicinal attributes and about 700 species of known medicinal properties (2,3). As a matter of fact, mushrooms need antibacterial and antifungal compounds to survive at ease in their natural environment in order to combat the attack of pathogenic microbes. This property of mushrooms can prove handy to be exploited for human welfare. The large number of wild mushrooms both edible and inedible, on scrutinization for their antimicrobial activities has come out with marvelous results (4-8). Many workers from different corners of the world advocated the usage of many Polypore and Agaric mushroom extracts to inhibit the growth of structurally and functionally diverse groups of bacterial and fungal strains (9,10). It has been found that nearly 75% of tested polypore mushrooms possess antimicrobial activity (11).

Despite advances in modern medicine, infectious diseases caused by various microbes still are one of the major threats to human health. Though a large number of synthetic commercial drugs are available but due to their hazardous impacts on health, novel antimicrobial agents from different biological sources is continually sought (12). Also, the alarming increase in bacterial resistance to existing antibiotics due to their inappropriate and indiscriminate use forced scientists to search for new antimicrobials from various alternative sources (13). The belief that green medicine is safer and more reliable than the costly synthetic drugs has renewed interest in traditional medicine. The situation provided the impetus to the research for new antimicrobial substances from various biological sources (12).

The research upon mushrooms related to their antimicrobial potential revealed that they have strong tendency to retard the growth of a number of pathogenic microbes, but the evaluation of the antimicrobial potential of mushrooms is still in an exploratory stage and there are only a handful of species subjected to pharmacological screening (14,15). Kashmir cherishes a wide range of mushrooms of glorious medicinal importance. There are about 250 species of wild mushrooms reported from Kashmir Himalayas having nutrition and medicinal attributes (16,17), but their scientific scrutinization for pharmacological potential is yet to be evaluated. Therefore, the present study was aimed to investigate the antibacterial and antifungal activity of some mushroom species against various pathogenic bacteria and fungi.

## Materials and Methods

**Collection of material:** Four species of mushrooms, viz. *Lentinus tigrinus* (Bull.) Fr., *Fomitopsis pinicola* (Sw.) P.Karst, *Inonotus hispidus* (Bull.) P.Karst and *Ramaria formosa* (Pers.) Quel. were selected for a screening of their antimicrobial activity. Field trips were carried out from May to September to different sites of Kashmir Himalayas during the year 2014-2015 for the collection of fresh fruiting bodies of these mushrooms. The list of sites visited during the study is shown in Fig. 1. After collection and recording of certain morphological characters in the field, the mushrooms were wrapped in paper bags and brought to the laboratory for further studies. Identification was carried out by referring to field guides of mushrooms, recent monographs and keys of experts. Experts help was also taken from Indian scientists like Dr T. N Lakhanpal, Dr. R. C. Upadhya, and Dr. N. S. Atri for correct identification. Identification was also confirmed by thorough comparison with museum collections of Sher-e-Kashmir University of Agricultural Sciences and Technology-Kashmir (SKUAST-K), Indian Institute of Integrative Medicine (IIIM) Srinagar. The vernacular name, accession number,

**Table 1:** Vernacular name, accession number, edibility and sites of collection of screened mushrooms.

| S. No. | Mushroom Species | Vernacular name | Accession number | Edibility | Site of collection |
|---|---|---|---|---|---|
| 1 | *Lentinus tigrinus* (Bull.) Fr. | Vire haddur | SH.KASH-28791M | Edible | Duksum, Shopian and Dudhpatheri |
| 2 | *Fomitopsis pinicola* (Sw.) P.Karst | Yaade lassh | SH.KASH-28776M | Inedible | Gulmarg, Dudhpatheri, Pahalgam and Tangmarg |
| 3 | *Inonotus hispidus* (Bull.) P.Karst | Chunth lash | SH.KASH-28792M | Inedible | Shopian and Pulwama |
| 4 | *Ramaria formosa* (Pers.) Quel. | Panze ungje | SH.KASH-28833M | Edible | Gulmarg, Tangmarg, Pahalgam and Yusmarg |

**Table 2:** List of bacterial and fungal species screened for antimicrobial activity.

| S.No | Test organism | Source |
|---|---|---|
| 1 | *Bacillus subtilis* (gram-positive) | MTCC-441 |
| 2 | *Staphylococcus aureus* (gram-positive) | MTCC-96 |
| 3 | *Escherichia coli* (gram-negative) | MTCC-407 |
| 4 | *Proteus vulgaris* (gram-negative) | MTCC-426 |
| 5 | *Klebsiella pneumoniae* (gram-negative) | MTCC-19 |
| 6 | *Pseudomonas aeruginosa* (gram-negative) | MTCC-1688 |
| 7 | *Saccharomyces cerevisiae* | MTCC-1023 |
| 8 | *Candida albicans* | MTCC-6258 |
| 9 | *Penicillium chrysogenum* | MTCC-1380 |
| 10 | *Aspergillus fumigatus* | MTCC-9001 |

edibility and sites of collection for each mushroom species are shown in Table 1.

**Preparation of extracts:** The fresh fruiting bodies of the mushrooms were wiped off any adhering impurity followed by shade drying and then heat drying in the electric oven at 45°C for 2-3 hours. The dried material was pulverized in an electric blender to get a coarse powder. 100 grams of the powdered material was subjected to Soxhlet extraction by using 1000 ml of three different solvents namely ethyl acetate, methanol and water successively in the increasing order of their polarity. The extracts were concentrated by evaporating the solvent on a water bath and the dry crude extract obtained was stored in air tight vials at 4°C till used for antimicrobial screening.

