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Effect of Chitosan in Radical Scavenging and Bactericidal Activity Isolated from *Agaricus bisporus* Mushroom

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ABSTRACT
In the present study, Chitin has been extracted from *Agaricus bisporus* (Button mushroom). The obtained chitin was converted into the more useful chitosan and the crude chitosan extract was measured for its absorption maxima by UV Spectrophotometer and the maximum peak at 265 nm was observed. FT-IR spectroscopy was done to identify the functional groups presented in the chitosan, which was analyzed between the ranges of 4000–400 cm^{-1}. Chitosan was characterized by significant amide bands at 3265.49 cm^{-1}. The absorbance bands of 1402.25, 1153.43, 900.76 and 445.56 cm^{-1} indicated CH2 stretching, CH stretching, C=O stretching in secondary amide respectively, which confirms the structure of chitosan. The antioxidant activity of chitosan was determined by DPPH free radical scavenging assay and the value gained was 65.90% at 250 mg/ml, which was due to the presence of rutin, gallic acid, caffeic acid and catechin in the phenolic composition of *Agaricus bisporus.* Finally, *in vitro* antibacterial screening of chitosan from *Agaricus bisporus* was performed against selected clinical isolates and the zone of inhibition shown highest activity in *Bacillus subtilis, P. aeruginosa* followed by *K. pneumonia,* and *Acinetobacter baumannii.* These finding suggested that the *Agaricus bisporus* act as the potential source produce eco-friendly chitin and chitosan in the development of drugs, artificial bone, and raw material for food industries in near future.

Key-words: *Agaricus bisporus,* Antioxidants, Antibacterial activity, Chitosan, UV spectroscopy, FTIR spectroscopy

INTRODUCTION

Chitosan (poly-*N*-acetyl glucosamine) is a natural and biodegradable biopolymer and this natural polymer is obtained from the renewable resources like exoskeletons of shellfish, prawn, crab and the wastes of the seafood industry. Chitin recognizes as the second most important natural polymer in the world. Chitin is the most bounteous natural amino polysaccharide and it can be produced annually as cellulose. The main sources of chitin are marine crustaceans like shrimp and crabs but mushroom is also act as the potential source to extract chitosan [1].

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By deacetylation of strong alkalis at high temperatures for long periods of time, Chitosan is commercially produced from shells of shrimp and crab. But the problem lies in the supply of raw materials, seasonal and also the process is laborious and costly. Furthermore, the chitosan derived from such a process is heterogeneous with respect to their physiochemical properties. Recent advances in fermentation technology suggest that the cultivation of selected fungi can provide an alternative source of chitosan.

Fungal cell walls and septa contain mainly chitin, which is responsible for maintaining their shape, strength and integrity of cell structure. These microorganisms can be readily cultured in simple nutrients and chitosan present in their cell wall can be easily recovered. Edible mushrooms are considered to be rich in protein and contain mainly chitin, glucagon and protein in the cell wall and are best source of dietary fibers [2]. Hence, mushroom act as the potent origin for chitin and

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chitosan extraction and which is similar to the animal origin.

The role of chitosan is significant since, it possess excellent medicinally active therapeutic effects as anti-inflammatory, anti-tumour, anti-viral, anti-parasitic, anti-bacterial, blood pressure regulator, cardiovascular disease, immuno-modulating, kidney tonic, hepato product nerve tonic, sexual potentiator, chronic bronchitis, cholesterol, wound healing and some antigenic properties [3]. It is used for food swelling as a food thickener, film forming agent, sterilizer and overall as an important health ingredient [4]. Chitosan and its derivatives can be variously used as a permeability control agent, an adhesive, a paper-sizing agent, a fining agent, flocculating and chelating agents, an antimicrobial compound and a chromatographic support. It is also used to immobilize enzymes or to deliver drugs to their target [5]. Chitosan, a polycationic polymer comprising of D glucosamine and N-acetyl-D-glucosamine linked by (1-4) glycosidic bonds, has been exploited as a carrier for the delivery of anticancer drugs, genes, and vaccines [6,7]. Due to their bioactive nutrient content mushrooms are considering as nutraceuticals and act as functional food for human concern. The current work was done with the purpose to extract chitosan from Button mushroom (*Agaricus bisporus*) and analyzing it antioxidant and antibacterial activity.

