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Research Article

# Phytochemical Analysis and *In vitro* Antibacterial Activity of *Russula lepida* and *Pleurotus ostreatus* from North West Himalayas, India

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**ABSTRACT**

This research aimed at screening and determination of phytochemical compounds and examine *in vitro* antibacterial activity of the aqueous and methanolic extracts of two wild edible mushroom (*Russula lepida* and *Pleurotus ostreatus*) found in Himachal Pradesh. The phytochemical analysis reveals that the extracts were a rich source of phytoconstituents containing saponins, phenols, steroids, glycosides, terpenoids and flavonoids. Methanol extract was shown to be more effective against all the tested pathogens followed by aqueous extract. *B.subtilis* was found to be more sensitive organism followed by *S.aureus* and *E.coli.* Methanol extract have higher solubility for more active antimicrobial and phytochemical constituents, consequently displaying the highest antimicrobial activity. The extract could be potential source of new antimicrobial agents and scientifically validates the use of the macro fungi in traditional medicine.

**Keywords:** *Bacillus subtilis/ Escherichia coli*/ filter paper disc diffusion/ Mushroom extract/ *Staphylococcus aureus*

## INTRODUCTION

Antibiotic resistance has become a global concern[1].The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens[2].The increasing failure of chemotherapeutics and antibiotics resistance exhibited by pathogenic microorganisms has led to the screening of several medicinal plants for their potential antimicrobial activity[3, 4]. Worldwide spending on finding new anti-infective agents is increasing. The alternative forms of medical treatments are being investigated by researchers.

Mushrooms possess high contents of qualitative protein, crude fiber, minerals and vitamins. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites with high therapeutic value. Health promoting properties, e.g. antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects have been reported for some species of mushrooms[5, 6, 7, 8]. This study was designed to evaluate the antibacterial activity of *Russula lepida* and *Pleurotus ostreatus* mushroom extracts (aqueous and methanolic) on bacterial isolates (*E.coli, S.aureus* and *B. subtilis*) and also determine the phytochemical properties of these two wild edible mushrooms.

## MATERIAL AND METHODS

Sample Collection: The edible mushrooms *Pleurotus ostreatus* and *Russula lepida* were collected from the forests of Distt. Shimla in Himachal Pradesh. They were identified at department of Biosciences Himachal Pradesh University, Shimla. The fruiting bodies were thoroughly cleaned and were sun dried. The dried mushrooms were weighed and ground to fine powder.

Microorganisms and Media: The bacteria used in the present study were *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. All the bacteria used were obtained from IGMC, Shimla. Bacterial cultures were maintained on nutrient agar. Cultures were stored at 4ºC and subcultured every 15 days.

Extraction of mushrooms: 50 gm of the each mushroom powder were soaked in 200 ml of methanol and cold water respectively, and then left for 36 hours at room temperature with occasional shaking. Each portion was filtered using whatman filter paper. The filtrates were collected in different beakers and labeled accordingly. The filtrates were evaporated to dryness in a steady air current for about 24 hours in a previously weighed evaporation dishes. After evaporation the dishes were re-weighed and differences in weight before and after evaporation were calculated[9]. The extracts were stored at 4ºC for further use.

Phytochemical analysis: Qualitative phytochemial analysis of the crude powder of the mushrooms was determined following [10, 11, 12].

Antibacterial assay: Antibacterial activities of mushroom extracts were carried out by filter paper disc diffusion technique[13]. The bacterial colony was transferred into a tube containing 5-6 ml of nutrient broth (HIMEDIA, India) and incubated at 37±1ºC. To standardize the inoculums density for a susceptibility test, a $BaSo_4$ turbidity standard, equivalent to a 0.5 McFarland standard was used (where, turbidity was 0.5 which is equal to approximately 1 to 2 X $10^8$ cfu/ml) of bacterial cells. One milliliter suspension of known turbidity was applied on the dried surface of the nutrient agar. The inoculated plates were left for 15-20

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Table 1

| S.no. | Mushroom | YIELD (mg) of crude extract | |
|---|---|---|---|
| | | Aqueous Extract | Methanolic Extract |
| 1. | *Russula Lepida* | 3.56 | 3.89 |
| 2. | *Pleurotus ostreatus* | 2.10 | 2.62 |

