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ORIGINAL ARTICLE

Studies on Antibacterial Potential of *Agaricus bisporus*

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ABSTRACT
Mushrooms are well known for their texture, flavour, nutrition and therapeutic characters such as antiviral, antibacterial, antithrombotic and immunomodulating properties. Along with their medicinal properties, mushrooms are necessary in our daily food due to their low fat and high protein content. Agaricus bisporus commonly known as Button Mushroom is a member of Agaricaceae family and is considered most important edible mushroom due to its high medicinal and nutritional values contributing to human health. In the present study antibacterial activity of Agaricus bisporus was tested against some pathogenic bacteria viz. E. coli, Enterobacter aerogenes, Streptococcus mutans, Staphylococcus aureus, Pseudomonas syringae, Pseudomonas putida and Bacillus subtilis procured from IMTECH, Chandigarh. Disc diffusion method was used to determine the antimicrobial activity of different extracts (Aqueous, Ethanol, Chloroform and Petroleum Ether). Ciprofloxacin was used as positive control whereas DMSO and water as negative controls. All the extracts exhibited significant antibacterial activity and highest activity was observed in petroleum ether extract against P. putida and S. mutans. Similarly chloroform extract was found highly effective against B. subtilis. Phytochemical analysis for all the extract was also done for testing the presence of tannins, saponins, steroids, terpenoids, glycosides, alkaloids, phenols and flavonoids. The current study is an attempt to prove the antibacterial potential of button mushroom against pathogenic bacteria. Isolation of phytoconstituents from the mushroom may lead to discovery of novel antibacterial compounds without any adverse effect on human health.
Key Words: *Antibacterial, Agaricus, Disc Diffusion, Enterobacter, Alkaloids*

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INTRODUCTION

Globally, in recent years, mushrooms have become one of the most important sources of functional food and medicines for human health and their demand is increasing due to their taste, flavor and beneficial nutrient content. Edible mushrooms are highly rich in proteins consisting of essential amino acids and fiber. They are also significantly rich in important fatty acids and vitamins B, C, D and E [1]. *Agaricus bisporus* is a macro fungi; commonly known as Button mushroom and belongs to family Agaricaceae. It is an important edible mushroom and a significant reservoir of many bioactive compounds contributing to human health [2-3]. Important amino acids present in button mushroom are serine, glycine, valine, aspartate, leucine, isoleucine, lysine, hystidine, proline, tyrosine and arginine [2,4]. Button mushrooms aids in human health by boosting the immune system and possess significant antibacterial, antioxidant, anticancer, anti-obesity and anti-inflammatory properties leading to treatment of many chronic diseases such as cancer, diabetes and even heart diseases [5-7]. The myochemical analysis of *A. bisporus* confirmed the presence of alkaloids, proteins, poly-phenols, saponons and tannins resulting in its antimicrobial potential against pathogens [8-9].

Agaricus bisporus is the wildest and cultivated mushroom and constitute more than 40% of total mushrooms in the world. Button mushroom is characterized by high biological activity, less toxicity and has significant folklore and ethanopharmacological importance. With its use in food, beverages and medicines, it also has role in perfumery and cosmetic industries [10]. Button mushroom also effects carbon cycle in ecosystem as a saprophytic decayer of plant litter [11]. Utilization of edible fungi to fulfill human nutrition has been a general denominator in the history. A. bisporus species are macro fungi

which treasures broad qualities to cure various diseases in future and a key principle for various researches involving pharmacology, microbiology and biotechnology [12]. Owing to such great medicinal properties the present study is an attempt to study the antibacterial potential and phytochemical analysis of *A. bisporus* i.e. button mushroom.

Systematic Position:

Scientific Name: *Agaricus bisporus* (L.) Imbach
Kingdom: Fungi
Divison: Basidiomycota
Class: Agaricomycetes
Order: Agaricales
Family: Agaricaceae
Genus: *Agaricus*
Species: *bisporus*

MATERIAL AND METHODS

Preparation of Fungal Extracts: Fungi (*A. bisporus*) were collected from different localities during their growing season. The mushrooms were thoroughly washed and then dried under shade at 28+2°C for about 10 days. The dried samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried powder of mushroom was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for extraction were petroleum ether, chloroform, acetone, ethanol and water as solvent and the extracts were dried. The dried extracts were stored in a refrigerator at 4^0C. Finally, concentration of 5 mg per disc was loaded on each disc.

