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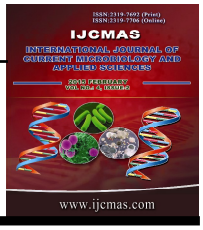

Original Research Article

Study on Antibacterial Activity of *Agaricus bisporus (Lang.)* Imbach

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Keywords

Agaricus bisporus, Antibacterial, *in-vitro*, *Staphylococcus aureus*, *Escherichia coli*

A B S T R A C T

Antibacterial activity of methanolic and acetone extracts of *Agaricus bisporus* were determined *in-vitro* against two pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* following agar well diffusion method using different concentrations (25, 50, 75 and 100%). Methanolic and acetone extracts showed potent antibacterial activity against tested bacteria. Methanolic extract showed maximum inhibitory effect against growth of each of the test bacterium. There is a need for further studies to isolate and characterize the antibacterial moieties in this fungus for practical disease control measures.

Introduction

The arising awareness of the relationship between diet and diseases has evolved the concept of functional foods and the development of a new scientific discipline, Functional Food Science (Sadler, 1998). A food may be considered to be functional if it contains a food component (whether a nutrient or not) which affects one or more identified functions in the body in a positive manner, which are in different name forms, e.g. dietary supplements, nutraceuticals, medicinal foods, vita foods, pharma foods, phytochemicals, mycochemicals and foods for specific health uses (Hasler, 1996). Mushroom is a macro fungus with a distinctive fruiting body that is large enough to be seen by the naked eyes. It includes both edible and non edible species. Some mushrooms serve as food because of their nutrient contents while some have been used extensively in traditional medicine (Stamets, 2000). Of the hundreds of known mushroom varieties, several have been studied for their ability to enhance the human immune system and fight infections.

In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents (Karaman *et al.,* 2003). Researchers showed antimicrobial activity of several mushrooms (Gezer *et al.,* 2006; Mercan *et al.,* 2006*;*

Turkoglu, *et al.,* 2007). Extracts from fruiting bodies and the mycelia of various mushrooms have been reported for antimicrobial activity against wide range of infectious bacteria (Hirasawa, *et al.,* 1999; Dulger *et al.,* 2002).

Agaricus bisporus, known as table mushroom, cultivated mushroom or button mushroom, is an edible basidiomycete fungus which naturally occurs in grasslands, fields and meadows. It has spread much more widely and is one of the most widely cultivated mushrooms in the world. The original wild form bears a brownish cap and dark brown gills but more familiar is the current variant with a white form, having white cap, stalk and flesh and brown gills (Jagadish *et al.,* 2009). *Agaricus* is the most cultivated mushroom and accounts for the 38% of worlds cultivated mushrooms. Thus, the present study focused on evaluation of antibacterial activities of methanolic and acetone extracts of *Agaricus bisporus* using agar well diffusion method against two clinical isolates *Escherichia coli* and *Staphylococcus aureus*.

Materials and Methods

Materials used

Materials used in the present study were fruiting bodies of *Agaricus bisporus* procured from DMR, Solan (H.P.) and two bacterial pathogens (*Escherichia coli* and *Staphylococcus aureus*). Pathogenic strains of bacteria were procured from IGMC, Department of Microbiology, Shimla.

Isolation of pure culture of *Agaricus bisporus*

The cultures were raised from the stipe and stroma portion of healthy, sun-dried and fresh specimen. The specimen was first washed with distilled water and then the tissue from the stipe and stroma portion were cut with the help of a sterilized blade. The bits of tissue (2-3 mm) were taken up with a sterilized forceps and dipped in 0.1% mercuric chloride solution for 5-10 seconds. Now the tissue was placed on filter paper to remove the excess moisture. The small bit of *Agaricus* tissues was then transferred aseptically into the petriplates containing Potato Dextrose Agar (PDA) medium with the help of a sterilized forceps (Chandra *et al.,* 2012; Pala *et al.,* 2013). These were then incubated at 25^0C for at least 8-10 days and observed regularly for appearance of culture. The actively growing mycelial colonies were sub cultured to obtain pure cultures.

