International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Original Article

EVALUATION OF PHYTOCONSTITUENTS, *IN VITRO* ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF EDIBLE WHITE BUTTON MUSHROOM *AGARICUS BISPORUS*

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Received: 21 Aug 2015 Revised and Accepted: 13 Jan 2016

ABSTRACT

Objective: The present study was aimed to investigate the phytochemicals, antimicrobial and antioxidant potential of wild edible mushroom (*Agaricus bisporus*).

Methods: Initially, phytochemical screening was carried out to identify the major phytoconstituents present in *A. bisporus*. Fourier transform infrared spectroscopy (FTIR) and GC-MS analysis were executed to study the phytochemical profile of *A. bisporus* methanolic extract. On the other hand, *in vitro* antioxidant activity was evaluated by reducing power assay and H_2O_2 scavenging assay. Also, Antibacterial activity was assessed by the agar-well diffusion method.

Results: The total phenol content was found to be 5.8 mg GAE/g in aqueous extract and 7.4 mg GAE/g in the methanolic extract of *A. bisporus*. Antioxidant assays clearly indicate that the methanolic extract of *A. bisporus* displays highest H_2O_2 scavenging activity than aqueous extract. The methanolic extract of *A. bisporus* gave a higher reducing power than the aqueous extract depicts its antioxidant potential. Similarly, methanolic extract of *A. bisporus* gives excellent antimicrobial activity against tested human pathogens whereas aqueous extract showed comparatively less action. A Higher level of the zone of inhibition was measured against *P. aureginosa* and *S. aureus* whereas *E. coli* and *B. subtilus* displayed a moderate level of inhibitory action.

Conclusion: The overall results suggest that methanolic extract of *A. bisporus* has shown stupendous antioxidant and antimicrobial potential. This study can be useful to develop commercially viable natural drugs for biomedical applications.

Keywords: *Agaricus bisporus,* GC-MS analysis, Antioxidant, Human pathogens, Phenolic compounds.

INTRODUCTION

Free radicals are atoms, molecules or ions with unpaired electrons that are highly unstable and active towards chemical reactions with other molecules. The generation of different free radicals like superoxide, hydroxyl and lipid peroxides mainly responsible for several pathological processes such as certain tumors (prostate and colon cancers) coronary heart disease and etc. [1]. In addition, antioxidant compounds are substances that directly scavenge ROS, inhibit ROS production [2]. Interestingly, the chemical constitution and metabolic fluxes of bioresources from different ecosystem shows the presence of various biologically active compounds like antioxidants. These antioxidants involve in quenching activity which protects the organism from different environmental stress [3].

Similarly, the occurrence of pathogenic diseases and prevalence of multiple drug resistance (MDR) leads to the development of novel drug candidates from natural products. Usually, the intake of natural antioxidants is providing protection to oxidative damage by free-radical molecules [4]. Such properties lead to the growing interest of using mushrooms in various nutraceutical products [5]. Mushrooms are valuable health foods since they are low in calories, fats and essential fatty acids. It also enriched with vegetable proteins, vitamin and minerals [6]. More than 2,000 species of mushrooms were present in nature, however, less than 25 species have been widely accepted as food and only a few were attained the level of an item of commerce [7]. The knowledge of phytoconstituents would provide a within reach into its biological functions beyond nutrition when consumed. To date, there is no report available on detailed antioxidant properties of mushroom collected from the Kodaikanal region in literature. In the present study, extracts of edible white button mushroom *A. bisporus* was subjected to phytochemical screening, on the other hand, the extracted products were examined for its antimicrobial and antioxidant activities under *in vitro* condition towards essential biomedical applications.

MATERIALS AND METHODS

Collection of samples

The specimen of investigated species (*A. bisporus*) was collected from Kodaikanal, Dindigul district of TamilNadu, India. They were collected using paper bags and packed loosely with the provision of aeration. The collected samples were identified and authenticated with the help of Prof. Rajangam, Horticulture Research Station, Kodaikanal.

