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ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF WILD MUSHROOMS COLLECTED FROM HIMACHAL PRADESH

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ABSTRACT

The methanolic and ethyl acetate extract of fruit body of four wild mushroom species collected from forests of Himachal Pradesh were tested for their ability to inhibit the growth of four pathogenic bacterial strains. Antibacterial activity was assayed by using agar well diffusion method. The ethyl acetate extract of all mushrooms inhibited the growth of *S. aureus* with zone inhibition diameter ranges from 10.3± 0.5 to 11.3±1.2 mm. Ethyl acetate extract of Polypore showed activity against *E.coli* having zone inhibition diameter 11.6 ±0.5 mm and no activity was shown by extracts of *Agaricus* sp. *Morchella* sp. and *Canthrellus* sp. Against *K. pneumoniae* except *Agaricus* sp. all three showed activity ranges from 11.3±0.5 to 10.6±0.5 .Methanolic extract of fruit body of Polypore and *Canthrellus* sp. showed antibacterial activity against *E. coli* ranges from11.3±0.57 to 11±1 and *K. pneumoniae* ranges from 12.3±0.57 to 12.3± 1.1, against *S. aureus* methanolic extracts of all fruiting bodies showed activity which ranges from 13±1 to 10.3 ± 0.5 while no activity was observed against *P. aeruginosa*. All extracts exhibited DPPH (1,1 – diphenyl -2- picrylhydrazyl) free radical scavenging activities with Polypore having highest antioxidant activity in both methanolic and ethyl acetate extract (IC_{50} 98.82±4.27 and 104.9±3.36 mg /ml). The mushrooms extracts investigated in this study have antibacterial and antioxidant activities that warrant further studies as potential dietary supplement to improve health and wellbeing.

Key words: Wild mushrooms, Antibacterial activity, Antioxidant activity, DPPH.

INTRODUCTION

Antimicrobial resistance to disease causing pathogenic microorganisms has become a serious worldwide problem recent days this is due to indiscriminate and inappropriate use of antibiotics.[1] It has an economic concern and have drastic impacts not only on patients but also on pharmaceutical producers, physicians, health care administrators, and the public.[2]

Due to rise in multiple drug resistance in pathogenic microorganisms, it has become important to develop new and effective therapeutic agents to counter resistant pathogens.[3] Although there is a huge diversity of antibacterial compounds, bacterial resistance to first choice antibiotics has been significantly increasing. Microorganisms such as *Klebsiella spp*. and *Escherichia coli* produce broad spectrum beta –lactamase or present resistant to third generation cephalosporins.[4] Another examples include MRSA(Multi drug resistant *S. aureus*), which is resistant to vancomycin, *Acinetobacter spp*. with an increasing resistance to carbapenems and colistin and *Pseudomonas spp.* resistant to carbapenemics. Orcephalosporins and aminoglycosides.[5]

The researchers in various studies found that antibiotic consumed in low dosage may not be effective against killing bacteria but they stress them which results in the production of free radicals. These free radical demage the bacterial DNA and might cause mutation leading to the development of resistance.[6] Whilst it is impossible to prevent both bacterial and fungal evolution, it is important to choose most appropriate antibiotics and use them appropriately to minimize the development of drug resistant strains.[7] In search of new antibiotics which prove to be fruitful in getting rid of from this problem several herbs, plants which are known to have antimicrobial properties are being used. In addition to herbs and plants, mushrooms are also used as source of medicine against resistant microorganisms and against free radical damage. These are diverse range of basidiomycete possessing the macroscopic reproductive structures and have been utilizing for medicinal and curative purpose not from present but since from prehistoric times. Mushrooms are known to be a veritable treasure - house of several bioactive compounds that possess antimicrobial, hypolipidemic, hypoglycaemic and antitumorgenic properties.[8]They have a low fat content and contain vitamins, proteins, polysaccharide and many other nutritive compounds.[9] It is also believed that mushrooms need antimicrobial compounds

in order to protect themselves and survive in their natural environment. These compounds could be isolated from many mushroom species and some proved to be of beneficial for humans.[10] Mushrooms contain bioactive compounds with wide range of activity against pathogenic microorganisms. They are known to be the rich sources of natural antibiotics, where the cell wall glucans are well known for their immune-modulatory properties, and for externalized secondary metabolites combat fungi, bacteria and viruses. [11,12,13]

The effects of mushroom extracts against pathogenic microorganisms are studied in different parts of the world by a number of researchers.[14,15]

The present study therefore sought to investigate antimicrobial activities and antioxidant activity of methanolic and ethyl acetate extracts of fruit body of four wild mushrooms collected from Himachal Pradesh.

