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Extraction & Characterization of Chitin & Chitosan from *Bionetria CBNR BKRR*, Synthesis of their Bionanocomposites and Study of their Application

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ABSTRACT: Chitin and chitosan hold a great economic value due to their versatile biological activities and chemical applications, mainly in medical and pharmaceutical. *Bionectria CBNR KRRR*, 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 Andarde et al medium with a mycelial dry weight of 15.21g/L. The best yields of the polysaccharides (mg per gram of dry mycelia biomass) are obtained with Sabouraud sucrose broth for chitosan (204 mg/g or 20%) and for chitin (2189.78 mg/g or 218%).From the SEM image, Chitin AgNP's exhibited smaller powder particle size. Ag/Chitosan BNCs showed show strong needle shaped structures. The EDS spectrum of *Bionectria CBNR KRRR* CS AgNP composite shows the peaks of C K, O K, Cl K and Ag L. The atomic ratio of was found to be 85:13:0.34:1.45 wt %. The EDS spectrum of *Bionectria CBNR KRRR* CS AgNP composite shows the peaks of C O K, and Ag L. The atomic ratio of was found to be 15:17:67 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 94.5% dye inhibition in 72 hours and Chitosan AgNP showed 97.5% inhibition.

KEYWORDS: Bionectria; Chitin; Chitosan; Pichavaram; FTIR; SEM; EDS; Antibacterial Activity; Methylene Blue.

INTRODUCTION

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 (Tan *et al.*, 2002; Pochanavanich and Suntornsuk 2002; Andrade *et al.*, 2003; Synowiecki *et al.*, 2003). 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 (Synowiecki *et al.*, 1997; Pochanavanich and Suntornsuk 2002; Nemtsev *et al.*, 2004). Usually, the Zygomycetes Class has higher amounts of chitin and chitosan in their cell walls when compared to other classes of fungi (Andrade *et al.*, 2003; Campos-Takaki *et al.*, 2005; Franco *et al.*, 2004).

Chitin and chitosan show peculiar properties, such as: biodegradability, biocompatibility, bioactivity, selective permeability, polieletrolic action, chelation, ion exchange

properties, antitumor and antimicrobial activity (Dos Santos *et al.,* 2003; Chung *et al.*, 2004; Yadav *et al*., 2004), and adsorption capacity (Shigemasa *et al.,* 1996; Tharanathan *et al.,* 2003; Franco *et al.*, 2004).

The antimicrobial activity of zerovalent silver is strictly dependent on the surface development of the solid phase. When the solid phase is in a nanoparticle form, the resulting antimicrobial activity can be significantly increased, and smaller Ag NPs may be several orders of magnitude more active than the corresponding bulk solid. Therefore, AgNPs adsorbed onto surfaces of various biomaterials are a potentially great choice when fabricating materials with antimicrobial properties (Sosa *et al.*, 2003; Sondi *et al.*, 2004). In this study, Chitin and Chitosan were used as the solid phase.

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 (Buitron *et al.*,2004). 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 (Sokmen *et al.,* 2001). 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 *Bionectria CBNR KRRR* grown in three different traditional culture media, synthesis of their bionanocomposites , comparative of the antibacterial activity of the polysaccharides and AgNP's against MDR pathogens and their ability to degrade the dye-methylene blue.

MATERIALS & METHODS

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 using hand lens 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.

Isolation and Identification of Test Fungus

Individual fungal colonies were picked and further purified by subculturing on potato dextrose agar medium. Further identity of fungus was confirmed by nuclear ribosomal DNA internal transcribed spacer (ITS) sequencing using ABI-Big Dye Termintor v3.1 Cycle Sequencing Kit in the ABI 3100 automated sequencer by National Fungal Culture Collection of India (NFCCI), Pune, India. ITS region was amplified by using universal fungal primer set, (Forward Primer) 5'-GACTCAACACGGGGAAACT-3' and (Reverse primer) 5'-AGAAA GGAGG TGATC CAGCC-3'. Polymerase chain reaction amplified regions were sequenced. The analysis of nucleotide sequence was done in Blast-n site at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) .The alignment of the sequences was done by using CLUSTALW (www.ebi.ac.uk/clustalw).

Extraction and characterization of Chitin and Chitosan
Culture medium

Bionectria CBNR KRRR 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 *Bionectria CBNR KRRR* 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 (Franco *et al.* 2004).

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 [19]: 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 30 mins 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 & DISCUSSION

Morphological identification of the fungal isolates obtained from the soil sample

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 [20, 21] .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 98% homology with *Bionectria* spp. The sequence was submitted to the Gene Bank under the accession number KF680540. The entirely new species was tentatively named as *CBNR BKRR.*

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.

Bionectria sps have velvety appearance and are whitish in colour on the surface. The reverse of the fungi has distinct yellowish pigmentation. Microscopic identification showed that the fungi are verticilliate. Conidia borne on verticillate conidiophores are hyaline, ellipsoidal, slightly curved, aseptate.

