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Extraction and characterization of Chitin and Chitosan from *Aspergillus terreus sps,* synthesis of their bionanocomposites and study of their productive applications

B. Krishnaveni[1] and R. Ragunathan*[2]

[1]*Department of Biotechnology, Maharaja Co-education Arts and Science College, Perundurai, Erode, India*
[2]*Synkromax Biotech Private Limited, Chennai, India*

ABSTRACT

Chitin (CH) and Chitosan (CS) have wide-scale biomedical applications and are being studied extensively for their antimicrobial activity. Aspergillus terreus CBNRKR KF529976, isolated from the marine soils of Pichavaram, Tamil Nadu was used for the economic production of Chitin and Chitosan using three different media-Sabouraud sucrose broth, Hesseltine and Anderson medium, Andrade et al medium. The polysaccharides were extracted by alkali-acid treatment, and characterized by infrared spectroscopy. The highest growth rate was with Henderson and Anderson medium with a mycelial dry weight of 24.83g/L. The best yields of the polysaccharides are obtained with Sabouraud sucrose broth for chitosan (48.32 mg/g) and for chitin (344.8 mg/g). The peak was observed to be at 390.5 nm in case of A.terreus CH AgNP and at 450 nm in case of CS AgNP for UV-Spectroscopy. From the SEM image, Chitin AgNP's exhibited smaller powder particle size and a nanoscale fiber-like surface structure. Ag/Chitosan BNCs showed show strong needle shaped structures. The EDS spectrum of A.terreus CH AgNP composite shows the peaks of C K, O K and Ag L. The atomic ratio of was found to be 87:11:0.75 wt %. The EDS spectrum of A.terreus CH AgNP composite shows the peaks of C K, NK, O K, Na K, Mg K, Cl K and Ag L. The atomic ratio of was found to be 20:3:19:1:1:0.46:53 wt %. The antibacterial activity of Chitin and Chitosan solution was found to be less than the Bionanocomposites indicating that the presence of the silver ion thereby increases the antibacterial strength of the polysaccharides. Chitin AgNP showed 91.5% dye inhibition in 72 hours and Chitosan AgNP showed 95.5% inhibition.

Keywords: *A.terreus*, Chitin, Chitosan, FTIR, SEM, EDS

INTRODUCTION

Chitin, the insoluble linear β 1, 4- linked homopolymer of N- acetyl-D-glucosamine (GlcNAc), is the second most abundant natural polysaccharide (after cellulose). Chitosan is a cationic amino polysaccharide, essentially composed of β-1,4 D-glucosamine (GlcNAc) linked to N-acetyl-D- glucosamine residues ,derived from de-N-acetylation of chitin [1-3]. These polysaccharides are found in a wide range of natural sources, such as crustaceans, insects annelids, molluscs, coelenterates and is a common constituent of fungal cell walls [4-6].

Recent advances in fermentation technologies suggest that the cultivation of selected fungi can provide an alternative source of chitin and chitosan. The amount of these polysaccharides depends of the fungi species and culture conditions [7-9].Filamentous fungi have been considered an attractive source of chitin and chitosan for

industrial applications because their specific products can be manufactured under standardized conditions [8-10]. Usually, the Zygomycetes Class has higher amounts of chitin and chitosan in their cell walls when compared to other classes of fungi [4-6].

The chemical properties of Ag nanoparticles (Ag NPs) are significantly different from those of silver ingot or Ag ion, and thus Ag NPs have been studied by many researchers due to their wide variety of potential applications [11, 12]. The special and unique properties of AgNPs can be attributed to their smaller size and the larger specific surface area relative to bulk materials, and many preparation processes have been proposed for controlling the physical and/or chemical characteristics of Ag NPs [13-17].

Chitosan and its derivatives have attracted considerable interest due to their antimicrobial and antifungal activity [18]. Chitosan is a mucoadhesive polymer that is able to open tight junctions and allow the paracellular transport of molecules across mucosal delivery of vaccines. Chitosan microparticles and nanoparticles loaded with DNA plasmids were reported to induce protective immune responses in mice [19].

Most dyes used in the pigmentation of textiles, paper, leather, ceramics, cosmetics, inks and food-processing products are derived from azo dyes, which are characterised by the presence of one or more azo groups (-N=N-) in their structure [20]. Approximately 15% of the dyes produced worldwide are lost within waste water during synthesis and processing. This waste represents a great hazard to human and environmental health due to the toxicity of azo dyes [21]. Hence an efficient system for degradation of these dyes is required which is eco-friendly.

The present paper aims to investigate chitin and chitosan production using *Aspergillus terreus* CBNRKR KF529976 grown in three different traditional culture media, synthesis of their bionanocomposites and their ability to degrade the dye-methylene blue.

EXPERIMENTAL SECTION

Isolation and characterization of Marine fungus

Collection of Samples

Pichavaram (Lat.11°428'E; Long.79°798`E), Cuddalore (dt) of Tamil nadu is home to the second largest Mangrove forest in the world, is rich in *Avicennia officinalis, Rhizophora mucaronata, Acanthus illicifoliu* and *Excoecaria agallocha* plants. Marine Mangrove sediments were collected from rhizosphere as well as non rhizosphere region of various parts of Pichavaram. The surface layer of the sediment was removed and the central portions of sediments were transferred into sterile plastic bags.

Isolation of fungi

The samples were taken separately for serial dilution. Ten grams of sample was suspended in 90 ml of sterile distilled water. The suspension was considered as 10^{-1} dilution. About 0.1 ml of the serially diluted sample was spread over the Potato Dextrose Agar (Potato Infusion 200, Dextrose 20, Agar 15 g/L) pH was adjusted to 5.6 ± 0.2.The medium was supplemented with 20 µg ml^{-1} Ciproflaxin to minimize the fungal and yeast contaminations respectively . After inoculation, the plates were incubated in an inverted position for 5-7 days at 25 ± 2^0C.

Microscopic Observation

The fungal isolates were observed and the colony morphology was recorded with respect to color, shape, size and nature of colony. Fungal isolates were microscopically characterized by Lactophenol Cotton Blue mounting. The cell morphology was recorded with respect to spore chain morphology, hyphae and mycelium structure.

Extraction and characterization of Chitin and Chitosan

Culture medium

A. terreus CBNRKR KF529976 was grown, for chitin and chitosan production, in three different culture media: a) Sabouraud sucrose (SS broth)-(bacteriological peptone (10 g) and sucrose (20 g) per litre of distilled water, pH 5.7); b) Hesseltine and Anderson (HA medium)- (glucose (40 g); asparagine (2 g); chloridrate of thiamine (0.05 mg); potassium phosphate (0.50 g) and magnesium sulphate (0.25 g) per litre of distilled water, pH 5.2); c) Andrade et al.(AD medium) (2000)- (glucose (60 g); asparagine (3 g); chloridrate of thiamine (0.08 mg); potassium phosphate (0.50 g) and magnesium sulphate (0.25 g) per litre of distilled water, pH 5.1)

Microbiological methods

Growth profile. The sporangioles of *A. terreus* CBNRKR KF529976 were harvested from cultures grown for seven days at 28ºC on Petri dishes containing PDA medium. A suspension was prepared and adjusted to 10^8 sporangioles/mL, using a hematocytometer for counting. For fungal submerse cultivation, 10 mL sporangioles suspension (10^8sporangioles/mL) were inoculated in Erlenmeyer flask of 1000 mL containing 290 mL of culture media, and the flasks were incubated at 28ºC in an orbital shaker at 150 rpm, during 96 hrs. The mycelia were harvested, washed twice in distilled and deionised water by filtration, utilizing a silkscreen nylon membrane (120 F), and were submitted to lyophilization process. After lyophilization the biomass was maintained in a vacuum dissecator until constant weight.

Chitin and chitosan extraction. The process of extraction involved deproteination with 2% w/v sodium hydroxide solution (30:1 v/w, 90ºC, 2 hrs), separation of alkali insoluble fraction (AIF) by centrifugation (4000 rpm,15 min), extraction of chitosan from AIF under reflux (10% v/v acetic acid 40:1 v/w, 60ºC, 6 hrs), separation of crude chitin by centrifugation (4000 xg, 15 min) and precipitation of chitosan from the extract at pH 9.0, adjusted with a 4 M NaOH solution. Crude chitin and chitosan were washed on a coarse sintered-glass funnel with distilled water, ethanol and acetone and air-dried at 20ºC [22].

Chitin and chitosan characterization

Infrared spectroscopy (Deacetylation degree – DD %).

The degree of deacetylation for microbial chitin and chitosan were determined using the infrared spectroscopy using the absorbance ratio A1655/A3450 and calculated according to equation [23]: A (%) = (A1655/A3450) x 100 / 1.33
Two milligrams sample of fungal chitin and chitosan, which had been dried overnight at 60ºC under reduced pressure were thoroughly blended with 100 mg of KBr, to produce 0.5 mm thick disks. The disks were dried for 24 hrs at 110ºC under reduced pressure. Infrared spectrometer was recorded with a Bruker 66 Spectrometer, using a 100 mg KBr disks for reference. The intensity of maximum absorption bands were determined by the baseline method.