**Sterilization:** In order to prevent the microbial contamination, sterilization of glassware, cultural media and various other equipments was carried out by dry heat, wet heat, direct flaming and UV sterilization methods depending upon the nature of the material prior to their use.

**Test organisms:** Six strains of bacteria, including both gram positive and gram negative, and four fungal strains received from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH) Chandigarh, India, were used to test the antimicrobial activity of selected mushroom extracts. The list of the bacterial and fungal strains along with their source is mentioned in Table 2.

**Antimicrobial screening by agar well diffusion method:** Separate cultural media were used for the evaluation of antibacterial and antifungal activity. Muller Hinton Agar and its broth were used for screening of antibacterial activity while Sabouraud Dextrose Agar and its broth were used for screening of antifungal activity. The antibacterial and antifungal activity of mushroom extracts was tested using Agar Well Diffusion method (19). The culture tubes containing 20-25 ml of molten media were inoculated by adding 100 µl of 0.5 Mac-Farland standard inoculum from the freshly prepared microbial suspension. 0.5 Mac-Farland standard inoculums contains approximately 1.5 x $10^8$ CFU/ml for bacteria and and 5 x $10^5$ CFU/ml for fungi. The tubes were then homogenized by rubbing between the hands and poured into 90 mm flat bottomed petri plates. The plates were then allowed to solidify under laminar air flow for about 15 minutes and thereafter wells were dug with the help of 6 mm cork borer. The solidified extract was dissolved in sterile dimethyl sulfoxide (DMSO) and 50 µl of each extract was added to the respective wells. Gentamicin (10µg/disc) and Nystatin (50µg/disc) were used as positive control for bacterial and fungal screening respectively, while DMSO was used as negative control. All the bacterial and fungal strains were susceptible to Gentamicin and Nystatin respectively. The plates were then sealed and incubated 37±1°C for 24 hours for bacterial and 27±1°C for 48 hours for fungal activity. After the incubation period, plates were observed for the clear zone formation around the wells, called zone of inhibition. The antimicrobial activity of the extracts was calculated by measuring the zone of inhibition in mm including the well diameter using standard scale (20). The results were

**Table 3:** Screening of antimicrobial activity of various mushroom extracts against selected bacterial and fungal strains.

| Mushroom species | Crude extract | Bacteria | | | | | | Fungi | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **BS** | **EC** | **SA** | **KP** | **PA** | **PV** | **CA** | **SC** | **AF** | **PC** |
| *Lentinus tigrinus* | Ethyl Acetate | ++ | ++ | ++ | ++ | ++ | ++ | + | + | - | - |
| | Methanol | ++ | + | + | + | + | + | - | - | - | - |
| | Aqueous | - | - | - | - | - | - | - | - | - | - |
| *Fomitopsis pinicola* | Ethyl Acetate | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | Methanol | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | Aqueous | - | - | - | - | - | - | - | - | - | - |
| *Inonotus hispidus* | Ethyl Acetate | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | Methanol | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| | Aqueous | - | - | - | - | - | - | - | - | - | - |
| *Ramaria formosa* | Ethyl Acetate | ++ | ++ | ++ | ++ | ++ | ++ | - | + | - | - |
| | Methanol | ++ | + | + | + | + | + | - | + | - | - |
| | Aqueous | - | - | - | - | - | - | - | - | - | - |

**-** (No activity, + (Zone of inhibition 9-12 mm), ++ (Zone of inhibition 13-18 mm), **BS** *(Bacillus subtilis),* **EC** *(Escherichia coli),* **SA** *(Staphylococcus aureus),* **KP** *(Klebsiella pneumonia),* **PA** *(Pseudomonas aeruginosa),* **PV** *(Proteus vulgaris),* **CA** *(Candida albicans),* **SC** *(Saccharomyces cerevisiae),* **AF** *(Aspergillus fumigatus),* **PC** *(Penicillium chrysogenum)*.

calculated as the mean ±SD of three independent experiments.

Initially three different extracts, i.e. ethyl acetate, methanol and aqueous extracts of each mushroom species at the concentration of 100mg of extract per ml DMSO were screened for antimicrobial activity. The extracts which showed significant antimicrobial activity were also evaluated for their activity against these test microbes at three different concentrations i.e. 50, 100 and 150mg of mushroom extract per ml of DMSO.

**Determination of minimum inhibitory concentration (MIC):** MIC was checked out for the extracts which showed significant antimicrobial activity in agar well diffusion method. MIC was determined by agar dilution method recommended by Wiegand *et al.* (19). A series of two-fold dilutions of the mushroom extracts ranging from 0.2-25.6 mg/ml was carried out to the respective bacterial and fungal media in the culture tubes prior to pouring into Petri plates. The treated media of the culture tubes were poured into the sterilized plates and allowed to solidify in laminar air flow, followed by spot inoculation with 3µl and 2µl of aliquots of the bacterial and fungal culture of 0.5 Mac-Farland standard inoculum containing approximately 1.5 x $10^8$ CFU/ml and 5 x $10^5$ CFU/ml respectively. The plates were sealed and incubated at 37±1°C for 18-24 hours for bacterial activity and 27±1°C for 48 hours for fungal activity. After the incubation period the plates were observed for the growth of test organisms and the lowest concentration at which there is no growth of test organisms is called MIC of that tested extract.

