Preparation of Nanocomposite Based Film from Fungal Chitosan and Its Applications

[1]A.Shajahan, [1]A. Sathiyaseelan, [1] Karthik. S. Narayan, [1]Esalkiel Raj,
[2]V. Narayanan and [1]V.Kaviyarasan
[1]CAS in Botany, University of Madras, Guindy Campus, Chennai
[2]Department of Inorganic Chemistry, University of Madras, Guindy Campus, Chennai - 25
E-mail: shajaahaan@gmail.com

Abstract:- Chitosan the second most abundant polysaccharide is biodegradable, biocompatible, and non-toxic. These properties makes chitosan widely applicable in the pharmaceutical and biomedical fields for controlled release of drugs for wound management and space filling implants etc. In the present study fungal chitosan is isolated form *Cunninghamella elegans*, characterized by FT-IR spectroscopy by confirming the functional groups exhibiting the main characteristic bands of carbonyl (C=O-NHR) and amine group (-NH2) at 1632 cm-1. The characteristic absorption bands of chitosan are at 1076.09–1155.61 cm−1, confirms that the monomers in chitosan are linked by d-glycosidic linkages, the same holds for the bonds between the monomers in the glucan component. The obtained fungal chitosan was bound with tetracycline to form nanocomposite based films and examined under FeSEM and AFM. Prepared nanocomposite films were further subjected to antibacterial activity against Gram positive and Gram negative bacteria.

Keywords: **fungal chitosan; tetracycline; nanocomposite film; SEM; Antibacterial activity.**

I.INTRODUCTION

Advances in nanotechnology are potential to produce drug loaded nanoparticles that can be utilized in a wide range of innovative ways. Polymeric nanoparticles are prospective drug carriers in which the active ingredient is dissolved, dispersed, entrapped, encapsulated, and absorbed or chemically attached. More prominently polymeric nanoparticle has great potential applications in pharmaceutical industry [7, 3]. Among the different kinds of polymers chitosan has received a great attention in both the medical and pharmaceutical arenas [5]. Chitosan a biopolymer is deacetylated form of chitin. It is the second most abundant polysaccharide after cellulose in nature found in crustaceans such as shrimp, crab shell as well as in the cell wall of fungi [20]. Commercially, chitosan is obtained through the chemical deacetylation of crustacean chitin under strong acidic treatment. These are inconsistent in its physical-chemical properties due to the variability in raw materials, the harshness of the isolation and conversion processes, and variability in the level of deacetylation and protein contamination. The use of biomass from fungi has demonstrated great advantages, such as it is independence from the seasonal factors, ease of large scale production, and the possible simultaneous extraction of chitin and chitosan. In ordered to obtain chitosan of more consistent quality, filamentous fungi have been considered an attractive source for industrial application [15, 20].

Antimicrobial activity of chitosan has been demonstrated against many bacteria, filamentous fungi and yeasts [10, 16, 20]. Chitosan has developing a low toxic and side effect along with the optimized pharmaceutical action of drug delivery system. Drug Carriers like liposomes and nanoparticles have been investigated most extensively, comparing to others drug carriers the chitosan nanoparticles can be act as a good polymeric carrier [6].

The present study deals with extraction of chitosan from fungi - *Cunninghamella elegans*, the obtained fungal chitosan was bound with tetracycline to prepare chitosan based nanocomposite films. The fugal chitosan was characterized by FTIR spectrum, and chitosan based nanocomposite films was examined under FeSEM. These films were subjected to antibacterial activity against Gram positive and Gram negative bacteria.

Fig.1 Chitosan - Nanocomposite film

II. MATERIALS AND METHODS

A. Chemicals, Materials, and Instrumentation

Cunninghamella elegans (*C. elegans*) was obtained from the Fungal Culture Collection Centre, Centre for Advanced Study in Botany, University of Madras. Four strains of bacteria were used as test microorganisms: *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia and Salmonella typhi* obtained from ATCC culture collection, tetracycline was procured from Himedia, sodium hydroxide, acetic acid were of Analytical grade. Malt extract, yeast extract, peptone and glucose were obtained from Himedia.

B. Cultivation and collection of fungus biomass

The fungus was grown in nitrogen enriched medium containing malt extract (11g), yeast extract (1g) and peptone (10g), whereas glucose (20g) is a carbon source.

