WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 844-863. Research Article ISSN 2277– 7105

BIOLOGICAL EXTRACTION OF CHITIN AND CHITOSAN FROM MARINE FUNGI, IT' S CHARACTERIZATION, ANTIMICROBIAL ACTIVITY, ANTITEXTILE ACTIVITY AGAINST MDR PATHOGENS AND ANTICANCER ACTIVITY

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Article Received on
29 Sep. 2017,
Revised on 20 Oct. 2017,
Accepted on 09 Nov. 2017
DOI: 10.20959/wjpr201715-10121

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ABSTRACT

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. These polysaccharides are found in a wide range of natural sources such as crustaceans, insects, annelids, molluscans, coelenterates and it's a common constituent of fungal cell wall. In the present study chitin/chitosan was extracted from *Aspergillus spp.* and *Trichoderma spp.* isolated from Pitchavaram a mangrove forest soil. The chitin and chitosan was extracted using 2% w/v sodium hydroxide solution for 2 hours, followed by acetic acid treatment. Crude chitin/chitosan was collected (yield was 500mg of mycelium) 0.1g of chitin/chitosan was obtained. Silver and Gold nanoparticles were synthesized by using 1mm Ag No_3 & 1mm Au Cl_4 respectively. The Bionanocomposites were characterized using UV-visible, FT-IR and SEM (Scanning Electron Microscopy) study. Antibacterial study was also conducted against Multi Drug Resistant pathogens. Further antitextile and anticancerous activities were carried out.

KEYWORDS: Chitin, Chitosan, Fourier Transform Infrared Spectroscopy(FT-IR), SEM (Scanning Electron Microscopy), MDR (Multi Drug Resistant), KBr- Potassium bromide.

INTRODUCTION

MDR or Multiple drug resistance is an antimicrobial resistance shown by a species of microorganisms to multiple antimicrobial drugs. MDR bacteria are most threatening to public health, resist to multiple antibiotics. Other types include MDR viruses, fungi and parasites. Here MDR bacteria *Bacillus* and *Escherichia coli* were used for antimicrobial activity. *Bacillus* is a Gram positive, rod shaped bacteria and member of Phylum Firmicutes. *Bacillus* can cause anthrax disease. The death rate due to anthrax was 25% to 60% annually. For anthrax currently many antibiotics are given such as Ciprofloxacin, Doxycycline, Quinolone, Aminoglycoside, Vancomycin, Chloramphenicol. But those antibiotics can cause many side effects like affect central nervous system including dizziness, confusion and hallucination, depression, swelling of lips, tongue or faces.

Escherichia coli are a Gram- Negative, Gammaproteobacterium commonly found in lower intestine of warm-blooded organism. *E.coli* can cause urinary tract infection (UTI). It has been estimated that every year, more than 13,000 patients are died due to this infection. But, in the year 2011 the death rate has been more high (i.e., 93,300 patients). For UTI currently many antibiotics like Levaquin oral, Cipro oral, Amoxicillin- potassium clavulanate, Ciprofloxacin, etc are used. These antibiotics can cause nausea, vomiting, diarrhoea as side effects.

Biopolymer is a term commonly used for polymers which are synthesized by living organisms (Khor, 2001). Chitin is the most abundant biopolymer on earth next to cellulose. It is estimated that its annual biosynthesis reaches 100 billion tons (Muzzerelli *et al*.,1997and 2004; Tharanathan *et al.*,2003). These polysaccharides are found in a wide range of natural sources, such as crustaceans, insects, annelids, molluscus, coelenterates and is a common constituent of fungal cell walls. In fungi, chitin exists in the cell wall of spores and hyphae. It is associated with glucan molecules in form of microfibrils, which are embedded in an amorphous matrix and provide the framework in cell wall morphology (Ruiz-Herrera, 1992).

Chitosan is not native to animal sources, but a small number of fungi, such as *Mucor*, *Absidia* and *Rhizopus* species have Chitosan as one of the structural components in the cell wall (Ruiz-Herrera, 1992). Chitosan is generally produced from chitin by treatment with concentrated sodium or potassium hydroxide solution (40-50%) at 80 - 150°C (No *et al*., 2002).

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 (White *et al.*,1979), fungal mycelia have lower level of inorganic materials compared to crustacean wastes, and thus no demineralization treatment is required during the processing (Teng *et al.*,2001),crustacean chitin and chitosan may vary in the physico-chemical properties, while fungal chitin and chitosan have relatively consistent properties because of the controlled fermentation conditions (Rane and Hoover.,1993).