## Results

During the present study, it was observed that the antimicrobial activity of these mushroom species against the given selected pathogenic bacteria and fungal strains varies with the nature of the solvent. The aqueous extract of all the four mushroom species was found to lack or show insignificant antimicrobial activity against the bacterial and fungal strains (Table 3). Both methanolic and ethyl acetate extract of *Fomitopsis pinicola* and *Inonotus hispidus* showed significant antimicrobial activity against all the bacterial and fungal strains while the ethyl acetate extract of *Lentinus tigrinus* and *Ramaria formosa* showed significant antimicrobial activity against the bacterial strains while mild or no activity against the fungal strains. Also, the methanolic extract of *Lentinus tigrinus* and *Ramaria formosa* showed significant antimicrobial activity against *Bacillus subtilis*, mild activity against the rest of the bacterial strains and no activity against all the fungal strains.

The assessment of the antimicrobial potential of ethyl acetate extract of *Lentinus tigrinus* at different concentrations revealed that there was a considerable increase in the zone of inhibition subsequent to the increase in the concentration of extract (Table 4). It is quite evident from the results that the extract showed the highest activity against *Bacillus subtilis* followed by *Proteus vulgaris*, *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus,* while the growth of *Klebsiella pneumoniae* was least inhibited. The extract produced the 18 mm zone of inhibition in case of *Bacillus subtilis* culture at the concentration of 150mg/ml while the same extract produced 15 mm zone of inhibition in case of *Klebsiella pneumoniae* culture at the corresponding concentration.

Screening of antimicrobial activity of ethyl acetate and methanolic extract of *Fomitopsis pinicola* revealed that both the extracts possess considerably significant antimicrobial potential against the selected set of bacterial and fungal microbes (Table 5). The extracts of this mushroom exhibited the highest antimicrobial activity among

**Table 4:** Antibacterial activity of ethyl acetate extract of *Lentinus tigrinus* against selected bacterial strains.

| S. No | Bacterial strains | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | Concentration (mg/ml) | | | Standard |
| | | 50 | 100 | 150 | Gentamicin (10µg/disc) |
| 1 | *Bacillus subtilis* | 12.66±0.57 | 15.66±0.57 | 18.00±1.00 | 26.66±2.08 |
| 2 | *Escherichia coli* | 11.66±0.57 | 14.66±0.57 | 16.66±1.15 | 25.66±1.52 |
| 3 | *Staphylococcus aureus* | 11.33±1.15 | 13.66±0.57 | 16.33±0.57 | 24.33±0.57 |
| 4 | *Klebsiella pneumonia* | 10.33±0.57 | 12.33±0.57 | 14.66±1.15 | 24.00±2.00 |
| 5 | *Pseudomonas aeruginosa* | 11.33±0.57 | 14.66±1.15 | 16.33±1.52 | 25.66±1.52 |
| 6 | *Proteus vulgaris* | 12.00±1.00 | 14.66±1.52 | 16.66±0.57 | 26.66±1.52 |

**Table 5**: Antimicrobial activity of ethyl acetate and methanolic extract of *Fomitopsis pinicola* against selected bacterial and fungal strains.

| S. No | Bacterial and fungal strains | Extract | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Concentration (mg/ml) | | | Standard | |
| | | | 50 | 100 | 150 | Gentamicin (10µg/disc) | Nystatin (50µg/ disc) |
| 1 | *Bacillus subtilis* | EA | 17.66±1.15 | 21.66±0.57 | 24.66±1.52 | 27.00±1.00 | NT |
| | | M | 18.00±1.00 | 22.33±1.15 | 25.66±1.52 | 26.66±1.15 | NT |
| 2 | *Escherichia coli* | EA | 15.33±0.57 | 18.33±1.15 | 21.66±1.15 | 26.00±1.00 | NT |
| | | M | 17.00±1.00 | 21.66±1.15 | 23.00±1.00 | 25.66±0.57 | NT |
| 3 | *Staphylococcus aureus* | EA | 15.00±1.00 | 18.66±1.15 | 20.00±1.00 | 24.66±1.15 | NT |
| | | M | 17.66±0.57 | 20.66±0.57 | 24.33±1.15 | 24.33±0.57 | NT |
| 4 | *Klebsiella pneumonia* | EA | 14.66±0.57 | 17.66±0.57 | 20.33±1.15 | 23.33±1.52 | NT |
| | | M | 14.00±1.00 | 16.66±0.57 | 19.66±1.15 | 23.66±1.15 | NT |
| 5 | *Pseudomonas aeruginosa* | EA | 15.00±1.00 | 18.00±1.00 | 20.33±0.57 | 25.00±1.00 | NT |
| | | M | 15.00±1.00 | 18.66±0.57 | 21.33±1.15 | 25.33±0.57 | NT |
| 6 | *Proteus vulgaris* | EA | 17.33±0.57 | 21.00±1.00 | 24.00±1.00 | 26.66±0.57 | NT |
| | | M | 16.66±0.57 | 21.33±0.57 | 23.66±1.15 | 26.66±0.57 | NT |
| 7 | *Candida albicans* | EA | 13.00±1.00 | 15.66±1.15 | 18.33±0.57 | NT | 24.66.±1.15 |
| | | M | 13.66±0.57 | 16.66±1.52 | 20.00±1.00 | NT | 24.00±1.00 |
| 8 | *Saccharomyces cerevisiae* | EA | 15.00±1.00 | 18.33±0.57 | 20.66±0.57 | NT | 26.66±0.57 |
| | | M | 16.00±1.00 | 19.66±1.15 | 22.00±1.00 | NT | 27.00±1.00 |
| 9 | *Aspergillus fumigates* | EA | 12.66±0.57 | 15.66±1.15 | 17.00±1.00 | NT | 19.00±1.00 |
| | | M | 13.33±0.57 | 16.33±0.57 | 18.00±1.00 | NT | 18.66±0.57 |
| 10 | *Penicillium chrysogenum* | EA | 13.00±1.00 | 15.66±1.15 | 17.66±0.57 | NT | 18.33±1.15 |
| | | M | 13.33±1.15 | 16.00±1.00 | 17.66±0.57 | NT | 18.00±1.73 |