Antimicrobial Susceptibility Test: All the fungal extracts were screened against seven pathogenic bacterial strains. The tested organisms were *E. coli*, *Enterobacter aerogenes*, *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas putida* and *Pseudomonas syringae* obtained from IMTECH, Chandigarh, India. The Disc Diffusion method (Bauer *et al*, 1966) was used to test the antibacterial activity of the fungal extracts [13]. 20 ml of sterilized nutrient agar medium were poured into each sterile petri dish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) loaded with 5 mg/ disc, of dry extract were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37^0C for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Phytochemical screening: The fungal extracts were subjected to preliminary phytochemical screening for presence of terpenes, saponins, steroids, glycosides, alkaloids, flavonoids and tannins. [14-15]. GC-MS analysis was done from Cultivator phyto lab, Jodhpur.

RESULTS AND DISCUSSION

Worldwide excessive use of antibiotics has promoted emergence of antibiotic-resistant pathogens and rising numbers of antibiotic unresponsive infectious disease agents confronting patients on global scale. There is an alarming need to control antimicrobial resistance by improved antibiotic usage. Mushrooms are valued all over world both as food and medicine from times unknown and several researchers are working on antibacterial potential of macro fungi [16-18]. Secondary metabolites obtained from fungi are diverse in structure and functions. Fungal metabolites are well known to show a range of biological properties, as a result, screening and isolation of fungal metabolites are an interesting alternative to revel novel bioactive compounds [19].

In the present investigation antibacterial potential and phytochemical analysis of A. bisporus was done. The antimicrobial action of extracts was observed against some pathogenic bacteria viz. *E. coli*, *Enterobacter aerogenes*, *Streptococcus mutans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas putida* and *Pseudomonas syringae*. All the extracts were found effective against all the tested bacteria and highest activity was exhibited by petroleum ether extract against *P. putida* followed by petroleum ether extract against *S. mutans.* Similarly Ren *et al* (2014) studied the antibacterial and antioxidant properties of edible mushrooms against *B. subtilis* and *Streotococcus epidermis* [20]. In the present study, in case of alcoholic extract highest activity was found against *P. syringae* and *P. putida*. Similarly, Gebreyohannes *et al* (2019) found that *S. aureus*, *P. aeruginosa* and *MRSA* were the most susuceptible to chloroform extract

of *Trametes spp* [21]. In the current study all the acetone extracts were also able to inhibit tested bacteria with most susuceptible being *S. mutans* and *B. subtilis*. Yakobi *et al* (2023) screened the antimicrobial properties and bioactive compounds of *Pleurotus ostreatus* extracts against *S. aureus*, *E. coli* and *Neisseria gonorrhoeae* [22]. Asri et al [23] also found that S. aureus was the most susuceptible bacteria when tested with the ethanolic extracts of mushrooms.

The preliminary phytochemical analysis of methanolic extract of *A. bisporus* indicated the high presence of terpenoids, phenols and glycosides. Other phytochemicals present were flavonoids, alkaloids, tannins, saponins and steroids. Similarly, Bose *et al* (2019) studied the phytochemical and pharmacological potential of *A. bisporus* [24]. Chandra *et al* (2017) worked on preliminary phytochemical composition of methanolic extract between *A. bisporus* and *Volvareilla volvacea* [25]. In GC-MS analysis total 58 compounds were observed out of which 11 compounds were found to be antimicrobial in action as described in literature available. Antimicrobial potential and phytochemical analysis of many plants is done by many researchers [26-30].

