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Phytochemical Screening and Evaluation of Antibacterial Potential of Silver Nanoparticles of White Button Mushroom (*Agaricus bisporus*)

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ABSTRACT
The current research investigation was conducted with the main purpose of phytochemical screening of edible mushroom Agaricus bisporus, synthesis of silver nanoparticles of aqueous (aq.) extract of *Agaricus bisporus* and to evaluate their antibacterial potential. The crude extract A. bisporus was prepared by maceration method by boiling continuously for 30 minutes in water bath. The silver nanoparticle (AgNPs) of *A. bisporus* was prepared by mixing 1ml of aq. extract of *A. bisporus* and 9 ml of 1mm stock solution of $AgNO_3$. The antibacterial activity assay was carried out by agar well diffusion method. Results depicted that the phytochemical analysis of aq. extract of *A. bisporus* disclosed the presence of secondary metabolites viz. terpenoids, saponins, steroids, alkaloids, phenols, insulin and glycosides. Antibacterial activity assay revealed that aqueous extract of *A. bisporus* was effective against *S. aureus* and *B. amyloliquefacien*. Furthermore, antibacterial activity assay results depicted the highest efficacy of AgNPs of *A. bisporus* as compared to aq. extract of *A. bisporus* alone. In conclusion, AgNPs of *A. bisporus* could be recommended to use for biomedical and pharmaceutical applications.
Keywords— *Agaricus bisporus*, AgNPs, Antibacterial activity

1. Introduction

Herbs are used all over the world. Medicinal plants are easy to find and have a variety of natural chemicals due to their therapeutic properties and uses. Most drugs in the past i.e., allopathic, ayurvedic and homeopathic medicines were made from plants. Medicinal plants have secondary metabolites, and these secondary metabolites are the main source of medicinal products with therapeutic effects [1]

Since the discovery of traditional antibiotics (such as penicillin), many microorganisms are now resistant to one or more antibacterial drugs. Antimicrobial resistance proves to cause fatal injuries to thousands of people every year, leading to high medical costs and serious economic losses. The increasing failure of chemotherapeutic drugs and the antibiotic resistance of pathogenic microorganisms has led to some medicinal plants being studied for their potential antibacterial activities [2].

Edible mushrooms are nutrient-rich fungi (mainly Basidiomycetes) that naturally grow on trunks, leaves and roots of trees, as well as on rotting wood materials [3]. Mushrooms have been traditional remedies since ancient Greece and Rome. It is believed that fungi require antibacterial compounds in order to survive in their natural environment [4]. Scientific research on shiitake mushrooms like shiitake mushrooms, maitake mushrooms like *Grifola frondosa*, chanterelles like *Chaterellus carius*, white button mushrooms like *Agaricus bisporus* and oyster mushrooms have shown that they can be used as B vitamins (like niacin, flavin and pyridoxine) [5], reservoirs for organic acids (such as ascorbic acid, shikimic acid, malic acid and fumaric acid); Carbohydrates such as dextran; Monoterpene and diterpene lipids; Proteins such as hydrophobins and trace elements such as selenium [2].

Research into natural remedies in various edible mushrooms has always attracted a lot of attention. Interestingly, silver nanoparticles (AgNPs) can also be synthesized from plants [6-8]. The development of a reliable synthesis process for green silver nanoparticles is an important aspect of

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current nanobiotechnological research. The biosynthesis of green synthetic nanoparticles is superior to chemical and physical processes because it is inexpensive and environmentally friendly and does not require the use of high pressure, energy, temperature or toxic chemicals. Plants provide a better platform for nanoparticle synthesis because they do not contain toxic chemicals and provide natural covering agents. In addition, the use of plant extracts also reduces the cost of microbial isolation and culture media and increases the cost competitiveness of microbial synthesis of nanoparticles.
With this viewpoint, the present study was designed for phytochemical screening of edible mushroom *A. bisporus*, synthesis of AgNPs of aq. extract of *A. bisporu*s and to evaluate their antibacterial potential.

2. Experimental Methods or Methodology

2.1 Collection and identification of plant materials

The samples of *A. bisporus* were procured from the local market, Bengaluru, India. Collected samples were identified and authenticated by the Dr. Suresh Kumar, Associate Professor, Department of Botany, Maharani Cluster University, Bengaluru, Karnataka, India.

2.2 Collection of pathogens

The multiple antibiotic-resistant isolates viz. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *B. amyloliquefacien* were isolated from clinical samples of local hospital in and around Bangalore and confirmed by various microscopic evaluation like Gram's staining [9]. Motility, capsule and spore formation was confirmed as per the procedure prescribed by Collins and Lyne [10]. All the bacterial pathogens were further confirmed by suitable biochemical tests [11], and used for antimicrobial activity studies.

2.2 Sample preparation

Plant samples were washed, dried and crushed to make fine powder. The 50g of dried rhizome powder of *A. bisporus* was weighed in a high precision weighing balance and dissolved in 500 ml of double distilled water to form crude extract by maceration method by boiling continuously for 30 minutes in water bath. The conical flasks of the extracts were covered by cotton plugs to avoid the evaporation. The extracts were placed in shaking incubator at 250 rpm for 48 h. After shaking they were filtered with muslin cloth and with filter paper twice. Prepared crude extract was evaporated to dryness, amount was measured [12], and was stored at 4°C [13].