Values represent mean ±SD of three separate experiments, EA=Ethyl acetate, M=methanol, NT= Not tested.

all the four species. It is quite evident from the results that methanolic extract of *Fomitopsis pinicola* exhibited more antimicrobial activity than its ethyl acetate extract against the bacterial and fungal microbes except for *Klebsiella pneumoniae* and *Proteus vulgaris* where ethyl acetate extract displayed slightly better results than methanolic extract. The ethyl acetate extract resulted highest growth inhibition for *Bacillus subtilis* followed by *Proteus vulgaris, Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and lowest that of *Klebsiella pneumonia*, while the methanolic extract exhibited highest growth inhibition against *Bacillus subtilis* followed by *Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* and lowest that of *Klebsiella pneumoniae* among the bacterial strain. The results clearly indicate that there is a difference of only 1-4 mm in the zone of inhibition displayed by the extracts at the concentration of 150mg/ml and the 10µg pure gentamycin against most of the bacterial strains. However, in case of *Staphylococcus aureus*, the methanolic extract at the concentration of 150mg/ml and the pure 10µg gentamycin exhibited the same results and 24 mm inhibition zone was observed in each case. Regarding the antifungal activity of *Fomitopsis pinicola* against the given set of fungal microbes, methanolic extract proved slightly more effective than ethyl acetate extract except in *Penicillium chrysogenum* where both the extracts produced more or less similar results. Both ethyl acetate and methanolic extracts exhibited maximum antifungal activity against *Saccharomyces cerevisiae* followed by *Candida albicans,* while *Penicillium chrysogenum* and *Aspergillus fumigatus* showed more or less equal but lowest susceptibility to these extracts. Also, in restricting the growth of *Penicillium chrysogenum* and *Aspergillus fumigatus,* both ethyl acetate and

**Table 6:** Antimicrobial activity of ethyl acetate and methanolic extract of *Inonotus hispidus* against selected bacterial and fungal strains.

| S. No | Bacterial and fungal strains | Extract | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Concentration (mg/ml) | | | Standard | |
| | | | 50 | 100 | 150 | Gentamicin (10µg/disc) | Nystatin (50µg/ disc) |
| 1 | *Bacillus subtilis* | EA | 13.66±0.57 | 17.33±0.57 | 19.00±1.00 | 26.66±1.54 | NT |
| | | M | 15.33±0.57 | 18.66±0.57 | 21.00±1.00 | 27.33±0.57 | NT |
| 2 | *Escherichia coli* | EA | 12.33±0.57 | 15.66±0.57 | 16.66±1.15 | 25.66±0.57 | NT |
| | | M | 12.66±0.57 | 15.00±1.00 | 17.66±1.15 | 26.00±1.00 | NT |
| 3 | *Staphylococcus aureus* | EA | 12.00±1.00 | 14.66±0.57 | 16.00±1.00 | 24.33±1.15 | NT |
| | | M | 12.33±0.57 | 14.66±1.15 | 16.00±1.00 | 25.00±1.00 | NT |
| 4 | *Klebsiella pneumonia* | EA | 10.33±0.57 | 12.00±1.00 | 13.66±0.57 | 23.66±0.57 | NT |
| | | M | 11.33±0.57 | 13.00±1.00 | 15.00±1.00 | 23.66±0.57 | NT |
| 5 | *Pseudomonas aeruginosa* | EA | 11.33±0.57 | 13.66±1.15 | 15.00±1.00 | 26.00±1. 00 | NT |
| | | M | 11.66±1.15 | 13.66±0.57 | 15.66±0.57 | 25.66±1.15 | NT |
| 6 | *Proteus vulgaris* | EA | 11.66±0.57 | 14.33±1.15 | 15.66±0.57 | 26.66±1.15 | NT |
| | | M | 12.00±0.00 | 14.33±1.15 | 15.66±0.57 | 26.66±0.57 | NT |
| 7 | *Candida albicans* | EA | 11.66±0.57 | 13.66±0.57 | 15.00±1.00 | NT | 24.00.±1.00 |
| | | M | 12.00±1.00 | 14.33±0.57 | 17.33±1.15 | NT | 23.66.±1.15 |
| 8 | *Saccharomyces cerevisiae* | EA | 13.33±0.57 | 16.33±1.15 | 18.33±0.57 | NT | 27.00±1.00 |
| | | M | 15.00±1.00 | 18.66±0.57 | 20.33±1.15 | NT | 27.66±0.57 |
| 9 | *Aspergillus fumigates* | EA | 12.00±1.00 | 14.66±1.15 | 16.33±0.57 | NT | 18.66±1.52 |
| | | M | 12.33±1.15 | 14.66±0.57 | 16.66±0.57 | NT | 19.00±1.00 |
| 10 | *Penicillium chrysogenum* | EA | 11.66±0.57 | 13.33±1.15 | 14.66±1.52 | NT | 18.00±1.00 |
| | | M | 11.66±0.57 | 14.66±0.57 | 16.00±1.00 | NT | 18.00±1.00 |

Values represent mean ±SD of three separate experiments, EA=Ethyl acetate, M=methanol, NT= Not tested.