Cultivation for chitin and chitosan isolation is usually carried out in the yeast peptone glucose broth (YPG), potato dextrose broth (PDB) or molasses salt medium (MSM) (Chatterjee *et al.*, 2004; Marguerite Riaudo, 2006).

It has been shown that chitosan possess strong antimicrobial activity against both gram-positive and gram-negative bacteria, including foodborne pathogens(No, *et al.*,2002, Joen *et al.*,2001, Sudharshan *et al.*,1992, Hadwiger *et al.*, 1986,Kumar *et al.*,2000).

The present paper aims to investigate chitin and chitosan production using *Aspergillus terreus* CBNRKR KF529976 and *Trichoderma viridae* CBNRKR 14grown in three different traditional culture media, synthesis of their bionanocomposites and their ability to degrade the MDR Pathogens, and also aims to synthesize silver and gold nanoparticles and to characterize these nanoparticles using UV-VIS Spectrophotometer, SEM and FT-IR and to test their antitextile activity and anticancer activity.

MATERIALS AND METHODS

Isolation and Characterization of Marine Fungus

Collection of Samples

Pichavaram (Lat.11□428'E; Long.79□798`E), Cuddalore (Dt.) of Tamilnadu is home to the second largest Mangrove forest in the world. 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. After inoculation, the plates were incubated in an inverted position for 5-7 days at 25 ± 2ºC.

Microscopic Observation

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

Extraction of Chitin and Chitosan

Culture Medium: *A. terreus* CBNRKR KF529976 and *T.viride* CBNRKR 14 was grown for chitin and chitosan production, in three different culture media: a)Sabouroad sucrose (SS broth)-(bacteriological peptone (10 g) and sucrose (20 g) per litre of distilled water, pH5.7); b) Hesseltine and Anderson (HA medium)- (glucose (40 g); asparagine (2 g); chloridrate of thiamine (0.05mg); 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).

Extraction: The process of extraction involved deproteinization with 2% w/v sodium hydroxide solution (30:1 v/w, 90ºC, 2hours) and incubated. Extraction of chitosan from filter paper under reflux (10% v/v acetic acid 40:1 v/w, 60ºC, 6 hours), separation of crude chitin by centrifugation (4000x g, 15 minutes) and precipitation of chitosan from the extract at pH 9.0, adjusted with a 4M NaOH solution. Crude chitin and chitosan were washed on a coarse sintered-glass funnel with distilled water and air-dried. Chitin and Chitosan were extracted.

Antimicrobial Activity

Nutrient agar (0.56 g) was dissolved in 20ml of distilled water and brings to boil. Agar was then autoclaved for 15 min at 121^0C and left to cool at room temperature. Once the medium was cooled (about 45^0C), it was poured into Petri dishes. Petri dish was left on the flat surface

for 30–40 minute until completely set. The test microorganism (*E.coli)* were seeded into respective medium by spread plate method. Further 20 µl was spread onto 20 ml of sterile agar plates by using a sterile cotton swab. The surface of the medium was allowed to dry for about 3 min. The wells (10mm) were punched over the agar plates using sterile gel puncher. Various extracts and filtrates were added to the wells. The plates were incubated for 24 hours at 37 ^{0}C. After incubation the diameter of inhibition zones formed around each wells were measured in mm and recorded.

Chitin and Chitosan Characterisation

Infra Red Spectroscopy (Deacetylation degree – DD %)

The degree of deacetylation (DD%) (Brugnerotto *et al.*,2001) for microbial chitin and chitosan were determined using the infrared spectroscopy (Brugnerotto *et al*., 2001) using the absorbance ratio A1655/A3450 and calculated according to equation 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 hours at 110ºC under reduced pressure. Infrared spectrometer was recorded with a Shimadzu. Using a 100 mg KBr disks for reference. The intensity of maximum absorption bands were determined by the baseline method.

Preparation and Characterization of Chitin/ Chitosan Bio Nanocomposites

Synthesis of Nano Particles (Sharma *et al.*, 2010)

A 0.5g of extracted chitin and chitosan were taken in separate vials. Mixed with 500µl of 1mm & 2mm AgNo3 into the respective vials. The mixture was stored in the dark room at 4°C. Readings were noted at 300-600nm.

Acid Hydrolysis to Purify Chitin and Chitosan Nano Particles

20mg of chitin was mixed with 500µl of Ag NPs suspension and 1ml of hydrochloric acid solution. The mixture were mixed well (at pH 7.0) for 30 min. The insoluble AgNPs/chitin composites were centrifuged at 6000 rpm for 10 minutes. Pellet weight was noted and supernatant were discarded.