Preparation of *A. bisporus* methanolic extract

Preparation of methanolic extract of *A. bisporus* was followed by Jose *et al.* [8]. Briefly, the mushrooms were shade dried and powdered using a kitchen blender. The powdered sample was extracted using methanol by soxhlet apparatus. After extraction, the solvent was evaporated to dryness under reduced pressure in a rotary evaporator.

Phytochemical screening

The phytochemical analysis of mushroom extract was carried out as per the method of Adebayo *et al.*, 2012 [9].

Total phenol content

The presence of phenolic compounds was estimated in the methanolic extracts by a colorimetric assay as per Barros *et al.* 2007 [1]. Briefly, 1 ml of sample was added into 1 ml of Folin and Ciocalteu's phenol reagent. The mixture was incubated at room temperature for 3 min followed by addition of 1 ml of saturated Na_2CO_3 solution. The final volume was made up to 10 ml with sterilized water. The reaction mixture was incubated for 90 min in dark condition. The absorbance was measured at 765 nm using a spectrophotometer. Gallic acid at concentrations of 0.01-0.4 mM was used to make a standard curve.

FTIR and GC-MS analysis

FTIR analysis was performed to study the major phytoconstituent present in the methanolic extract of *A. bisporus*. Briefly, the samples for FTIR analysis were prepared by mixing with KBr powder and pelletized after drying the spectra were recorded using Perkin Elmer make model spectrum RX1 (Wavelength range between 4000 cm-1 to 400 cm-1). For GC-MS analysis, 2 µl of the methanolic extract of *A. bisporus* was injected into Perkin-Elmer GC Clarus 500 system. The spectrum of unknown compounds was compared with the known compounds stored in National Institute Standard and technology (NIST) library to ascertain the name, molecular weight, and structure of the compounds.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986) [10] with some modifications. Reaction was carried out in a mixture containing 2.5 ml of sample (100, 200, 300, 400 and 500 µg/ml), 2.5 ml of 0.1 M sodium phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1%, w/v) by incubating at 50 °C for 20 min. After addition of 2.5 ml trichloroacetic acid (10%, w/v), the mixture was centrifuged at 5000g for 10 min. The upper layer (5 ml) was mixed with 0.5 ml of fresh $FeCl_3$ (0.1%, w/v) and the absorbance was measured at 700 nm. Vitamin C was used as the positive control. The percentage reducing power was calculated by using the following formula:

% reducing power = [Test OD–Control OD/Test OD] × 100

H_2O_2 scavenging assay

H_2O_2 scavenging activity was determined as per the method of Ruch *et al.*, 1989 [11]. Briefly, the mixture containing 1 ml of sample (100, 200, 300, 400 and 500 µg/ml), 2.4 ml of phosphate buffer (0.1 M, pH 7.4) and 0.6 ml of H_2O_2 solution (40 mM) was shaken strongly and incubated at room temperature for 10 min. Then, the absorbance of the reaction mixture was determined at 230 nm. Vitamin C was used as the positive control. The H_2O_2 scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [1-A_1-A_2]/A_0] \times 100$$

Where A_0 is the absorbance of the control, A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample only (phosphate buffer instead of H_2O_2 solution). The IC_{50} value corresponds to the concentration of the compounds that caused 50% inhibition of H_2O_2.

Screening of antimicrobial activity

The antimicrobial effect of *A. bisporus* aqueous and methanolic extracts was evaluated against human pathogens such as Gram positive (*S. aureus* and *B. subtilis*) and Gram negative (*E. coli* and *P. aureginosa*) by disc diffusion method. Cultures were maintained at −80 °C on glycerol stock. Clinically isolated strains were sub-cultured in nutrient broth for 24 h at 30 °C. Each strain was swabbed uniformly into the individual nutrient agar plates using sterile cotton swabs. Using sterile micropipette different dosage of extracts (50 µl, 75 µl 100 µl and 150 µl) were loaded onto a sterile paper disc, and it was allowed to dry. The sample loaded discs along with standard antibiotic disc (Ampicillin) were impregnated onto the nutrient agar medium. Doses of the sample were selected based on the preliminary data obtained from the earlier studies in our laboratory. After incubation at 37 °C for 24 h different levels of the zone of inhibition were measured using meter rulers.