Table 2

| S.no. | Name of constituents | *Russula lepida* | | *Pleurotus ostreatus* | |
|---|---|---|---|---|---|
| | | Aqueous extract | Methanolic extract | Aqueous extract | Methanolic extract |
| 1. | Tannins | + | + | + | ++ |
| 2. | Saponins | + | +++ | + | +++ |
| 3. | Flavonoids | ++ | - | + | ++ |
| 4. | Alkaloides | + | + | + | - |
| 5. | Cabohydrates | + | + | ++ | ++ |
| 6. | Phenols | + | ++ | + | + |
| 7. | Steroidal Glycosides | + | + | + | + |
| 8. | Terpenoides | + | + | + | + |
| 9. | Resins | - | - | - | - |
| 10. | Cyanogenic Glycosides | - | - | - | - |

*- = Not present*
*+ = Present in small amount (concentration)*
*++ = Moderately present*
*+++ = Present in large amount*

Table 3

| S.No. | Microorganism | *Russula lepida* | | *Pleurotus ostreatus* | | Standard |
|---|---|---|---|---|---|---|
| | | Aqueous Extract | Methanolic Extract | Aqueous Extract | Methanolic Extract | Tetracycline |
| 1. | *E.coli* | - | - | - | 2.6±0.1 | 8.0±0.0 |
| 2. | *S.aureus* | 2.3±0.1 | 4.8±0.0 | 5.2±0.2 | 8.7±0.1 | 21.0±0.0 |
| 3. | *B.subtilis* | 3.4±0.1 | 5.7±0.1 | 4.5±0.1 | 9.8±0.2 | 17.0±0.0 |

minutes at room temperature. Each disc was 6 mm in diameter. As reference antibiotic tetracycline (30µg/ ml) was used. All the plates were incubated in Biological Oxygen Demand incubator (YORCO, India) at 37±1ºC. After 24 hours each plate was examined.

**RESULTS**

In the preset investigation, two wild edible mushrooms (*Russula lepida* and *Pleurotus ostreatus*) were evaluated for their phytochemical contents and anti bacterial activity. We present in Table 1 total yields of the mushroom extraction. The yields of extraction with methanol were higher as compared to water. The total yield of crude extracts of *R. lepida* and *P. ostreatus* were 3.89mg and 2.62mg in methanolic extraction respectively.

Phytochemical analysis revealed the presence of bioactive compounds as shown in Table 2. The phytochemicals of the mushrooms were present at varying levels. Saponins were present in large amount in methanolic extracts of both mushrooms. Tannins, Flavonoids, alkaloids, carbohydrates, Phenols, steroidal glycosides and terpenoids were present in low to moderate amount. Resins and Cyanogenic glycosides were not detected in aqueous and methanolic extracts of the mushrooms.

Results of antibacterial activity of different mushroom extracts of concentration (30µg/ml) were determined. Preliminary antibacterial testing of the aqueous and methanolic extracts of *R. lepida* and *P. ostreatus* by disc diffusion method produced zones of inhibition which are shown in Table 3. Of all the common bacterial isolates tested showed higher sensitivity to methanolic extract of *P. ostreatus*. *R. lepida* had a narrow antibacterial spectrum against the tested bacteria. The antimicrobial potential of the mushrooms against *S.aureus* and *B.subtilis* were comparable with the tested antibiotic.

**DISCUSSION**

The total yield of the crude extracts obtained from each of the mushroom was relatively low and this could probably be due to the extraction methods employed. Extraction by cold water has generally been reported to produce low amount of extracts compared to organic solvent extraction[14]. The phytochemical analysis of *R. lepida* and *P. ostreatus* disclosed the presence of major phytoconstituents viz. Saponins, Tannins, Flavonoids, alkaloids, carbohydrates, Phenols, steroidal glycosides and terpenoids. Among two solvents used for extraction, methanolic extracts showed more number of

phytoconstituents as compared to aqueous extracts. Methanol extracts obtained in this study might have higher solubility for more active antimicrobial constituents, consequently displaying the highest antimicrobial activity. This is in agreement with the research findings that some phytochemicals are more soluble in alcohol than water[15, 16]. This also confirmed the suggestions of Fujita *et al.*[17] who suggested that methanol was better than water as extracting solvent. Different mushroom species posses different constituents and in different concentration, which account for the differential antimicrobial effect. As microorganisms get resistant to the antibiotics after some time. Therefore, the potential of developing antimicrobials from mushrooms appears rewarding.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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