Coating of Plant Extract (*Albizia Amara*) with Chitin/Chitosan Nano Particles

Silver & Gold chitin/chitosan nanocomposites500µl were taken in separate vials and a pinch of plant extract was added. Mix it well and incubated at room temperature for 45minutes and readings were taken at 254nm.

Characterization of nanoparticles(Islem Younes *et al.*,2015)

Uv-Visible Spectrophotometer

UV-Vis absorption spectra of the samples were recorded in the wavelength range of 300 to 500 nm using UV spectrophotometer (UV-Visible Perkin Elmer Lambda) at the Bioscience and Nanoscience Research Centre, Coimbatore, India.

Fourier transform-infrared spectroscopy (Duarte *et al*.,2002**)**

FT-IR (FT-IR Perkin Elmer) in which samples was recorded in the wavelength of absorption on the region 400-4000cm at the Bioscience and Nanoscience Research Centre, Coimbatore, India.

Scanning Electron Microscopy (Mcmullan, 2006)

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.

Cell Death Measurment Using Turbidity Method

Nutrient broth with *E.coli* culture 0.1ml, 10µl of 1mm *A.terrus* gold nanoparticles and 10µl of plant extract sample were added in tube .*A.terrus* silver nanoparticles were added in another tube with the above same preparation. Likewise *T.viride* silver and gold nanoparticles was prepared in two separate tubes. The tubes were incubated at 37°C for 24 hours and readings were taken at 600nm, then cell death rate was calculated.

Textile Application

The medical gauze cloth was pre-coated with synthesized chitosan (20µl) and placed on the pre swabbed pyogenic cultures on Nutrient agar plates. Before that test microorganisms (*Escherichia coli, Bacillus subtilis*) were swabbed by using spread plate technique. It was found that the chitin nano composites coated gauze cloth had more antimicrobial activity than chitosan against the pathogens. The zone of inhibition was measured and recorded.

Anti Cancer Activity (Zhang and Liu., 2015)

DMEM (Dulbecco's Modified Eagels medium, high glucose), DMEM (Dulbecco's Modified Eagels medium, low glucose), FBS (Foetal Bovine Serum) media was used.MTT (3 - [4, 5-Dimethyl thiazol -2-yl] - 2, 5- Diphenyl tetrazolium bromide) is a yellow coloured water soluble tetrazolium dye. Mitochondrial enzyme lactate dehydrogenase, produced by metabolically active cells reduces MTT to water-insoluble formazan crystals. When dissolved in appropriate solvent, these formazan crystals exhibit purple color.

In Cytotoxicity assay HeLa Cells were grown in RPMI-1640 medium (Hi Media, Mumbai) supplemented with 10% foetal bovine serum (FBS) (Hi Media, Mumbai), 100 U/ml penicillin and 100 µg /m*l* streptomycin (Hi Media, Mumbai). Cells were incubated in a humidified incubator contain 5% CO2 at 37 ºC. After 24hrs the cells were seeded in to 96 well. The cell culture suspension was washed with 1 X PBS (Phosphate Buffered Saline) and then added with 200µl MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium Bromide] solution to the culture flask. It is then incubated at 37°C for 3 hours, removed all MTT solution, washed with 1X PBS and added with 300µl DMSO to each culture flask and incubated at room temperature for 30 minutes until all cells get lysed and homogenous color was obtained. The solution was then transferred to centrifuge tube and centrifuged at top speed for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD was measured at 540 nm using DMSO blank. Then the percentage viability was calculated using the percentage of viability formulated.

RESULTS

The Mycelia mat was collected from MGYP broth medium and Czapek dox broth medium after 48 hours of incubation. (Fig:1, Plate:1 and Plate:2).

Extraction of Chitin and Chitosan

The sodium hydroxide solution containing mycelia mat and this were incubated for 24 hours. The residues are collected by using filter paper. Then it is immersed in acetic acid solution overnight and the Chitin and Chitosan were collected. (Fig:2 and Fig:3).

Antimicrobial Activity

Here the efficacy study of the extracted chitin and chitosan from *A.terrus* and *T.viride* were tested against *E.coli* bacteria and it showing high antibacterial activity against the sample (Table:1, Fig: 4 and Plate :3).

Preparation and characterization of bio nanocomposites

Synthesis of silver & gold nanoparticles

A synthesised Silver nanoparticle was confirmed by using UV-Visible spectra of reaction mixture after regular time intervals at a range of 300nm-600nm. The spectrum showed a peak which is assigned to SPR effect of silver 301nm. Synthesis of gold nanoparticles was confirmed by taking UV-Visible spectra of reaction mixture after regular time intervals at a range of 400-700nm. The spectrum showed a peak which is assigned to SPR effect of gold 580nm. The UV-Visible spectrum of Silver & Gold nanoparticles was obtained by using UV-Visible spectrophotometer. The dispersion of silver nanoparticles displays intense Colour due to the Plasmon resonance absorption (Fig:5 and Fig:6).