2.3 Phytochemical screening

The crude aq. extract of *A. bisporus* was tested for the phytoactives such as steroid, tannins, phenols, flavonoid, alkaloids, glycoside, triterpenoids, carbohydrates and proteins using standard procedures as described by Adebayo et al [14].

2.4 Antibacterial activity assay

Agar well diffusion method

Muller Hinton Agar media were prepared and autoclaved at 121°C for 15 minutes at 15 lbs and poured in sterile petri plates up to a uniform thickness of approximately 10-15 minutes and the agar was allowed to set at ambient temperature. This method is suitable for the organism to grow rapidly overnight at 35-37°C. The wells were made in medium after inoculation with the microorganism. 200 µl of inoculums were spread over Muller Hinton Agar plates using sterile spreader, after few minutes four wells were made in each petri plated and loaded with different concentration of aqueous plant extract with control. Plates were incubated at 37°C for 24 h. Antibacterial activity was observed by measuring its inhibition length. Inhibition length against bacteria was calculated [15]. The experiments were done in triplicate.
Inhibition length = Zone of Inhibition (mm) – Well diameter (mm)

2.5 Synthesis of silver nanoparticles (AGNPS)

50 ml stock solution of 1mm AgNO3 was prepared in distilled water. 1ml of aq. extract of A. bisporus each was added to 9 ml of stock solution of $AgNO_3$ in conical flask. The mixture was stirred

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continuously for 5-10 minutes and then incubated in dark room at 37ºC under static condition. Suitable controls were maintained throughout the experiment. Reduction of silver nitrate to silver ions was confirmed by the color changes from light yellow color to brown color. Simultaneously, the positive control was maintained with the extract and de-ionized water was used as negative control containing only silver nitrate solution [16].

2.6 Characterization

UV-Visible spectra analysis

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles [17]. UV-Visible absorption spectrophotometer with a resolution of 1 nm between 300 to 700 nm was used [18]. The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting small aliquot of the sample into deionized water. 1 ml of the sample was pipetted into test tube and diluted with 4 ml of deionized water and subsequently analyzed at room temperature, UV-Vis spectral analysis was done by using UV-Vis spectrophotometer 117 by recording the absorbance from 200-700 nm and the strong Plasmon absorbance band was observed at 420-430 nm in positive mushroom samples and sweet flag rhizomes indicating the production of silver nanoparticles from the extracts [19].

3. Results and Discussion

3.1 GGBS and Cement

The phytochemical analysis of aq. extract of *A. bisporus* disclosed the presence of secondary metabolites viz. terpenoids, saponins, steroids, alkaloids, phenols, insulin and glycosides. Whereas, tannins, flavonoids, amino acids, carbohydrates, phlobatannins, starch, reducing sugars and naphthoquinone were absent (Table 1). These phytoconstituents play a vital role in medicinal properties of plants. *A. bisporus* and have vital role in promoting health. The presence of essential nutrients and minerals in the edible mushroom (*A. bisporus*) imply they could be utilized to improve health [20]. Saponins for instant comprise a large family of structurally related compounds containing a steroids or triterpenoid. They are reported to have a wide range of pharmaceutical properties, such as anti-inflammatory and anti-diabetic effects. Thus *A. bisporus* can be used in the management of diabetes and inflammation related diseases. Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anticancer effects among others. Phenolic compounds are antioxidant and exhibit a wide range of spectrum of medicinal properties such as anti-cancer and anti-inflammatory. *A. bisporus* can be therefore being harnessed in the management of oxidative stress induced disease since phenol have been shown to possess various anti-oxidant functions [21].

Table. 1. Phytochemical screening for *aq.* extract of *Agaricus bisporus*

S. No.	Phytochemical Screening	Aq. extract of *Agaricus bisporus*
1	Tannins	-
2	Flavonoids	-
3	Terpenoids	+
4	Saponins	+
5	Steroids	+
6	Alkaloids	+

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7	Phenols	+
8	Amino acids	-
9	Carbohydrates	-
10	Phlobatannins	-
11	Starch	-
12	Insulin	+
13	Glycosides	+
14	Reducing sugars	-
15	Naphthoquinones	-

Plants are a source of a large number of drugs that have affirmed the properties of antibiotics in the traditional system. These plants have a sort of secondary metabolites that are responsible for their antibacterials, antifungals, antiulcer, antifeedant, repellents and pesticides and, therefore, take care of a large number of diseases. The antibacterial activity of aq. *A. Bisporus* rhizome extracts for Agar well diffusion method with different concentration. The length of the inhibition was calculated to reveal its inhibitory effect against two positive grams (*B. amyrochefaciens, S. aureus*) and two Gram negative bacteria (*E. coli, P. aeruginosa*) and its antibacterial activity (Table 2).

