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Original Article

A detail study of phytochemical screening, antioxidant potential and acute toxicity of *Agaricus bisporus* extract and its chitosan loaded nanoparticles

*Ganesan Dhamodharan, Sankaran Mirunalini**

Department of Biochemistry & Biotechnology, Annamalai University, Annamalai Nagar, 608 002 Tamil Nadu, India

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ABSTRACT

Aim: Scrutinize the phytochemical, antioxidant and acute toxicity of *Agaricus bisporus* extract (ABE) and *A. bisporus* loaded chitosan nanoparticles (ABCNPs).
Methods: Phytochemical such as total phenol and flavonoid, and terpenoid, alkaloid, steroid, carbohydrate, tannins and proteins where determined. The antioxidant activity such as DPPH, ABTS and reducing power of ABE and ABCNPs where estimated by spectrophotometry assay. The estimation of total phenolic content was determined by Folin–Ciocalteu method. The acute toxicity studies of ABE and ABCNPs were investigated in male Sprague Dawley rats. Results: In the DPPH, $ABTS^{\bullet+}$ and reducing power scavenging assays, of ABE displayed significant antioxidant activities with the inhibition values of 27.78, 27.62 and 81.97% and ABCNPs inhibition values 27.78, 27.62 and 78.13% respectively. The reducing power of the extract increased dose-dependently, and the ABE and ABCNPs reduced the most Fe^{3+} ions. The amount of total phenolics was reported 1 g of sample contains 8.19±1 mg of gallic acid and total flavonoid analysis by the assay of aluminum chloride spectrophotometric reported 1 g of sample contains 10.3 ± 1 mg of quercetin in ABE and ABCNPs. The acute toxicity was found bellow 2747.25 mg/kg b.w. in ABE and 3178.86 mg/kg b.w. of ABCNPs.
Conclusion: The white button mushroom *A. bisporus* could be considered as a dietary natural food products with antioxidant activity. Thus our results provide evidence that AB and ABCNPs proves to have a potent antioxidant and intermittent therapy against cancer.

1. Introduction

Free radicals are chemically unstable atoms or molecules that can cause extensive damage to cells as a result of imbalance between the generation of reactive oxygen species (ROS) and the antioxidant enzymes.[1] ROS or reactive nitrogen species (RNS) and their excess have a harmful effect, such as the peroxidation of the membrane lipids, aggression to tissue

* *Corresponding author*. Tel.: +91 9442424438 (mobile).
E-mail address: mirunasankar@gmail.com (S. Mirunalini).
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proteins and membranes, on damage to DNA and enzymes.[2] The beneficial effects of antioxidants on promoting health is believed to be achieved through several possible mechanisms, such as direct reaction with and quenching free radicals, chelation of transition metals, reduction of peroxides, and stimulation of the antioxidative enzyme defense system.[3]

Currently, there is a great interest in the study of antioxidant substances mainly due to the findings concerning the effects of free radicals in the organism. Phenolic plant compounds have attracted considerable attention for being the main sources of antioxidant activity, in spite of not being the only ones. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant activities of phenolics play an important role in the adsorption or neutralization of free radicals.[4]

Mushrooms have been a part of the human diet for thousands of years. They also have been used normally in homeopathic medicine.[5] *Agaricus bisporus* is usually called button mushroom, the nutritional value of the *A. bisporus* originates from its chemical composition such as crude protein, carbohydrates, fat, dietary fiber, sugars, fat, protein, water, pantothenic acid (B5), riboflavin (Vit. B2), niacin (Vit. B3), vitamin C, iron and ash contents as well as the amino acid composition are favorable.[6] The phytochemicals of AB using direct for cytotoxicity in relation with antioxidant compounds like phenol and flavonoids have demonstrated that chemotherapy induced apoptosis and subsequent phagocytosis of cancer cells.[7] The present study, whose aims were to investigate the antioxidant activity, phytochemicals and acute toxicity of the ethanol extracts of *A. bisporus* and its loaded chitosan nanoparticles.