MATERIAL AND METHODS

2.1 Source of Macrofungi:

Mushroom fruiting bodies were collected from forest around District Solan and Mandi of Himachal Pradesh. The identification of fruiting bodies of wild mushrooms was based on sporocarp morphology and macroscopic characteristics.

2.2 Source of Test microorganisms used and their maintenance

Four bacterial strains *Klebsiella pneumonia* MTCC109, *Pseudomonas aeruginosa* MTCC741, *Staphylococcus aureus* MTCC 737and *E. coli* MTCC739 were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh (INDIA) and stored at 4 ^{o}C in refrigerator and sub cultured at regular intervals of 48 h until use.

2.3 Extract preparation from of fruiting body:

Dried fruiting body of mushrooms was crushed using pestle and mortar to fine formed powder. 100mg powder of each mushroom fruiting body powder was extracted in 1ml of methanol and ethyl acetate separately. The mixtures were sonicated for 30 minutes then left at room temperature overnight. The extracts were filtered over Whatman No.1 filter paper, and the filtrates were sterilized by membrane filtration using 0.45µm pore size filters.[16]

2.4 Antibacterial activity:

Pure cultures of inoculated bacterial strains were seeded into nutrient agar plate. Well (7mm diameter) were made on petri dish using sterile cork borer. About 25µl extract were introduced into bore agar wells using sterile dropping pipette. These plates were kept inside the refrigerator at 4°C for 6

hours to allow proper diffusion of extracts into medium. The plates were then examined for antibacterial activities of extracts after 24 hours of incubation at 37°C.[17]

2.5 Determination of antioxidant activity:

DPPH method was used to determine the free radical scavenging activity of mushrooms extracts.0.1mM of DPPH solution in methanol was prepared and 0.5 ml of it was added to 0.5 ml of extract. The mixture was vortexed thoroughly and left for 45 minutes in dark at room temperature and absorbance was measured at 515 nm against blank. A lower absorbance represents a higher DPPH scavenging activity. The capability of scavenging DPPH radical was calculated using following equation DPPH scavenging effect (%) = (1-AS/AC) x 100 where AC is the absorbance of control containing DPPH solution and AS is absorbance of extract solution containing DPPH.[18]

RESULTS

3.1 Antibacterial activity

Methanolic and ethyl acetate were used as solvent for the preparation of extracts of fruit body.Screening for antimicrobial activity against four bacteria was done by agar well diffusion methods. Results were showed in **table1 and table 2and fig 1 and 2**respectively. The antimicrobial activity was showed by ethyl acetate extract of Polypore against *E.coli* 11.6±0.5 mm in diameter and no activity was shown by extracts of *Agaricus* sp. *Morchella* sp. and *Canthrellus* sp. against *S. aureus* all fruit body extracts showed antimicrobial activity ranges from 11.3±1.2 to 10.3± 0.5 and against *K. pneumoniae* except *Agaricus* sp. all three showed activity while no activity was showed against *P .aeruginosa.* Methanolic extract of fruit body of Polypore and *Canthrellus* sp. showed antibacterial activity against *E. coli* ranges from11.3±0.57 to 11±1 and *K. pneumoniae* ranges from 12.3±0.57 to 12.3± 1.1, against *S. aureus* all fruiting body extract showed activity which ranges from 13±1 to 10.3 ± 0.5 while no activity against *P. aeruginosa*.

