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ORIGINAL ARTICLE **OPEN ACCESS**

Study on Phytochemical Secondary Metabolites of Five Wild Mushroom Extracts and Their Antimicrobial Activity

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ABSTRACT

Wild mushrooms are a vital rich source of natural nutrients and they are occasionally consumed for their supposed medicinal value. There are numerous reports on wild edible mushrooms, which doubt and confusion persist regarding which species are safe and suitable to consume. They are known as highly valued non-wood products today, thus wild mushrooms have played an important role in providing new sources of medicine in the whole World.. Our review highlights the need for further information on wild species in a clear. They can be used in the treatment of disease through their antimicrobial properties. Five different wild mushrooms were identified and collected from the campus of St. Ann's college for women, Malkapuram, Visakhapatnam. The result revealed that all mushroom extracts were having antimicrobial activity with high potential effectiveness in suppressing bacterial cell growth when compared with fungal cell growth. The antibacterial activity was ranging from 0.1mm to 0.8mm against Staphylococcus aureus, 0.1mm to 1mm against E. coli and 0.2mm to 1mm against K. pneumoniae. The antifungal activity was ranging from 0.1mm to 0.3mm against Aspergillus niger and Trichoderma harizianum. The maximum zone of inhibition was 1mm against on E.coli by Brown wild mushroom and minimum zone of inhibition was 0.1mm on Staphylococcus aureus. In the present study the presence of phytochemicals like flavonoids, alkaloids and terpenoids were also analysed by using standard methods. The need for greater clarity on wild species of mushrooms is further underlined to know there nutritional values and phytochemical analysis with their molecular interactions.

Keywords: *Wild Mushrooms, phytochemicals, Antimicrobial activity and medicinal values.*

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INTRODUCTION

Mushroom is a general term utilized mostly for the macrofungal cell and mainly belongs to higher fungi. They have only a short reproductive stage in their life cycle with their nourishment source for human beings and animals. Fungi are eukaryotic, heterotrophic, and osmotrophic. They develop a rather diffuse, branched, tubular body (radiating hyphae making up mycelia or colonies), and reproduce by means of spores. Wild mushrooms are a popular food source. The high humidity level during almost all season provides ideal atmospheric conditions for the mushrooms. The group includes mainly terrestrial species of diverse forms and habitat and is a general term used mainly for the fruiting bodies of macrofungal (Ascomycota and Basidio mycota) and represents only a short reproductive stage in their life cycle [2]. They are untapped resources of nutrition and palatable food of the future. Due to high protein content they can be used to bridge the protein malnutrition gap. Edible mushrooms are sources of food and are cogitated as one of the delicious food all over the world. They have a high nutritional value almost twice that of any vegetable or fruit [6]. As microbial resistance to antibiotics is becoming more and more prevalent, mushrooms are seen as a good source of new classes of compounds with antimicrobial activity, some of which, such as pleuromutilin, have led to the synthesis of new drugs that have been recently approved for use in humans [5].

MATERIAL AND METHODS

Extraction by Maceration

Five different wild mushroom were included in this study (Figure 1) identified and collected from campus of St. Ann's college for women, Malkapuram, Visakhapatnam. The collected mushrooms were watery washed, disinfected, rinsed with distilled water and finally dried. The dried mushrooms of each type was homogenised into fine paste using mortar and pestle separately (Figure 2). 50g of the fine paste was soaked in 200 ml of ethanol with stirring for 72h and then filtered through double layers of muslin, centrifuged at 9000 rpm for 10 min and finally filtered again through Whatman filter paper No.1 to attain a clear filtrate (Figure 3). The extract yields were stored in a small bottles in fridge at 5^0C for future phytochemical and antimicrobial analysis.

Figure A. ***Turbaria furfuracea***

Figure B. ***Tylopilus sps***

Figure C. ***Termitomyces eurrhizus***

Figure D. ***Leucoagaricus rhodocephalus***

Figure E. ***Collybia tuberosa***

Figure 1. Images of five wild mushroom from campus of St. Ann's college for women, Malkapuram, Visakhapatnam.