RESULTS

Phytochemical analysis

The preliminary phytochemical analysis of *A. bisporus* methanolic extract shows the presence of active metabolites such as phenol, flavnoids, tannins, glycosides, carotenoids, saponin and reducing sugar [table 1]. Also, the total phenol content of aqueous and methanolic extract of *A. bisporus* was found to be 5.8±0.4 and 7.4±1.3 respectively using gallic acid standard [table 2].

Table1: Phytochemical analysis of metholic extract of *A. bisporus*

Test	Result
Phenol	+
Flavonoid	+
Tannin	+
Terpenoids	-
Alkaloids	-
Saponin	+
Carotenoids	+
Glycosides	+
Reducing sugar	+
Phlobatanins	-

(+) = Presence (-) = Absence

Table 2: Total phenolic content in aqueous and methanolic extract of *A. bisporus*

Extract	Total phenol (µg/ml)
Aqueous	5.8±0.4
Methanol	7.4±1.3

FTIR analysis

The IR spectrum of *A. bisporus* methanolic extract was shown in fig. 1 which manifests prominent transmittance located at 3373.61, 2926.11, 1629.9, 1448.59, 1410.01, 1132.25, 1053.17, 929.72, 868, 825.56 and 599.88 cm^{-1}.

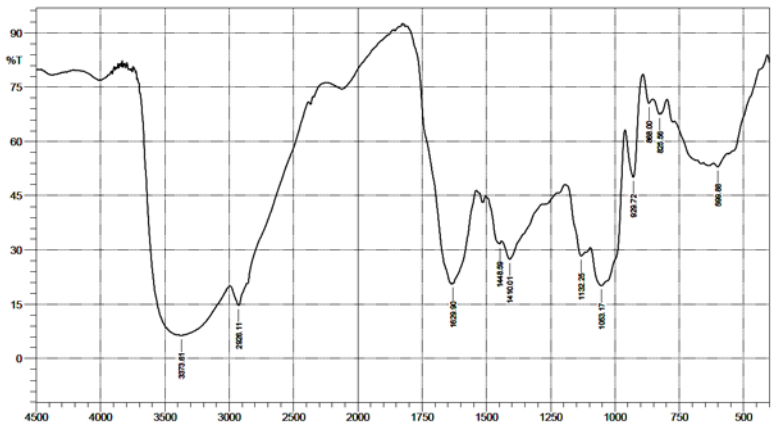

Fig. 1: FTIR analysis of crude methanolic extract of *A. bisporus*

Table 3: FTIR peak values and functional groups of crude extracts of *A. bisporus*

S. No.	FTIR peak values	
	Peak value	Functional groups
1	599.88	OH out-of-plane bend
2	825.56	N-H bending
3	868	Si-H stretching
4	929.72	C-H bending
5	1053	C-O stretching
6	1132.25	OH-stretching
7	1410.01	C-O Amino acids
8	1448.59	NO_2
9	1629.9	C=O
10	2926.11	C-H stretching
11	3373.61	OH-stretching

Strong bands at 3373.61 cm^{-1} correspond O-H streching of phenolic compounds whereas transmittance at 1629.9 and 1053 cm^{-1} attributes C=O and C-O respectively. Details of the remaining transmittance and their corresponding functional groups were given table 3.

GC-MS analysis

GC-MS analysis of the *A. bisporus* methanolic extract was shown in fig. 2, the spectra of each bio-compounds were compared with those of NIST libraries and their retention indices were shown in table 4.

