Research Article

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES AND PHYTOCHEMICAL ANALYSIS OF WHITE BUTTON MUSHROOM *AGARICUS BISPORUS*

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ABSTRACT

In the present study, evaluation of antimicrobial activity of *Agaricus bisporus* against human pathogenic microorganisms revealed that *Agaricus bisporus* was effective against five human pathogenic microorganisms out of six taken for the evaluation and in many cases was found to be better than the standard used i.e. Ciprofloxacin and Fluconazole. Petroleum ether extract was most effective with maximum zone of inhibition of 28mm against *Pseudomonas aeroginosa*, and Candida *albicasn* was the most sensitive pathogen taking all solvent and aqueous extract at 20mg/ml concentration. Antioxidant activity of white button mushroom has been proved with its DPPH free radical scavenging activity. At 9mg/ml concentration of methanol extract, scavenging activity was 85.2% and phytochemical analysis of white button mushroom has revealed the presence of bioactive molecules i.e. saponins, flavonoids, tannins and cardiac glycosides which might be responsible for its antioxidant and antimicrobial activity, therefore it can be concluded that *Agaricus bisporus* has great medical potential and further purification and structural elucidation of bioactive molecule is required.

Keywords: White Button Mushroom, *Agaricus bisporus*, Antimicrobial Activity, Antioxidant Activity, Phytochemical Analysis.

INTRODUCTION

Since the discovery of the traditional antimicrobials (e.g., penicillin), a lot of micro-organism are now resistant to one or more antimicrobial drugs. Anti-microbial resistance is proving fatal for thousands of people each year resulting in high medical cost and heavy economic losses[1]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the screening of several medicinal plants for their potential antimicrobial activity[1]. Edible mushrooms are nutritionally endowed fungi (mostly *Basidiomycetes*) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials[2]. Mushroom is a traditional medicines since Greek and Roman antiquity. It is believed that mushrooms need antibacterial compounds to survive in their natural environment[3]. Scientific explorations of Shiitake mushrooms such as *Lentinus edode*s, Maitake mushrooms such as *Grifola frondosa*, chanterelles such as *Chaterellus carius,* white button mushrooms such as *Agaricus bisporus*, and oyster mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine[1,4]; organic acids such as ascorbate, shikimate, malate and fumarate; carbohydrates such as the Glucans; monoterpenoid and diterpenoid lipids; proteins such as hydrophobins and trace elements such as selenium[1].

The degenerative disease associated with aging include cardiovascular, cancer, immune system decline, brain dysfunction and cataract they are also associated with free radicals because oxidative damage to DNA, protein and other macromolecule accumulated with age and has been postulated to be a major type of endogenous damage leading to age[5-8]. Intake of exogenous antioxidants is crucial to maintain an adequate level of antioxidants in order to balance the Reactive Oxygen Species especially when human *in vivo* antioxidant defense and repair systems are considered to be insufficient to totally prevent the damage vasodilation[9, 10]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylated hydroxyquinine (TBHQ) have been reported to be carcinogenic thus, their use has been restricted[10, 11]. This situation has prompted the search for potential antioxidant from natural sources. The phenolic compounds in mushrooms have been found to be an excellent antioxidant and synergist that is not mutagenic. Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage in humans[12]. The present study investigates the antimicrobial and antioxidant activity as well as phytochemical analysis of edible white button mushroom *Agaricus bisporus*.

MATERIALS AND METHODS

Collection and identification of Mushroom

Edible mushroom *Agaricus bisporus* was collected from local market of kurukshetra and was identified by Dr. Rupesh Arora Scientist from Krishi Vigyan Kendra (K.V.K), Tepla, Ambala, Haryana, India.

Preparation of Mushroom extract

Fresh mushrooms were thoroughly washed with clean water. Fruiting body of mushroom was separated and

dried in oven at 58 degree Celsius for 48 hours and grounded into fine powder 5gm of mushroom powder was soaked in 50 ml of each solvent namely petroleum ether, ethyl acetate, methanol (80%), water, ethanol (96%) and incubated for 36 hr at room temperature. extracts were filtered with Whatman filter no 4, extra solvent from the filtrate were evaporated using water bath at different temperature according to boiling point of different solvent (ethanol at 78°C, methanol at 64°C, petroleum ether at 55°C, ethyl acetate at 77°C and water at 100°C) After evaporation, the dishes were re-weighed and the differences in weights before and after evaporation were calculated[13,14]. The extracts (residues) were stored (4°C) in a clean sterile container for further use[14].