Acid Hydrolysis to Purify Chitin and Chitosan

The HCl solution contains chitin and chitosan material was centrifuged and weight of the pellet was calculated. The *A.terrus* chitin/chitosan (sample 1) has 0.12g and *T.viride* chitin/chitosan (sample 2) has 0.17g respectively.

Coating of Plant Extract (*Alzibia Amara)* With Chitin/Chitosan Nanoparticles

Synthesis of nanoparticles with plant extract was confirmed by taking UV-visible spectra reaction mixture after measuring at 254nm. The silver nanoparticle (sample 1) has 0.855nm and gold nanoparticle (sample 2) has 0.735nm respectively (Fig:5 and Fig:6).

Cell Death Measurment Using Turbidity Method

10 µl of 1mm gold & silver nanoparticles and 10 µl of plant extract sample in nutrient broth medium and readings were taken at 600nm. The blank has 0.00nm and the corresponding samples 1, 2, 3 & 4 have 0.139nm, 0.126nm, 0.115nm and 0.075nm respectively.

Formula = C-T /c x 100 Control reading: 0.189 mm

The percentage of cell death such as 26.4% (*A.terrus* AgNP), 33.3% (*A.terrus* Au NP), 39.1% (*T.viride* AgNP) and 60.3% (*T.viride* AuNP). This confirms that the *T.viride* gold NP (sample 4) has high cell death rate (Table: 3).

Fourier Transform Infrared (Ft-Ir) Spectroscopy

Further characterization of nanoparticle was done by FT-IR, to identify possible interaction of bio molecules with silver nanoparticles. FT-IR provides the information about functional groups present in the sample which is responsible for the transformation of $AgNO_3$ from

simple inorganic to elemental silver. The FT-IR studies showed sharp absorption peaks located at 725.23cm-1 and 3903.92cm^{-1}.

The peak appeared at 1500cm^{-1} shows that the stretching of bonded hydroxyl (-OH) group and H-bonded. It is the characteristics of N-H stretching in primary/secondary amines. The band seen at 1589.34cm-1 is characteristics of -C=O carbonyl groups and -C=C- stretching. The FT-IR studies showed sharp absorption peaks located at 725.23cm-1 and 3942.50cm^{-1} (Fig:8).

The peak appeared at 1600cm^{-1} shows that the stretching of bonded hydroxyl (-OH) group and H-bonded. It is the characteristics of N-H stretching in primary/secondary amines. The band seen at 1705.07cm^{-1} is characteristics of -C=O carbonyl groups and -C=C- stretching (Fig. 9).

Scanning Electron Microscopy (Sem)

The samples for SEM analysis were prepared by solvent casting on Petri dish. The chitin/chitosan sample in gold nanoparticle shows good result. Surface morphology of polymer and Gold bionano composites are illustrated in (Fig. 7). The size was in the range of 60nm to 145nm under 15,000X.

Antitextile Activity: Antitextile activity of the chitin chitosan nanoparticle were tested against gram positive and gram negative bacteria (Table2), which showing high antibacterial activity against gram negative bacteria *E.coli.*(Table:2,Plate5 and Plate 6).

Anticancerous Activity

Cancer is a life threatening and dreadful disease characterized by the abnormal proliferation of the cells that invade the adjacent tissues and causes the destruction of these tissues. Cancer spreads to distant organs through blood stream and lymphatic vessels. The cell viability was calculated and thus Chitosan gold has high number of viable cells (Table:4 and Fig:10).

Culture Plate

Plate 1. ***Aspergillus terrus.***

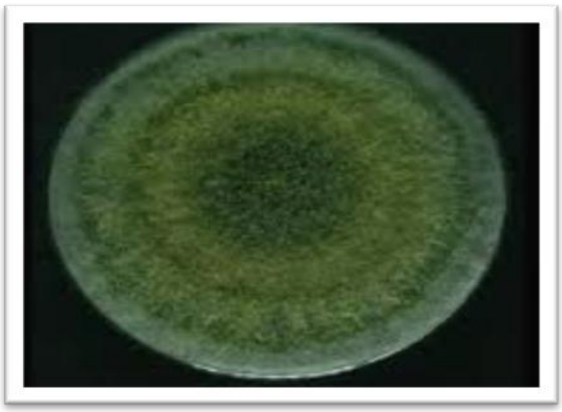


Plate 2. ***Trichoderma viride.***

Collection of Mycelial Mat

1 **2** **3** **4**

Fig. 1. Formation of mycelial mat.

