

## Bioactive potential of different solvent extracts of some woody and fleshy mushrooms

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### ABSTRACT

A plate culture and disc diffusion method was performed to evaluate the bioactive potential of 20 different types of naturally growing mushrooms collected from different locations like gardens, plantations and protected habitats of Bhubaneswar, Odisha. These mushrooms belong to *Clavaria*, *Coltricia*, *Coniophora*, *Daldinia*, *Fomitopsis*, *Geastrum*, *Grifola*, *Langermannia*, *Lentinus*, *Lycoperdon*, *Microporous*, *Peniophora*, *Pisolithus*, *Polyporous*, *Schizophyllum*, *Suillus*, *Trametes* and *Tricholoma*. Extracts were prepared by using distilled water, methanol, ethyl alcohol, isopropanol, ethyl acetate, acetone and chloroform. The concentrated extracts were used for the preparation of bioactive bioassay and placed singly on the media plates, inoculated with bacteria and fungus separately and incubated for 24hr and 4 days, respectively. Observation was recorded for the clear zone of inhibition against the bioassay as indicator of growth inhibition due to bioactive compounds extracted from the individual mushroom fruit bodies. Data recorded on zone formation around the bioactive bioassay exhibited the antibacterial properties of *Coniophora puteana*, *Grifola frondosa*, *Peniophora incarnate*, *Schizophyllum commune* and *Trametes versicolor* against gram negative bacteria where as most of the fleshy mushrooms showed antimicrobial properties except *Lycoperdon pyriformi*, *Pisolithus arrhizus*. Among tested mushroom species, no one found with antifungal properties. The preliminary study on screening of bioactive potential of these mushrooms revealed the suitability of different solvent system for extraction of bioactive compounds.

**Keywords:** Bioactive potential, concentrated extracts, bio-disc, mushrooms

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Mushrooms are known as functional food and endowed with nutraceuticals and pharmaceutical properties. They are also reported with the bioactive properties like antimicrobial, antitumor, antiviral, anti-inflammatory etc (Sharma and Annepu, 2018). Apart from this, mushrooms have been prescribed for treatment of various human diseases such as gastrointestinal disorder, bleeding, high blood pressure and various microbial infections. Many of them are known for good source of antibiotics. *Lycoperdon*, *Tricholoma*, *Fomitopsis*, *Trametes*, *Schizophyllum* and others have found to have good antimicrobial

activity. The demand of traditional medicine also known as green medicine, encourages to search for new sources. Mushrooms are good alternative for chemosynthetic drugs and are being used as curing agent for many diseases (Gbolagade and Fasidi, 2005; Nair and Chanda, 2007; Guler *et al.*, 2009; Akyuz, 2010; Hleba *et al.*, 2014; Mirfat *et al.*, 2014). In view, the unexplored urban environment of Bhubaneswar as far as mushroom bioactivity is concerned, the mushrooms of local environment has been collected and evaluated for bioactivity against some gram +ve and gram -ve bacteria and *Colletotrichum sp.*

## MATERIALS AND METHODS

### Macrofungi and test organism

In this study, 20 mushroom species were selected belonging to different genus. Samples were collected within the city including protected green campus, public gardens and road side plantation of Bhubaneswar, Odisha and identified morphologically. These 20 mushrooms namely- *Clavaria vermiculari*, *Coltricia cinamomia*, *Coniophora puteana*, *Daldinia concentrica*, *Fomitopsis pinicola*, *Geastrum fimbriatum*, *Grifola frondosa*\*\*\*\*, *Langermannia gigantean*, *Lentinus betulina*, *Lentinus fusipes*, *Lentinus torulosus*, *Lycoperdon pyriformi*, *Microporous xanthopus*, *Peniophora incarnate*, *Pisolithus arrhizus*, *Polyporous sulphurous*, *Schizophyllum commune*, *Suillus luteus*, *Trametes versicolor* and *Tricholoma lobayense* were used for antimicrobial screening against 4 bacteria and one fungi (4 bacteria, PPB-9 and MLB-6 (gram +ve), another two PPB-1 and PPB-5 (gram -ve) and single fungi i.e., *Colletotrichum sp.* used in this study).