Procurement and Maintenance of Test Pathogens

The various human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC): Institute of Microbial Technology (IMTECH), Chandigarh; which included Gram positive bacteria: *Streptococcus mutans* (MTCC 497)*; Bacillus subtilis* (MTCC 441); Gram-negative bacteria: *Escherichia coli* (MTCC 5704) *Pseudomonas aeruginosa* (MTCC 2295)*;* and yeasts: *Candida albicans* (MTCC 3017); C*andida parapsilosis* (MTCC 1965); The Muller Hilton broth was made to preserve the cultures. All the test tubes containing broths were kept at 4ºC in the refrigerator for further studies.

Agar well diffusion assay

The antimicrobial activity of white button Mushroom was determined by Agar well diffusion method. 100µl of test microbes were aseptically introduced and spread using cotton swabs on surface of gelled sterile Muller Hilton agar plates. A well of about 6.0mm diameter with sterile cock borer was aseptically punched on each agar plate. 100µl of the petroleum ether, ethanol, methanol, water and ethyl acetate extracts of *Agaricus bisporus* were introduced into the wells in the plates. A negative control well was made too with 100µl of the extracting solvent (DMSO). A positive control was made by placing Antibiotic disc (Ciprofoxacin for bacteria and Fluconazole for yeast) on the agar plate. Plates were kept in laminar flow for 30 minutes for pre-diffusion of extract and then incubated at 37ºC for 24 hours. Resulting Zone of Inhibition was measured using a Hi media zone scale[15].

Phytochemical screening

The crude extract of mashroom was subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents using the methods described by Parekh and Chanda[15], Trease and Evans[16], Karumi *et al*.,[17] and Fasoyira and Adegoke[18].

Determination of Antioxidant activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The scavenging activity of methanol extract was measured according to method of and Cheung *et al*.[8] and Chu *et al*.[19]. For determination of antioxidant activity following methods were followed:-

0.1 mM DPPH was prepared by adding 398mg of DPPH in 100ml of water Different concentration of extract were prepared as indicated in table 1.

Table 1: Preparation of different concentration of extract

Concentration of Mushroom	Volume of mushroom extract	Volume of methanol
1.5 mg/ml	0.015ml	0.985ml
3.0mg/ml	0.03ml	0.970ml
4.5mg/ml	0.045ml	0.955ml
6.0mg/ml	0.06ml	0.940ml
7.5mg/ml	0.075ml	0.925ml
9.0mg/ml	0.09ml	0.910ml

Test tubes were labelled for difference in concentration of mushroom to, each test tube 0.5 ml of 0.1mM DPPH radical was added, the reaction was carried out in dark room and test tubes were wrapped with black paper the reaction mixture was vortex mixed. Absorbance for different concentration was measured immediately after mixing by measuring at 520 nm with a spectrophotometer. Methanol was used instead of the mushroom extract as a control. The steps were repeated three times and mean value was obtained as per following equation

SA= (1-Abs in presence of sample/Abs in the absence of sample) x 100

Where SA= Scavenging Activity

RESULTS AND DISCUSSION

In the present investigation, antimicrobial activity of ethyl acetate, methanol, Ethanol, petroleum ether and aqueous extract of white button mushroom *Agaricus bisporus* was evaluated. While investigating the antimicrobial activity of solvent and aqueous extract of *Agaricus bisporus,* it was found that amongst all the extract used petroleum ether extract of the mushroom was more effective in inhibiting the test pathogen with zone of inhibition ranging between 14mm to 28mm as shown in table 3. Petroleum ether showed activity against four test pathogen out of five whereas methanol and aqueous extract showed activity against one test pathogen and ethyl acetate was inactive against all the test pathogens, *C. albican* was found to be the most sensitive test pathogen against *A. bisporus* solvent and aqueous extract used.

The activity of Petroleum ether extract on *C.parapsilosis* (16 mm) as shown in table 2 was slightly greater than control (fluconazole showed inhibition zone of 14mm, as shown in Table 3 and significantly greater as compared to the work of Iwalokun *et al*.[1] which showed inhibition zone ranging from 8.1 mm to 8.2 mm, whereas our study showed a inhibition zone of 16m (table 2). Activity of Petroleum ether against *E. coli* was almost half the activity shown by control (Ciprofloxacin showed inhibition

zone of 33 mm, as shown in table 2 whereas in our study the zone of growth inhibition was observed to be 14mm (table 2). However our study showed better activity compared to work of Iwalokun *et al.*[1] (zone of inhibition for *E. coli* 7.1mm–8.9mm). The largest zone of inhibition i.e. 28 mm (table 2) was shown against *P. aeruginosa* by Petroleum ether extract.