Preparation of crude mushrooms extract

The fresh fruiting bodies were dried in shade conditions and the dried material (50 g) was pulverized in a blender to get a coarse powder and soaked separately in 300 ml of methanol and acetone in Erlenmeyer flask for methanol and acetone extracts. The flasks were covered with aluminium foil and allowed to stand for 7 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator (Jonathan and Fasidi, 2003; Balakumar *et al.,* 2011). The extracts were collected and stock solution of conc.10 mg/ml was prepared.

Screening of extracts of *Agaricus bisporus* for antibacterial activity

Screening of mushroom extracts (methanol and acetone) of *Agaricus bisporus* was done using agar well diffusion method. Nutrient agar medium (Beef extract 1g, Yeast extract 2g, Sodium Chloride 1g, Peptone 5g, Agar 20g, Distilled Water 1000 ml) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes

and poured into Petri plates. Bacteria were grown in nutrient broth for 24 hours. A 100 µl of bacterial suspension was spread on each nutrient agar plates. Five agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared extracts of *Agaricus bisporus.* The control well containing pure solvent only. The plates were incubated at 37 ± 2^0C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all three replicates and the average values were tabulated. Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control from the values of inhibition diameter using control as standard (Hemashenpagam and Selvaraj, 2010).

Percentage of growth inhibition= (Control-Test/Control) x100

Control=average diameter of bacterial colony in control.

Test=average diameter of bacterial colony in treatment sets (Kannan *et al.*, 2009).

Results and Discussion

Morphological and mycelial characteristics

The fruiting bodies of *Agaricus bisporus* were white in colour (Fig.1A). Spores were oval to round having brown colour spore print (Fig. 1C). Mycelial growth of *Agaricus bisporus (Lang.)* Imbach was longitudinally radial, aerial initially, creamish white, becoming densely matted and cottony in texture (Fig. 1B).

Antibacterial activity of *Agaricus bisporus* against *S. aureus* and *E. coli*

The methanolic and acetone extracts of *Agaricus bisporus* were screened against *S. aureus* and *E. coli*. Stock solutions were prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution.

The methanolic and acetone extracts of *Agaricus bisporus* showed considerable growth inhibition of two test bacteria in different concentrations (25%, 50%, 75%, 100%). The methanolic extract of *Agaricus bisporus* showed maximum inhibition of 16.67% and 20.00% at 100% concentration of the extract against *S. aureus* and *E. coli* respectively (Table 1, Fig. 2A, C) and the acetone extract showed maximum inhibition of 16.67% and 17.77% at 100% concentration against *S. aureus* and *E. coli* respectively (Table 2, Fig. 2B, D). It is evident from the results that methanolic and acetone extracts of *Agaricus bisporus* showed maximum percent inhibition against *E. coli* and methanolic extract was more effective than acetone extract against both the test bacteria.

The results of the present study are in agreement with the work of the earlier workers (Nasim and Ali, 2011; Kamra and Bhatt, 2012), who have also reported strong antibacterial activity of methanolic extract of *G. lucidum* against gram negative bacteria (*E. coli*) and comparatively less activity against gram-positive (*S. aureus*) bacteria. Similar trend in antibacterial activity of methanolic extract of *Lactarius delicious* (Sagar and Thakur, 2012), *Sparassis crispa* (Sagar and Tandon, 2012), *Morchella esculenta* (Sagar and Kumari, 2012) and *Ganoderma lucidum* (Sagar and Kumari, 2012) have been reported against *S. aureus*

and *E. coli*. Ramesh and Patter (2010) have reported that extract of *Clavaria vermicularis* and *Marasmium oreades* offered more inhibition to gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) as compared to gram-positive bacteria (*Bacillus sutilis* and *Staphylococcus aureus*). Neelam and Singh (2013) also reported the antibacterial potential of ethanolic extract of *Pleurotus florida* and *Pleurotus ostreatus.*

Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from mushrooms and distributed worldwide. Mushroom based products either from the mycelia or fruiting bodies are consumed in the form of capsules, tablets or extracts (Filipa, *et al.*, 2013. This study has revealed that the edible mushroom *Agaricus bisporus* exhibited various levels of antimicrobial activity in different solvents. The bioactive contents of the mushrooms are promising natural antimicrobial agents that can be harvested as potential antibacterial substances.