Figure 2. Ethanol extraction by maceration

Filtration **Mushroom dry pellet** **Dry pellet in DMSO Solvent**

Figure 3. Processing the crude mushroom extract for analysis of Antimicrobial Activity

Antimicrobial activity of the Ethanol mushroom extracts

Bacterial and fungal strains

The antimicrobial potency of each extract was evaluated using three bacterial strains and two fungal strains. One strains of Gram positive *Staphylococcus aureus* and two strains of Gram negative *Escherichia coli* and *Klebsiella pneumoniae* bacteria. Two fungal strains used for antifungal activity was *Aspergillus niger* and *Trichoderma harizianum*. The bacterial and fungal strains were provided from the Microbial type culture collection (MTCC), Chandigarh, India.

Inoculum preparation

Each bacterial and fungal strains was sub-cultured overnight at 35^0C in Mueller-Hilton agar slants and PDA slants. The microbial growth was harvested using 5 ml of sterile broth kept overnight in orbital shaker at 37^0c for 24 hours. Separate bacterial and fungal lawn plates were prepared by inoculating fresh broth by using spread plate technique on solidified Mueller-Hilton (Bacterial Media) and PDA agar plate (Fungal media).

Antibacterial Activity

The well diffusion method is used to evaluate antibacterial activity of the each mushroom extract. The extract residues (50 mg) were then loaded in the well on lawn of bacterial culture plates (Figure 4). The plates were kept in incubator at 37^0C for 24 h. The presence of inhibition zones were measured by using Hi-Media zone scale, recorded and considered as indication for antibacterial activity.

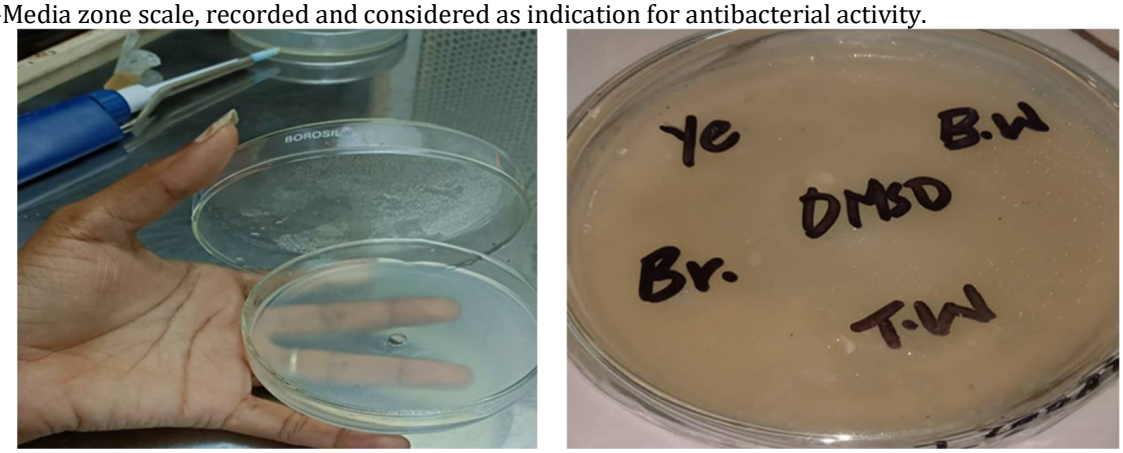

Figure 4. Loading the crude mushroom extract in the well on microbial lawn containing plate

Antifungal Activity

The well diffusion method is used to evaluate antifungal activity of the each mushroom extract. The extract residues (50 mg) were then loaded in the well on lawn of fungal culture plates. The plates were kept in incubator at 27^0C for 24 h. The presence of inhibition zones were measured by using Hi-Media zone scale, recorded and considered as indication for antifungal activity.