Preparation, characterization of Chitin Bionanocomposites

Preparation of AgNPs

Briefly, 0.50 g of silver-containing glass powder was dispersed in 50 mL of an aqueous solution of 0.25, 1, or 4.0 wt% glucose in a 100 mL glass vial. The mixture was at 121° C and 200 kPa for 20 min. The mixture was then gradually cooled to room temperature and centrifuged at 3000 rpm for 10 min. The supernatant containing the Ag NP suspension was removed and stored in the dark at 4° C.

Preparation of Ag NP/ Chitin Composites.

In this study, 10 mg of chitin (<5% DAc) was added to 1 mL of each Ag NPs suspension (about 60 μg/mL). The mixture was mixed well (at pH 7.0) on a shaker for 30 min. The insoluble Ag NP/chitin composites were centrifuged at 6000 rpm for 10 min. The centrifuged composites were washed twice with distilled water by centrifugation at 6000 rpm for 10 min. The washed composites were dried up at 70ºC on a blockheater for 2 h.

Preparation, characterization of Chitosan Bionanocomposites

Preparation of silver-chitosan nanocomposites

A solution of chitosan (1 - 3 mg/ml) in acetic acid solution (1 - 2 %) was first prepared. Due to the poor solubility of chitosan, the mixture was vortexed to achieve complete dissolution, and then kept overnight at room temperature. The solution was filtered through a 0.22 µm millipore syringe filter to remove any impurity before use. Silver-chitosan nanocomposites were obtained by chemical reduction of the silver salt to yield the corresponding zero valent silver nanoparticles with $NaBH_4$. To ensure complete reduction, the concentration of $NaBH_4$ was 10 times that of the silver salt. The silver nanoparticles were separated by centrifugation at 15000 rpm and dried at 60 ºC for 24 h on a Petri dish, yielding a thin layer.

Characterization of nanoparticles

Scanning electron microscope (JEOL/EO, JSM-6390, Japan, magnification range 1500, acceleration voltage 20 kv) was used to evaluate the surface and shape characteristics of the particles after prior coating with gold. Elemental film composition was analyzed using Energy Dispersive Spectrometer (JEOL, JED-2300) at SAIF, Kochi, India. UV-Vis absorption spectra of the samples were recorded in the wavelength range of 300 to 500 nm using UV

spectrophotometer (UV-Visible Perkin ElmerLambda) at the Center for Bioscience and Nanoscience Research, Coimbatore, India.

Comparative Study-Antimicrobial Activity of the polysaccharides and their bionanocomposites
Preparation of Chitin and Chitosan Solution
Chitin and Chitosan solution 1% (w/v) was prepared in 1% (v/v) acetic solution. The chitosan solutions and chitin suspensions were stirred overnight at room temperature, and the chitosan solutions were filtered using miracloth to remove potential impurities. Then solutions were then diluted by physiologic serum (0.9% NaCl solution) to get final concentration of 0.1% (w/v^{-1}). pH of the solution was adjusted to 5.5 by addition of 2M NaOH and the solutions were autoclaved at 121º c for 20 mins.

Antimicrobial Activity
The antibacterial activity of the polysaccharides and nanoparticles was evaluated against *E.coli, S.aureus, C.albicans and K.pneumoniea* by the agar diffusion method with Mueller Hilton agar as the medium. The four microbial cultures were procured from Department of Microbiology, Maharaja Co-education Arts and Science College, Erode. An aliquot of polysaccharide solution and nanoparticle dispersion (10 µl) was added into each of two wells in a plate, and then incubated for 24 h at 37°C. Amoxicillin was used as reference standard.

Photo catalytic Degradation of Dye
Typically 10mg of Methylene Blue dye was added to 1000 mL of double distilled water used as stock solution. About 10 mg of synthesized Chitin and Chitosan Nanoparticles were added to 100 mL of dye solutions. A control was also maintained without addition of silver nanoparticles. Before exposing to irradiation, the reaction suspension was well mixed by being magnetically stirred for 30min to clearly make the equilibrium of the working solution. Afterwards, the dispersion was put under the sunlight and monitored from morning to evening sunset. At specific time intervals, aliquots of 2-3 mL suspension were filtered and used to evaluate the photocatalytic degradation of dye. The absorbance spectrum of the supernatant was subsequently measured using UV-Vis spectrophotometer at the different wavelength. Concentration of dye during degradation was calculated by the absorbance value at 660 nm.

Percentage of dye degradation was estimated by the following formula:

$$\% \text{ Decolourization} = 100 \times [(C_0 - C)/ C_0]$$

Where C_0 is the initial concentration of dye solution and C is the concentration of dye solution after photocatalytic degradation.

RESULTS AND DISCUSSION

Morphological identification of the fungal isolates obtained from the soil sample
Fungi also represent a dominant component of the soil microflora in terms of biomass. The isolated fungi were purified by repeated sub-culturing on the Potato Dextrose Agar medium at regular intervals and incubating at 29°C. The isolates were identified based on the colony morphology, microscopic observation and molecular identification [24, 25].

Aspergillus sps colonies have granular appearance, are brownish in colour and the colour darkens with time. Reverse side of the fungi is also brown in colour. Microscopic identification of the fungus showed compact, biseriate conidial heads and hyaline septate hyphea. Smooth elliptical conidia form long chains.

Culture-independent molecular techniques, comprising of direct DNA extraction from soil followed by PCR and electrophoresis or cloning, have been introduced to investigate soil fungal communities [26]. The identification was done based on 18S rRNA gene sequencing. The 18S rRNA sequences of the isolates were compared with the data present in NCBI. The BLASTn of the isolates was showing 100% homology with *Aspergillus* spp. The sequence was submitted to the Gene Bank under the accession number KF529976.

Extraction and characterization of Chitin and Chitosan

Biomass Production

The growth of the fungus *A. terreus* CBNRKR KF529976 in three different media was observed for 14days at RT. The highest growth rate was with Henderson and Anderson medium with a mycelial dry weight of 24.83g/L while the next highest growth rate was observed with Andarde et al medium 21.42g/L followed by Sabouraud sucrose broth with a mycelia dry weight of 16.76g/L. These results are in agreement with *C. elegans* (UCP 542) grown in Hesseltine and Anderson medium with a biomass yield of 10.3 g/L. The result is also in agreement with the growth curve of *C. elegans* (IFM 46109) established by Andrade et al. (2000) and Franco et al. (2005) using the same culture medium, which referred biomass yield of 11.0 and 11.6 g/L, respectively [1, 6].

Chitin and Chitosan Extraction

The best yields of the polysaccharides (mg per gram of dry mycelia biomass) are obtained with Sabouraud sucrose broth for chitosan (48.32 mg/g or 4.8%) and for chitin (344.8 mg/g or 34%) In addition, the next best yield of chitin and chitosan per 1 g of biomass from *A. terreus* CBNRKR KF529976 are obtained using Henderson and Anderson medium and Andrade et al. medium for chitin 122 mg/g or 12% and 80.76 mg/g or 8% and chitosan 24.96 mg/g or 2% and 49.95 mg/g or 4% respectively. The results are superior to those reported by Andrade et al. (2000) and Franco et al. (2005) which proposed *C. elegans* as a promising chitin source, obtaining higher chitin yields 280 mg/g and 240 mg/g, respectively [1,6]. Andrade *et al*.,reported a maximum chitin yield of 239 mg/g from *Mucor javanicus* [4].

Chitin and chitosan characterization

Infrared spectroscopy

In this study, the IR spectra of the three isolated samples of chitin and chitosan were analyzed and compared with the IR spectrum of commercial chitosan (Fig. 1-8).

The presence of bands at 3339 cm^{-1} in chitin samples from SS broth, 3281 cm^{-1} in HA Medium and 3434 cm^{-1} from AD medium and in the extracted Chitosan samples from SS broth at 3425 cm^{-1}, HA medium in 3438 cm^{-1}, AD medium in 3370 cm^{-1} indicate strong dimeric OH stretch. Chitin from SS broth have spectra in the Amide I region 1655 cm^{-1}, those from HA medium showed spectra at 1657 cm^{-1} and those from AD medium showed spectra at 1642 cm^{-1} , while Chitosan from SS broth have bands at 1631 cm^{-1},HA medium at 1638 cm^{-1},AD medium at 1638 cm^{-1} indicating presence of C=C stretch. The peaks around 1557 cm^{-1} in chitin samples from SS broth, 1558 cm^{-1} in HA medium and 1556 cm^{-1} in AD medium and bands around 1552 cm^{-1} in Chitosan samples from HA medium and 1551 cm^{-1} in AD medium are due to stretching vibrations of C-O group (Amide II). Amide III region presence was indicated by bands at 1378 cm^{-1} in chitin samples from SS broth, 1376 cm^{-1} in HA medium and 1377 cm^{-1} in AD medium. Chitosan samples from SS broth showed bands at 1415 cm^{-1}, HA medium at 1413 cm^{-1} and 1410 cm^{-1} indicating aromatic C-C stretch. Commercial Chitin samples showed similarity with the extracted samples by exhibiting bands at 3473 cm^{-1}, 1653 cm^{-1}, 1560 cm^{-1} and 1380 cm^{-1}. Similar results were obtained with commercial Chitosan samples which revealed bands at 3429 cm^{-1}, 1651 cm^{-1} and 1417cm^{-1}. The results are in agreement with Shigemasa and Minami [27] Andrade *et al*. and Franco *et al.* which reported that chitin structure containing two types of amide group and both form C = O N-H intermolecular bonds, but one is also an acceptor for the CH_2OH group [4, 6].