MATERIALS AND METHODS

Materials and chemical used- All the chemicals required for this work was purchased from Hi-media chemical laboratories, Mumbai, India and analytical grade. This complete work was done in the Department of Zoology, Holy Cross College, Trichy, Tamilnadu, India from December 2017 to March 2018.

Agaricus bisporus (Button mushroom) was widely used for human consumption and thus this species was selected for isolating chitosan. Button mushrooms were collected from commercial vendors in Trichy, Tamilnadu, India. Whole fruit bodies were used. The collected mushrooms were cleaned, cut into small pieces and dried. The dried pieces were ground to a powder and stored in a container at room temperature for further analyses and extraction.

Isolation and extraction of chitosan

Deproteinization- The sample was obtained soaking 10 gm of dried, powdered mushroom in boiling 4% sodium hydroxide for 1 hr. The sample was removed and then allowed to cool at room temperature for 30 minutes.

Demineralization- The sample obtained was demineralized using 1% hydrochloric acid with 4 times its quantity. They were then soaked for 24 hrs to remove minerals. The above samples were treated with 50 ml of 2% sodium hydroxide for 1 hr. The remaining sample was washed with deionized water and then drained off.

Deacetylation- The process was then carried out by adding 50% sodium hydroxide to the obtained sample on a hot plate and boil it for 2 hrs at 100 degree Celsius. The sample was then allowed to cool at room temperature for 30 minutes. Then they were washed continuously with 50% sodium hydroxide. The sample obtained is filtered (chitosan is obtained). The sample was left uncovered, and oven dried for 6 hrs at 110 degree Celsius.

Characterization of chitosan using spectral analysis techniques

UV-visible spectroscopy- The sample was subjected to uv/visible spectroscopy using Perkin Elmer Lambda 35, double beam uv/visible spectrophotometer. Distilled water was used as the blank solution and 10% glacial acetic acid was used as the reference solution. The absorbance of the sample was measured in the range of 200–800 nm. The peaks obtained for the sample was compared with Spectral properties of the Standard chitosan and the results were interpreted.

FT-IR Spectroscopy- The prepared biopolymer chitosan was analyzed by FTIR 8300 spectrophotometer (Shimadzu) in the wavelength between 400cm^{-1} and 4000cm^{-1} and in the solid state using potassium bromide pellets.

In vitro antimicrobial activity

Test microorganisms- Seven bacterial strains used in the present study were *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Bacillus subtilis,* Methicillin-Resistant *S. aureus* and *Acinetobacter baumannii* were maintained on nutrient agar.

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Vairamuthu *et al*., 2018
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Antibacterial assay- The effect of chitosan extracts from *A. bisporus* was tested for their antibacterial activity on the several bacterial strains by agar well diffusion method [8].

DPPH radical scavenging activity method- The scavenging activity of mushrooms was estimated according to the procedure described by Shimada *et al.* [9]. An aliquot of 1.5 ml of sample extracts at different concentrations (100, 150, 250 mg/ml) was added to test tubes with 3.5 ml of 0.1mM DPPH radical in methanol. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The reaction mixture was determined at 515 nm with UV-visible spectrophotometer. Extraction solvent was used as a blank while mixture without extract served as control. Ascorbic acid was used as a standard. The scavenging effect was calculated based on the following equation-

Scavenging effect (%)=

1- [(Absorbance sample/ Absorbance control) x 100]

RESULTS

Natural products are the important source of biopolymer material as polysaccharides, polyphenols, polyamides and proteins. All of these play important roles in biomedicine *A. bisporus* was commonly consumed mushroom and chitosan were isolated (Fig. 1) by three step process of deprotenization, demineralization and deacetylatation. The total extract of chitosan from 10gm of dried powered mushroom is 4 gm. The extracted chitosan was pale yellow and hygroscopic in nature with flabby texture.

Fig. 1: *A. bisporus* (Button Mushroom)

Fig. 2: Extracted Chitosan from *Agaricus bisporus*

Solubility- Chitosan was noted to be insoluble or sparingly soluble in water. Chitosan was found to dissolve in acetic acid and hydrochloric acid. It was insoluble in sulphuric acid. The solubility of chitosan in various acids agreed with the observation of Guibal [10].