C. Extraction of Chitosan

Mycelia from fungus harvested by vacuum filtration, washed with distilled water and freeze dried. 1gm of dried powdered mycelia was ground and soaked with 2M NaOH solution for overnight was autoclaved. The pellet was washed thrice with distilled water and was followed by ethanol and centrifuged at 10000 rpm for 15 minutes at 4^{o} C. The pellet was dissolved in 200 mL of 1% of acetic acid solution. Addition of 2M NaOH solution into the suspension yields precipitated chitosan and it was further centrifuged at 10000 rpm for 15 minutes. The pellet obtained and freeze dried.

D. Characterization of Chitosan

Chitosan was analyzed by FTIR spectrometer (Perkin Elmer). Approximately 2mg of Freeze dried sample and 100 mg of potassium bromide was prepared by KBr disc method. The instrument was operated with resolution of 4 cm^{-1} with frequency range of 400–4000 cm^{-1}.

E. Preparation of Chitosan based Tetracycline bound Nanocomposite films

Hundred microgram of extracted chitosan nanoparticles were dissolved in 100 ml of 2% Acetic acid. Tetracycline hydrochloride (10%) was slowly added into the chitosan solution [25]. Thereafter the blended sample was stirred for12 hours. Later the samples were poured into Teflon coated plate. And it's kept for 60°C in hot air oven. After evaporation of water the drug bounded chitosan film was collected.

F. Morphological Characterization

The surface morphology of the dried chitosan blended film was observed with the scanning electron microscope (SEM) and atomic force microscope (AFM).

G. Antibacterial Properties of Chitosan based Nanocomposite films

The chitosan bound tetracycline film was cut into 10mm*10mm size to check the bactericidal properties of Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) gram negative bacteria (*Klebsiella pneumonia*, *Salmonella typhi)* in solid state condition. The activity was examined by zone formation.

III. EXPERIMENTAL RESULTS

A. Effect of growth medium with enriched nitrogen source

Growth the *Cunninghamella elegans* under submerged fermentation condition in the MGYP medium. The mycelium was harvested by vacuum filtration and dried by using lyophilizer. The lyophilized dry mass is then made into powdered form. The obtained yield of biomass was weighed as 4g/L with enriched nitrogen source1gm of dried biomass was 10.5%. These values are higher than those reported by [18].

B. FTIR Analysis of Chitosan

The FT-IR spectra for chitosan from fungus of *Cunninghmella elegans* in comparison with standard chitosan from Sigma are illustrated in Fig. 2 (A) The main characteristic peaks of fungal chitosan[4,20] are at 3362 (–OH stretch), 2978 (C–H stretch), 1632 and (N–H bend), 1378 (bridge O stretch), and 1076 cm-1(C–O stretch). Whereas the main corresponding peaks of standard chitosan were at 3379, 2880, 1658 and 1595, 1378 and 1076 cm-1 respectively Fig.2 (B).In general, the complexity of the IR spectra of chitosan is due to the complicated and specific network of H-bonds in which the OH, C-O and NH groups are involved for each polymorphic form [14, 21, 26], and to the typical broadness of the IR bands of natural polymers. The amide I band at (1655 cm^{-1}) is clear in the spectrum of fully N-acetylated chitin. Its intensity decreased with a decrease in DA and finally disappeared in completely deacetylated chitosan [1, 2].