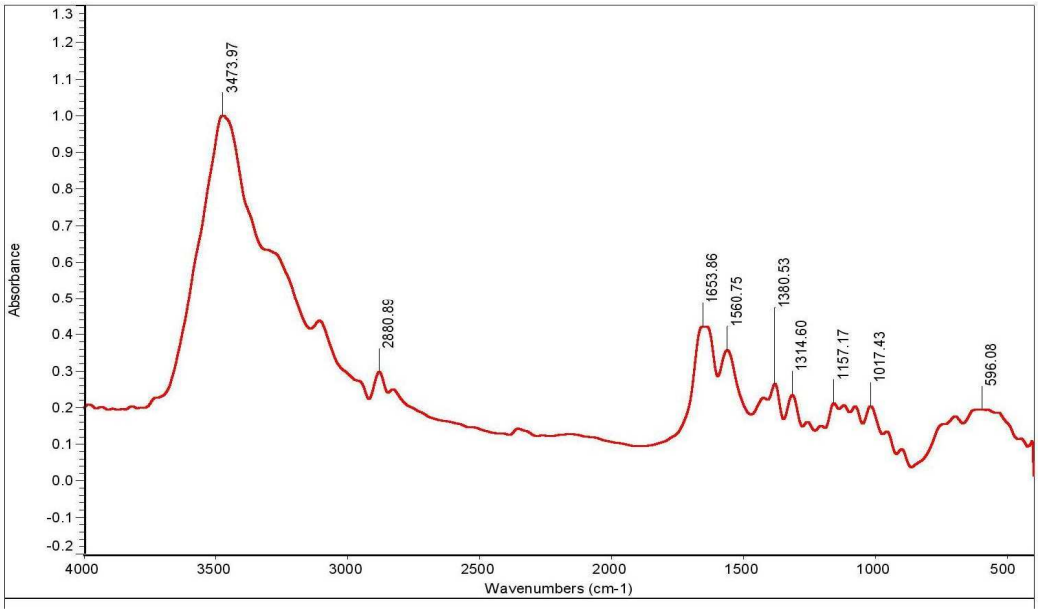

Fig.1 FTIR Spectrum of Commercial Chitin

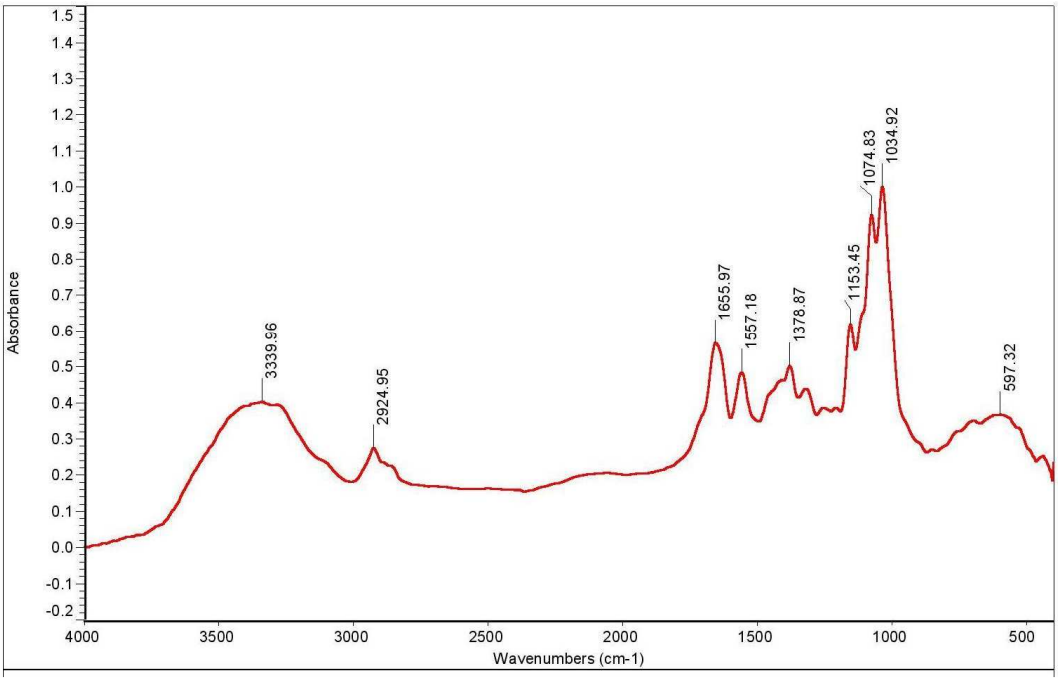

Fig. 2 FTIR Spectrum of Chitin extracted from *Aspergillus terreus* CBNRKR KF529976 using Sabouraud Sucrose broth

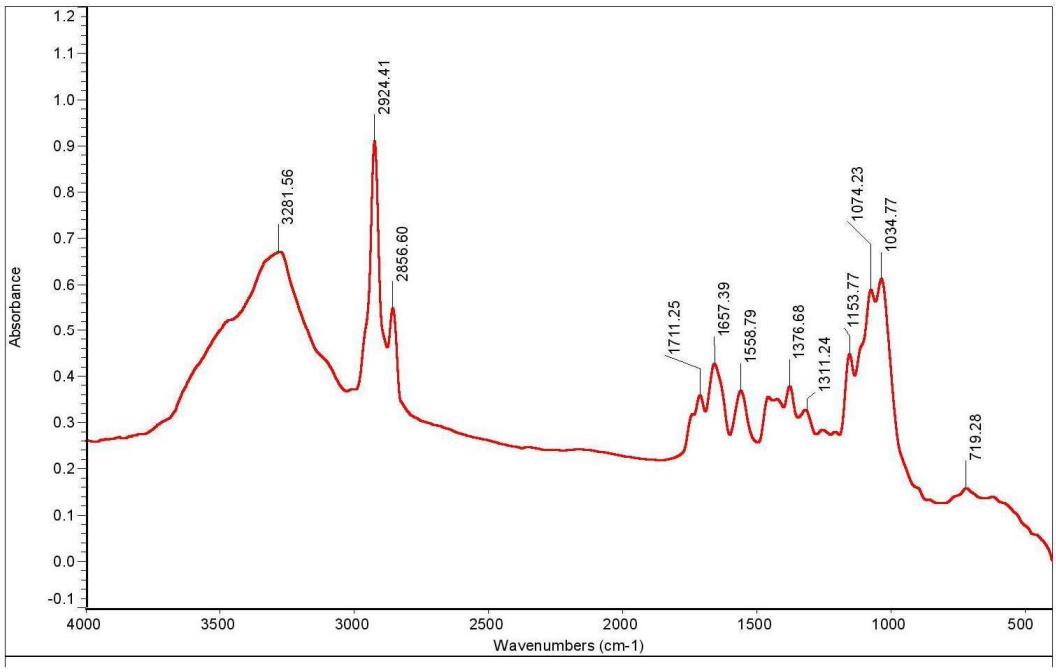

Fig.3 FTIR Spectrum of Chitin extracted from *Aspergillus terreus* CBNRKR KF529976 using Henderson and Anderson medium

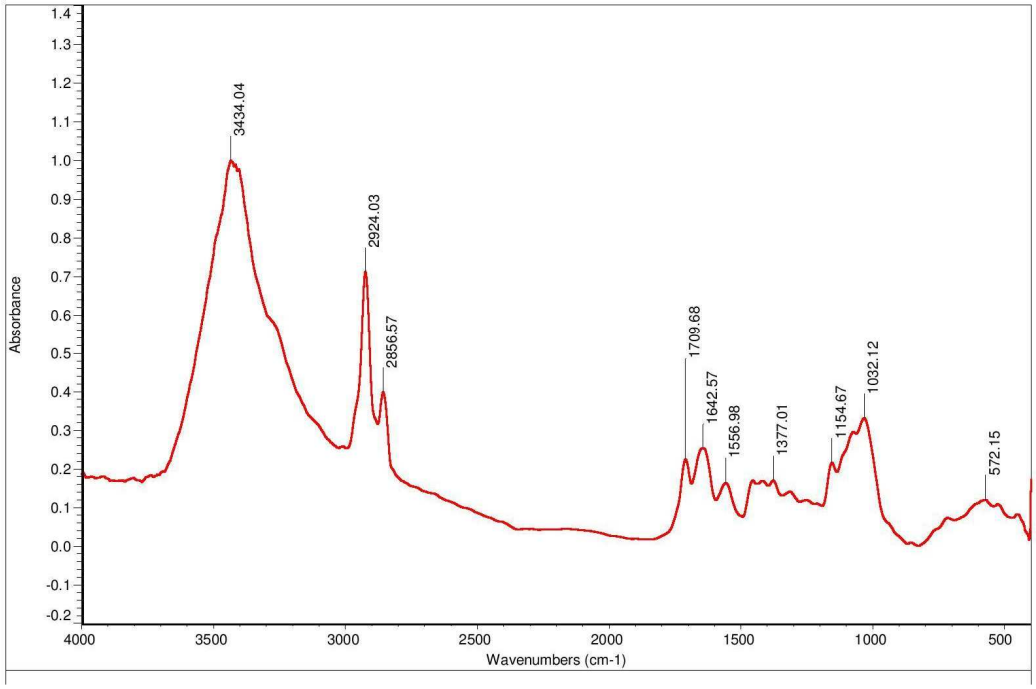

Fig.4 FTIR Spectrum of Chitin extracted from *Aspergillus terreus* CBNRKR KF529976 using Andarde et al medium