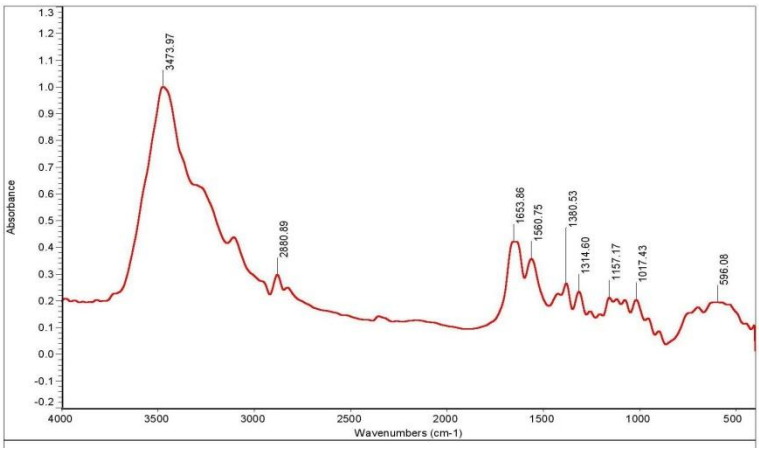

Figure 1 FTIR Spectrum of Commercial Chitin

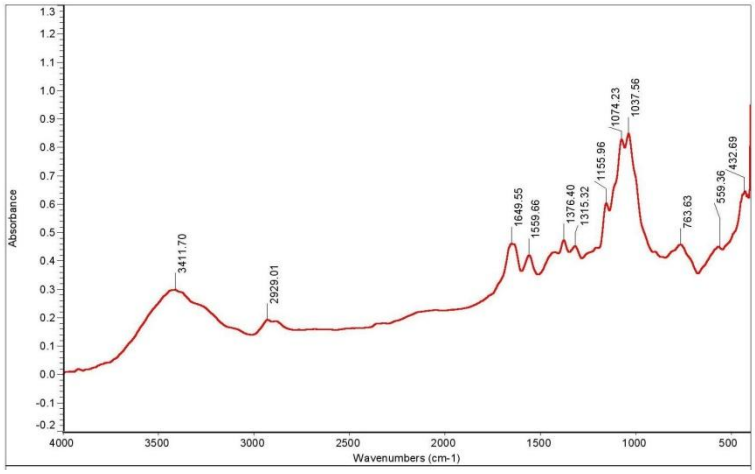

Figure 2 FTIR Spectrum of Chitin extracted from using Sabouraud Sucrose broth

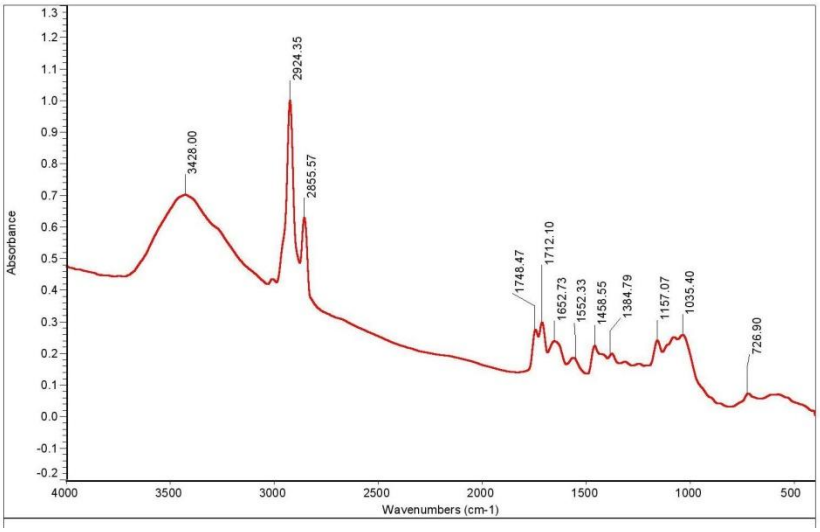

Figure 3 FTIR Spectrum of Chitin extracted from using Henderson and Anderson medium