Phytochemical Analysis

Test for Flavonoids

The stock solution (1 mL) of ethanol extract of mushroom was taken in a test tube and added few drop of dilute 2 % NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid.

Test for alkaloids

One gram of mushroom dry pellet were taken in a conical flask and added 100ml distilled water and 20ml acetic acid. Hagar's reagent was added to the prepared crude solution and allow it for 8-10hours.

Test for terpenoids

The dry crude mushroom extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution.

RESULTS

Antibacterial activity

Five wild species of mushroom were investigated to evaluate their antibacterial activity against three bacterial strains. One strains of Gram positive *Staphylococcus aureus* and two strains of Gram negative *Escherichia coli* and *Klebsiella pneumoniae* bacteria. Evaluation of antibacterial activity of these extracts was recorded in Table 1 and illustrated in Figure 5. The results revealed that all mushroom extracts were potentially effective in suppressing microbial growth with variable potency. All mushroom extracts have effective in retarding microbial growth of all tested pathogenic bacteria. *Termitomyces eurrhizus* mushroom extracts exhibited highest inhibitory effect against *Staphylococcus aureus, Turbaria furfuracea* mushroom extracts exhibited highest inhibitory effect against *E. coli*, *Leucoagaricus rhodocephalus* and *Collybia tuberosa* mushroom extracts exhibited highest inhibitory effect against *Klebsiella pneumonia.*

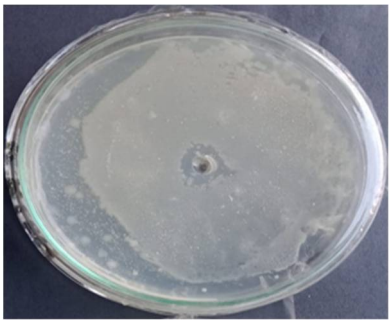

Figure A. *Staphylococcus aureus*

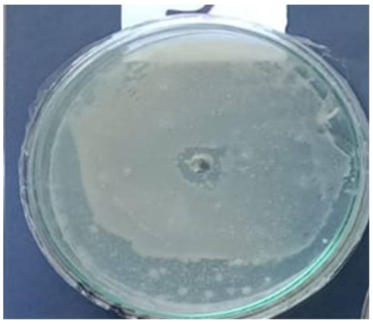

Figure B. *E. coli*

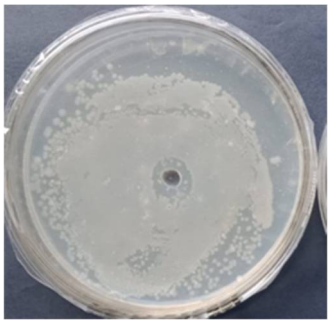

Figure C. *Klebsiella pneumoniae*

Figure 5. Maximum Zone of inhibition on bacterial cell

Table 1. Zone of inhibitions (mm) of five mushroom extracts on three bacterial cells

S/N	Name of the Wild Mushroom	*S. aureus*	*E. coli*	*K. pneumoniae*
1	*Turbaria furfuracea*	0.1mm	1mm	0.5mm
2	*Tylopilus sps*	0.5mm	0.1mm	0.2mm
3	*Termitomyces eurrhizus*	0.8mm	0.2mm	0.5mm
4	*Leucoagaricus rhodocephalus*	0.5mm	0.2mm	1mm
5	*Collybia tuberosa*	0.1mm	0.5mm	1mm

Antifungal activity

Five wild species of mushroom were investigated to evaluate their antifungal activity against two fungal strains *Aspergillus niger* and *Trichoderma harizianum.* The mushroom extracts have very less potential activity on fungal cell. *Termitomyces eurrhizus* mushroom extracts exhibited highest inhibitory effect against *Aspergillus niger* and *Leucoagaricus rhodocephalus* and *Tylopilus sps* mushroom extracts exhibited highest inhibitory effect against *Trichoderma harizianum.*

Results of antimicrobial activity of the five mushroom extracts can suggested that *E.coli* was the most resistant strain to mushroom extracts followed by *S. aureus* and *K. pneumonia*. Moreover, *Termitomyces eurrhizus, Leucoagaricus rhodocephalus* and *Turbaria furfuracea* extracts were the most effective extracts and showed a strong antibacterial activity. Very less potential effect on fungal cell when compared with bacterial cell (Figure 6, Table 2).