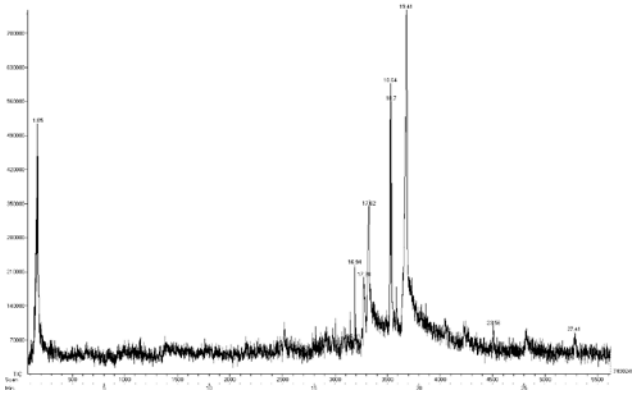

Fig. 2: GC-MS analysis of crude methanolic extract of *A. bisporus*

Totally 9 compounds were identified in the crude extract based on their peak area, retention time, molecular weight and molecular formula. In the present investigation, a variety of compounds has been detected in *A. bisporus* including β-sitosterol, 1-Eicosanol, n-Hexadecanoic acid, 7,11-Epoxymegastigma-5(6)-en-9-one, Tride-canoic acid, methyl ester, Hexadecanoic acid, ethyl ester**.**

***In vitro* antioxidant activity**

Antioxidant activity of *A. bisporus* methanolic extract was assessed by Reducing power assay, H_2O_2 scavenging assay was shown in fig. 3. Different concentrations of the extracts showed free radical scavenging activity. In the present study, the extracts of *A. Bisporus* have shown significant antioxidant and radical scavenging potential.

Reducing power assay

Reducing power assay demonstrates the reducing power of methanolic extract, aqueous extract, and Vitamin C. It was observed that the reducing power of all tested samples was increased in a dose-dependent manner. However, the reducing power of methanolic extract was lower than that of Vitamin C [fig. 3a]. The methanolic extract (88.2%) of *A. bisporus* gave a higher reducing power than the aqueous extract (87.5%).

H_2O_2 scavenging activity

It has been reported that H_2O_2 plays an important role as the radical-forming intermediate in the production of ROS molecules. Thus, H_2O_2 is considered as one of the main inducers of cellular aging and could attack many cellular energy-producing systems.

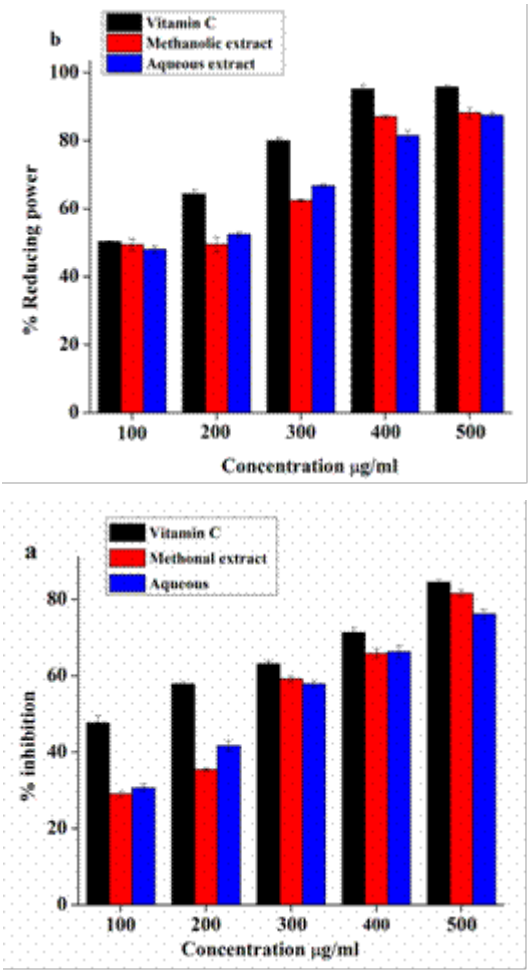

Fig. 3: Antioxidant assay of *A. bisporus* a. H_2O_2 scavenging assay b. Reducing poweer assay. All the results were given as the triplicate of mean±SD

Methanol, aqueous extract and Vitamin C exerted dose-dependent H_2O_2 scavenging activities [fig. 3b]. The results indicate that the methanolic extract (81.56%) has higher H_2O_2 scavenging activity than aqueous extract (76.19%) of *A. bisporus*. However, Vitamin C (84.48%) also exhibited higher H_2O_2 scavenging activity than the methanolic extract of *A. bisporus.*

Antibacterial activity

A. bisporus extract was observed to inhibit bacterial pathogens under *in vitro* condition to reveal its antimicrobial potential.