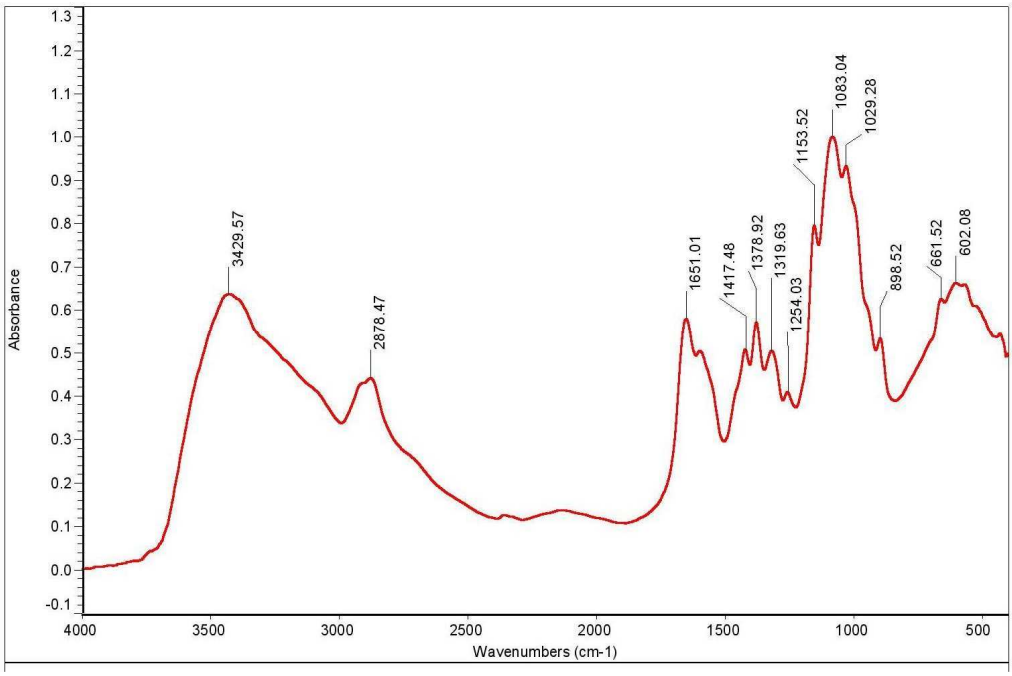

Fig.5 FTIR Spectrum of Commercial Chitosan

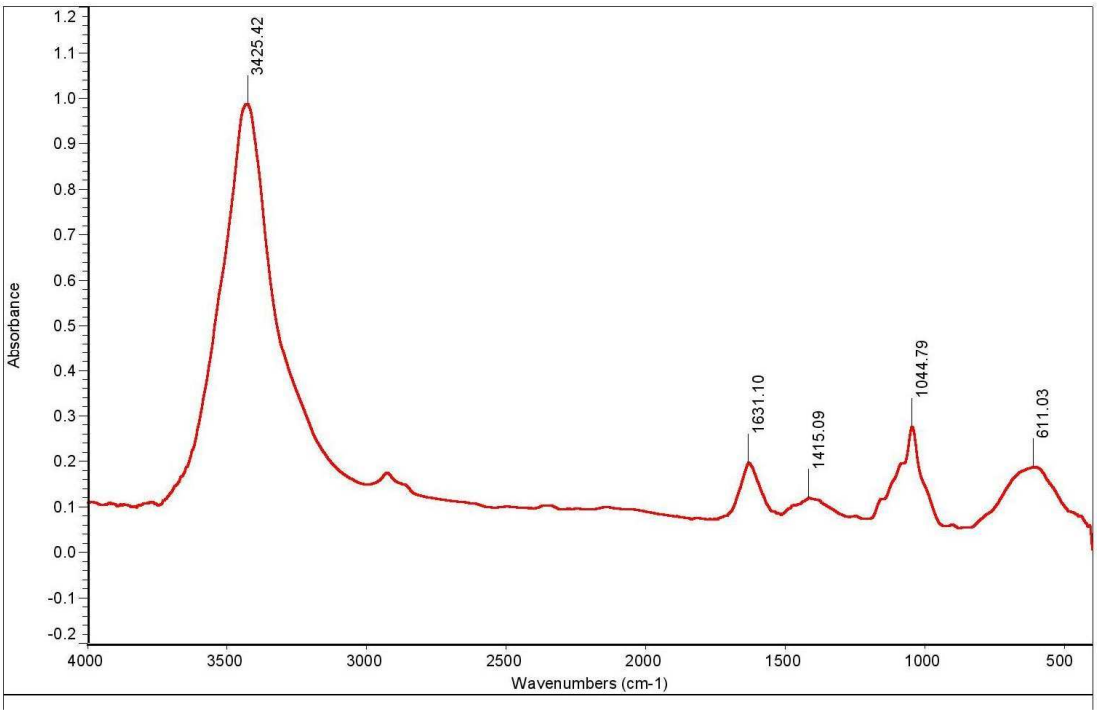

Fig.6 FTIR Spectrum of Chitosan extracted from *Aspergillus terreus* CBNRKR KF529976 using Sabouraud Sucrose broth

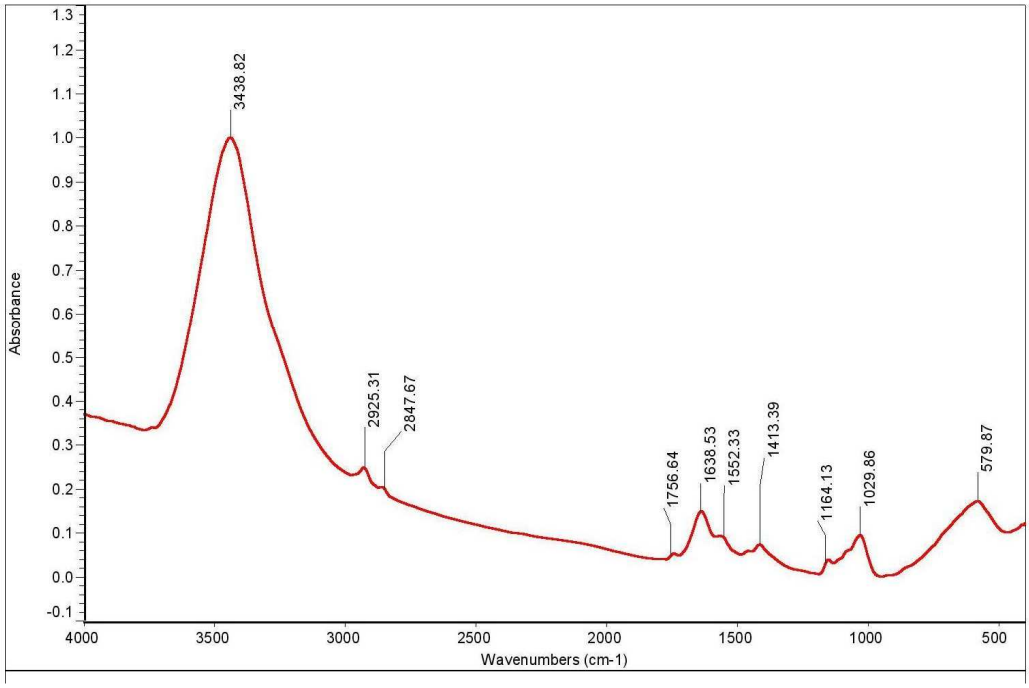

Fig.7 FTIR Spectrum of Chitosan extracted from *Aspergillus terreus* CBNRKR KF529976 using Henderson and Anderson medium