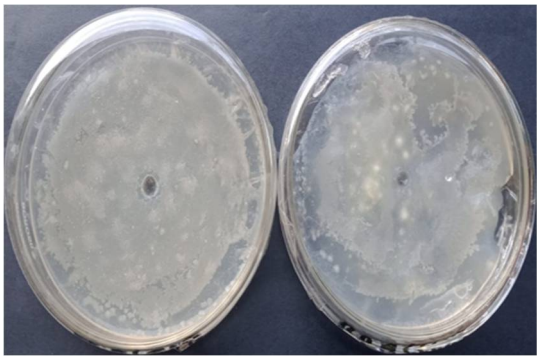

Figure A. *Aspergillus niger*

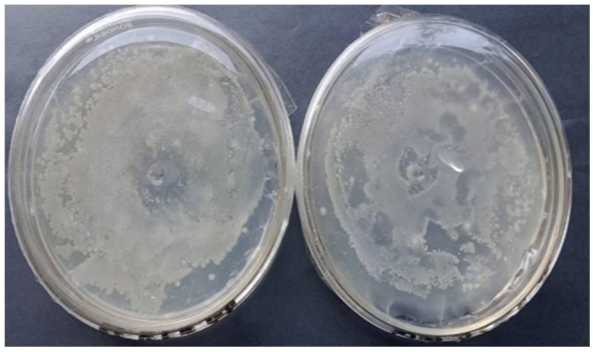

Figure B. *Trichoderma harizianum*

Figure 6. Antifungal activity of mushroom extracts

Table 2. Zone of inhibitions (mm) of five mushroom extracts on two fungal cells

S/N	Name of the Wild Mushroom	*Aspergillus niger*	*Trichoderma harizianum*
1	*Turbaria furfuracea*	0.2mm	0.3mm
2	*Tylopilus sps*	0.1mm	0.2mm
3	*Termitomyces eurrhizus*	0.3mm	0.1mm
4	*Leucoagaricus rhodocephalus*	0.0mm	0.2mm
5	*Collybia tuberosa*	0.0mm	0.1mm

Phytochemical Analysis

Disappearance of formed colour when treated few drop of dilute acid indicates the presence of flavonoids in the *Leucoagaricus rhodocephalus* Orange, *Turbaria furfuracea* brown, *Termitomyces eurrhizus* big white and *Tylopilus sps* yellow. Where there is no colour formation in tiny white and small white indicate the absence of flavonoids. Precipitate crystals were formed after the incubation period along with Hagar's reagent revealed the presence of alkaloid in five wild mushroom extracts. The result for phytochemical screening of ethanol extracts were showed the presence of flavonoids, alkaloids in five different wild mushroom extracts but terpenoids not present in the crude extract (Figure 7, Table 3).