Table 2: Antimicrobial activity of various solvent extracts of mushroom against human pathogens.

Solvent	Zone of inhibition (mm) (Approx. mean value calculated from triplicates)					
	C.Parapsilosis (MTCC 1965)	*E. Coli* (MTCC 5708)	*P. Aeruginosa* (MTCC 2295)	*B. substilis* (MTCC 441)	*S. mutans* (MTCC 497)	*Candida albicans* (MTCC 307)
Methanol	NA	NA	20	NA	NA	NA
Ethanol	NA	NA	NA	11	NA	20
Petroleum Ether	16	14	28	14	NA	NA
Ethyl Acetate	NA	NA	NA	NA	NA	NA
Aqueous	NA	NA	NA	NA	NA	18
Ciprofloxacin	ND	33	24	24	22	ND
Flucanazole	14	ND	ND	ND	ND	12
DMSO	NA	NA	NA	NA	NA	NA

Where "NA" denotes No Activity and "ND" denotes Not Determined

Ethanolic extract of *Agaricus bisporus* showed better activity on fungus in comparison to bacteria i.e. it showed inhibition zone of 11mm against *B.subtilis* whereas our positive control, ciprofloxacin showed much greater zone of inhibition (i.e. 24 mm) (table 2). On other hand Ethanolic extract showed a zone of inhibition of 20 mm against fungus *C. albicans* which is significantly greater than zone of inhibition 12 mm shown by fluconazole. Result of ethanol extract of Jagadish *et al.,*[20] against *B. subtilis* (i.e zone of inhibition of 12±1 mm) is in agreement with our result of Ethanolic extract against *B. subtilis* (11mm, table 2) whereas their result against *C.albican* was 14±0.5mm, which is not in agreement with our zone of inhibition of 20mm (table 2).

Abah and Abah[21] showed antimicrobial activity of methanol extract (100mg/ml) of *Agaricus bisporus* against *E.coli.* (7.30 ± 1.53mm), against *C. albicans* (8.33 ± 0.58mm) *P. aeruginosa* (9.00 ± 1.00mm) whereas our study showed activity against just one test pathogen .i.e. *P. aeruginosa*, which was almost the double activity i.e zone of inhibition was 20 mm, (table 2) shown by earlier study of Abah and Abah[21].

Activity of aqueous extract against *C. albican* was greater (18 mm) than the activity shown by the positive control, fluconazole (12mm). Work of Surekha *et al*.[2] revealed that ethyl acetate extract of *Agaricus bisporus* is active against all five pathogen taken for study namely *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris* with zone of inhibition ranging from 15-24mm, whereas in our study ethyl acetate extract showed no activity.

Phytochemical Analysis

The result of phytochemical analysis of Petroleum ether extract of *Agaricus bisporus* revealed that Cardiac glycosides and saponin were present (table 3) while in a similar analysis of petroleum ether extract of *P. ostreatus* undertaken by Iwalokun *et al*.,[1] revealed the presence of terpenoids, tannins, steroidal glycosides and carbohydrates which is in agreement with our result. Our result also indicated the presence of Flavonoid, Cardiac glycosides and Tannin in aqueous extract, presence of Saponin, Flavonoid and Tannin in ethyl acetate, presence of flavonoid in methanol, but none of the bioactive compound was found in ethanol extract of edible mushroom *Agaricus bisporus* (table 3). The observed disparity in the susceptibilities of bacteria and fungus tested with petroleum ether extract eliciting greater effect provides an indication that the organic solvents and water used have varying abilities to extract bioactive substances from *Agaricus bisporus*, this is further evidenced by the different levels of phenolics observed in the different organic extracts and aqueous extract.