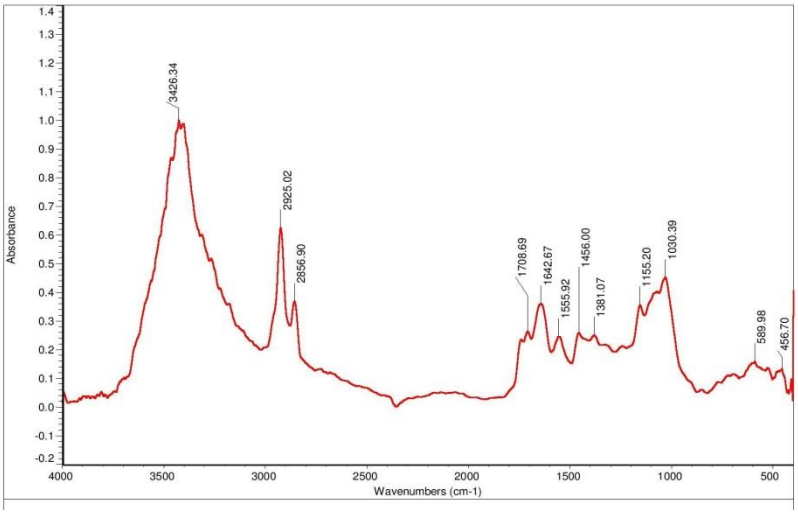

Figure 4 FTIR Spectrum of Chitin extracted from using Andarde et al., medium

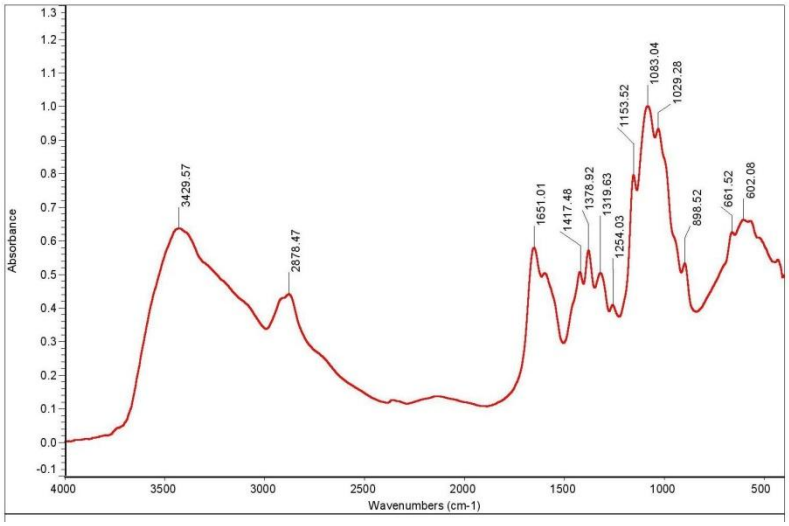

Figure 5 FTIR Spectrum of Commercial Chitosan

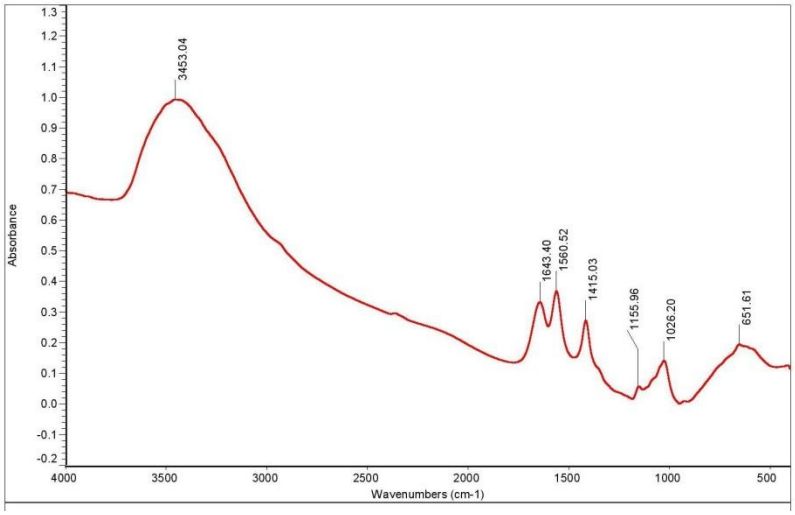

Figure 6 FTIR Spectrum of Chitosan extracted using Sabouraud Sucrose broth