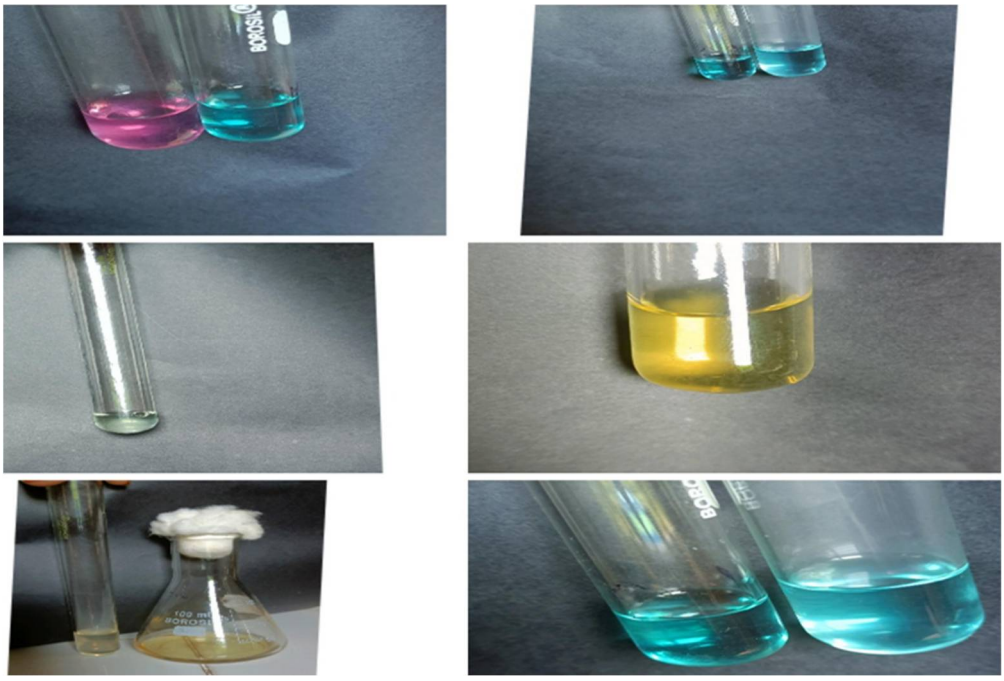

Figure 7. Phytochemical analysis- Test for Flavonoids

Table 3. Summary of Phytochemical analysis (secondary metabolites) in wild mushrooms extracts

S/N	Name of the Wild Mushroom	Flavonoids	Alkaloids	Terpenoids
1	*Turbaria furfuracea*	Present	Present	Absent
2	*Tylopilus sps*	Present	Absent	Absent
3	*Termitomyces eurrhizus*	Present	Present	Absent
4	*Leucoagaricus rhodocephalus*	Present	Present	Absent
5	*Collybia tuberosa*	Absent	Present	Absent

DISCUSSION

The chemical constituents in the mushroom extracts are known to be biologically active ingredients. Some chemical constituents are considered as secondary metabolites components. They are directly responsible for different activity such as antioxidant, antimicrobial, antifungal and anticancer [3]. Therefore, the detected different bioactive compounds is very essential to know their antimicrobial activity. Several authors already reported on flavonoids groups exhibited a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, antiangionic, anticancer and anti-allergic [1,4]. Medicinal plants are the best sources for chemical ingredients, antimicrobial and antioxidant agents for cure of different diseases. Here we can also consider mushroom extracts for their potential effective parameters against microbial cells. These crude extracts could be used as antibiotics or different aliments in pharmaceutical fields. The present study suggested that the extracts which proved to be potentially effective can be used as natural preservatives to control health hazards. These t extracts considered as natural sources of antimicrobial agents, regarded as nutritionally safe and easily degradable. The collected wild edible mushrooms are nutritious and therapeutic. Therefore, wild edible mushroom can be a source of nutritional components of food such as protein, carbohydrate, fats, inorganic compounds and essential vitamins. Hence terms like mushroom nutraceuticals, dietary supplements have emerged. Due to deforestation and urbanization, existence of different groups of the organisms including mushrooms are threatened and has resulted in the loss of traditional knowledge about their uses which is acquired over hundreds years of experience and understanding of environment. In this regard, ethnomycological survey to be conducted future. As microbial resistance to antibiotics is becoming more and more prevalent, mushrooms are seen as a good source of new classes of compounds with antimicrobial activity, some of which, such as pleuromutilin, have led to the synthesis of new drugs that have been recently approved for use in humans [5].

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