2. Materials and methods

2.1. Chemicals

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-Diphenly-2-picrylhydrazyl (DPPH), ascorbic acid, butylated hydroxyanisol (BHA) and trolox (6-hydroxy-2-5-8 tetra methyl chroman-2-carboxilic acid) were purchased from Sigma (Sigma, Aldrich GmbH, Sternheim, Germany). All other unlabeled chemicals and reagents were analytical graded.

2.2. Edible fungal material

A. bisporus (AB) were commercially purchased from Cuddalore in vegetable markets, Tamil Nadu. A voucher specimen (No. 217) was deposited in Department of Botany, Annamalai University.

2.3. Preparation of ethanol extracts

Powder of AB (50 g) were extracted by stirring with 500 ml of ethanol (30 °C) at 150 rpm for 24 h and filtered through Whatman No. 4 filter paper. The residues of ethanol extract was then rotary evaporated at 40 °C to dryness, re-dissolved in ethanol to a concentration of 10 mg/ml and stored at 4 °C for further use.

2.4. Phytochemical analysis

The terpenoids content of the *A. bisporus* extracts were determined by the method of Puncal D Test. The flavonoid content of the sample were detected with few ml of ammonia shows the presence of fluorescence at 366 nm indicates the presence of flavonoids. The steroids content of the sample were detected by added a few ml of concentrated sulfuric acid solution to the extract. Formation of green color indicates the presence of steroids. The Carbohydrates and Sugars content of the sample were detected by added a few ml of concentrated sulfuric acid solution to the extract and heated formation of charring indicates the presence of carbohydrates. The alkaloids content of the sample were detected by the method of Dragandorff's test. The proteins content of the sample were detected by the method of Ninhydrin test. The Tannins content of the sample were detected by 1 ml of Aluminum chloride.

The total phenolic concentration in ABE and ABCNPs was expressed as gallic acid equivalents and was measured according to the method described by Bandoniene et al[8] with slight modifications. The Total flavonoid contents (TFC) of the *A. bisporus* were extracted with 5% $NaNO_2$, 10% AlCl3 and 1 M NaOH were measured at 510 nm with a known quercetin concentration as a standard. The results were expressed as milligrams of quercetin equivalents (CE) per gram of sample.

2.5. Preparation of AB loaded chitosan nanoparticles

AB loaded chitosan nanoparticles were synthesized by ionic gelation method using tripolyphosphate as a gelating agent. A known amount of chitosan was dissolved in 1% (v/v) acetic acid and allowed to stir for 1 h 3 mg/ml AB ethanol extract have prepared already was then added to the freshly prepared chitosan dispersion. The pH of the medium was maintained at 5.0 using 1 M NaOH and then further stirred for 1 h. Finally, 1 mg/ml of TPP was added to the chitosan- AB ethanol extract under mild magnetic stirring. The resulting mixture was allowed to stir for 2 h to form AB encapsulated chitosan nanoparticles. The AB loaded chitosan nanoparticles were collected after the centrifugation of 10,000 rpm for 45 min with 4 °C.[9] The powdered samples were collected with the help of lyophilizer and stored at 4 °C for further use.

2.6. Antioxidant activity assays

The ABE and ABCNPs were used for analyzing their DPPH radical scavenging activities where determined by the method of Chen.[10] The ascorbic acid equivalent ABTS radical of ABE and ABCNPs was estimated method for total antioxidant activity.[11] The reductive potential of the ABE and ABCNPs are determined according to the method of Oyaizu.[12] Varying concentration of ethanol extract of ABE were used and tested against standard antioxidant. Inhibition of free radical by scavenging activity in percent (I %) was calculated in following way: I (%) = [(A blank−A sample)/A blank] × 100; Where A blank is the absorbance of the control reaction and A sample is the absorbance of the test compound. The values of inhibition

Table 1 – Proximate analysis of *Agaricus bisporus* and its loaded chitosan nanoparticles.

S. No	Name of the phytochemical	ABE	ABCNPs
1	Terpenoid	+	+
2	Alkaloid	+	+
3	Steroid	+	+
4	Carbohydrate	+	+
5	Tannins	+	+
6	Proteins	+	+
7	Flavonoids	+	+

+ = Present, – = Absent.

were calculated for the various concentrations of ethanol extracts. Tests were carried out in triplicates.