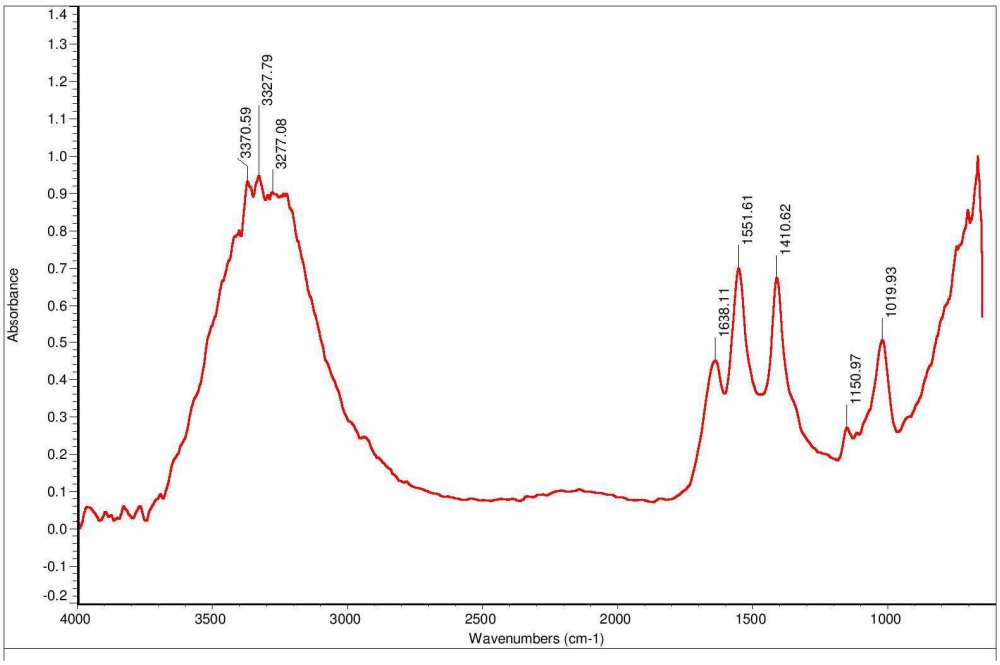

Fig.8 FTIR Spectrum of Chitosan extracted from *Aspergillus terreus* CBNRKR KF529976 using Andarde et al medium

Deacetylation degree – DD %

In the present study, chitin and chitosan from *A.terreus* grown in Sabouraud sucrose broth was found to have 98% DD and 11% DD, respectively. Chitin and Chitosan grown in Hesseltine and Anderson medium were found to have 49% DD and 10% DD. Andarde medium provided chitin and chitosan with 16% DD and 44% DD. Amorim *et al.*,

Pochanavanich and Suntornsuk and Franco *et al.*, reported deacetylation degree of chitosan from fungi between 80 to 90% DD [6,8].

Synthesis of Bionanocomposites from Extracted Polysaccharides

Visual Appearance

Nano-science is the study of phenomena and manipulation of materials at atomic molecular and macromolecular scales. Since the highest yields for both the polysaccharides were obtained from Sabouaraud Sucrose broth, the same were used for synthesis of bionanocomposites. Chitin (<5% DAc) was added as stabilizer to the AgNPs suspensions to remove the generated caramel and to prevent agglomeration and precipitation of the AgNPs. The composites so formed were twice with water to remove the caramel. The composites were brown coloured. That brown colour indicated that surface plasmon vibrations, typical of silver nanoparticles. Similarly, addition of $NaBH_4$ leads to reduction of $AgNO_3$ whereby chitosan is added as stabilizer for synthesis of AgNP's. The AgNP's so produced are dark brown in colour.

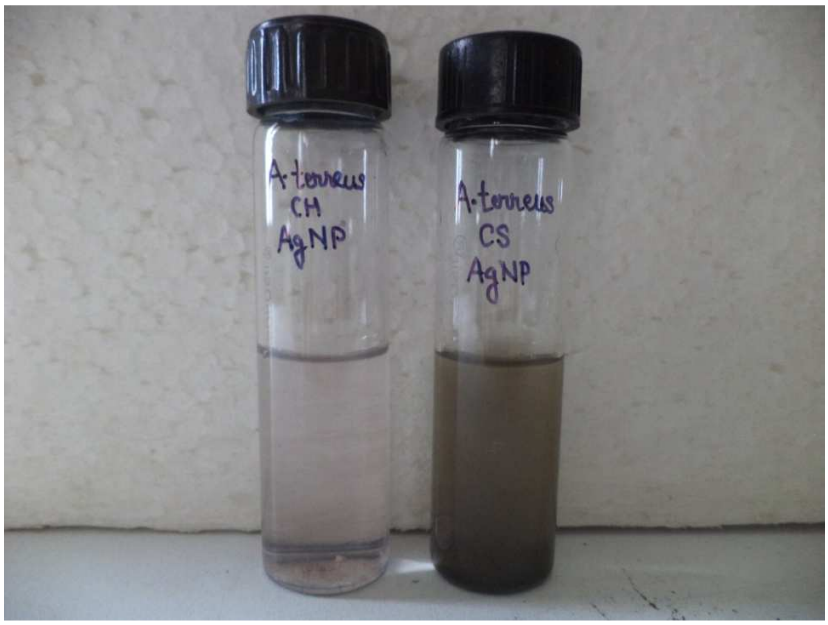

Fig.9 Bionanocomposites synthesized from Chitin and Chitosan extracted from *Aspergillus terreus* CBNRKR KF529976

UV-VIS Spectroscopy

Chitin Bionanocomposites

The UV-Vis spectra were recorded for the supernatants of the post-reaction mixtures in which chitin reacted with the AgNP. The peak was observed at 390 nm in case of the *A.terreus*. This is representative of the spherical Ag NPs used in this work. Vinh *et al* (2013) reported peak at 390.5 nm which representative of the spherical Chitin AgNP's used in their work [29] (Fig.10)

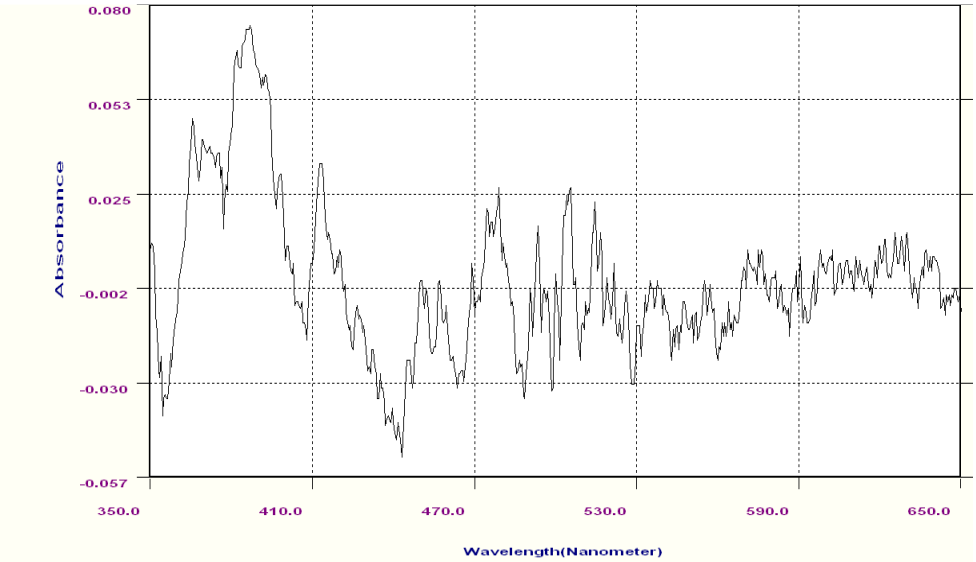

Fig.10 UV-Visible Spectroscopy of Chitin AgNP

Chitosan Bionanocomposites

During the $NaBH_4$ reducing process, color of the $AgNO_3$ / Chitosan suspensions changed from colorless to brown. The color changes due to the formation of Ag NPs are proven by UV-visible spectra. After adding $NaBH_4$, the maximum absorbance bands for *A.terreus* CH AgNP were detected at 450 nm respectively. Honary *et al* reported similar results with peaks in the range of 400-420 nm which is typical of surface Plasmon band indicating formation of Silver nanocomposites with Chitosan [30]. (Fig.11)

