INTRODUCTION

The name `chitin' is derived from the Greek word `chiton', meaning a coat of mail. and was apparently first used by Bracconnot in 1811 [1] and [2]. Chitin is a polysaccharide, made of N-acetyl-D-glucosamine units connected by β (1→4) linkage and it is a second most abundant natural biopolymer after cellulose, with this difference that in chitosan one hydroxyl group on each monomer has substituted with an acetylamine group. The chemical structure of chitin is similar to that of cellulose with 2-acetamido-2-deoxy-D-glucose (NAG) monomers attached β (1→4) linkages [3].

Chitin is a hard, inelastic, and white polysaccharide found in the exoskeleton of insects, crabs, shrimps and lobsters, in the internal structure of other invertebrates such as prawns and insects, as well as, on the cellular wall of fungi and yeasts. It is the second most abundant biopolymer after cellulose [4]. Chitin is an n-acetyl-d-glucosamine linear polymer joined by β (1→4) glycosidic linkage: (1→4, 2-acetamide-2-deoxy-b-d-glucose) and there are three different polymeric forms a, b and c [5]. It has relevant properties including antimicrobial, antifungal, and antiviral activities; it is non-toxic, biocompatible, biodegradable, emulsifying, grease-absorbent, the adsorbent of contaminating metals, and fibrogenic; therefore, it is considered that chitin has wide-ranging applications in various industrial fields [6].

Chitosan is the deacetylated form of chitin, which, unlike chitin, is soluble in acidic solutions Chitosan (1→4)-2-amino-2-deoxy-glucose is a cationic biopolymer produced by alkaline N-deacetylation of chitin, which is the main component of the shells of crab, shrimp, krill and other crustaceans [3].

Chitin and chitosan are the biopolymers that have received much research interests due to their numerous potential applications in agriculture, food industry, biomedicine, paper making and textile industry. The most common source of chitin and chitosan is the crustacean shell; however, mushrooms are an alternative source for isolating these biopolymers because their cellular wall has a high content of chitin, which may be transformed into chitosan through a deacetylation reaction [7].

Chitosan is a polymer derived from chitin and is used in applications from health care to agriculture to dyes for fabrics. There are even medical applications. Chitosan itself is the major source of the nutritional supplement glucosamine. Chitosan is, at the pH of the gastrointestinal tract, a positively charged polymer and can bind to negatively charged substances. Chitosan might bind to some dietary lipids. It may also bind to the fat-soluble vitamins A, D, E, and K, as well as flavonoids, carotenoids and some minerals such as zinc, found in foods [8].

Production of chitin and chitosan from fungal mycelium has recently received increased attention due to significant advantages. For example, while crustacean waste supplies are limited by seasons and sites of fishing industry, fungal mycelium can be obtained by convenient fermentation process that does not have geographic or seasonal limitations, fungal mycelia have lower level of inorganic materials compared to crustacean wastes, and thus no demineralization treatment is required during the processing, crustacean chitin and chitosan may vary in the physicochemical properties, while fungal chitin and chitosan have relatively consistent properties because of the controlled fermentation conditions, fungal chitin and chitosan are apparently more effective in inducing the plant immune response and are potentially more suitable for agricultural applications [9].

Many fungal species, including Abisidiaglaucua, Abisidiacericulosa, Aspergillus niger, Mucor rouxii, Gongronellabutleri, Phycomyces-
Recently increasing attention has been paid to develop and test active biomolecules such as chitosan and its derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi. chitosan has a great potential for a wide range of application due to its biodegradability, biocompatibility, antimicrobial activity, nontoxicity, and versatile chemical and physiological properties. All the chemicals and reagents used were of AR grade from Hi-Media (Mumbai). The culture of *Pleurotus* sp. 14 h and *Rhizopus* sp. 14 h were separately filtered through Whatman No.1 paper and used for further studies. A modified protocol of [18], confirmed by infrared spectroscopy using KBr pellet method in FTIR (Shimadzu FTIR-8200, Japan). In FTIR spectra were recorded in the middle infrared region (4000 cm⁻¹ to 400 cm⁻¹) with a resolution of 4 cm⁻¹.

The structure of extracted mycelia chitin and chitosan was examined and photographed in SEM using ICON ANALYTICAL, FEI with Model QUANTA 200 instruments. The agar well diffusion method was used for the antibacterial assay. The extracted chitin and chitosan samples were examined and photographed in SEM using ICON ANALYTICAL, FEI with Model QUANTA 200 instruments.