Characterization of chitosan

UV-Visible spectroscopy- The uv-visible spectrum of chitosan was obtained and it was compared with that of standard chitosan [11]. The Fig. 3 showed the spectral peaks of chitosan respectively.

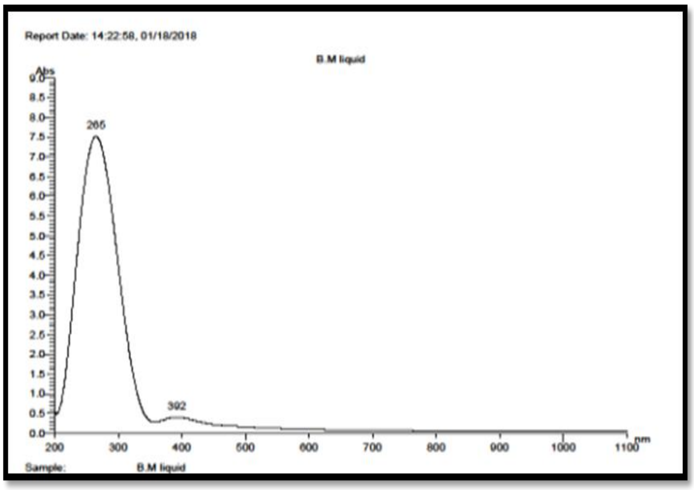

Fig. 3: UV–visible spectral data of chitosan from *A. bisporus*
(Maximum Absorbance peak was observed at 265 nm for chitosan)

FTIR Spectroscopy- The FT-IR spectrum of chitosan was obtained and it was compared with that of standard chitosan [11]. The Fig. 4 & Fig. 5 as well as Table 1 & Table 2 shows the spectral peaks of chitosan respectively.

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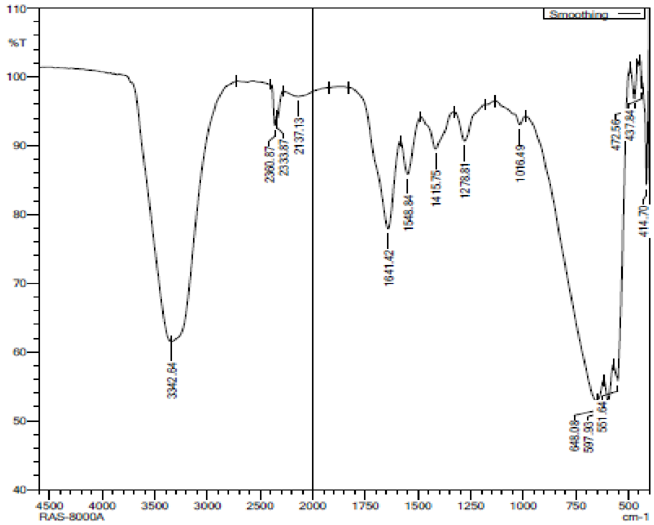

Fig. 4: FTIR spectral data of powdered chitosan from *Agaricus bisporus*

Table 1: Functional groups of powdered chitosan from *A. bisporus*

Wave length	Functional group
3342.64	Hydroxyl group (O-H)
2360.87	Cycloalkane
1641.42	Aromatic ring
1548.84	Phenol ring
1016.49	Polysaccharides
597.93	Halogen compound (chloro compound C-Cl)
472.56	Halogen compound (Iodo compound C-I)
1278.81	Ester carbonyl