2.7. Animals

All animal studies were conducted in central animal house after approval from the Institutional Animal Ethics Committee endorsed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (No. 930; dated: 29.05.2012), Government of India guidelines. 6-week-old male Sprague Dawley rats were obtained from National Institute of Nutrition, Hyderabad, India and maintained in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University.

2.8. Experimental design for acute toxicity study

Acute toxicity of a drug can be determined by the calculation of LD_{50}, i.e., the dose that will kill 50% of animals of a particular species. Recently, we reported the LD_{50} of *A. bisporus*, in male rats described by the method Lorke.[13] Rats were divided into separate groups, comprising of ten rats in each groups as follows: Animals were kept without food for 18 h prior to dosing the ABE and ABCNPs was dissolved in DMSO and water to administered orally using gavages. The acute toxicity studies of ABE and ABCNPs were investigated in male Sprague Dawley rats, were oral administered the extracts of ABE at the single dose of 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000 and 4500 mg/kg b.w. and ABCNPs at the dose of 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 and 5000 mg/kg b.w. for 72 h respectively. All animals were monitored continuously on the day of treatment and surviving animals were scrutinized daily for 3 days for signs of acute toxicity. Recovery and weight gain were seen as indications of having survived the acute toxicity. The rats were observed for signs of intoxication and lethality.

2.9. IC_{50} determination

The extract concentration that exhibited 50% inhibition (IC_{50}) is calculated is calculated by according to the method of is calculated by according to the method of Aderogba et al.[14]

2.10. Statistical analysis

All the analyses were performed in triplicate, and these results were reported as means ± standard derivation (SD). The significance of differences among treatment means were determined by one-way analysis of variance (ANOVA) using SPSS Program with a significant level of 0.05.

3. Results and discussion

3.1. Phytochemical screening of A. bisporus

Qualitative analysis carried out for ethanol extract of AB and ABCNPs showed in Table 1 have the presence of major phytochemicals such as terpenoid, alkaloid, steroid, carbohydrates, tannins, proteins and flavonoids that can also influence the biological effects.

3.2. Antioxidant activity of ethanolic extracts of A. bisporus and its loaded chitosan nanoparticles

The DPPH method was used to investigate the free radical scavenging activity of the edible mushrooms. The antioxidant potential of ABE and ABCNPs was investigated in the search for new bioactive compounds from natural resources. It has been used to evaluate the potential of various natural plants and vegetable extracts as antioxidants.[15] The inhibition values were originate at 27.78%, 27.78% and 25.51% for ABE, ABCNPs and ascorbic acid were observed at a concentration of 50, 100, 150, 200 and 250 μg/ml, respectively (Fig. 1(A)). For $ABTS^{\bullet+}$ radical cation was generated by the interaction of $ABTS^{\bullet+}$ (250 mM) and K2S2O8 (40 mM) and observed different concentration of 50, 100, 150, 200 and 250 μg/ml, respectively (Fig. 1(B)). In ABE and ABCNPs the inhibitory concentration (IC_{50}) was found to be 250 μg/ml. This suggests that antioxidant activity was retained even after the encapsulation of