**Materials and Methods**

**Chemicals**

All the chemicals and reagents used were of AR grade from Hi-Media (Mumbai).

**Microorganism**

The culture of *Pleurotus* sp. and *P. eous* were grown at 25 °C for 7 d on potato dextrose agar (PDA), and was periodically transferred onto a new PDA medium. The strain was maintained at 4 °C and this was used for further studies.

**Production medium**

Actively growing mycelia were obtained from a newly prepared PDA agar plate culture after being incubated for 7 d at 25 °C. The inoculums were prepared as follows about 1 cm², 1 cm of mycelia was inoculated into a 250 ml Erlenmeyer flask, which contained 50 ml of MGYP medium (meat extract 2%; glucose 1.5%; yeast extract 0.3% and peptone 0.5%). The inoculated Erlenmeyer flask was incubated at 28 °C for 15 d, without any disturbance.

After 15 d of incubation, the grown fungal mycelium of *Pleurotus* sp. and *P. eous* were separately filtered through What Man No.1 filter paper. The mycelium mat was washed with sterilized distilled water until getting a clear filtrate. The mat was dried at 60 °C to get a constant weight for the extraction of chitin and chitosan.

**Chitin extraction**

Chitin was extracted from the modified protocol of [17]. The dried fungal cell mass was finely homogenised and subjected to alkali treatments to extract soluble alkali material like glucan and protein, present in fungal biomass. Fungal mycelia mat was subjected to alkali treatment with 1M NaOH in the ratio of 1:30 (v/v). Then the mixture was mixed, and this was incubated at 45 °C for 2 h. Then this suspension was centrifuged at 8000 rpm for 10 min, and washed with deionized water. Decolorization was done with 10g L⁻¹ potassium permanganate for 90 min, and then this was treated with 10g L⁻¹ oxalic acid for 1 h. After decolorization technique again centrifuged and washed until getting neutral pH. This was dried at 45 °C for 12 h. The amount of chitin was determined by a dry weight method.

**Chitosan extraction modified protocol of [18]**

The mycelial chitosan samples were prepared by grinding the dry mycelial chitosan powder with powdered KBr, in the ratio of 1:5 (sample: KBr) and then compressed to form KBr pellet and subjected to FTIR analysis.

**Characterization of chitin and chitosan**

**FTIR**

The structure of extracted mycelia chitin and chitosan was confirmed by infrared spectroscopy using KBr pellet method in FTIR. The structure of extracted mycelia chitin and chitosan was confirmed by infrared spectroscopy using KBr pellet method in FTIR. In FTIR spectra were recorded in the middle infrared region (4000 cm⁻¹ to 400 cm⁻¹) with a resolution of 4 cm⁻¹. The mycelial chitosan samples were prepared by grinding the dry mycelial chitosan powder with powdered KBr, in the ratio of 1:5 (sample: KBr) and then compressed to form KBr pellet and subjected to FTIR analysis.

**SEM**

The extracted chitin and chitosan samples were examined and photographed in SEM using ICON ANALYTICAL, FEI with Model QUANTA 200 instruments.

**Antimicrobial assay**

The agar well diffusion method was used for the antibacterial assay. The agar well diffusion method was used for the antibacterial assay. Petri plates were prepared by pouring 20 ml of Mueller Hinton Agar medium and allowed to solidify. Plates were solidified and 20 µl of 24 h old bacterial culture *E. coli, S. aureus, P. aeruginosa, K. pneumonia* and *B. Subtilis* were poured and uniformly spread. The inoculum was obtained from a freshly grown culture and subjected to each well was loaded with 20 µl of extract. The plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of zones of inhibition against the tested bacteria.

**PDA agar medium**

The PDA agar medium was prepared from commercially available dehydrated base according to the manufacturer instructions. The wells were made using cork borer on PDA agar plate. The borer was deepened into the alcohol for sterilisation and then was used to make wells. Wells with 10-mm diameter were punched in the agar and filled with 20 µl of the fungal spores. The plates were incubated at room temperature and the zone of inhibition were calculated.