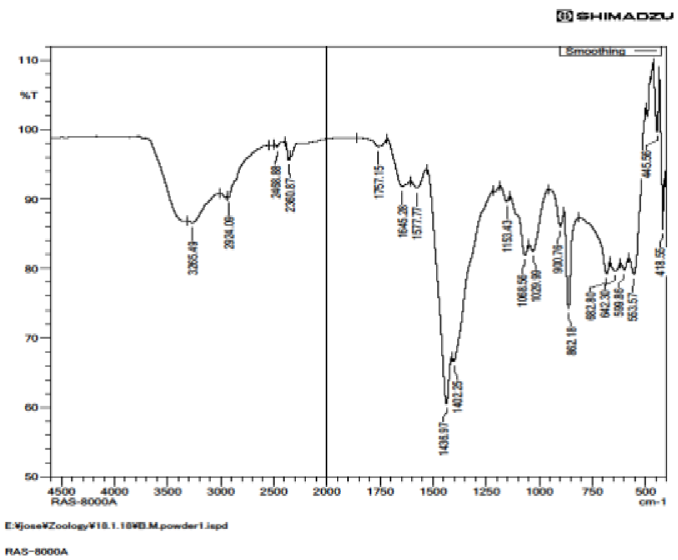

Fig. 5: FTIR spectral data of Chitosan soluble in 10% of glacial acetic acid

Table 2: Functional groups of chitosan soluble in 10% of glacial acetic acid

Wave length	Functional group
3265.49	Amide
1402.25	C-O bond
1153.43	Polysaccharides
900.76	Aromatic compound
445.56	Halogen compound Iodocompound (C-I)

Antioxidant activity of chitosan- The antioxidant activity of chitosan was determined by DPPH free radical scavenging assay and the values are presented in the Fig. 6.

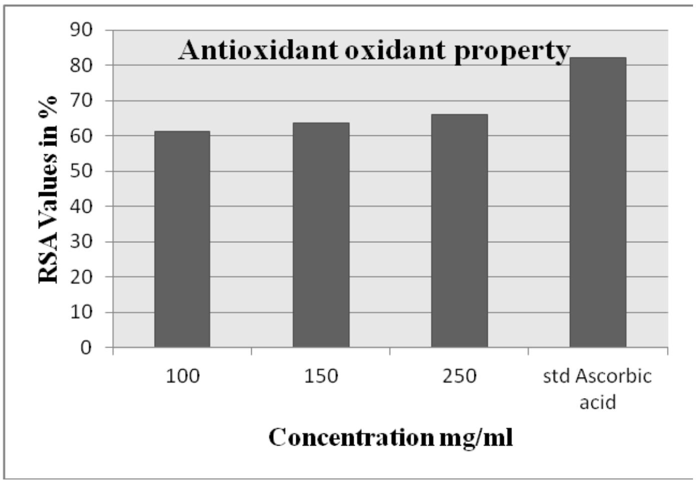

Fig. 6: Showing *in-vitro* antioxidant activity by DPPH assay

Antibacterial activity of the Chitosan- The *in vitro* antibacterial activity of the chitosan extracts of button mushroom was tested against the several organisms like *S. aureus, A. baumannii, K. pneumoniae, B. subtilis, P. aeruginosa,* methicillin resistant *S. aureus* where 10% glacial acetic acid was incorporated as a control. It was done by the well diffusion method. The antibacterial activity potentials were assessed by presence or absence of inhibition zone in diameters around the well. The antibacterial assay was analyzed at 100 mg/ ml, 200 mg/ml, 300 mg/ml of the extracts and showed wider zone of inhibition (Fig. 7 to 12).

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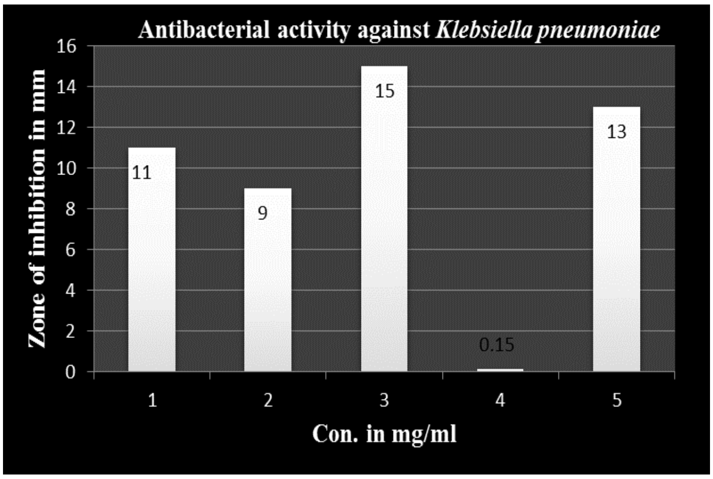