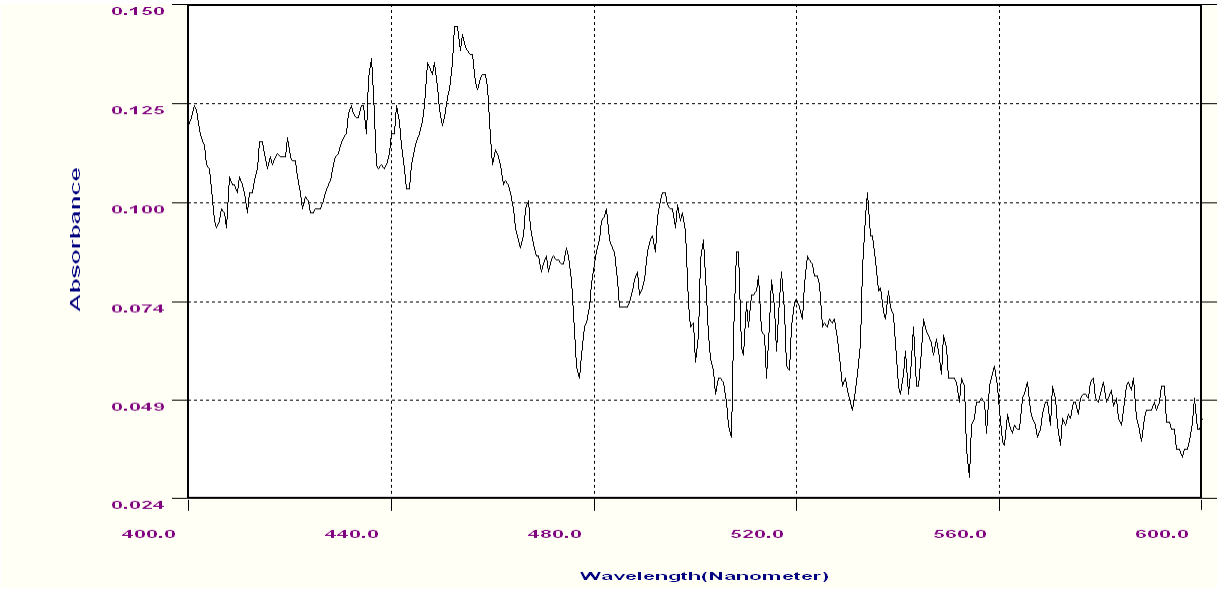

Fig.11 UV-Visible Spectroscopy of Chitosan AgNP

Surface topography by Scanning Electron Microscopy

Chitin Bionanocomposites

The structural morphology of Chitin bionanocomposite was characterized by Scanning Electron Microscope. The samples were prepared by taking thin sections with a scalpel blade. The sections were platinum sputtered in vacuum. Chitins AgNP'S exhibited smaller powder particle size and a nanoscale fiber-like surface structure indicating that they are well dispersed. The pore size was reported to be in the range of 10-30 µm. Sowmya *et al* reported that SEM

images of the β-chitin/nBGC composite scaffold were found to have porous structures with smooth surface morphology. The pore size of β-chitin/nBGC composite scaffold is in the range of 100-150 µm [31] (Fig.12)

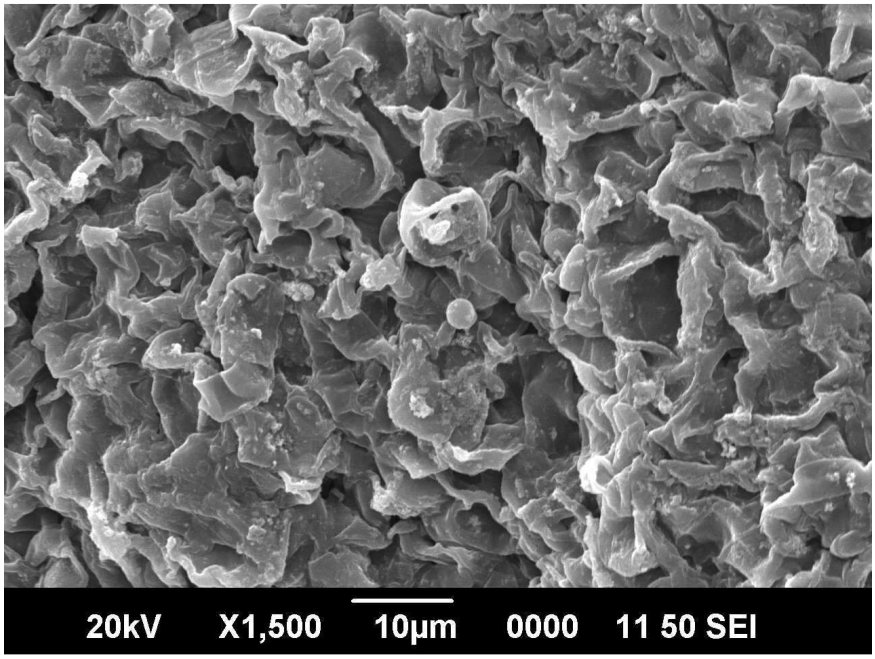

Fig.12 SEM Image showing the porous surface of Chitin AgNP

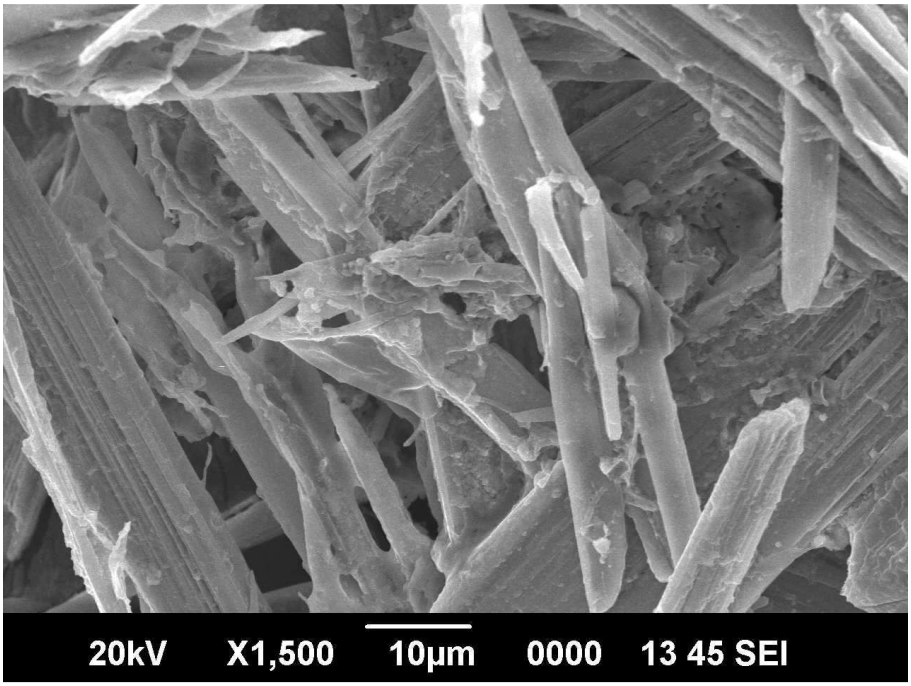

Fig.13 SEM Image showing the needle shaped surface of Chitosan AgNP

Chitosan Bionanocomposites

Surface morphology of polymer and Ag BNCs are illustrated in Figure 13. The samples for SEM analysis were prepared by solvent casting on petridish. From the SEM image, Ag/Chitosan BNCs showed strong needle shaped

structures. Bin Ahmad *et al* [32] stated that the SEM image of Ag/Cts BNCs in their work showed show layered surfaces with small flakes

Elemental Composition Analysis

Chitin Bionanocomposites

Energy-dispersive Spectroscopy (EDS) analysis was performed on JEOL, JED-2300. Thin section of scaffold was placed on carbon tape coated stub. The sample was then platinum coated. The EDS spectrum (Fig.) of *A.terreus* CH AgNP composite shows the peaks of C K, O K and Ag L. The atomic ratio of was found to be 87:11:0.75 wt %. Similar results were suggested by Sowmya *et al* for the EDS spectrum of β-chitin/nBGC composite scaffold shows the peaks of Ca, P, Si and O. The atomic ratio of Si:Ca:P:O was found to be 29:13:8:48 wt %. (Fig.14) [31].