**Table 7**: Antibacterial activity of ethyl acetate extract of *Ramaria formosa* against selected bacterial strains.

| S. No | Bacterial strains | Zone of Inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | Concentration (mg/ml) | | | Standard |
| | | 50 | 100 | 150 | Gentamicin (10µg/disc) |
| 1 | *Bacillus subtilis* | 13.33±1.15 | 16.00±1.00 | 18.66±1.15 | 26.66±1.52 |
| 2 | *Escherichia coli* | 12.00±1.00 | 14.66±0.57 | 16.00±1.00 | 25.66±0.57 |
| 3 | *Staphylococcus aureus* | 11.33±1.15 | 14.00±1.00 | 15.66±0.57 | 24.33±1.54 |
| 4 | *Klebsiella pneumonia* | 11.33±0.57 | 13.33±0.57 | 14.66±1.15 | 24.33±1.15 |
| 5 | *Pseudomonas aeruginosa* | 11.66±0.57 | 14.00±1.00 | 16.00±1.00 | 25.33±1.15 |
| 6 | *Proteus vulgaris* | 13.00±1.00 | 15.66±0.57 | 18.00±1.00 | 26.66±0.57 |

Values represent mean ±SD of three separate experiments.

methanolic extracts at the concentration of 150mg/ ml exhibited almost as good results as 50µg pure nystatin.

The ethyl acetate and methanolic extracts of *Inonotus hispidus* also displayed substantial antimicrobial activity against the selected bacterial and fungal microbes at the given set of concentrations (Table 6). As evident from the results, the methanolic extract of this mushroom proved slightly more effective in restricting the growth of all the bacterial strains than ethyl acetate extract. Among the bacterial strains, the growth of *Bacillus subtilis* was most inhibited followed by *Proteus vulgaris, Escherichia coli, Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa,* while the growth of *Klebsiella pneumoniae* was least inhibited by both methanolic and ethyl acetate extracts. The screening of antifungal activity of *Inonotus hispidus* also revealed that methanolic extract proved slightly more effective in inhibiting the growth of fungal microbes than ethyl acetate extract, except *Aspergillus fumigatus* where both the extracts came out with almost the same results. The ethyl acetate extract exhibited maximum antifungal activity against *Saccharomyces cerevisiae* followed by *Aspergillus fumigates Candida albicans* and *Penicillium chrysogenum,* while the methanolic extract exhibited maximum antifungal activity against *Saccharomyces cerevisiae* followed by *Aspergillus fumigatus* and *Penicillium chrysogenum,* and least against *Candida albicans*.

The assessment of the antibacterial activity of ethyl acetate extract of *Ramaria formosa* at different concentrations also yielded good result, particularly at the concentration of 150mg/ml (Table 7). It was found that the extract shows



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**Table 8:** Minimum inhibitory concentration (MIC) of mushroom extracts (in mg/ml) for the selected bacterial and fungal strains.

| Name of Mushroom | Extract type | Test organism | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **BS** | **EC** | **SA** | **KP** | **PA** | **PV** | **CA** | **SC** | **AF** | **PC** |
| *Lentinus tigrinus* | Ethyl Acetate | 3.2 | 6.4 | 6.4 | 12.8 | 6.4 | 6.4 | NT | NT | NT | NT |
| *Fomitopsis pinicola* | Ethyl Acetate | 0.8 | 1.6 | 1.6 | 1.6 | 1.6 | 0.8 | 3.2 | 1.6 | 3.2 | 3.2 |
| | Methanol | 0.8 | 0.8 | 0.8 | 1.6 | 1.6 | 0.8 | 3.2 | 1.6 | 3.2 | 3.2 |
| *Inonotus hispidus* | Ethyl Acetate | 3.2 | 3.2 | 3.2 | 6.4 | 3.2 | 3.2 | 6.4 | 3.2 | 6.4 | 6.4 |
| | Methanol | 1.6 | 3.2 | 3.2 | 6.4 | 6.4 | 3.2 | 6.4 | 1.6 | 6.4 | 6.4 |
| *Ramaria formosa* | Ethyl Acetate | 3.2 | 6.4 | 6.4 | 6.4 | 6.4 | 3.2 | NT | NT | NT | NT |

**BS**=*Bacillus subtilis,* **EC**=*Escherichia coli,* **SA**=*Staphylococcus aureus,* **KP**=*Klebsiella pneumonia,* **PA**=*Pseudomonas aeruginosa,* **PV**=*Proteus vulgaris,* **CA**=*Candida albicans,* **SC**=*Saccharomyces cerevisiae,* **AF**=*Aspergillus fumigatus,* **PC**=*Penicillium chrysogenum, **NT**= Not tested*.

maximum activity against *Bacillus subtilis* followed by *Proteus vulgaris*, *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus, Klebsiella pneumonia.* The results obtained from this extract were found parallel to the ethyl acetate extract of *Lentinus tigrinus*.