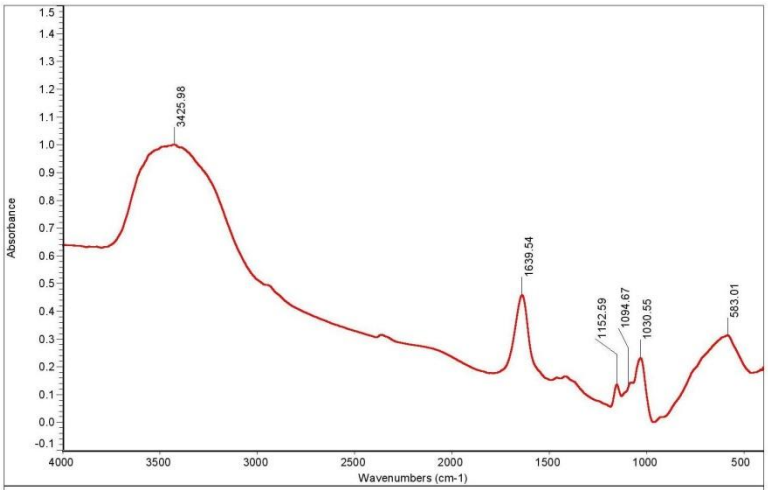

Figure 7 FTIR Spectrum of Chitosan extracted using Henderson and Anderson medium

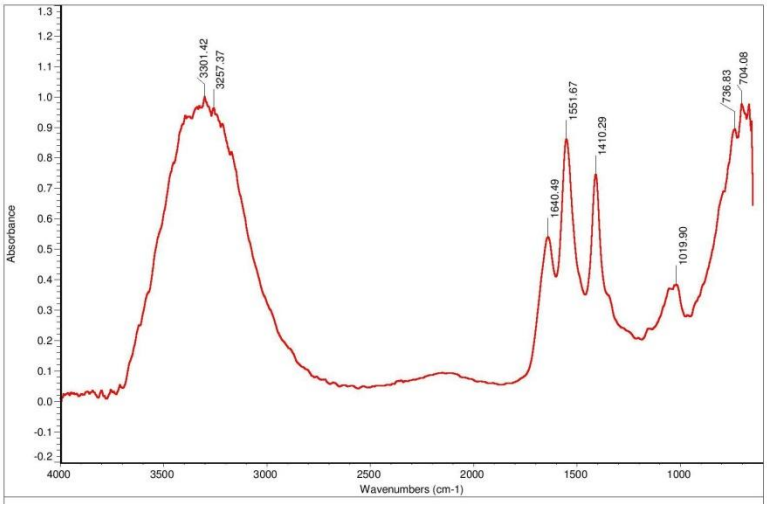

Figure 8 FTIR Spectrum of Chitosan extracted using Andarde et al medium

Figure 9 Bionanocomposites synthesized from Chitin and Chitosan extracted from *Bionectria*