Table 3: Phytochemical analysis of various solvent extract of *Agaricus bisporus*

Extract/ solvent	Phytoconstituents				
	Saponin	Flavonoid	Cardiac Glycosides	Steroid	Tannin
Petroleum ether	+	_	+	_	_
Aqueous	_	+	+	_	+
Ethanol	_	_	_	_	_
Methanol	_	+	_	_	_
Ethyl acetate	+	+	_	_	+

+ Indicates presence of bioactive compound
– Indicates absence of bioactive compound

Antioxidant activity

The molecule of 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour. Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

Z• + AH = ZH + A•

where ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant. The reaction is therefore intended to provide the link with the reactions taking place in an oxidising system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule Z• is thus intended to represent the free radicals formed in the system whose

activity is to be suppressed by the substance AH. The free radical RS• evidently then reacts with another molecule of the same kind that was produced by a parallel reaction to: RS• + RS• = RS–SR

This therefore leads to the observed reduction of two molecules of DPPH by two molecules of cysteine, that is, a 1:1 stoichiometry. If however the molecule has two adjacent sites for hydrogen abstraction which are internally connected, as is the case with ascorbic acid[22]. Hence absorbance should decrease with increase in the amount of reductant added. As shown in Fig. 1 and table 4, with increase in the concentration of mushroom in the methanol extract decrease in absorbance was observed which clearly shows that DPPH free radical are reduced with greater efficiency when concentration of mushroom, *Aagaricus bisporus* (reductant in this case) increases. Increase in reducing power of DPPH can be seen with increase in the concentration (Fig :2 and Table 4).

Table 4: Antioxidant activity of white button mushroom *Agaricus bisporus*

Concentration of extract	Concentration of extract after dilution	Mean value absorbance of extract	Absorbance of blank	Scavenging activity(%) on DPPH radical
1.5 mg/ml	0.15 mg/ml	0.31 ×10=3.1	0.4×10=4	22.5
3.0 mg/ml	0.30 mg/ml	0.27×10= 2.7	0.4×10=4	32.5
4.5 mg/ml	0.45 mg/ml	0.24×10= 2.4	0.4×10=4	40.0
6.0 mg/ml	0.60 mg/ml	0.20×10= 2.0	0.4×10=4	50.0
7.5 mg/ml	0.75 mg/ml	0.13×10= 1.3	0.4×10=4	67.5
9.0 mg/ml	0.90 mg/ml	0.07×10= 0.7	0.4×10=4	82.5

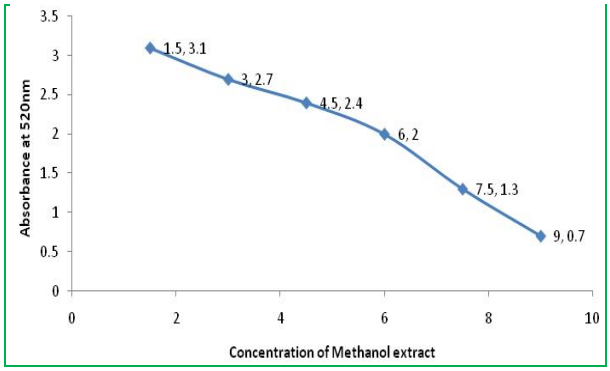

Figure 1: Absorbance v/s Concentration

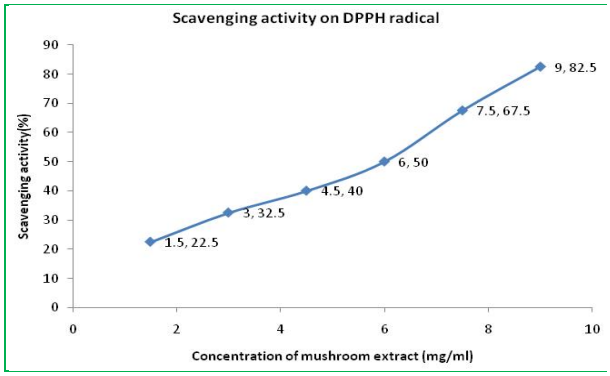

Figure 2: Scavenging activity (%) on DPPH radicals

CONCLUSION

Evaluation of antimicrobial activity of *Agaricus bisporus* against human pathogenic microorganism has revealed that *Agaricus bisporus* is effective against five test pathogen out of six taken for the evaluation and in many cases better than the standard used i.e. Ciprofloxacin and Fluconazole. Petroleum ether extract was most effective with maximum zone of inhibition of 28mm against *P. aeroginosa*, and *C. albicans* was the most sensitive pathogen taking all solvent and aqueous extract at 20mg/ml conc. Antioxidant activity of white button mushroom has been proved with its DPPH free radical scavenging activity, at 9mg/ml conc. of methanol extract scavenging activity was 85.2% and phytochemical analysis has revealed the presence of bioactive molecules i.e. saponin, flavonoid, tanin and Cardiac Glycosides which is responsible for its antioxidant and antimicrobial activity, therefore it can be concluded that *Agaricus bisporus* have great medical potential and further purification and structural elucidation (using NMR/Crystallography) of biomolecule is required.