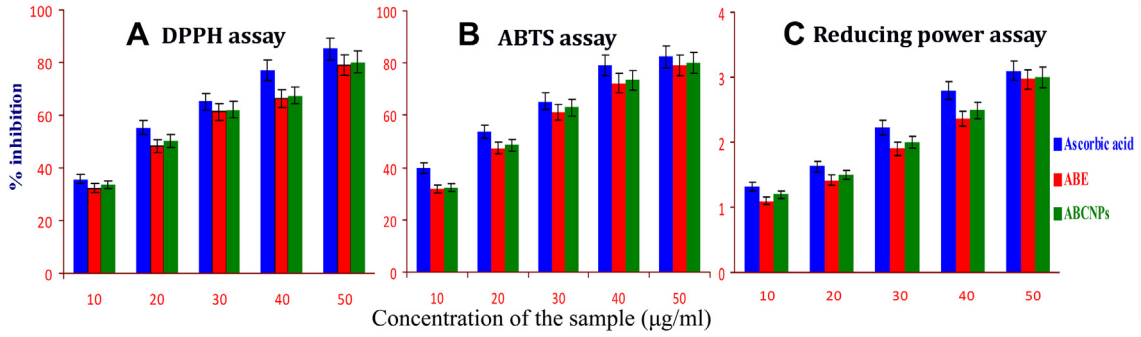

Fig. 1 – Antioxidant activity of ethanolic extracts of *A. bisporus* and its loaded chitosan nanoparticles.

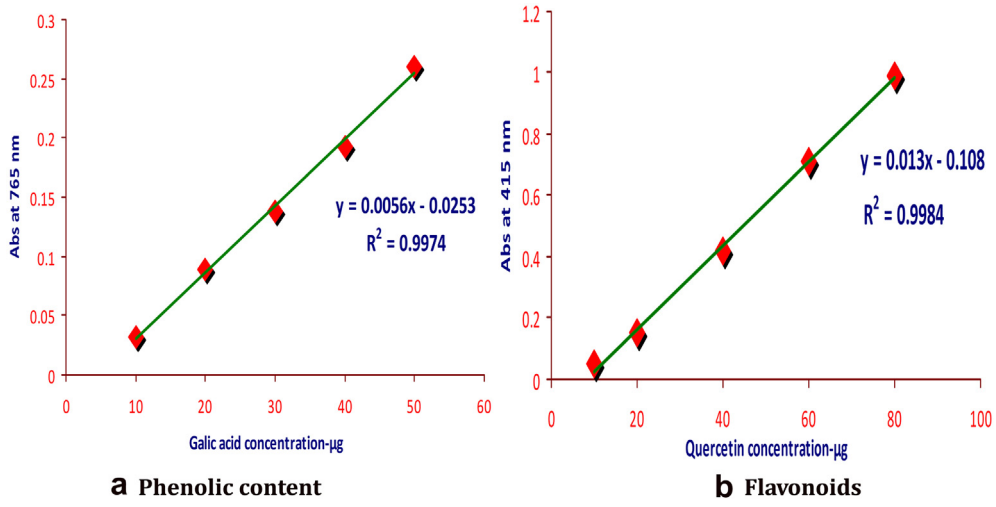

Fig. 2 – Amounts of total phenolic and flavonoids present in the ABE and ABCNPs.

Table 2 – Results of the lethal doses of ABE and ABCNPs for the determination of LD50 after oral administration in rats ($n = 10$).

Group	ABE & ABCNPs dose (mg/kg) b.w.	Log dose	ABE % dead	ABCNPs % dead	Corrected %		Probits	
					ABE	ABCNPs	ABE	ABCNPs
1	500	2.69	0	0	2.5	2.5	3.04	3.04
2	1000	3.0	0	0	2.5	2.5	3.04	3.04
3	1500	3.18	10	0	10	2.5	3.72	3.04
4	2000	3.30	20	0	20	2.5	4.16	3.04
5	2500	3.39	30	0	30	2.5	4.48	3.04
6	3000	3.47	40	10	40	10	4.75	3.72
7	3500	3.54	50	30	50	30	5.00	4.48
8	4000	3.60	80	40	80	40	5.84	4.75
9	4500	3.65	100	80	97.5	80	6.96	5.84
10	5000	3.69	–	100	–	97.5	–	6.96

chitosan with ABE. Fig. 1(C) shows the reducing ability of the ABE and ABCNPs compared to that of ascorbic acid and increased dose dependently. At the concentration of 250 µg/ml, the AB mushroom extracts and its loaded chitosan nanoparticles were determined to have 81.97% and 78.13% reducing power relative to the ascorbic acid 73.52%, respectively. The extracts showed more scavenging activity on hydroxyl radical and reducing power.