Fig. 7: Antibacterial assay of Chitosan against *K. pneumonia*

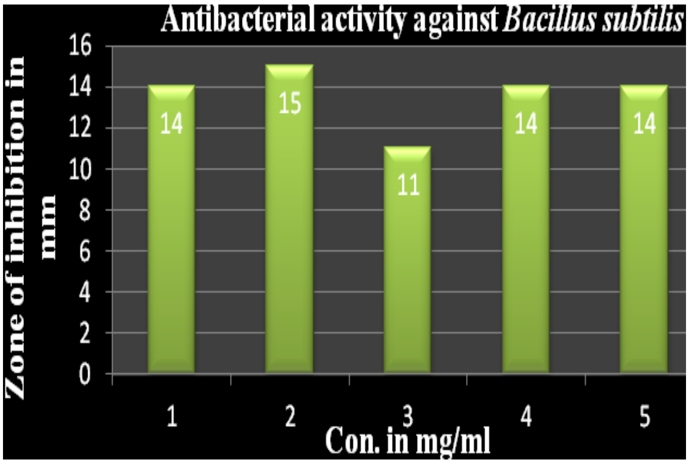

Fig. 10: Antibacterial assay of Chitosan against *B. subtilis*

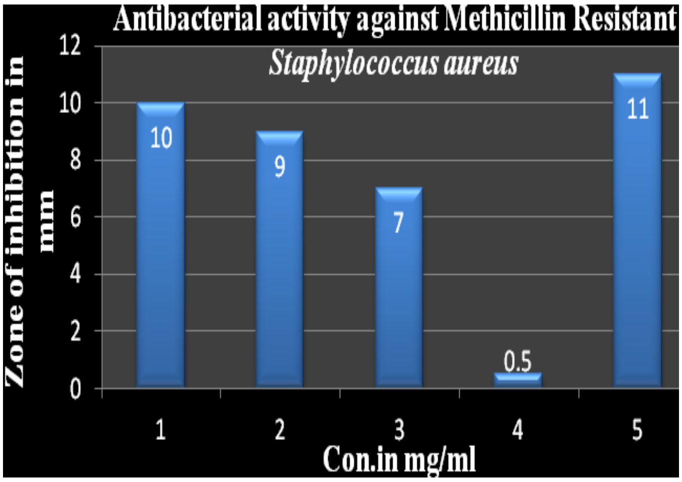

Fig. 8: Antibacterial assay of Chitosan against *Methicillin resistant S. aureus*