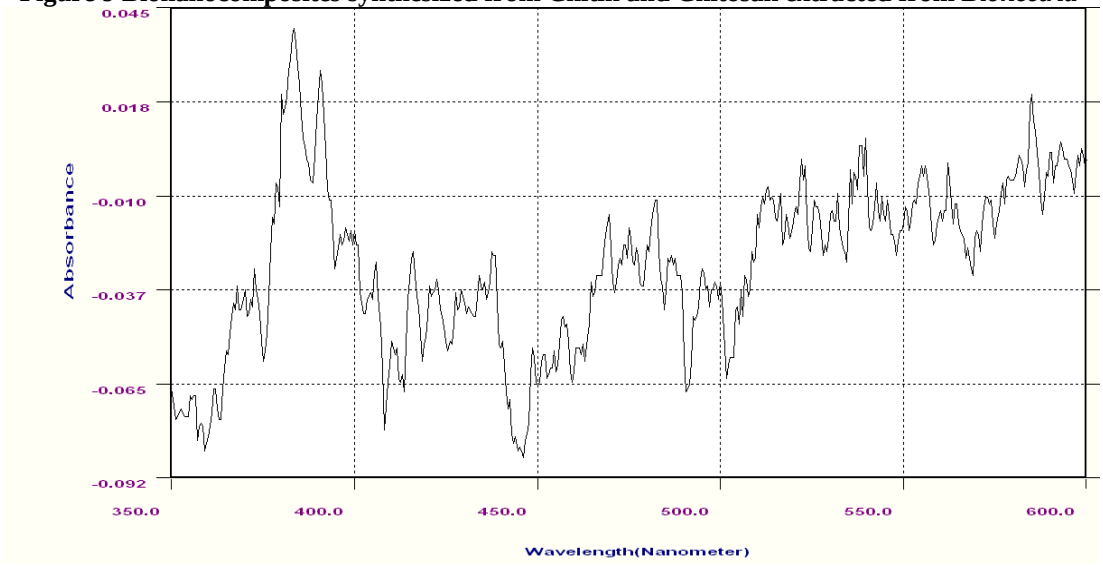

Figure 10 UV-Visible Spectroscopy of Chitin AgNP

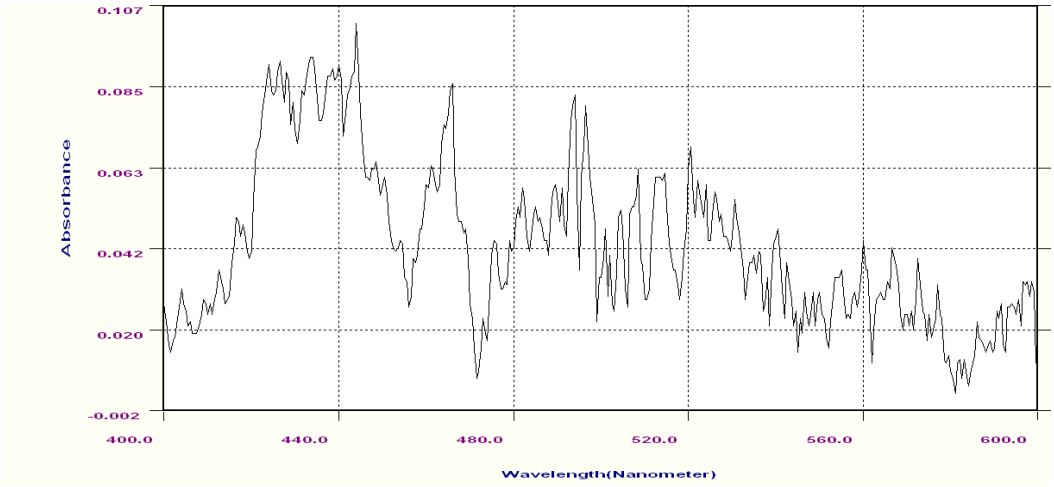

Figure 11 UV-Visible Spectroscopy of Chitosan AgNP

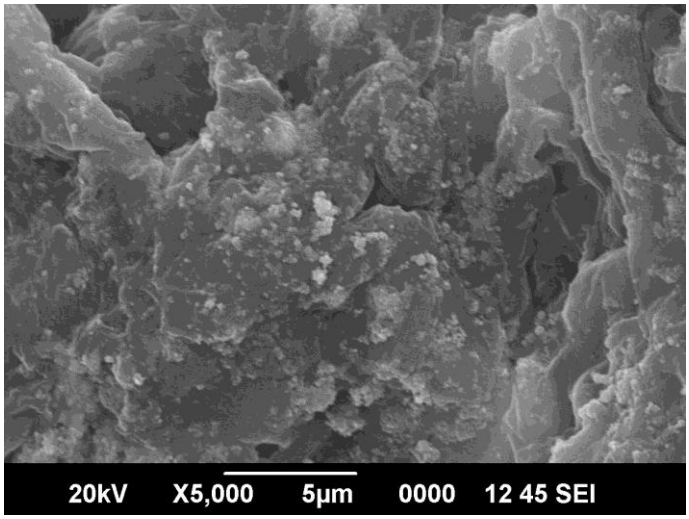

Figure 12 SEM Image showing the porous surface of Chitin AgNP

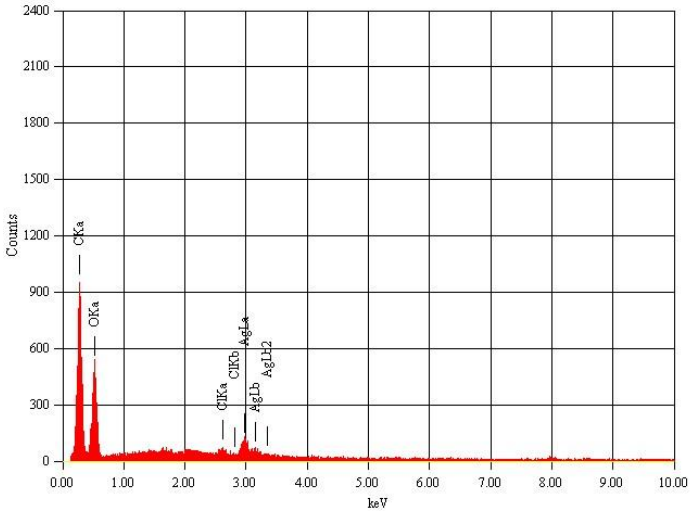

Figure 13 EDX analysis graph for Chitin AgNP

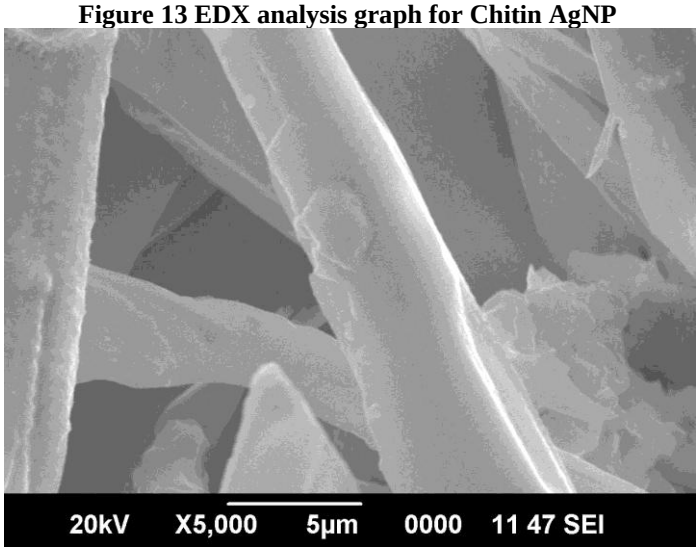

Figure 14 SEM Image showing the needle shaped surface of Chitosan AgNP

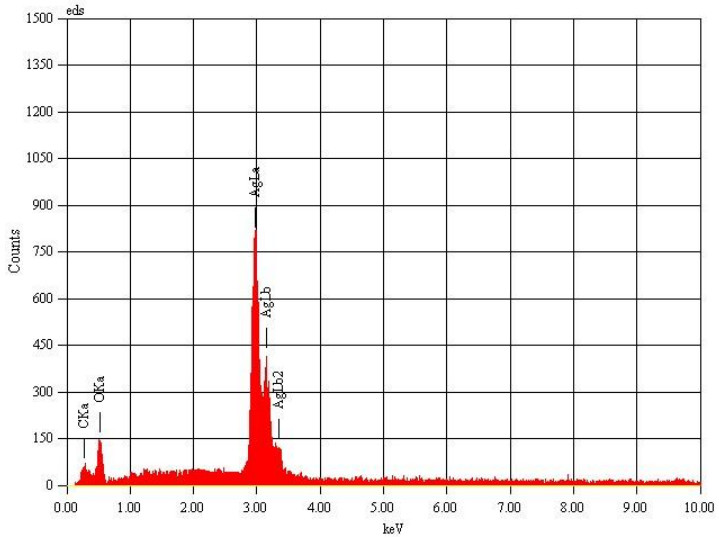

Figure 15 EDX analysis graph for Chitosan AgNP

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

	E.coli	*S.aureus*	*K.pneumoniea*	*C.albicans*