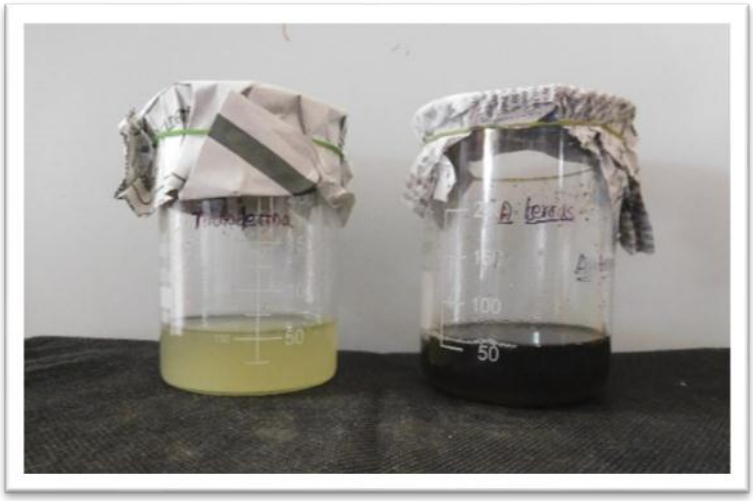
1- *Aspergillus terrus* (MGYP medium)

2-*Trichoderma viride* (MGYP medium)

3-*Aspergillus terrus* (Czapek dox medium)

4-*Trichoderma viride* (Czapek dox medium)

Extraction of Chitin and Chitosan

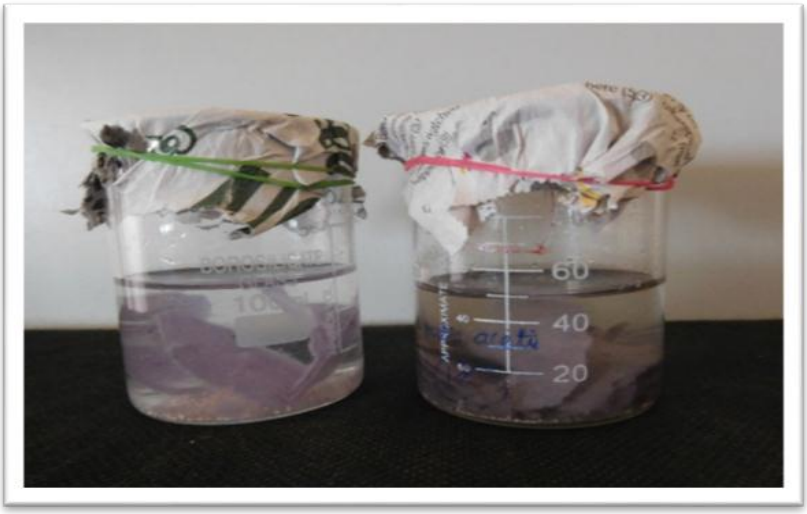


1 **2**

Fig. 2. Mycelial Mat in NaOH.

1 - *Trichoderma viride*

2 - *Aspergillus terrus*



1 **2**

Fig. 3. Mycelial mat residues treated in acetic acid for the separation of Chitin / chitosan.

1 -*Trichoderma viride*

2 -*Aspergillus terrus*

1 **2**

Fig. 4. bio nanocomposites of chitin/chitosan.

1-*Aspergillus terrus* (Silver)

2-*Trichoderma viride* (Gold)

Uv-Visible Spectrophotometer

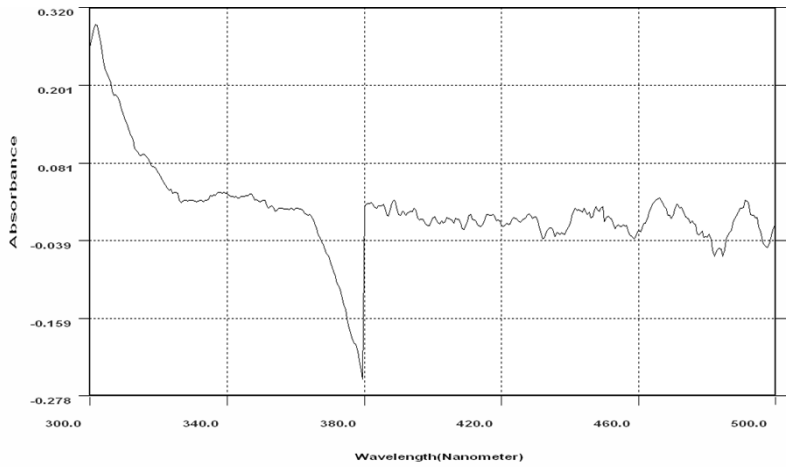


Fig. 5. *Aspergillus terrus* (1mm silver).