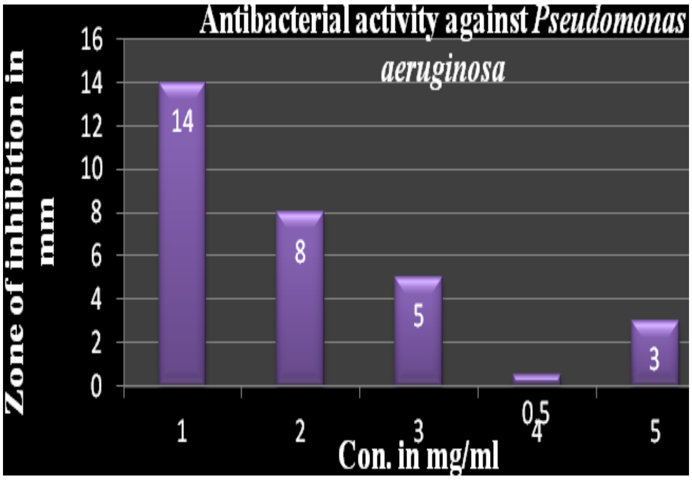

Fig. 11: Antibacterial assay of Chitosan against *P. aeruginosa*

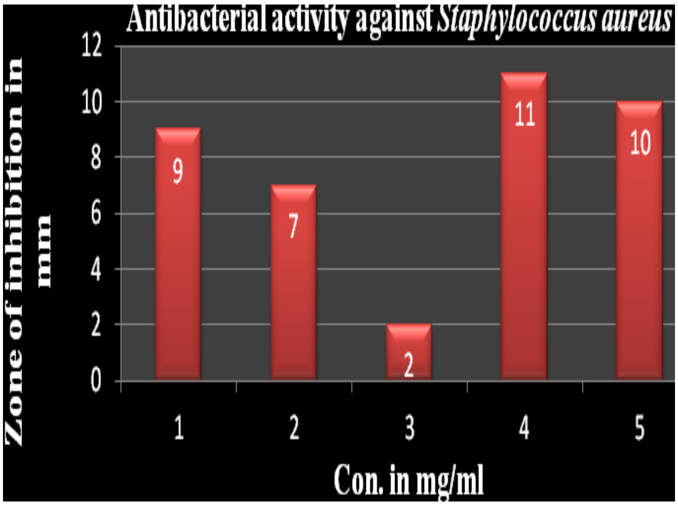

Fig. 9: Antibacterial assay of Chitosan against *S. aureus*

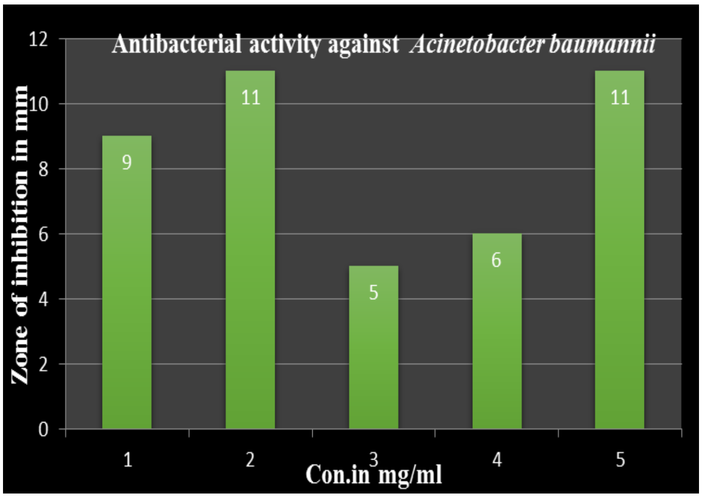

Fig. 12: Antibacterial assay of Chitosan against *A. baumannii*

1. 100 mg/ml**, 2.** 200 mg/ml**, 3.** 300 mg/ml**, 4.** Antibiotic (Amoxycillin100 mg/ml)**, 5.** Control (10% acetic acid)

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Vairamuthu *et al*., 2018
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DISCUSSION

Chitosan is a biopolymer, which has a potential role as functional biomaterial in various fields such as, pharmaceutical, agricultural, industrial and medicinal ones. The most common source of chitosan is obtained from the shells of crab and shrimps which are considered as waste from food processing units. But they face certain problems like seasonal supply and species variations. So, in current scenario the chitosan extraction from fungal cells gained its importance and here *Agaricus bisporus* (button mushroom) is chosen for the study. The production of biopolymer from fungi depends on its species and culture conditions. The total yield of chitosan from 10gm of dried powered mushroom is 4 gm. The synthesis of chitosan involves several chemical steps. Initially, the removal of proteins is done by demineralization; subsequently the removal of mineral is done by demineralization. Finally, chitin was obtained and removal of carbon and other salts are done by deacetylation of chitin, where they are converted to chitosan a biopolymer. The extracted chitosan was characterized based on colour, nature, texture and its solubility. The colour of the extracted chitosan is pale yellow, hygroscopic in nature and has a flabby texture. They are insoluble or sparingly soluble in water. Chitosan was found to dissolve in 10% acetic acid.

UV-Visible Spectrophotometric analysis represents the absorption spectrum of the chitosan from *Agaricus bisporus.* The spectrum shows strong band between 260 nm and 390 nm with maximum absorption at 265 nm. This band is due to the electronic transitions produced by the secondary amide fragment of the chitosan. The λ max of chitosan (265 nm) shifts to longer wave length which indicates the chemical interaction of chitosan and acetic acid at room temperature. A similar effect was reported by Ifuku *et al.* [12], who had studied, the potential source of chitosan and its interaction.