Table 1: Zone of inhibition of Ethyl acetate exracts of fungal fruit bodies against pathogenic bacteria

Test bacteria	Isolates(Solvent used Ethyl acetate)				Control
	Polypore	*Canthrellus* sp.	*Agaricus* sp	*Morchella* sp.	DMSO
P.aeruginosa	`-	-	-	-	-
E.coli	11.6±0.5	-	-	-	-
S. aureus	11±1	11.3±1.2	11±1	10.3±0.5	-
K. pneumoniae	11.3±0.5	11.6±2	-	10.6±0.5	-

Each value is expressed as mean±SD of three replicates, - indicates no activity

Table 2: Zone of inhibition of Methanol extracts of fungal fruit bodies against pathogenic bacteria

Test bacteria	Isolates(Solvent used Methanol)				Control
	Polypore	*Canthrellus* sp.	*Agaricus* sp.	*Morchella* sp.	DMSO
P.aeruginosa	-	-	-	-	-

E.coli	11±1	11.3±0.57	-	-	-
S. aureus	10.3±0.5	11.6±0.57	11.3±0.57	13±1	-
K. pneumoniae	12.3±1.1	12.3±0.57	-	-	-

Each value is expressed as mean±SD of three replicates, - indicates no activity

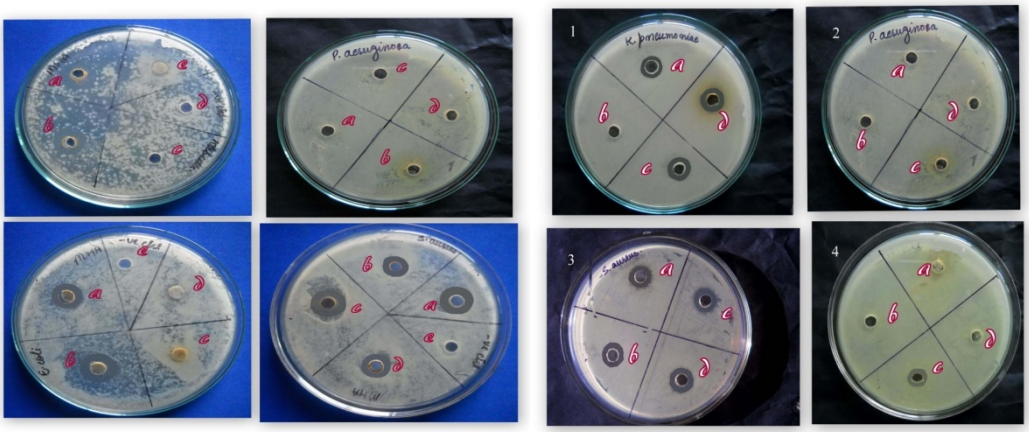

Fig 1 and Fig 2. Antibacterial activity of methanolic and ethylacetate extracts 1.*K. pneumoniae* 2. *P.aeruginosa* (no activity was shown) 3.*S. aureus* 4.*E.coli*

3.2 Antioxidant activity:

3.2.1 DPPH free radical scavenging assay

The antioxidant activities of both methanolic and ethyl alcohol extracts were expressed as IC_{50} values of DPPH. The values varied from 98.82±4.27 in Polypore to 121.3±1.93 in *Agaricus* sp. for methanolic extract while for ethyl acetate the values varied from 104.9±3.36 in Polypore to 127.14±3.420 in *Agaricus* sp. as shown in **table no. 3**.

Table 3: IC50 value of extracts of different samples of macro fungi for DPPH free radical scavenging activity:

S. no.	Macrofungi name	IC_{50} (DPPH) (Methanolic extracts)	IC_{50} (DPPH) (Ethyl acetate extracts)
1.	Polypore	98.82±4.27	104.9±3.36
2.	*Canthrellus* sp.	110.40±1.91	113.69±3.42
3.	*Agaricus* sp.	121.3±1.93	127.14±3.420
4.	*Morchella* sp.	109.1±2.84	111.85±2.42
5.	Ascorbic acid	93.1±2.82	