Chitin Solution	11	8	8	10
Chitin AgNP	12	10	10	10
Antibiotic	18	19	10	12

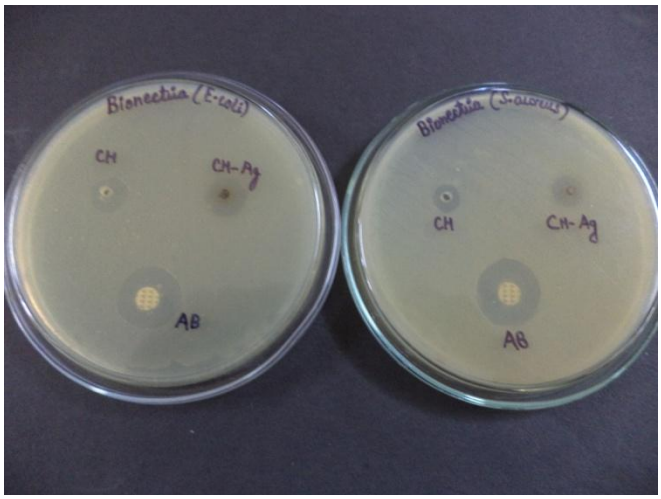

Figure 16 Comparative study of the antimicrobial activity of the polysaccharides (Chitin) and its bionanocomposites against *E. coli* and *S. aureus*

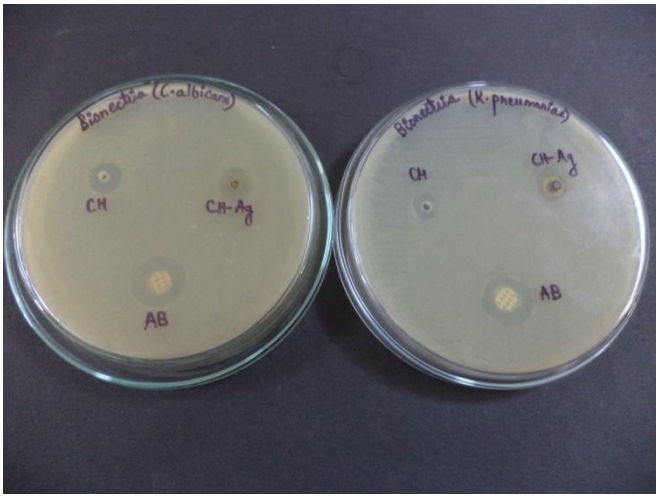

Figure 17 Comparative study of the antimicrobial activity of the polysaccharides (Chitin) and its bionanocomposites against *C. albicans* and *K. pneumoniae*

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

	E.coli	*S.aureus*	*K.pneumoniea*	*C.albicans*
Chitosan Solution	10	10	9	8
Chitosan AgNP	12	10	12	15
Antibiotic	20	17	14	26