While figuring out the minimum inhibition concentration (MIC) of different mushroom extracts for the given set of bacterial and fungal microbes, it was found that MIC values vary from extract to extract for a common set of microbes (Table 8). For ethyl acetate extract of *Lentinus tigrinus,* the MIC value was found to vary from 3.2-12.8 mg/ml against bacterial strains. The MIC value for methanolic and ethyl acetate extract of *Fomitopsis pinicola* was found to vary from 0.8-1.6 and 1.6-3.2 mg/ml against bacterial and fungal microbes respectively. The MIC value for ethyl acetate extract of *Inonotus hispidus* was found to vary from 3.2-6.4 mg/ml against both bacterial and fungal microbes. Similarly, MIC value for methanolic extract of this mushroom was found to vary from 1.6-6.4 mg/ml against both bacterial and fungal microbes. Likewise, the MIC value for ethyl acetate extract of *Ramaria formosa* was found to vary from 3.2-6.4 mg/ml against the bacterial strains.

After observing the zone of inhibition and MIC values of different mushroom extract against the given set of bacterial and fungal microbes it was found that *Fomitopsis pinicola* exhibited the highest zone of inhibition and lowest MIC value, followed by *Inonotus hispidus*, *Ramaria formosa* and *Lentinus tigrinus,* thereby indicating the relative antimicrobial potential of these mushrooms in the same sequence. It was also found that all the mushroom extracts exhibited the highest zone of inhibition and lowest MIC value towards *Bacillus subtilis* and *Saccharomyces cerevisiae* among the bacterial, fungal microbes respectively, thereby indicating the most susceptibility of these two microbes to the applied mushroom extracts. The zone of inhibition and MIC values also revealed that the growth of *Klebsiella pneumoniae* was least inhibited by the mushroom extracts among the bacterial strains, while *Aspergillus fumigates, Candida albicans* and *Penicillium chrysogenum* responded differently to different mushroom extracts among the fungal strains. In negative control, DMSO was found lacking antimicrobial activity against all the bacterial and fungal strains.

## Discussion

All the four mushrooms were found to possess the varying degrees of antimicrobial activity against the selected set of bacterial and fungal microbes. The aqueous extract was found less effective in restricting the growth of different pathogenic bacterial and fungal microbes as compared to ethyl acetate and methanolic extract. Also, the ethyl acetate extract of *Lentinus tigrinus* and *Ramaria formosa* was found highly effective against the bacteria, but showed mild inhibition of fungal microbes. However, both ethyl acetate and methanolic extract produced strong antimicrobial activity against both fungal and bacterial microbes. The high antimicrobial activity of ethyl acetate and methanolic extracts than aqueous extract can be explained because of the fact that most of the antimicrobial molecules are insoluble in water, and diffusibility of antimicrobial compounds gets enhanced in organic solvents (21). Methanolic extract also has the ability to dissolve both polar and nonpolar molecules. The extracts of mushroom in different solvent systems have been reported to possess considerable antimicrobial activity by the number of researchers (5,14,22). The antimicrobial potential of an extract is mainly determined by the nature of the solvent used for extraction, as the active antimicrobials vary in solubility (23). Alves *et al*. (5) and Singh *et al.* (24) reported that aqueous extract of mushrooms possesses less antimicrobial activity as compared to organic solvents. Our results also agree favorably with the findings of Moglad and Saadabi (25) who suggested that bioactive components of mushrooms differ in their solubility depending upon the extractive solvents used and found water is not a good solvent to extract antimicrobials from the mushrooms. The difference in the antimicrobial activity of different extracts could also be absolved due to the difference in their diffusion rate in the cultural medium Venturini *et al.* (26).

Generally, it was observed that the antimicrobial values for all extracts against the fungi were found low as compared to bacteria. This supports the suggestion of Moglad and Saadabi (25) and Takazawa *et al.* (27) that antifungal compounds are less common in the Basidiomycetes. The difference in sensitivity of bacteria and fungi towards the mushroom extracts could be attributed to the difference in transparency of cell wall (28). Some other researchers from different parts of the world also reported that mushroom extracts are more effective in restricting the growth of bacteria than fungal pathogens (22,23,29). Shameem et al. (30) evaluated the antimicrobial activity of crude fractions of *Morchella esculenta* and *Verpa bohemica* of Kashmir Himalayas and found that ethyl acetate extract of both the mushroom species show strong antibacterial activity than antifungal activity against the common set of pathogens.

Both ethyl acetate and methanolic extracts of all the four mushroom species were found to retard the growth of all bacterial strains with varying degree of effectiveness. Generally, the mushroom extracts proved more antagonistic towards the gram-positive bacteria than gram-negative bacteria. The difference in the relative composition of the cell wall of gram-positive and gram-negative bacteria possibly assert the different responses (28,29). Also we know that most of the antimicrobial molecules target intracellular processes. There is an outer membrane in gram negative bacteria, which excludes certain drugs and antibiotics from penetrating the cell (31). The lack of this outer membrane in gram positive bacteria can possibly contribute to their high susceptibility for antimicrobial molecules. Both *Bacillus subtilis* and *Staphylococcus aureus* were found highly susceptible to all concentrations of mushroom extracts. The high value of the zone of inhibition was also substantiated by MIC values. The high susceptibly of gram-positive bacteria than gram-negative bacteria towards mushroom extracts has also been reported by many other researchers (6,7,9,26). Khan et al. (32) also found strong antibacterial activity against gram positive bacteria than gram negative bacteria while evaluating the antibacterial potential of *Agaricus bisporus*, *Pleurotus ostreatus* and *Coprinus atramentarius* collected from Kashmir Himalayas against given set of bacterial microbes. Among gram-negative bacteria, the growth of *Klebsiella pneumoniae* was least affected. Quereshi *et al*. (10) and Kamra and Bhat (33) also found that *Klebsiella pneumoniae* was less susceptible to mushroom extracts. Zowawi *et al.* (34) reported that *Klebsiella pneumoniae* exhibits strong multidrug resistance because of its genetic plasticity. Among fungi *Saccharomyces cerevisiae* was found most susceptible to all extracts whereas others showed a mixed response. Similar results were observed by Singh *et al*. (24) and Waithaka *et al*. (35).