Aq. extracts showed maximum antimicrobial sensitivity against *B. amyloliquefacien* i.e., 19 mm (150 mg/ml) zone of inhibition & 20 mm (300mg/ml) compared to *S. aureus* i.e., 17 mm (150mg/ml) zone of inhibition & 18 mm (300mg/ml). Whereas there was no zone of inhibition observed against *P. aeruginosa* & *E. coli* (Table 2). Our results are comparable with the finding of Pipriya et al wherein zone of inhibition find out by them against *S. aureus* and *B. amyloliquefacien* was found to be 15 mm and 16 mm respectively. Whereas, *E. coli* and *P. aeruginosa* did not show zone of inhibition which is similar to our study [22]. Morchella esculenta and Ganoderma lucidum, have been reported against S. aureus and E. coli. [23]. Ramesh et al have reported that extract of *Clavaria vermicularis* and *Marasmium oreades* offered more inhibition to gram negative bacteria (*E. coli* and *P. aeruginosa*) as compared to gram-positive bacteria (*B. subtilis* and *S. aureus*) [24]. Furthermore, Neelam et al also reported that the antibacterial potential of ethanol extract of *Pleurotus florida* and *Pleurotus ostreatus* [25]. Overall results of antibacterial activity delineated that aq. extract of *A. bisporus* was effective against *S. aureus* & *B. amyloliquefacien*.

Nanotechnology provides a platform for the development of nanomaterials and has broad application prospects in various fields. This study revealed the traditional history of mushrooms used by ancient tribes and local Hakim people, and found that mushrooms have nutritional and medicinal values. This study shows that the production of silver nanoparticles from edible fungi and its strong inhibitory effect on *S. aureus, E. coli* and *P. aeruginosa* and *B. amyloliquefacien* strains. The AgNPs extracted from these mushrooms with medical and nutritional value will be used as a substitute for antibiotics, which may be effective, safe and inexpensive in the treatment of infections.

Nanotechnology provides a platform to develop nanomaterials with promising application in different fields. In the present study the history of conventional use of mushrooms in ancient times was revealed from tribals and local hakims and it was found that mushrooms were used for their nutritional and medicinal values. The present study reveals the production of silver nanoparticles from edible mushrooms and their potent inhibitory activity against *S. aureus, E. coli, P. aeruginosa*, and *B. amyloliquefacien* strains.

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Table. 2. Antibacterial activity of aq. extracts of *Agaricus bisporus*

Bacterial strains	Aq. Extract of *A. bisporus*					
	150 mg/mL			300 mg/mL		
	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)	Well Diameter (mm)	Zone of Inhibition (mm)	Inhibition Length (mm)
S. aureus	11 mm	17 mm	9 mm	11 mm	18 mm	9 mm
P. aeruginosa	11 mm	NIL	NIL	11 mm	NIL	NIL
E. coli	11 mm	NIL	NIL	11 mm	NIL	NIL
B. amyloliquefacien	11 mm	19 mm	10 mm	11 mm	20 mm	11 mm

Table. 3. Antibacterial activities of biologically synthesized AgNPs of *Agaricus bisporus*

Bacterial strain	Diameter of Zone of Inhibition (mm)		Control
	AgNPs of *A. bisporus*	*Aq.* Extract *A. bisporus*	Sterile Distilled Water
S. aureus	22	9	NIL
P. aeruginosa	13	NIL	NIL
E. coli	19	NIL	NIL
B. amyloliquefacien	21	11	NIL

The AgNPs extracts from these medicinally and nutritionally important mushrooms will act as an alternative to antibiotics which could be effective, safe and cost effective for the treatment of infections.

The antibacterial effects of biologically synthesized AgNPs have been investigated against *B. amyloiquefaciens*, *S. aureus*, *E. coli* and *P. aeruginosa*. The test was performed with loading the biologically AgNPs into the wells followed by the plant aq. extract *A. bisporus* and the sterile distilled water in the blank well. In this susceptibility test, *S. aureus* and *B. amyloiquefacien* showed more sensitive in biologically AgNPs i.e., 22 mm zone of inhibition of and 21 mm zone of inhibition respectively followed by *E coli* and *P. aeruginosa* showed 19 mm and 13 mm zone of inhibition respectively. Whereas, the aq. extract of *A. bisporus* showed 9 mm and 11 mm zone of inhibition against for *S. aureus* and *B. amyloliquefacien*. The aq. extract showed no inhibition against for *E. coli* and *P. aeruginosa* (Table 3). The results depicted that the highest efficacy of AgNPs of *A. bisporus* as compared to aq. extract of *A. bisporus* alone.

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CONCLUSION

The aq. extract of *A. bisporus* composed of the secondary metabolites like terpenoids, saponins, steroids, alkaloids, phenols, insulin and glycosides. Aq. extract of *A. bisporus* was quite effective against *S. aureus* and *B. amyloliquefacien*. Furthermore, biologically synthesized AgNPs of *A. bisporus* revealed highest efficacy as compared aq. extract of *A. bisporus* alone. Hence, AgNPs of *A. bisporus* could be recommended to use for biomedical and pharmaceutical applications.

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