Bacterial inhibitory action for a crude extract of mushroom has been shown in table 5. The zone of inhibition increases with increase in the concentration of the methanolic extract, aqueous extract of *A. bisporus*. The antimicrobial activity was higher in methanolic extract of than in aqueous extract. Antimicrobial activity was performed against human pathogenic bacteria such as *P. aureginosa, S. aureus, B. subtilis* and *E. coli.* Ampicillin was used as positive control presented a good inhibitory against all tested organisms. The zone of inhibition for *P. aureginosa* was 10 mm and 10.5 mm at 150 µl for aqueous and methanolic extract respectively. *P. aureginosa* was more susceptible to a methanolic and aqueous extract of *A. bisporus.* The crude extract of *A. bisporus* showed poor activity against *B. subtilis* the zone of inhibition at 150 µl was 6.5 mm.

Table 4: GC-MS analysis reveals the photo components identified in the crude methanolic extract of *A. bisporus*

S. No.	Retention time	Name of the compound	Peak area %	Molecular formula	Molecular weight
1	1.85	Butane, 2-methyl-	2.73	C_5H_{12}	72
2	16.94	Hexadecanoic acid, ethyl ester	1.69	$C_{18}H_{36}O_2$	284
3	17.38	1-Hexadecene	6.85	$C_{16}H_{32}$	224
4	17.62	Tridecanoic acid, methyl ester	0.79	$C_{14}H_{28}O_2$	228
5	18.64	7,11-Epoxymegastig ma-5(6)-en-9-one	1.08	$C_{13}H_{20}O_2$	208
6	18.7	n-Hexadecanoic acid	5.07	$C_{16}H_{32}O_2$	256
7	19.41	13-Docosenamide	0.28	$C_{22}H_{43}$ NO	337
8	23.56	1-Eicosanol	0.21	$C_{20}H_{42}O$	298
9	27.41	β-Sitosterol	1.85	$C_{29}H_{50}O$	414

Table 5: Antimicrobial activity of crude extract of *A. Bisporus*. The test was performed in triplicate and the results were given in mean±SD

Organisms	Zone of inhibition (mm)				
	Concentration	50 µl	75 µl	100 µl	150 µl
E. coli	Ampicillin	5±1.0	8.5±0.8	10±1.3	13.5±1
	Aqueous	4±0.5	5.5±0.2	6.5±0.5	7.5±0.5
	Methanol	5±0.7	6±1.5	7±1.2	8.5±1.2
S. aureus	Ampicillin	4.5±0.9	9±2	10±0.8	12.5±1.8
	Aqueous	5.5±0.5	6±0.3	7.5±0.2	9±0.3
	Methanol	6.5±1.2	7±1.7	8±0.9	10±0.7
B. subtilis	Ampicillin	9.5±0.5	12.5±1.2	14.5±1.5	17±1.2
	Aqueous	2±0.2	3±0.2	3.5±0.6	6.5±0.4
	Methanol	2.5±0.4	4.5±0.6	7.5±0.5	9±0.9
P. aureginosa	Ampicillin	11.5±1.5	12.5±1.2	17±1.7	20±1.2
	Aqueous	5±0.2	7±0.3	8.5±0.5	10±0.5
	Methanol	7.5±0.8	8±1.2	8.5±1.2	10.5±1.6

The zone of inhibition for *S. aureus* was 9 mm and 10 mm, whereas *E. coli* was 7.5 mm and 8.5 mm at 150 µl for an aqueous and methanolic extract of *A. bisporus* respectively. The antibacterial properties of *A. bisporus* were not as effective as the commercial drugs.