The present study revealed that mushrooms can act as a green and viable source of antimicrobials. All four species of mushrooms were found to show significant antimicrobial activity. In the case of *Fomitopsis pinicola*, the zone of inhibition at the concentration of 150mg/ml was found in between 20-26 mm against all the bacterial strains and the MIC values were found very low. The values were in close proximity with the values of pure 10µg gentamycin, thereby indicating that the extract is a rich source of broad-spectrum bacterial antibiotics. The affirmations put forth by various researchers regarding the antimicrobial potential of *Fomitopsis pinicola* (9,36), *Inonotus hispidus* (37), *Lentinus* sp. and *Ramaria* sp. (22,38-40) were found in balance with our results with slight variations. The differences could be accorded to the variance in environment, genetic structure, physical and biochemical constituents and nature of the substrate on which the mushroom grows (5).

## Conclusion

Mushrooms produce antimicrobial metabolites that can be exploited for the treatment of different bacterial and fungal diseases in human's animals and plants. All the extracts exhibit potent antimicrobial activity but *Fomitopsis pinicola* was found to harbor strong antibacterial and antifungal activity, therefore there is a need to carry further studies to identify and isolate the bioactive compounds from its extract.

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## Competing Interests

The authors have no conflict of interests.

## Authors' contribution

All authors contributed equally to carry out the research work.

## References

1. Wani BA, Bodha RH, Wani AH. Nutritional and medicinal importance of mushrooms. Journal of Medicinal Plant Research 2010; 4(24): 2598-2604. https://doi.org/10.5897/JMPR09.565
2. Deshmukh SK. Biodiversity of tropical Basidiomycetes as source of novel secondary metabolites. In: Jain PC (Eds) Microbiology for sustainable development, CBS Publishers and Distributors, New Delhi; 2004. p. 121-140.