**RESULTS AND DISCUSSION**

The synthesis of chitosan involves various chemical steps such as preparation of the chitin from the mycelial mat of *Pleurotus* sp which will be the initiation of the chitosan synthesis with the removal of the proteins in the shells followed by demineralization for the removal of the carbon and other salts present in the crude form which will be preceded by the decacetylation of the chitin that would result in chitosan. The regular chitosan is obtained by following the above steps, but a polymer of pharmaceutical grade has to fall in the region of its predetermined quality aspects, and usually commercial chitins are prepared by the first step of deproteinization followed by a second step of demineralization.
Fig. 1: Growth and mycelial mat formation of *Pleurotus florida* and *Pleurotus eous* in MGYP medium after 15 d of incubation

The fungal growth was monitored for 15 d. The growth rate of the different fungal species was varied and shown in Table 1. The greatest biomass, chitin and chitosan were harvested in the case of *P. Florida* and the lowest in the case of *P. eous*. [19] obtained chitosan from different fungus (*Aspergillus niger* TISTR3245, *Rhizopus oryzae* TISTR3189, *Lentinus edodes* no. 1, *Pleurotus sajocaju* no. 2, *Zygosaccharomyces rouxii* TISTR5058 and, *Candida albicans* TISTR5239). They carried out cultures fungus during different periods among 15–21 d, and then, they evaluated biomass and chitosan production yield. *Rhizopus oryzae* TISTR3245 was shown to give a maximal yield of chitosan at 138 mg g−1 dry weight.

In the present study, the maximum yield of the chitin and chitosan was obtained on 12th day of incubation by *P. Florida* (201.3 mg/g) followed by the *P. eous* (145.65 mg/g). [20] produced chitosan from *Rhizopus oryzae* PAS 17 in low-cost non-sterile conditions revealed the increment of chitosan and biomass yields of 14.45 and 8.58 folds from its an optimised condition, respectively [14].

**Table 1: Biomass and yield of chitin and chitosan extracted from *Pleurotus* spp**

| Day | Yield of Biomass (g/dry wt) | Chitin (mg/l) | Chitosan (mg/l) |
|-----|-----------------------------|---------------|-----------------|
|     | *Pleurotus florida*          | *Pleurotus eous* | *Pleurotus florida* | *Pleurotus eous* |
| 3   | 1.2                         | 0.89          | 25.63           | 0.124           |
| 6   | 4.89                        | 3.14          | 55.32           | 0.418           |
| 9   | 8.52                        | 6.58          | 75.62           | 0.602           |
| 12  | 10.52                       | 8.96          | 201.3           | 145.65          |
| 15  | 9.56                        | 8.24          | 65.61           | 45.23           |

Used different Zygomyces for the production of mycelial chitosan and *Absidialbutleri* NCIM 977 was found to produce the highest mycelial chitosan which is identical to our investigation. [21] extracted chitosan from *A. niger* under solid state fermentation after 12 d of fermentation with the yield at 10.9 g/kg of urea as substrate and 17.05g/kg of chitin using soyabean residue.

[13] chitosan extracted from 15 d old biomass of *Agaricus* spp *pleurotus* spp, *ganoderma* spp contain the yield of 0.944 mg/g of substrate. [22] extracted high yield at chitosan 29.7% using *P. waksmanii*. Production of chitosan from fungi of *A. niger*, *R. oryzae*, *L. edodes* and *P. sajorcaju*, (21%) [23].

**FTIR**

The FTIR spectrum was measured in transparent potassium bromide pellets with Shimadzu FTIR-8200, Japan Spectrum one FT-IR spectrometer to identify the structural groups in the raw material. The raw material was characterised by FT-IR in the middle region, in the range of 4000–400 cm−1. For comparison, commercial chitosan from shrimp shells (Hi Media, Mumbai) was used (fig. 2 to 4).

In our study, the infrared spectra of the chitins extracted from *P. florida* and *P. eous* were characterized by three significant amide bands at 1654, 1617 and 1550 cm−1, which corresponded to the C = O secondary amide stretch (Amide I), C O secondary amide stretch (Amide II), and N H bend and C N stretch(Amide III), respectively (fig. 2). The absorbance bands of 3268, 2930,2878,1563, and 1418 cm−1
indicated the N-H stretching, symmetric CH3 stretching and asymmetric CH2 stretching, CH stretching, C=O stretching in secondary amide (amide I) and C-N–stretching in secondary amide (amide II), respectively [24].