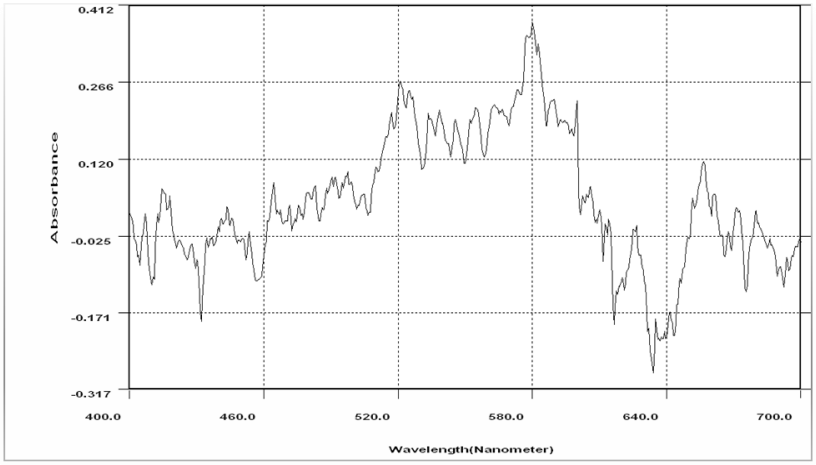


Fig. 6. *Trichoderma viride* (1mm gold).

Antimicrobial Activity of Chitin/Chitosan

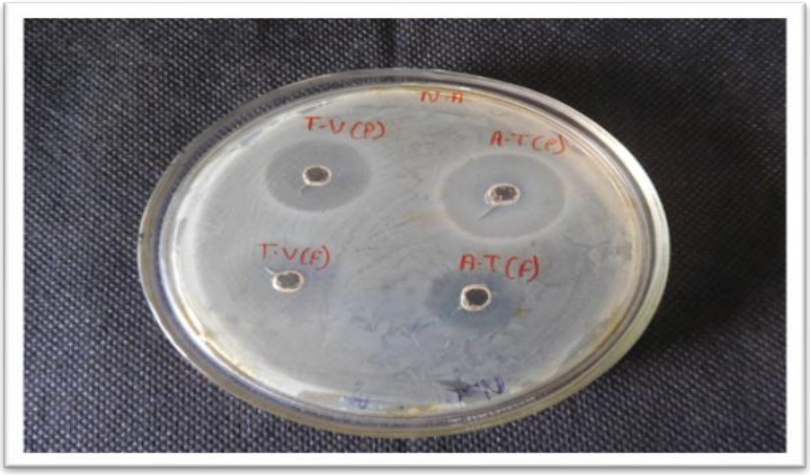


Plate 3: *Escherichia coli*

Scanning Electron Microscopy (Sem) of Chitin/Chitosan

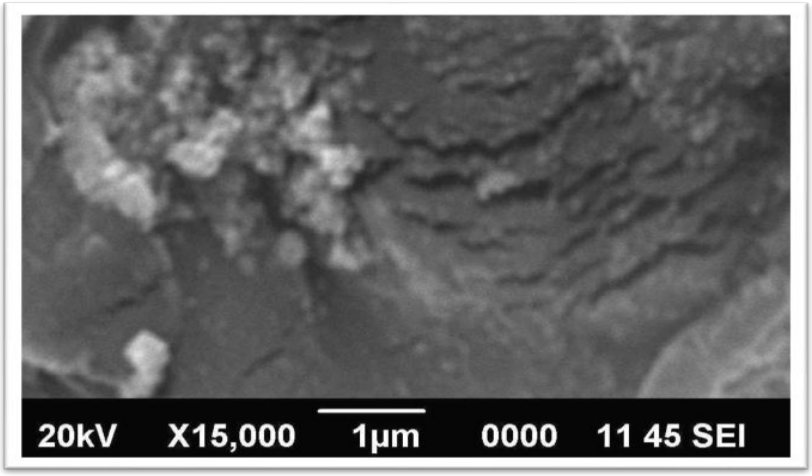


Fig. 7. Chitin/chitosan in gold nanoparticles.