3. Pala SA, Wani AH. Mushrooms: The entities with multifarious medicinal properties. Journal of Pharmacy Research 2011; 4(12): 4721-4726.
4. Gao Y, Tang W, Gao H, Chan E, Lan J, Li X, Zhou S. Antimicrobial activity of the medicinal mushroom *Genoderma*. Food Rev International 2005; 21: 211-229. https://doi.org/10.1081/FRI-200051893
5. Alves MJ, Ferreira ICFR, Dias J, Teixeira V, Martins A, Pintado M. A review on antifungal activity of mushroom (Basidiomycetes) extracts and isolated compounds. Current Topics in Medicinal Chemistry 2013; 13(21): 2648-2659. https://doi.org/10.2174/15680266113136660191
6. Zhang Y, Geng W, Shen Y, Wang Y, Dai YC. Edible mushroom cultivation for food security and rural development in China: Bio-innovation, technological dissemination and marketing. Sustainability 2014; 6: 2961-2973. https://doi.org/10.3390/su6052961
7. Prasad S, Rathore H, Sharma S, Yadav AS. Medicinal mushrooms as a source of novel functional food. International Journal of Food Sciences and Nutrition 2015; 4(5): 221-225.
8. Valverde ME, Perez TH, Lopez OP. Edible mushrooms: Improving human health and promoting quality life (review). International Journal of Microbiology 2015; 1: 1-15. https://doi.org/10.1155/2015/376387
9. Fagade OE, Oyelade AA. A comparative study of the antibacterial activities of wood-decay fungi to synthetic antibiotic discs. Electronic Journal of Environmental, Agricultural and Food Chemistry 2009; 8(3): 184-188.
10. Quereshi S, Pandey AK, Sandhu SS. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. People's Journal of Scientific Research 2010; 3(1): 5-15.
11. Zjawiony JK. Biologically active compounds from Aphyllophorales (polypore) fungi. Journal of Natural Products 2004; 67(2): 300–310. https://doi.org/10.1021/np030372w
12. Lindequist U, Niedermeyer THJ, Julich WD. The pharmacological potential of Mushrooms. Evidence-Based Complementary and Alternative Medicine 2005; 2(3): 285-299. https://doi.org/10.1093/ecam/neh107
13. Karaman I, Sahin F, Gulluce M, Ogutçu H, Sengul M, Adıguzel A. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. Journal of Ethnopharmacology 2003; 85: 213-235. https://doi.org/10.1016/S0378-8741(03)00006-0
14. Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology 2011; 89(5): 1323-1332. https://doi.org/10.1007/s00253-010-3067-4
15. Pala SA, Wan AH, Bhat MY. Ethnomycological studies of some wild medicinal and edible mushrooms in the Kashmir Himalayas (India). International Journal of Medicinal Mushrooms 2013; 15(2): 211–220. https://doi.org/10.1615/IntJMedMushr.v15.i2.100
16. Beig MA, Dar GH, Khan NA, Ganai NA. Seasonal production of epigeal fungal sporocarps in mixed and pure Fir (*Abies pindrow*) stands in Kashmir forests. Journal of Agricultural Technology 2011; 7(5): 1375-1387.
17. Pala SA, Wani AH, Parveen S. Some hitherto unreported macromycetes from coniferous forests of Kashmir Himalaya (India). Austrian Journal of Mycology 2013; 22: 21-29.
18. Pala SA, Wani AH, Bhat MY. Six hitherto unreported Basidiomycetic macrofungi from Kashmir Himalayas. Nusantra Bioscience 2011; 3(2): 92-97.
19. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols 2008; 3(2): 163-175. https://doi.org/10.1038/nprot.2007.521
20. Norrel SA, Messley KE. Microbiology laboratory manual principles and applications. Prentice Hall, Upper Saddle River New Jersey; 1997. p. 85-90.
21. Sharma A, Sharma K. Should Solubility and Zone of Inhibition Be the Only Criteria for Selection of Solvent in Antimicrobial Assay? Advances in Biological Research 20115; 5: 241-247.
22. Barros L, Calhelha RC, Vaz JA, Ferreira ICFR, Baptista P, Estevinho LM. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. European Food Research and Technology 2007; 225:151–6. https://doi.org/10.1007/s00217-006-0394-x
23. Alves MJ, Ferreira IC, Dias J, Teixeira V, Martins A, et al. A review on antimicrobial activity of mushrooms (Basidiomycetes) extracts and isolated compounds. Plant medicine 2012; 78: 1707-1718. https://doi.org/10.1055/s-0032-1315370
24. Singh J, Gupta S, Malviya S, Ahrwar B. In-vitro Evaluation of Antimicrobial Activity of *Ganoderma lucidum*. International Journal of Advanced Research 2014; 2(6): 460-466.
25. Moglad EHO, Saadabi AM. Screening of Antimicrobial Activity of Wild Mushrooms from Khartoum State of Sudan. Microbiology Journal 2012; 2(2): 64-69. https://doi.org/10.3923/mj.2012.64.69
26. Venturini ME, Rivera CS, Gonzalez C, Blanco D. Antimicrobial Activity of Extracts of Edible Wild and Cultivated Mushrooms against Foodborne Bacterial Strains. Journal of Food Protection 2008; 71(8): 1701–1706. https://doi.org/10.4315/0362-028X-71.8.1701
27. Takazawa H, Tajima F, Miyashita C. An antifungal compound from shiitake (*Lentinus edodes*). Yakugaku Zasshi 1982; 102: 489-491. https://doi.org/10.1248/yakushi1947.102.5_489
28. Yang Y, Anderson EJ. Antimicrobial activity of a porcine myeloperozidase against plant phatgenic bacteria and fungi. Journal of Applied Microbiology 2001; 86: 211-220. https://doi.org/10.1046/j.1365-2672.1999.00652.x
29. Kosanic M, Rankovic BR, Dasic M. Antioxidant and antimicrobial properties of mushrooms. Bulgarian Journal of Agricultural Science 2013; 19(5): 1040-1046.
30. Shameem N, Kamili AN, Ahmad M, Masoodi FA, Parray JA. Antimicrobial activity of crude fractions and morel compounds from wild edible mushrooms of north western Himalaya. Microbial Pathogenesis 2017; 105: 356-360. https://doi.org/10.1016/j.micpath.2017.03.005
31. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. The Journal of Infectious Diseases 2008; 197: 1079– 1081. https://doi.org/10.1086/533452
32. Khan AA, Gani A, Ahmad M, Masoodi FA, Amin F, Kousar S. Mushroom varieties found in the Himalayan

regions of India: Antioxidant, antimicrobial, and antiproliferative activities. Food Science and Biotechnology 2016; 25(4): 1095-1100. https://doi.org/10.1007/s10068-016-0176-6

33. Kamra A, Bhatt AB. Evaluation of antimicrobial and antioxidant activity of *Ganoderma Lucidum* extracts against human pathogenic bacteria. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4(2): 359-362.
34. Zowawi HM, Forde BM, Alfaresi M, Alzarouni A, Farahat Y, Chong TM, Yin WF, Chan KG, Li J, Schembri MA et al. Stepwise evolution of pandrug-resistance in *Klebsiella pneumoniae*. Scientific Reports 2015; 5: 15082. https://doi.org/10.1038/srep15082
35. Waithaka PN, Gathuru EM, Githaiga BM, Onkoba KM. Antimicrobial Activity of Mushroom (*Agaricus bisporus*) and Fungal (*Trametes gibbosa*) Extracts from Mushrooms and Fungi of Egerton Main Campus, Njoro Kenya. Journal of Biomedical Sciences 2017; 6(3): 20-25.
36. Keller AC, Maillard MP, Hostettmann K. Antimicrobial steroids from the fungus *Fomitopsis pinicola*. Phytochemistry 1996; 41: 1041-1046. https://doi.org/10.1016/0031-9422(95)00762-8
37. Akyuz M, Onganer AN, Erecevit P, Kirbag S. Antimicrobial activity of some edible mushrooms in the eastern and southeast Anatolia region of Turkey. Gazi University Journal of Science 2010; 23(2): 125-130.
38. Hatvani N. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture. International Journal of Antimicrobial Agents 2001; 17: 71–74. https://doi.org/10.1016/S0924-8579(00)00311-3
39. Imtiaj A, Lee TS. Screening of antibacterial and antifungal activities from Korean wild mushrooms. World Journal of Agricultural Sciences 2007; 3(3): 316-321.
40. Ramesh CH, Pattar MG. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka. Indian Pharmacognosy Research 2010; 2: 107-111. https://doi.org/10.4103/0974-8490.62953