In present study, we have reported the antibacterial activity of methanolic and acetone extract of *Agaricus bisporus* against *S. aureus* and *E. coli*. So, there is a need for further studies to isolate and characterize the bioactive compounds present in *Agaricus bisporus* and these metabolites can be used to develop effective drugs against these human pathogenic bacterial strains.

Table.1 Percent inhibition of growth of *S. aureus* and *E. coli* at different concentrations of methanolic extract of *A.bisporus*

Sr.no.	**Concentration of the methanolic extract (%)**	**Inhibition of growth of test bacteria (%)**	
		S.aureus	***E.coli***
1	Control	0.00	0.00
2	25	10.00±0.14	14.44±0.44
3	50	12.22±0.13	15.55±0.34
4	75	14.44±0.22	16.67±0.23
5	100	16.67±0.44	20.00±0.13

Table.2 Percent inhibition of growth of *S. aureus* and *E. coli* at different concentrations of acetone extract of *A.bisporus*

Sr.no.	**Concentration of the acetone extract (%)**	**Inhibition of growth of test bacteria (%)**	
		S.aureus	***E.coli***
1	Control	0.00	0.00
2	25	0.00±0.00	07.77±0.55
3	50	10±0.01	15.00±0.23
4	75	13.33±0.11	15.55±0.37
5	100	16.67±0.86	17.77±0.77

Fig.1 Fruiting bodies of *Agaricus bisporus*

(A) Pure culture of *Agaricus bisporus* **(B)** Basidiospores of *Agaricus bisporus* **(C)** Basidiospores of *Agaricus bisporus*

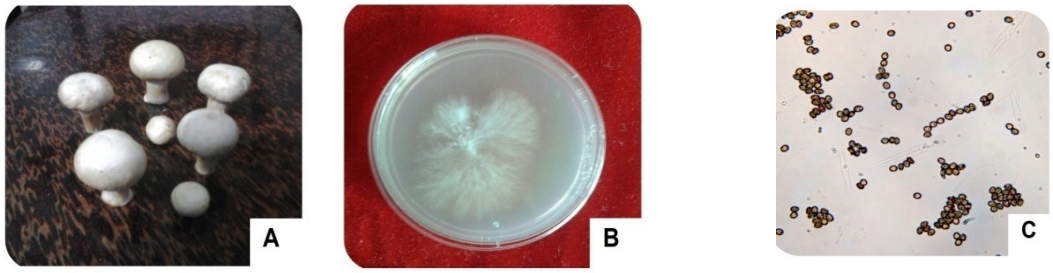
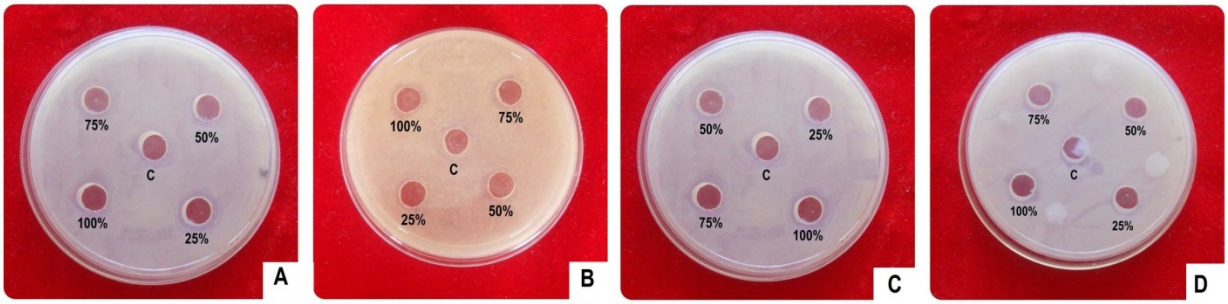


Fig.2(A) Inhibition in the growth of *S.aureus* at different concentrations of methanolic extract, **(B)** Acetone extract of *Agaricus bisporus.* **(C)** Inhibition in the growth of *E. coli* at different concentrations of methanolic extract, **(D)** Acetone extracts of *Agaricus bisporus.*



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