Results are expressed as mean± SD

DISCUSSION

Antimicrobial activity of fruiting body of wild mushrooms in the present study was carried out by agar well diffusion and it was revealed that both methanolic and ethyl acetate extract of fruit body of all mushrooms taken for study showed good antimicrobial activity against Gram positive bacteria *S. aureus* while few of the extract showed activity against Gram negative bacteria *E. coli* and *K. pneumoniae* and no activity was showed against *P. aeruginosa*. The sensitivity of *S. aureus* to mushrooms extracts agrees with previous studies of various researchers [19] Unlike Gram negative bacteria, Gram positive bacteria have outer membrane and periplasmic space surrounding the cell wall, inner leaflet of this outer membrane consist of phospholipids whereas outer leaflet is mainly comprised of lipo polysaccharides [20]. Lip polysaccharides are mainly

constructed from three parts namely, a proximal hydrophobic lipid A region, a core oligosaccharide region, connecting a distal O- antigen polysaccharide region to lipid A .The lipid polysaccharides molecules contains six or seven covalently linked fatty acid chains also. This results in asymmetric bilayer of bacterial outer membrane from lipid –like cell walls and serve an efficient barrier against rapid penetration by chemotherapeutic agents and antibiotics[21] and polysaccharides from various mushrooms extracts. Murin a peptidoglycan majorly contained by periplasmic space. The form of bacterial cell wall was determined by a rigid layer represented by peptidoglycan sacculus. The periplasmic space contains various molecules, such as mono and oligosaccharides and ammino acids apart from peptidoglycan. The higher concentration of these molecules results in making periplasmic space a gel like matrix with some holes.[22] The enzymes present in periplasmic space are capable of breaking down the foreign molecule introduced from outside further.[23] The periplasmic space act as protective barrier and thus crucial for osmotic stability. In contrast, the absence of outer membrane in Gram positive bacteria results in the absence of membrane bound periplasm. They contain multi-layered peptidoglycan covalently substituted with the anionic polymer teichoic acid or teichuronic acid. Gram positive walls possess the ability to retain large amount of proteins, lypoglycans and cation , large water capacity, due to thick hydrophilic porous structure.[24] Gram positive bacteria due to all these properties are more permeable to antibiotics, chemotherapeutic agents as well as more vulnerable to attack of mushroom polysaccharides.

The inability of the extracts to inhibit the growth of gram negative bacteria like *P. aeruginosa* could be that the organisms possess a mechanism for detoxifying the active components.[25] To the best of knowledge, least studies were made to reveal that extracts prepared from wild mushrooms collected from Himachal Pradesh have antibiotic activity against bacteria used in the present study.

DPPH free radical scavenging assay to assess free radical scavenging activity is widely accepted model.[26] The assay avoid side reactions such as enzyme inhibition and metal ion chelation, which complicate assay that are based on laboratory-produced free radicals such as super oxide anion and hydroxyl radical.[27] The reaction of antioxidants to scavenge free radical of DPPH is caused by hydrogen donating ability of these compounds.[28] Polysaccharides isolated from mushrooms

extracts have been proved for this ability, where the hydroxyl group of monosaccharaides unit can donate hydrogen to reduce DPPH radical.[29]

The pathophysiology of wide range of diseases are related to reactive oxygen and nitrogen species. Oxidative damage to DNA results in triggering carcinogenesis.[30] It has been suggested that the immunomodulation and antitumour activities of polysaccharides are lagerly related to their antioxidant properties.[31] The DPPH free radical scavenging assay performed in the present study is only a preliminary research tool to illustrate the antioxidant properties of extracts of wild mushrooms prepared in two different solvents. Other methods are required to assess the additional type of antioxidant activity of these mushrooms extracts possess.

CONCLUSION

The extracts extracted from two different extracts of wild mushrooms inhibited the growth of *S. aureus, E. coli* and *K. pneumoniae*, whereas *P. aeruginosa* is resistant to all extracts. Therefore, the results confirmed that only one Gram negative bacteria namely *P. aeruginosa*is more resistant to inhibitory effect of extracts of macrofungi. Extracts of all four wild macrofungi showed DPPH free radical scavenging activity also. Hence it is concluded from above study that further more work is required to understand the molecular basis of antibacterial activity of the extracts of macrofungi and for additional antioxidant activity other methods are required to assess.

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