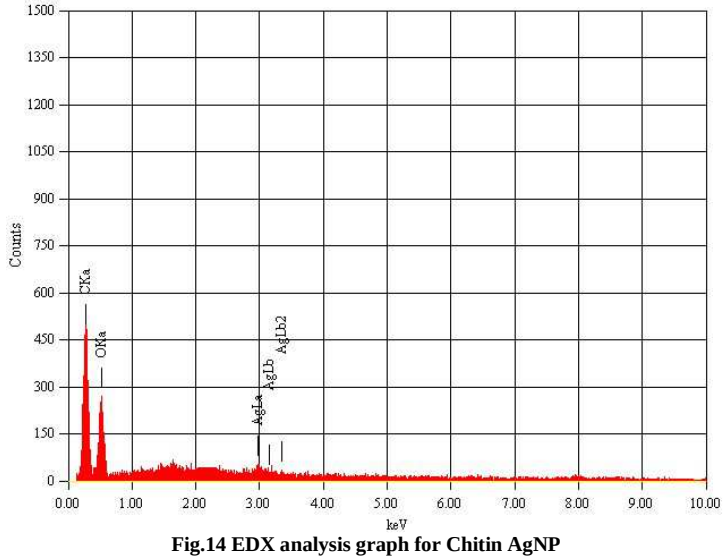

Fig.14 EDX analysis graph for Chitin AgNP

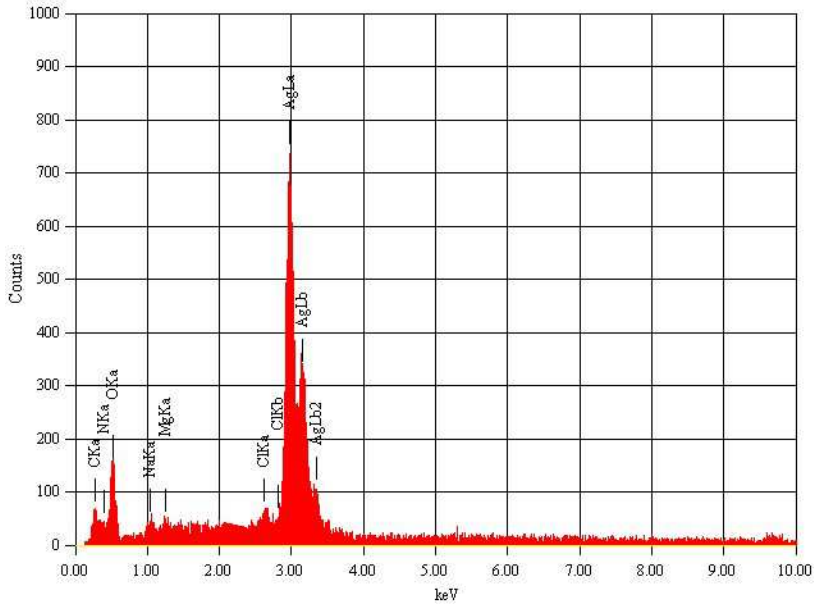

Fig.15 EDX analysis graph for Chitosan AgNP

Chitosan Bionanocomposites

The EDS spectrum (Fig.) of *A.terreus* CS AgNP composite shows the peaks of C K, NK, O K, Na K, Mg K, Cl K and Ag L. The atomic ratio of was found to be 20:3:19:1:1:0.46:53 wt %. This represents a very good adsorption by the large surface area of paramagnetic Ag, with good stability and high storage of the chitosan layer. The EDX spectra for the CS Ag BNCs had confirmed the presence of elemental compounds in the CS and Ag NPs without any impurity peaks. All the samples tested for EDX were coated with gold to prevent the accumulation of static electric fields during imaging [32]. The Ag BNCs film morphologies were dependent on several factors including polymer solubility, solvent evaporation, total thickness, molecular weight and surface composition [33]. (Fig.15)

Comparative Study-Antimicrobial Activity of the polysaccharides and their bionanocomposites

Inhibition zone values were obtained from the polysaccharides solution and the synthesized Ag NPs tested against Gram-negative bacteria (*E. coli and K.pneumoniea*) and Gram-positive (*S. aureus and C.albicans*). Figure (16-19) illustrate the images of each inhibition zones for the samples for antibacterial activity studies. Results of the inhibition zones are presented as average values in mm in the Table (1, 2).

The table shows that the Ag NPs had high and similar antibacterial activity against Gram-positive and Gram-negative bacteria. Due to their particle size, Ag NPs can easily reach the nuclear content of bacteria by disrupt the membranes of bacteria. The particle size smaller than 10 nm interact with bacteria and generate electronic effects that improve the reactivity of Ag NPs. The antibacterial activity of Chitin solution was found to be less than the Chitin AgNP's indicating that the presence of the silver ion thereby increases the antibacterial strength of the polysaccharides. Chitin AgNP's showed comparable antibacterial strengths as the antibiotic disks (Amoxicillin) employed. Similar results were obtained for Chitosan solution and Chitosan AgNP's with comparable antimicrobial activity to the antibiotic disk used.

Koide stated that chitin and chitosan *in vitro* showed antibacterial and anti-yeast activities [34]. One of chitosan derivatives, i.e., N-carboxybutyl chitosan, was tested against 298 cultures of different pathogenic microorganisms that showed bacteriostatic and bactericidal 16 activities, and there were marked morphological alterations in treated microorganisms when examined by electron microscopy according to Muzzarelli *et al* [35].

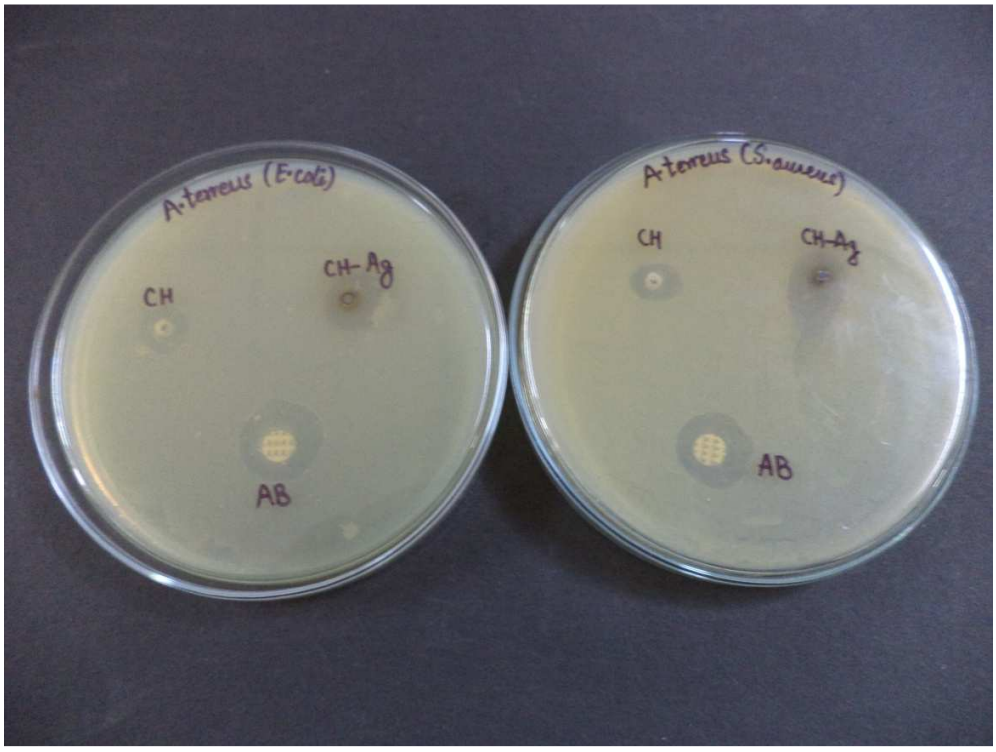

Fig.16 Comparative study of the antimicrobial activity of the polysaccharides (Chitin) and its bionanocomposites

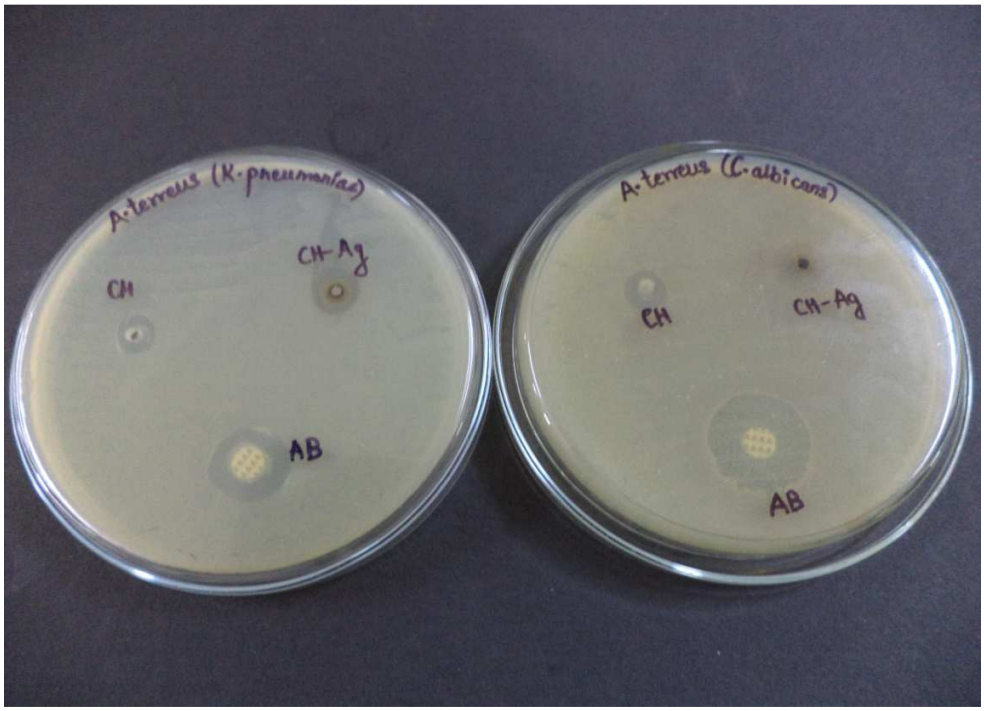

Fig.17 Comparative study of the antimicrobial activity of the polysaccharides (Chitin) and its bionanocomposites