Fourier Transform Infrared (Ft-Ir) Spectroscopy

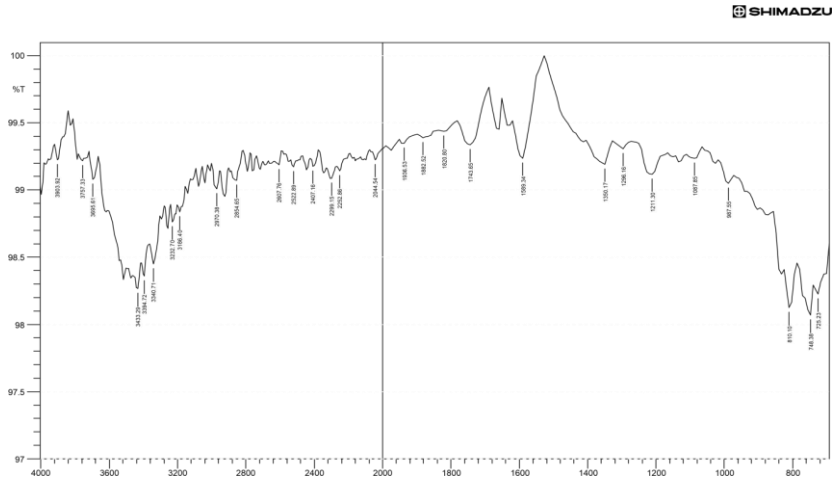


Fig. 8. ***Aspergillus terrus(silver).***

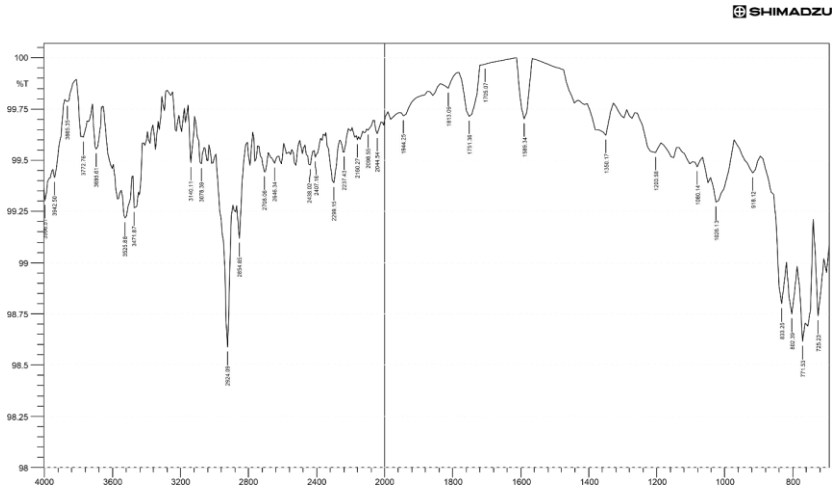


Fig. 9. ***Trichoderma viride (Gold).***

Antitextile Activity of Chitin/Chitosan

1 **2**

Plate 5. ***Escherichia coli.***

1-*Trichoderma viride*

2-*Aspergillus terrus*

1 **2**

Plate 6: ***Bacillus subtilis.***

1-*Aspergillus terrus*

2-*Trichoderma viride*

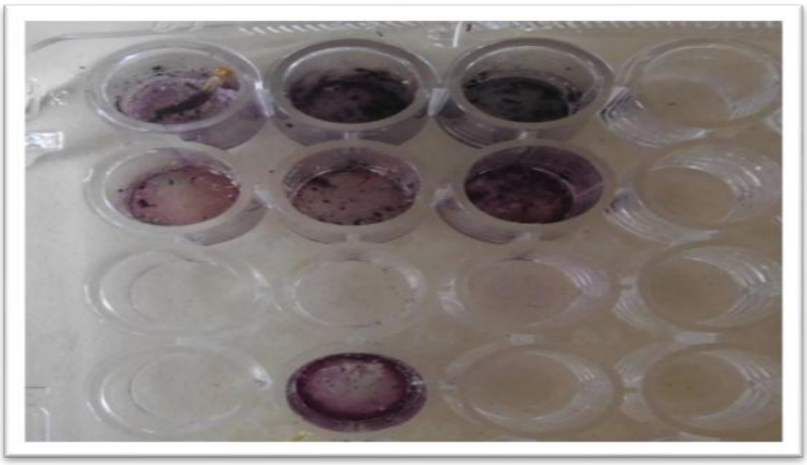


Fig. 10: Anticancer Activity.

Table. 1: Antimicrobial Activity.

Organism	**Zone Formation**			
E.coli	**A.T(F)**	**A.T(P)**	**T.V(F)**	**T.V(P)**
	5mm	8mm	2mm	7mm

Table. 2: Antitextile Activity.