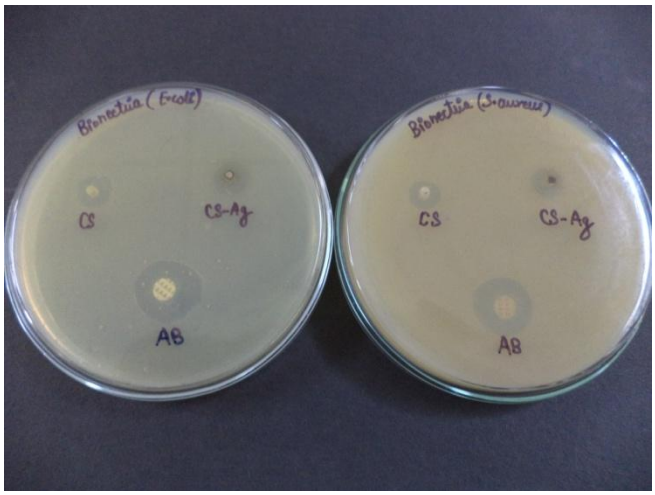

Figure 18 Comparative study of the antimicrobial activity of the polysaccharides (Chitosan) and its bionanocomposites against *E. coli* and *S. aureus*

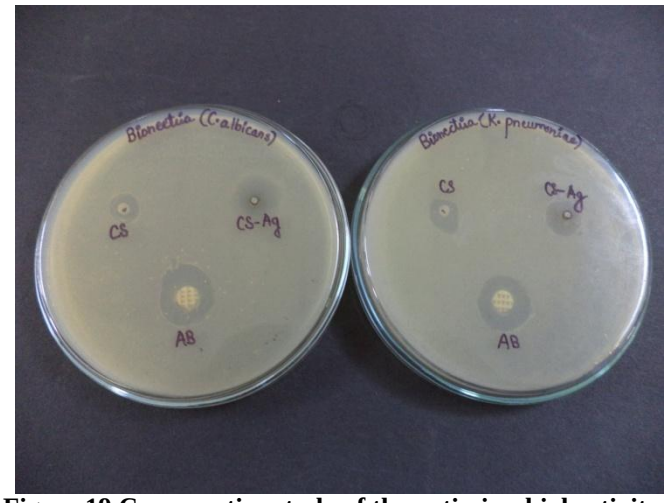

Figure 19 Comparative study of the antimicrobial activity of the polysaccharides (Chitosan) and its bionanocomposites against *C. albicans* and *K. pneumoniae*

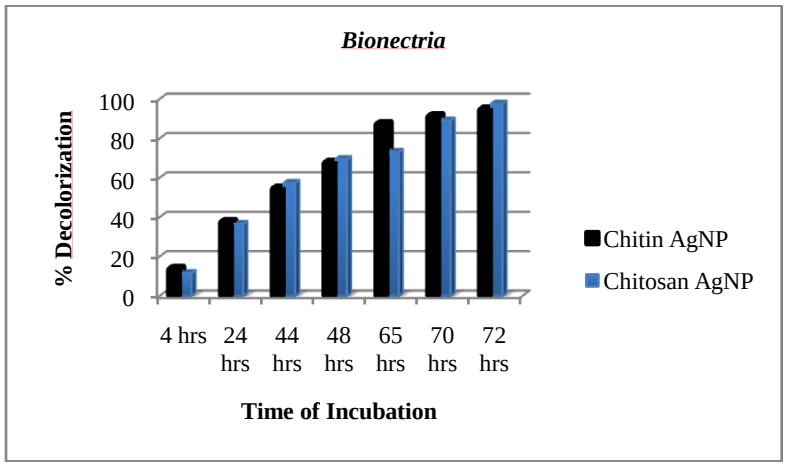

Figure 20 Dye degradation using Chitin and Chitosan AgNP's- *Bionectria*

Extraction and characterization of Chitin and Chitosan

Biomass Production

The growth of the fungus *Bionectria CBNR BKRR* in three different media was observed for 14days at RT. The highest growth rate was with Andarde *et al* medium with a mycelial dry weight of 15.21g/L while the next highest growth rate was observed with Henderson and Anderson *et al* medium 6.45g/L followed by Sabouraud sucrose broth with a mycelia dry weight of 2.74g/L. The result is superior to the value 10.41 g/L and 11.6 g/L reported by Andrade *et al.* 2000 and Franco *et al.* 2004, respectively, for C. *elegans* (URM 46109) grown during 96 hrs in Mucorales medium. This result is similar to the reported by Synowiecki and Al-Khatteb 1997 which obtained a yield biomass of *Mucor rouxii* grown in yeast extract and glucose 2% medium, for 48 hrs, to the 4 g, per litre of medium.

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 (204 mg/g or 20%) and for chitin (2189.78 mg/g or 218%) In addition, the next best yield of chitin and chitosan per 1 g of biomass from *Bionectria CBNR BKRR* are obtained using Henderson and Anderson medium and Andrade et al. medium for chitin 83.72 mg/g or 8% and 37.47 mg/g or 3% and chitosan 48.06 mg/g or 4% and 56.54 mg/g or 5% respectively. Thayza *et al.*, 2007 reported that the best yields of the polysaccharides (mg per gram of dry mycelia biomass) are obtained with 48 hrs of culture for chitosan (66 mg/g or 6.6%) and with 72 hrs for chitin (440 mg/g or 44%) . Similar results were reported to Tan *et al.* 1996, which studied different Zygomycetes strains and observed that *Cunninghamella echinulata* was the best chitosan producing strain, with a yield of approximately 7.0% of chitosan per mycelia dry weight .