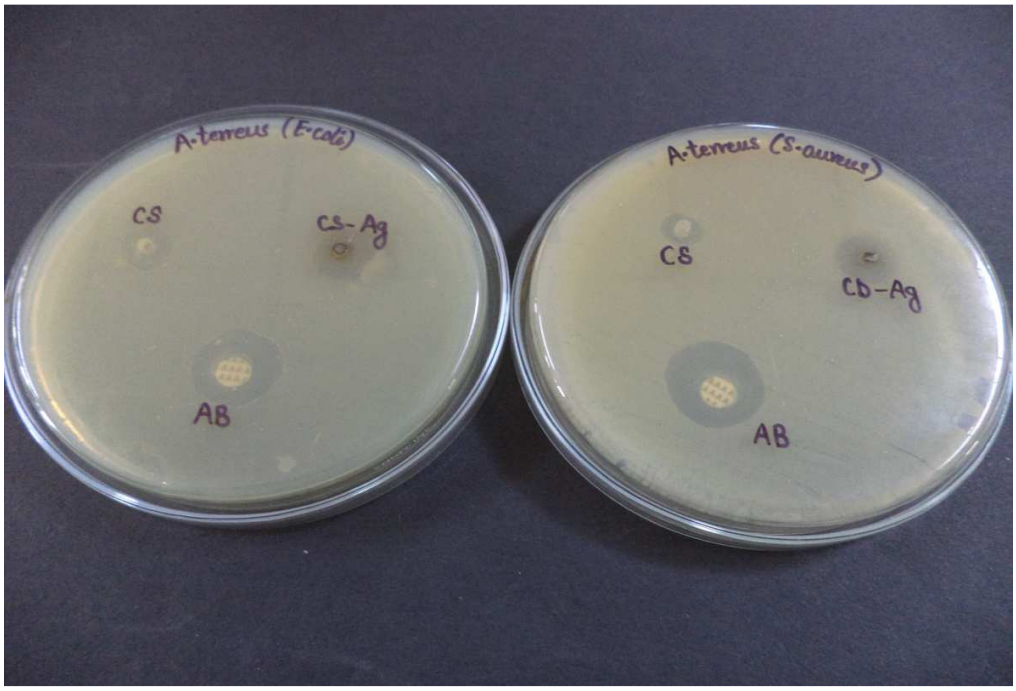

Fig.18 Comparative study of the antimicrobial activity of the polysaccharides (Chitosan) and its bionanocomposites

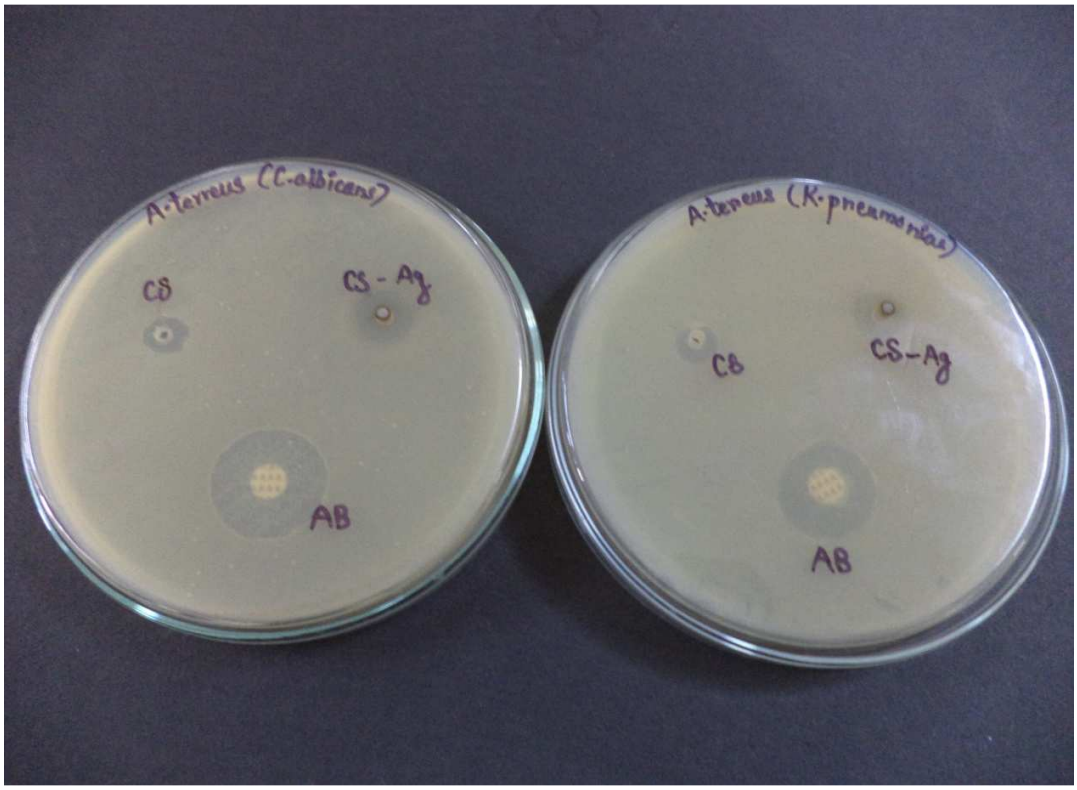

Fig.19 Comparative study of the antimicrobial activity of the polysaccharides (Chitosan) and its bionanocomposites

Table.1.Antimicrobial Activity of the Polysaccharide solution (Chitin- *A.terreus*) and the Bionanocomposites against MDR pathogens (in mm)

	E.coli	*S.aureus*	*K.pneumoniea*	*C.albicans*
Chitin Solution	**10**	**10**	**7**	**9**
Chitin AgNP	**12**	**12**	**10**	**12**
Antibiotic	**18**	**16**	**11**	**20**

Table.2.Antimicrobial Activity of the Polysaccharide solution (Chitosan- *A.terreus*) and the Bionanocomposites against MDR pathogens (in mm)

	E.coli	*S.aureus*	*K.pneumoniea*	*C.albicans*
Chitosan Solution	**10**	**8**	**7**	**8**
Chitosan AgNP	**11**	**12**	**10**	**11**
Antibiotic	**17**	**9**	**16**	**21**

Photocatalytic Degradation of Dye

Visual Observation

Photocatalytic degradation of methylene blue was carried out by using AgNP's synthesized from the bionanocomposites synthesized from Chitin and Chitosan under solar light. Dye degradation was initially identified by color change. The color of dye shows blue color changed into light blue after the 4 h of incubation while exposed to solar light .Thereafter light blue was changed into light sheen of blue. Finally, the degradation process was completed at 72 h and was identified by the change of reaction mixture to colorless. Similar results have been reported by Vanaja *et al*, for degradation of methylene blue by nanoparticles synthesized from *Morinda tinctoria* [36].

UV-VIS Spectrophotometer

Photocatalytic activity of AgNP's synthesized from the extracted Chitin and Chitosan on the degradation of dye was demonstrated by using the dye methylene blue, at different time in the visible region. The absorption spectrum

showed the decreased peaks for methylene blue at different time intervals. The percentage of degradation efficiency of Chitin and Chitosan AgNP was calculated to be 91.5% and 95.5% after 72 hrs. (Fig. 20). Absorption peak for methylene blue dye was centered at 660 nm in visible region which diminished and finally it disappeared which indicates that the dye had been degraded. The percentage of degradation efficiency of silver nanoparticles was calculated as 95.3% at 72h. The degradation percentage was increased as increasing the exposure time of dye silver nanoparticles complex in sunlight [36].

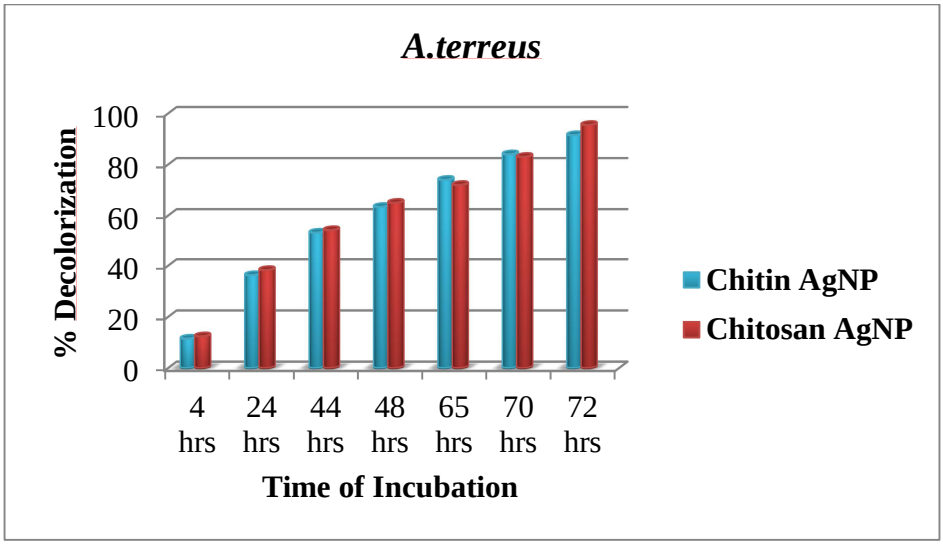

Fig.20 Dye degradation using Chitin and Chitosan AgNP's- *A.terreus*

CONCLUSION

Soil has plethora of microbial diversity which can be used for sustainable development of industrially important products in cost effective manner. These results present a viable methodology for production of the polysaccharides-Chitin and Chitosan from marine fungi. Further results have been used for synthesis of bionanocomposites from both the polysaccharides which have potential application as antibiotics and in bioremediation.

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