### Preparation of extracts

Mushrooms were dried properly at a controlled temperature 50°C through hot air oven. Dried mushrooms were powdered by using mixture grinder and treated with seven different solvent distilled water, methanol, ethyl alcohol, isopropanol, ethyl acetate, acetone and chloroform. To obtain active compounds, solutions were prepared with a ratio 0.05 g/ml in shaking condition at 120rpm for 24 hour, filtered, residue and solvent collected separately. Respective extractions were carried out till clear solvent obtained. Extracts containing crude compounds were evaporated and obtain in dried condition and stored in a refrigerator in air tight containers for antimicrobial analysis (Jonathan and Fasidi, 2003).

### Antimicrobial assay

Sterilised 6mm diameter filter paper discs were dipped into sets of extracts of different mushrooms overnight, dried and placed singly on respective inoculated plates of different test organisms. The experimental plates were incubated at 37°C for 24hr for bacteria and 4 days for fungi. Data was recorded for clear zone formation around the bioactive biodisc.

## RESULTS AND DISCUSSION

All the mushrooms tested for antimicrobial properties against for different bacterial organisms belonging to gram +ve and gram -ve and one fungus i.e., *Colletotrichum sp.* The present study revealed the bioactive potential of almost all mushrooms tested except *Pisolithus arrhizus*, *Lycoperdon pyriformi*, *Coltricia cinamomia*, *Daldinia concentric*, *Fomitopsis pinicola*, *Lentinus betulina* and *Polyporous sulphurous*. Interestingly, all mushroom extracts did not show any bioactive inhibitory zone against bacteria PPB-9 (gram -ve) and the fungal strain. *Coniophora puteana*, *Lentinus torulosus*, *Suillus luteus* were active against both gram -ve and gram +ve bacteria. Observations depicted in table-1 clearly indicate the poor antibacterial properties of woody mushrooms tested in the present study. However, water extract of few woody mushrooms -*Grifola frondosa*, *Schizophyllum commune* and *Trametes versicolor* exhibited the inhibition zone against gram -ve bacteria B2 (PPB-5). Surprisingly, the different solvent extracts of different woody mushrooms tested in the present study did not show antibacterial properties, though the woody mushroom are reported as good source of antibacterial properties (Siljegovic *et al.*, 2011; S<sup>3</sup>awińska *et al.*, 2013). It may be due to the different bacterial strains, solvent used and/or quantity of test material. Wasser and Weis (1999) reported the role of different solvent system for the extraction of bioactive compounds from different kinds of mushrooms. In similar way, different

Table 1. Antibacterial and antifungal activity of woody mushrooms

| Mushroom species             | Solvent systems |    |    |    |          |    |    |    |               |    |    |    |             |    |    |    |               |    |    |    |         |    |    |    |            |    |    |    |    |    |
|------------------------------|-----------------|----|----|----|----------|----|----|----|---------------|----|----|----|-------------|----|----|----|---------------|----|----|----|---------|----|----|----|------------|----|----|----|----|----|
|                              | Water           |    |    |    | Methanol |    |    |    | Ethyl alcohol |    |    |    | Isopropanol |    |    |    | Ethyl acetate |    |    |    | Acetone |    |    |    | Chloroform |    |    |    |    |    |
|                              | B1              | B2 | B3 | B4 | F1       | B1 | B2 | B3 | B4            | F1 | B1 | B2 | B3          | B4 | F1 | B1 | B2            | B3 | B4 | F1 | B1      | B2 | B3 | B4 | F1         | B1 | B2 | B3 | B4 | F1 |
| <i>Coltricia cinamomia</i>   | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Coniophora puteana</i>    | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Daldinia concentrica</i>  | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Fomitopsis pinicola</i>   | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Grifola frondosa</i>      | +               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Lentinus betulina</i>     | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Microporus xanthopus</i>  | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Peniophora incarnata</i>  | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Polyporus sulphureus</i>  | -               | -  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Schizophyllum commune</i> | -               | +  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |
| <i>Trametes versicolor</i>   | -               | +  | -  | -  | -        | -  | -  | -  | -             | -  | -  | -  | -           | -  | -  | -  | -             | -  | -  | -  | -       | -  | -  | -  | -          | -  | -  | -  | -  | -  |