FTIR analysis shows that chitin and chitosan represent the bands according to the report of [24] and [9], compared with standard chitin and chitosan.

SEM

SEM analysis showed that chitosan had a long thin crystal structure on a smooth surface. This was in accordance with previous data [27]. Nonhomogeneous and non-smooth surface structure of chitosan were also reported by. It is well documented in the literature that the surface morphology of chitin differs depending on insect species, gender and growth stage. [28] Observed that chitins extracted from adult and nymph grasshoppers (Dociostaurus maroccanus) consisted of long nanofibers with many big nanopores. A very uniform structure with a lamellar organisation and less dense structure was observed in the present study (fig. 8 and 9).

Antimicrobial activity

The antimicrobial activity of chitin, chitosan, and their derivatives against different groups of microorganisms, such as bacteria, yeast, and fungi, has received considerable attention in recent years. Two main mechanisms have been suggested as the cause of 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 [29]. In our present study, the extracted chitin and chitosan showed encouraging results against bacterial and fungal pathogens with maximum inhibitory activity against the fungal pathogens (fig. 10 to 13).
In vitro antibacterial screening of chitosan and water soluble chitosan from *Pleurotus* spp. against selected clinical isolates were performed and zone of inhibition were given in graph (Fig. 6 and 7). The highest zone of inhibition was observed in *E. coli*, *S. aureus*, *B. subtilis* followed by *P. aeruginosa*, *K. pneumonia* when compared with the commercial chitin and chitosan. The chitin and chitosan also exhibited the antifungal activity against *A. niger* (5 mm), *A. flavus* (5 mm) followed by the *F. solani* (5 mm). [30] reported that chitin and chitosan from shrimp shell waste have antibacterial activity against *E. coli* and *S. aureus*.

**ACKNOWLEDGEMENT**

The authors thank the Research and Development Centre of Bharathiar University, Coimbatore, Tamilnadu, India for their support in this research work.

**CONFLICT OF INTERESTS**

We declare that we have no conflict of interest.

**REFERENCES**

1. Lower SE. Polymers from the sea chitin and chitosan. Manuf Chem 1984;55:73-5.
2. Skaugrud O, Sargent G. Chitin and chitosan: crustacean biopolymers with potential application. International By-products Conference, Anchorage, Alaska; 1990. p. 61-72.
3. Fereidoon Shahidi, Janak Kamil Vidana Arachchi, You-Jin Jeon. Food applications of chitin and chitosan. Trends Food Sci Technol 1999;10:37-51.
4. Wan ACA, Tai BQI. CHITIN-A promising biomaterial for tissue engineering and stem cell technologies. Biotechnol Adv 2013;31:1776–85.
5. Dutta PK, Ravikumar MNV, Dutta J. Chitin and chitosan for versatile applications. Polym 2002;42:307–54.
6. Caprile MD. Obtencion y utilizacion de quitina y quitosano a partir de desechos de crustaceos. Congreso Mundial ISW: Hacia un sistema integral de residuos solidos urbanos, Argentina; 2005.
7. Natali Mesa Ospina, Sandra Patricia Ospina Alvarez, Diana Marcela Escobar Sierra, Diego Fernando Rojas Vahos, Paola Andrea Zapata Ocampo, Claudia Patricia Ossa Orozco. Isolation of chitosan from *Ganoderma lucidum* mushroom for biomedical applications. J Mater Sci: Mater Med 2015;26:135.
8. HK No, Meyers SP, W Prinyawiwatkul, Z Xu. Applications of chitosan for improvement of quality and shelf life of foods: a review. J Food Sci 2007;72:R87–100.
9. Teng WL, Khor E, Tan TK, Lim LY, Tan SL. Concurrent production of chitin from shrimp shells and fungi. Carbohydr Res 2001;332:305-16.
10. Chatterjee S, Adhya M, GUha, AK Chatterjee. BP chitosan from *Mucor macus* spp. production and physio-chemical characterisation. Process Biochem 2004.
11. Percot A, Viron C, Domard A. Optimization of chitin extraction from shrimp shells. Biomacromolecules 2003;4:12-8.
12. Dutta PK, Tripath S, Mehrrota GK, Dutta J. Perspective of chitosan-based antimicrobial films in food applications. Food Chem 2009;114:173–82.
13. Kannan M, Nesakumari M, Rajarathinam K, Singh AJAR. Production and characterization of mushroom chitosan under solid–state fermentation conditions. Adv Biol Res 2010;4:10-3.
14. Vaingankar PN, Juvekar AR. Fermentative production of mycelial chitosan from zygomycetes: media optimisation and physicochemical characterization. Adv Biosci Biotechnol 2014;5:940-56.
15. Yang N, Li W. Facile one-pot synthesis of chitosan oligosaccharide/silver nanocomposites and their antimicrobial properties. Mater Lett 2014;132:145-8.