3.3. *Amount of total phenol and flavonoids*

Free radical scavenging is a generally accepted mechanism for phenolic antioxidants to inhibit lipids oxidation. The antioxidative activity of phenolics is generally directed by their chemical structures, the activity increases with increasing the number of hydroxyl groups and their location in the molecules involved.[16] The amount of total phenolics was reported 1 g of

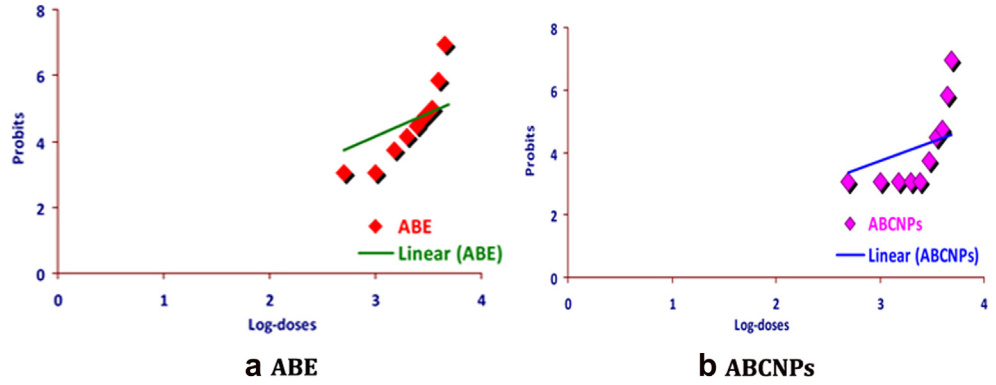

Fig. 3 – Plot of log-doses versus probits from Table 2 for calculation of LD_{50} of ABE and ABCNPs administered rats.

sample contains 8.19 ± 1 mg of gallic acid by Folin–Ciocalteu method and total flavonoid analysis by the assay of aluminum chloride spectrophotometric reported 1 g of sample contains 10.3 ± 1 mg of quercetin in ABE and ABCNPs shown in Fig. 2(a) and (b). Pekkarien et al attributed the antioxidant activity of phenolic acids in a bulk lipid system to their DPPH radical scavenging activity.[17] *A. bisporus* contained significant amounts of phenolic amino acids (tyrosine, L-glutaminyl-4-hydroxybenzene, 3, 4-dlhydroxyphenylalanine and L-glutaminyl-3,4-dihydroxybenzene), which may be responsible for the relatively high antioxidative activity.

3.4. *Acute toxicity studies*

The acute lethal effect of ABE and ABCNPs on rats (Table 2 and Fig. 3(a) and (b)) shows that number of animal died within 72 h. After the major signs of toxicity noticed within 72 h included change in physical activity, difficulty in breathing, mortality, loss of appetite, general weakness, respiratory suffering and convulsions or coma. These signs were not seen in bellow 2747.25 mg/kg b.w. in ABE and 3178.86 mg/kg b.w. of ABCNPs, but progressed and became increasingly pronounced as the dose increased towards 4000 mg/kg b.w. of ABE and 5000 mg/kg b.w. of ABCNPs. The LD_{50}, around 3000 mg/kg b.w. is thought to be safe as suggested by Lork.[13] Again, the absence of death among rats in some of the groups throughout the 3 days of the experiment seems to support this claim. However, LD_{50} of 4000 mg/kg bw has been reported for the methanolic extract of the leaves of *Salvia officinalis*; sage, in streptozotocin induced diabetic rats.[18] Azu et al, reported LD_{50} of 3981.07 mg/kg bw in methanolic fruit extract of *Kigelia africana*.[19]

4. Conclusions

The conversion of *A. bisporus* extract loaded chitosan nanoparticles has same antioxidant properties. Thus our results provide evidence that ABE and ABCNPs proves to have a potent antioxidant and very low toxic also might act as a potential intermittent therapy against cancer. From the results of this study, it is hypothesized that extracts of *A. bisporus* is safe for usage in traditional medicine. Higher doses should, however, be avoided and users should not rule out completely the possibility of chronic toxicity developing with the continual usage with higher concentration.

Conflicts of interest

All authors have none to declare.

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