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 3476 cm^{-1} in chitin samples from SS broth, 3378 cm^{-1} in HA Medium and 3426 cm^{-1} from AD medium and in the extracted Chitosan samples from SS broth at 3470 cm^{-1}, HA medium in 3423 cm^{-1}, AD medium in 3301 cm^{-1} indicate strong dimeric OH stretch. Chitin from SS broth have spectra in the Amide I region 1656 cm^{-1}, those from HA medium showed spectra at 1652 cm^{-1} and those from AD medium showed spectra at 1642 cm^{-1} , while Chitosan from HA medium at 1639 cm^{-1},AD medium at 1640 cm^{-1} indicating presence of C=C stretch. The peaks around 1556 cm^{-1} in chitin samples from SS broth, 1558 cm^{-1} in HA medium and 1555 cm^{-1} in AD medium and bands around 1552 cm^{-1} in Chitosan samples from SS broth show bands around 1565 cm^{-1}, 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, 1373 cm^{-1} in HA medium and 1381 cm^{-1} in AD medium. Chitosan samples from SS broth showed bands at 1410 cm^{-1} and 1410 cm^{-1}in AD medium 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 characterization of chitin and chitosan obtained from *Bionectria CBNR KRRR* by infrared spectra are similar to those reported in the literature (Andrade *et al.,* 2000; Amorim *et al.,* 2001; Franco *et al.,* 2005). The most significant parts of chitin and chitosan spectra are those showing the amide bands at approximately 1665, 1555 ,1313 cm^{-1} , which could be assigned to the C = O stretching, the N-H deformation in the CONH plane and the CN bond stretching plus CH_2 wagging.

Deacetylation degree – DD %

In the present study, chitin and chitosan from *Bionectria CBNR BKRR* grown in Sabouraud sucrose broth was found to have 51% DD and 59% DD, respectively. Chitin and Chitosan grown in Hesseltine and Anderson medium were found to have 95% DD and 10% DD. Andarde medium provided chitin and chitosan with 33% DD and 38% DD. Thayza *et al.,* 2007 that the chitin and chitosan from *C. elegans* grown in yam bean medium had 62% DD and 85% [23].

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 and 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)

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 380 nm in case of the *Bionectria CBNR KRRR*. 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 (Fig.10)

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 *Bionectria CBNR KRRR* CH AgNP were detected at 450 nm respectively. Honary *et al* 2011 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 . (Fig.11)

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 indicating that they are well dispersed. The pore size was reported to be in the range of 5-10 µm. Sowmya *et al.,* 2010 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 [27] (Fig.12)

Chitosan Bionanocomposites

Surface morphology of polymer and Ag BNC's are illustrated in Figure 14. 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.,* 2012 stated that the SEM image of Ag/Cts BNC's 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 of *Bionectria CBNR KRRR* CS AgNP composite shows the peaks of C K, O K, Cl K and Ag L. The atomic ratio of was found to be 85:13:0.34:1.45 wt %. Similar results were suggested by

Sowmya *et al*., 2010 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.13)

Chitosan Bionanocomposites:

The EDS spectrum of *Bionectria CBNR KRRR* CS AgNP composite shows the peaks of C O K, and Ag L. The atomic ratio of was found to be 15:17:67 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 (Mansor et al., 2012). The Ag BNCs film morphologies were dependent on several factors including polymer solubility, solvent evaporation, total thickness, molecular weight and surface composition (Puišo et al., 2008) (Fig.15)

Disc Diffusion Assay

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. The antimicrobial activity of chitosan is described to be associated with molecular weight, degree of acetylation, concentration of chitosan and bacterial inoculum size was described (Fernandes *et al.,* 2008).

Sondi *et al.,* 2004 reported that the inhibitory effect of silver on microorganisms tested is affected via two possible mechanisms. First, is the electrostatic attraction between the negatively charged cell membrane of the microorganisms and the positively charged Ag, and second, is the formation of 'pits' in the cell wall of bacteria related to Ag concentration. Since the zero valent metal nanoparticles were obtained by chemical reduction of metal salts, it seems the latter mechanism would have been mooted (Sondi *et al.,* 2004).

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*, 2014 for degradation of methylene blue by nanoparticles synthesized from *Morinda tinctoria*.

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 94.5% and 97.5% after 72 hrs. (Figure 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 (Vanaja *et al.,* 2014).

CONCLUSION

Fungi are abundantly available sources for the production of industrially important secondary metabolites. These results present an economically viable methodology for production of the polysaccharides-Chitin and Chitosan from marine fungi. Further results have been provided for cost effective synthesis of bionanocomposites from both the polysaccharides which have potential application as antibiotics and in bioremediation.

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