+ :- indicates partial zone of inhibition and - :- shows no activities. B1:- PPB1(gram +ve ) bacteria, B2:- PPB-5 (gram -ve ) bacteria, B3:- MLB-6 (gram +ve ) bacteria, B4:- PPB-9 (gram -ve ) bacteria and F1:- *Colletotrichum sp.*

Table 2. Antibacterial and antifungal activity of fleshy mushrooms grows on soil, leaf litter and grassland

| Mushroom species             | Solvent systems |    |    |          |    |    |               |    |    |             |    |    |               |    |    |         |    |    |            |    |    |    |    |    |    |   |
|------------------------------|-----------------|----|----|----------|----|----|---------------|----|----|-------------|----|----|---------------|----|----|---------|----|----|------------|----|----|----|----|----|----|---|
|                              | Water           |    |    | Methanol |    |    | Ethyl alcohol |    |    | Isopropanol |    |    | Ethyl acetate |    |    | Acetone |    |    | Chloroform |    |    |    |    |    |    |   |
|                              | B1              | B2 | B3 | B4       | F1 | B1 | B2            | B3 | B4 | F1          | B1 | B2 | B3            | B4 | F1 | B1      | B2 | B3 | B4         | F1 | B1 | B2 | B3 | B4 | F1 |   |
| <i>Clavaria vermicularis</i> | -               | -  | -  | -        | -  | -  | -             | -  | -  | -           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Lentinus fusipes</i>      | -               | -  | -  | -        | -  | ++ | -             | -  | -  | -           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Lentinus torulosus</i>    | -               | -  | -  | -        | -  | ++ | ++            | -  | -  | -           | -  | -  | -             | -  | +  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Tricholoma lobayense</i>  | -               | -  | -  | -        | -  | -  | -             | -  | -  | -           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Geastrum fimbriatum</i>   | -               | +  | -  | -        | -  | -  | -             | -  | -  | +           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Pisolithus arrhizus</i>   | -               | -  | -  | -        | -  | -  | -             | -  | -  | -           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Suillus luteus</i>        | -               | -  | -  | -        | -  | ++ | +             | -  | -  | +           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Langermannia gigantea</i> | -               | -  | -  | -        | -  | -  | -             | -  | -  | -           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |
| <i>Lycoperdon pyriformi</i>  | -               | -  | -  | -        | -  | -  | -             | -  | -  | -           | -  | -  | -             | -  | -  | -       | -  | -  | -          | -  | -  | -  | -  | -  | -  | - |

+ :- indicates partial zone of inhibition and - :- shows no activities. B1:- PPB1(gram +ve ) bacteria, B2:- PPB-5 (gram -ve ) bacteria, B3:- MLB-6 (gram +ve ) bacteria, B4:- PPB-9 (gram -ve ) bacteria and F1:- *Colletotrichum sp.*

extracts of *Coniophora puteana* prepared in isopropanol and ethyl acetate exhibited distinct antibacterial activity against B2 and B3, respectively. Ethyl alcohol extracts of *Lentinus torulosus* showed inhibitory zone against PPB1 (gram -ve) and PPB5 (gram -ve) bacteria. It is interesting to note that bioactive compounds of antibacterial nature could be extracted with methanol, ethyl alcohol, isopropanol and acetone. Different extracts of *Suillus luteus* was active against PPB1, PPB5, MLB6. Antibacterial activity of different mushrooms against PPB5 (gram -ve) showed extractable properties of different solvents used. It indicates that either mushrooms have different kinds of antibacterial compounds useful against PPB5 or different solvents are helpful in extracting different compounds having bioactivity against PPB5. To analyze the different factors, a detail study is required on mass scale extraction of bioactive substances, purification and characterization and wide spectrum antimicrobial analysis. However, the present preliminary data on antibacterial properties of woody and fleshy mushrooms against gram -ve and +ve bacteria has given a significant lead to proceed further for the detail study on this aspects.

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