Organism	*Aspergillus terrus*	*Trichoderma viride*
Escherichia coli	28 mm	30 mm
Bacillus subtilis	25 mm	22 mm

Table. 3: Cell Death Using Turbidity Method.

Samples	Silver	Gold
A.terrus	26.4%	33.3%
T.viride	39.1%	60.3%

Table. 4: Anticancer Activity.

Samples	% of Od Values			
	% of Viable Cells		% of Viable Cells	
Chitin	80.8%	19.2%	Chitin	80.8%
Chitin silver	82.3%	17.7%	Chitin silver	82.3%
Chitin gold	81.2%	18.8%	Chitin gold	81.2%
Chitosan	79.5%	20.5%	Chitosan	79.5%
Chitosan silver	84.5%	15.5%	Chitosan silver	84.5%
Chitosan gold	88.1%	11.9%	Chitosan gold	88.1%

DISCUSSION

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 Hesseltine 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. Andrade *et al*., reported a maximum chitin yield of 239 mg/g from *Mucor javanicus* (Krishnaveni and Ragunathan., 2015.

In the present investigation, the chitin and chitosan were extracted from two marine fungi.The extraction process involved using 2% sodium hydroxide followed by acetic acid and further NaOH treatment. Crude chitin and chitosan (150mg for 200g of fungal mycelium) was extracted.

Nano-science is the study of phenomena and manipulation of materials at atomic molecular and macromolecular scales. 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 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 AgNPs. The AgNPs so produced are dark brown in colour. Photo catalytic degradation of methylene blue was carried out by using AgNPs synthesized from the bio nano composites synthesized from chitin and chitosan under solar light. Dye degradation was initially identified by colour change **(**Kim and Kim., 2006).

In the present investigation, the synthesized silver and gold nanoparticles were incubated in dark room overnight. The colour of the nanoparticles turns greenish yellow to dark brown colour after incubation. Then the peak range was measured under UV- visible spectrophotometer. The range of the Plasmon peak was observed at 301nm in silver and 580nm in gold nanoparticles.

Due to their particle size, AgNPs can easily reach the nuclear content of bacteria by disrupt the membranes of bacteria. The particle size smaller than 10nm interact with bacteria and generate electronic effects that improve the reactivity of AgNPs(Jone *et al*.,2001). The antibacterial activity of chitin solution was found to be less than the chitin AgNPs indicating that the presence of the silver ion thereby increases the antibacterial strength of the polysaccharides. Chitin AgNPs showed comparable antibacterial strengths as the antibiotic disks (Amoxicillin) employed. Similar results were obtained for chitosan solution and chitosan AgNPs with comparable antimicrobial activity to the antibiotic disk used **(**Krishnaveni and Ragunathan., 2015).

In the present investigation a number of anti-microbial agents have been used to prevent the adverse effects caused by various microbes. It is very important to discover new antimicrobial agents because of the rate of antibiotic production are much lower than the rate at which the microbes are becoming resistant to them. Here the efficacy study of the extracted chitin and chitosan from *A. terrus* and *T.viride* were tested against *E.coli* bacteria and it showing high antibacterial activity against the sample.

Chitosan and its derivatives have been widely studied for potential tissue engineering biomaterials as they will be degraded at a reasonable rate without causing any inflammatory reaction or producing any toxic end-products when the new tissues are formed. They are porous in nature for diffusion of gases, nutrients, and metabolic wastes for the seed cells

along with increasing the surface area for cell attachment, migration, and differentiation. They can be molded easily into anatomical shape and volume; are biocompatible with the surrounding biological fluids and tissues, as well as providing temporary mechanical support. All these properties fit the special properties use as tissue engineering scaffold (Felt *et al.*, 2002).

In the present investigation, FT-IR provides the information about functional groups present in the sample which is responsible for the transformation of AgNO3 from simple inorganic to 3 elemental silver. The FT-IR studies showed sharp absorption peaks located at 725.23cm-1 and 3903.92cm-1.

The peak appeared at 1500cm-1 shows that the stretching of bonded hydroxyl (-OH) group and H-bonded. It is the characteristics of N-H stretching in primary/secondary amines. The band seen at 1589.34cm-1 is characteristics of -C=O carbonyl groups and -C=C- stretching. The FT-IR studies showed sharp absorption peaks located at 725.23cm-1 and 3942.50cm-1.

The peak appeared at 1600cm-1 shows that the stretching of bonded hydroxyl (-OH) group and H-bonded. It is the characteristics of N-H stretching in primary/secondary amines. The band seen at 1705.07cm-1 is characteristics of -C=O carbonyl groups and -C=C- stretching.

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