16. Krishnaveni B, Ragunathan R. Extraction and characterization of Chitin and Chitosan from Aspergillus terreus spp, synthesis of their bionanocomposites and study of their productive applications. J Chem Pharm Res 2015;7:115-32.

17. Sandra Patricia Ospina-Alvarez, David Alexander Ramirez Cadavid, Diana Marcela Escobar Sierrà, Claudia Patricia Ossa Orozco, Diego Fernando Rojas Vahos, Paola Zapata Ocampo, et al. Comparison of extraction method of chitin from Ganoderma lucidum mushroom obtained in submerged culture. Hindawi publishing corporation BioMed Res Int 2014;7. http://dx.doi.org/10.1155/2014/169071

18. Hu KJ, Hu JL, Ho RP, Yeunj KW. Screening of fungi for chitosan producers and copper adsorption capacity of fungal chitosan and chitosanaceous materials. Carbohydrpolymers 2004;58:45-52.

19. Pochanavanich P, Santomsuk W. Fungal chitosan production and its characterization. Department of Microbiology, King Mongkut's the University of Technology. Thon buri, Thailand. Appl Microbiol 2002;35:17–21.

20. Ozen Canli Tasara, Serkan Erdalb, Mesut Taskin. Chitosan production by psychrotolerant Rhizopus oryzae in non-sterile open fermentation conditions. Int J Biomacromol 2016;69:428–33.

21. Maghsoodi V, Yaghmaie S. Influence of different nitrogen sources on the amount of chitosan production by Aspergillus niger in solid state fermentation. Iran J Chem Eng 2008;27:47-52.

22. Mohammad Ali Ebrahimzadeh, Arona Cabzn, Eshrat Gharaei-Fathabad, Fereshteh Pourmord. Preparation of chitosan from Penicillium spp. And the determination of their degree of deacetylation. Indian J Biotechnol 2013;12:231-5.

23. Nadarajah K, Kader J, Mazmira M, Paul DC. Production of chitosan from fungi. Pak J Biol Sci 2001;4:263-5.

24. Paulino T, Simionato JL, Garcia JC, Nozaki J. Characterization of chitosan and chitin produced from silkworm chrysalides. Carbohydr Polym 2006;64:98–103.

25. Kasaai MR, Ardj L, Charlet G. Intrinsic viscosity-molecular weight relationship for chitosan. J Polym Sci Part B: Polym Phys 2000;38:2591-8.

26. Adam Wasuoa A, Piotr Bulkb, Magdalena Polak-Bereckaa, Katarzyna Nowakb, Gazey Polakowskib, Andrzej Bieganowski. The first report of the physicochemical structure of chitin isolated from Hermetia illucens. Int J Biomacromol 2016;92:316–20.

27. Suneeta Kumari, Pradip Kumar Rath. Extraction and characterization of chitin and chitosan from (Labeorohit) fish scales. Procedia Mater Sci 2014;6:482–9.

28. Erdogan S, Kaya M, Mol T, Baran T. Comparison of chitin structures isolated from seven Orthoptera species. Int J Biol Macromol 2015;72:797–805.

29. Helander I, Nurmiacho-Lassila E, Ahvenainen R, Rhoades J, Roller. Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. Int J Food Microbiol 2001;71:235-44.

30. KAbu Tareq, Masihul Alam, Salim Raza, Tanvir Sarwar, Z Fardous, Alamgir Z Chowdhury, et al. Comparative study of antibacterial activity of chitin and chemically treated chitosan prepared from shrimp (Macrobrachium Rosenbergii) shell waste. J Virol Microbiol 2013;9. Doi:10.5171/2013.369217

How to cite this article
• Jesteena Johny, Kannan Eagappan, RR Ragunathan. Microbial extraction of chitin and chitosan from Pleurotus spp, its characterization and antimicrobial activity. Int J Curr Pharm Res 2017;9(1):88-93.