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REFERENCES

1. Iwalokun, B. A., Usen, U. A., Otunba, A. A. and Olukoya, D. K. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus.* Afric. J. Biotechnol., 6 (15), 2007, 1732-1739.
2. Surekha, C., Kaladhar, D. S. V. G. K., Raju, S. J. R. and Haseena. Evaluation of antioxidant and antimicrobial potentiality of some edible mushroom. International J. Adv. Biotechnol. Res., 2, 2011, 130-134.
3. Manjunathan, J. and Kaviyarasan, V. Solvent Based Effectiveness of Antibacterial Activity of Edible Mushroom *Lentinus tuberregium.* Int. J. PharmaTech Res., 2 (3), 2010, 1910-1912.
4. Solomko, E. F. and Eliseeva, G. S. Biosynthesis of vitamins B by the fungus *Pleurotus ostreatus* in a submerged culture. Prikl. Biokhim . Mikrobio., 24, 1998, 164-169.
5. Harman, D. The aging process. Proc. Natnl. Acad. Sci. USA, 78, 1981, 7124-7128.
6. Fraga, C. G., Shinginaga, M. K., Park, J. W., Degan, P. and Ames, B. N. Oxidative damage to DNA during aging. 8-hydroxy-2-deoxyguanosine in rat organ DNA and urine. J. Sci. Food Agric., 9 (2), 1990, 98-104
7. Ames, B. N., Shigenaga, M. K. and Hagen, T. M. Oxidant, antioxidant and degenerative disease of aging. Proc. Natl. Acad. Sci. USA, 90, 1993, 7915-7922

8. Cheung, L. M., Cheung, P. C. K and Ooi, V. E. C. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem., 81 (2), 2003, 249–255.

9. Munzel, T. and Keaney, J. F. Are ACE inhibitors a magic bullet against oxidative stress. Am. Heart Assoc.,104 (13), 2001, 1571–1574.

10. Abdullah, N., Ismail, S. M., Aminudin, N., Shuib, A. S. and Lau, B. F. Evaluation of Selected Culinary-Medicinal mushrooms for Antioxidant and ACE Inhibitory Activities. Evidence Based Complementary and Alternative Medicine. Hindawi Publishing Corporation, 2011, pp 12.

11. Botterweck, A. A. M., Verhagen, H., Goldbohm, R. A., Kelinjans, J. and Brandt, P. A. V. D. Intake of butylated hydroxyanisole and butylatedhydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. Food Chem. Toxicol., 3, 2000, 599–605.

12. Adebayo, E. A., Oloke, J. K., Ayandele, A. A. and Adegunlola, C.O. Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius*. J. Microbiol. Biotechnol. Res., 2 (2),2012, 366-374.

13. Trease, G.E. and Evans, W. C. Pharmacognosy., Xii Ed London. Bailere London, 1994.

14. Ejikeme, N. and Henrietta O. U. Antimicrobial activity of some local mushrooms on pathogenic isolates. J. Med. Plant Res., 4 (23),2010, 2460-2465

15. Parekh, J. and Chanda, S. *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteria. Afric. J. Microbiol. Res., 1 (6), 2007, 92-99.

16. Trease, G. E., Evans, W. C. A Text Book of Pharmacognosy. Tindal, Oxford/ELSB, Bailliere, 1987.

17. Karumi, Y., Onyeyili, P. A. and Ogugbuaja, V. O. Identification of active principle of *M. balsamina* (Balsam apple) leaf extract. J. Med. Sci**.,** 4, 2004, 179-182.

18. Fasoyio, S. B. and Adegoke, G. O. Phytochemical characterization and the antimicrobial property of *Aframum danielli* extract. Afric. J. Agric. Res., 2, 2007, 76-79.

19. Chu, Y. H., Chang, C. L. and Hsu, H. F. Flavanoid content of several vegetable and their antioxidant activity. J. Sci. Food Agric., 80, 2000, 561-566

20. Jagadish, L. K., Krishnan, V. V., Shenbhagaraman, R. and Kaviyarasan, V. Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* before and after boiling. Afric. J. Biotechnol., 8 (4), 2010, 654-661.

21. Abah S.E. and Abah G. Antimicrobial and Antioxidant Potentials of *Agaricus bisporus.* Adv. Biol. Res., 4 (5), 2010, 277-282.

22. Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J. Sci. Tech., 26 (2), 2004, 211-219.

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