Table 1. Antibacterial Activities of *A. bisporus* against Pathogenic Bacteria

Agaricus bisporus extracts	Zone of Inhibition (mm)						
	E. coli	*Enterobacter aerogenes*	*Streptococcus mutans*	*Staphylococcus aureus*	*Pseudomonas syringae*	*Pseudomonas putida*	*Bacillus subtilis*
Aqueous	5.0+1.0	4.33+1.53	3.33+0.52	3.67+1.52	5.33+1.55	3.33+1.15	5.32+1.15
Alcoholic	4.67+0.58	6.67+1.53	6.1+2.0	5.23+1.52	7.67+1.53	7.5+1.53	6.30+1.25
Acetone	6.33+2.52	6.0+1.0	8.32+1.53	7.33+1.52	6.20+1.8	6.67+1.56	7.48+1.57
Chloroform	9.33+1.53	8.67+2.52	9.67+2.08	8.67+2.08	8.0+1.73	8.5+1.43	11.67+1.53
Petroleum Ether	8.67+2.52	7.67+3.51	13.67+1.53	9.52+1.73	9.2+0.58	14.33+0.58	10.12+1.15

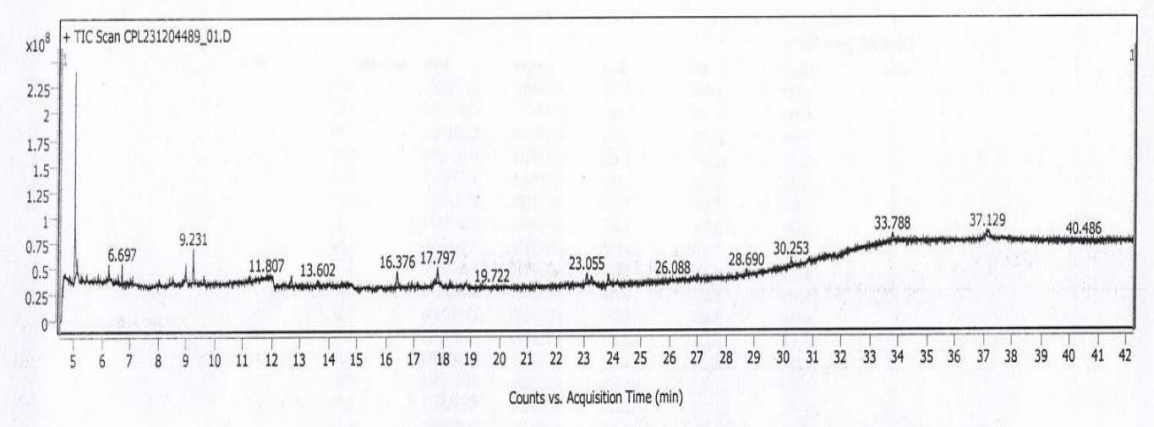
Table 2. Phytochemical Analysis plant part extracts of *Citrus limon* L.

Phytochemical Component	Agaricus bisporus extract (Methanolic)
Alkaloids	++
Glycosides	+++
Saponins	+
Flavonoids	++
Tannins	+
Phenols	++++
Terpenes	++++
Steroids	+

Table 3. Important Compounds from GS-MS analysis of *A. bisporus* extract

S.No.	Compound Name	Mass (Molecular Weight)
1.	Phosphinic Acid, diethyl-methyl ester	214.099
2.	Methyl stearate	386.32
3.	Mephenesin	182.09
4.	Phenol, 2-methyl	108.05
5.	Z-6-Tridecene	182.2
6.	D-Araninitol	152.06
7.	Gluconic Acid, δ Lactone	178.04
8.	Phenol, 2,5,-di-tert-butyl	206.16
9.	Chol-7-ene-12,24-diol	360.3
10.	Gallic Acid, tetra TMS	458.17
11.	Triptolide	358.14

Figure 1. GC-MS analysis (Chromatogram Peaks) of *A. bisporus*



CONCLUSION

In conclusion it was observed that all the extracts of *A. bisporus* had significant antibacterial potential against all the microbes tested and highest activity was observed in petroleum ether and chloroform extracts. This investigation proves the importance of fungal products in the treatment of different bacterial diseases. The present attempt may reveal a solution for antibacterial resistance by using products of plant origin, which are essentially important in reducing the global burden of infectious diseases.

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