DISCUSSION

Phytochemical compounds such as flavonoids, phenols, saponin, glycosides and tannins is considered to be major secondary metabolites in mushroom. In our study preliminary phytochemical screening shows the presence of various active constituents. These active metabolites are well known for their curative activities against several human problems such as diuretic choleretic, spasmodic, chronic eczema, diarrhoea, dysentery and menstrual disorders. Alkaloids, terpenoids and phlobatanins were absent in *A. bisporus* extract. Tannins are identified to inhibit bacteria and pathogenic fungi. The flavonoids and phenolic compounds in plants have been reported to put forth multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc [12]. Triterpenoids are a large class of natural isoprenoids present in higher plants, which demonstrate a wide range of biological activities. Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness [13]. FTIR characteristic absorption peaks attribute the presence of functional moites. 2926.11 peak values indicate the presence of saturated C-H stretching, 1053.17 cm^{-1} indicates C-O stretching of alcohols, carboxylic acids, esters and ethers [14].

Our GC-MS data reveals the presence of different bioactive compounds based on the peak area, retention time-molecular weight and molecular formula. In the present investigation, a variety of compounds have been detected in *A. Bisporus*, the identified major phytochemical compounds may be responsible for its antimicrobial and antioxidant properties. Different bioactive components; like phenol, flavonoids, ascorbic acid and β-carotene. polyphenols are considered to be major contributors to the antioxidant property of fruits, vegetables and mushrooms [15]. To consider the significance of polyphenolic compounds and its presence in many varieties of mushrooms, the total antioxidant activity of wild edible *A. bisporus* were determined in the present study. The antioxidant activity of *A. bisporus* methanolic extract was also due to these bioactive compounds. Flavonoids and phenolic compounds are potent water soluble and free radical scavenger which prevent oxidative cell damage. The presence of ascorbic acid and phenolic compound in A. bisporus confirms its antioxidant activity. A number of studies have been focused on biological activities of a phenolic compound as a potential antioxidant and free radical scavengers.

The antioxidant activities of compounds have been attributed to various mechanisms, such as prevention of chain initiation, binding with transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reducing capacity and radical scavenging ability [16]. Among them, the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In the present study, methanolic extract of *A. bisporus* gave a higher reducing power than previously reported methanolic extract of *A. bisporus* from Portugal [17, 1]. This clearly confirms the high level of antioxidant and reducing the capacity of *A. Bisporus* methanolic crude extract.

It has been reported that H_2O_2 plays an important role as the radical-forming intermediate in the production of ROS molecules. Although H_2O_2 is not very reactive, its high penetrability in cellular membrane leads to hydroxyl radical formation when it reacts with Fe2+or superoxide anion radical in the cell [16]. Thus, H_2O_2 is considered as one of the main inducers of cellular aging and could attack many cellular energy-producing systems. Methanolic extract

of *A. bisporus* has given higher radical scavenging ability than the ethanolic extract of *A. bisporus* from China, which has shown moderate H_2O_2 scavenging activity [18]. Methanolic extracts from mushrooms (commercial) scavenged hydroxyl free radicals by 38.0–52.6% [19] which is lower than the methanolic extract of wild *A. bisporus*. These results clearly depict that commercial mushrooms are not good scavengers for hydroxyl free radicals.

Apart from antioxidant potential, the crude methanolic extract of *A. bisporus* exhibited stupendous antimicrobial activity against human bacterial pathogens. It was observed that the antimicrobial potential of *A. bisporus* was mainly due to the presence of essential phenolic bioactive components like catechin which has been already known for its antimicrobial, antioxidant, anticancer and antiallergy properties [20]. Caffeic acid and rutin have shown high-quality antimicrobial activity [21]. Hence, the *A. bisporus* is found to be a good source of antibacterial agents.

CONCLUSION

To summarize, the preliminary phytochemical analysis of *A. bisporus* shows the presence of phenols as a major constituent. Further, FTIR and GC-MS analysis also confirm the presence of various phenolic bioactive metabolites in the methanolic extract of *A. bisporus*. On the other hand, methanolic fractions of *A. bisporus* displayed extensive antimicrobial and antioxidant potential. Further studies are essential to assess structural insights of bioactive compounds towards the development potential drug candidate for biomedical applications.

CONFLICT OF INTERESTS

Declare none

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