The infrared spectrum is obtained by passing infrared electromagnetic radiation to the sample having a permanent induced dipole moment. The IR spectrum and the bands are generally large due to the macromolar character of the chitosan and because of intermolecular binding of hydrogen in the solid and liquid state of the sample. The infrared spectra of the chitosan extracted from *A. bisporus* was characterized by three significant amide bands at 1641, 1644, 1548, 1577 cm^{-1}, which corresponded to the CO by three secondary amide stretch (Amide I), C-O secondary amide stretch (Amide I) and NH_2 deformation, C-N-stretching in secondary, respectively (Fig. 5 & 6). The absorbance bands of 3342, 2924, 1436cm^{-1} indicates the N-H stretching, OH and CH deformation ring, C-H stretching-alkane groups-CH_3, CH_2 and N-H stretch–primary amine group, respectively. From this data quantitative analyses and structure of the compounds can be employed [13]. In fact certain group of atoms presenting bands at or near the same frequency and there is unique IR finger print of molecules. By this technique, elucidate the structure of a compound and it was similar to the finding of Palaciosa *et al.* [13].

Free radical Scavenging is one of the important aspects of inhibiting the lipid oxidation and used to estimate antioxidant activity. DPPH is a stable free radical with absorption at 515 nm. Obviously, chitosan extract has antioxidant activity of 65.9% at 250 mg/ml, 63.6% at 150 mg/ml and 61.36% at 100 mg/ml. It has been described that the phenolic composition of *A. bisporous* methanolic extract was found to contain rutin, gallic acid caffeic acid and catechin, which contributes radical scavenging activity [14]. Chitosan reacts rapidly with DPPH and reduce the DPPH radicals, which can be noted visibly due to its colour reduction in the samples. This result indicates that the extract has free radical inhibition or scavenger [15]. This activity involves in termination of free radical reaction and indicates that chitosan from *Agaricus bisporus* has a noticeable effect on scavenging free radicals.

In vitro antibacterial screening of chitosan from *Agaricus bisporus* against selected clinical isolates were performed and zone of inhibition was given in the graph (Fig. 7 to 12). The highest zone of inhibition was observed in *B. subtilis, P. aeruginosa* followed by *K. pneumonia* and *A. baumannii.* The antimicrobial activity of chitosan and their derivatives against gram positive and gram negative bacteria has received considerable attention in recent years. Several mechanisms are responsible for the inhibition of microbial cells by chitosan. The interaction with anionic groups on the cell surface, due to its polycationic nature, causes the formation of an impermeable layer around the cell, which prevents the transport of essential solutes. It has been demonstrated by electron microscopy that the site of action is the outer membrane of gram-negative bacteria. Recently, the bactericidal effect is also partially mediated by ompA, an outer protein of bacteria that is responsible for cell surface integrity [16]. It is also due to decreases in pH level and change in osmotic pressure in peptidoglycan layer of cell wall, which does cellular destruction and cell lysis [17]. These results also suggest that higher molecular weight of the polymer chitosan shows greater antibacterial activity because the positive charge present in amino group of chitosan may interact with the sites on the cell surface causing disturbances in cellular permeability [18]. In our present study, the extracted chitosan showed encouraging results against bacterial with maximum inhibitory activity.

The importance of chitin and chitosan are increasing day by day due to its renewable, biodegradable property. Search for new natural health supporting fungi including mushroom paves way for curing many health related diseases and enhance immune function. Hence, based on the results, it may conclude that chitosan isolated from *A. bisporus* is mushroom should has significant antioxidant and antibacterial activity.

CONCLUSIONS

According to the results of this study, it's clearly confirmed that extract of *A. bisporus* (Button mushroom) has potent chitosan. The importance of chitin and chitosan increased lately on one hand due to the fact that they represent sources of renewable and the biodegradable materials and on the other hand for that purpose to a better knowledge of their functionality through application in domains such as biology, pharmacy, biotechnology, medicine and the chemistry of materials. Based on the result of antioxidant and antibacterial activity, it clearly indicated that chitosan from *A. bisporus* can inhibit the lipid oxidation by free radical Scavenging activity and it shows good resistance against pathogenic bacteria. Hence, based on the above properties of chitosan this work can be further carried to Nano conversion for drug delivery and also for hydrogen preparation with respect to the wound healing activity.

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CONTRIBUTION OF AUTHORS

Research concept and work design of the article was done by the corresponding author. Data collection, experimentation, data analysis and interpretation for the work were done together by Vairamuthu GM and Peter JJR. Drafting of the article, Critical revision of the article for important intellectual content, and final approval of the version to be published were done by Jerley A and Dhandapani S. Finally, all contributed equally and successfully completed the work.

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