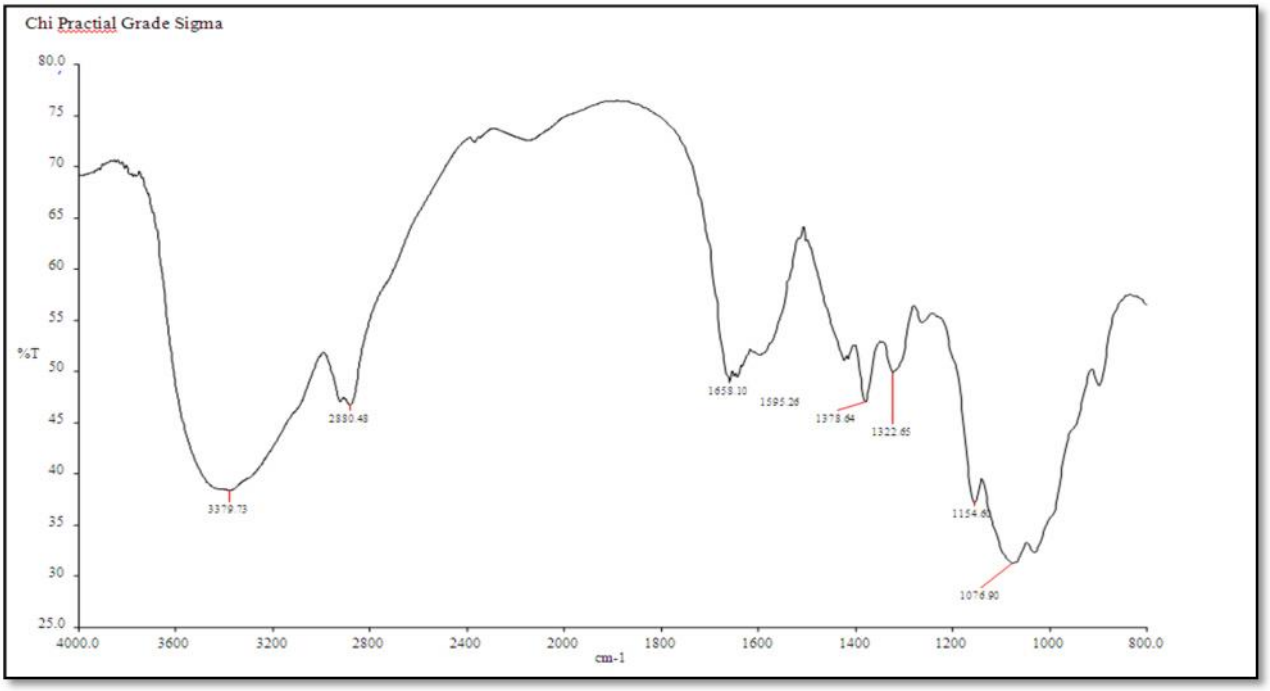

Fig. 2(A) FTIR spectrum of chitosan standard (Sigma chemicals)

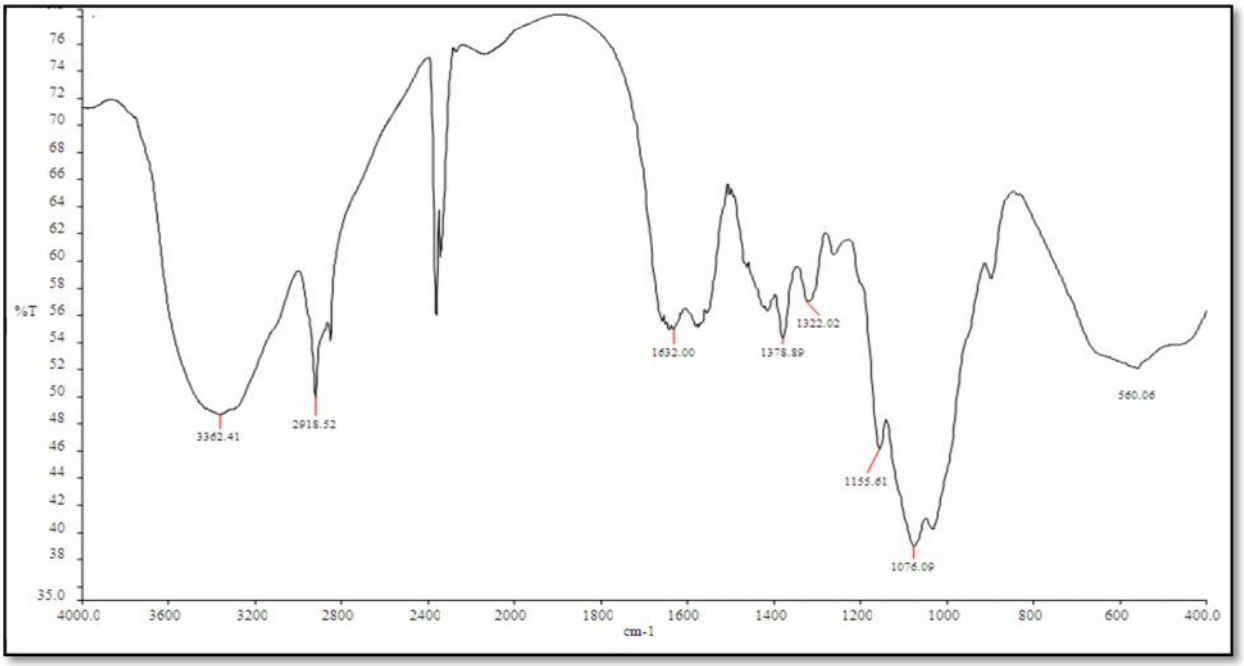

Fig.2 (B) FTIR spectrum of fungal chitosan

E. Morphological Characterization

The gold coated fungal chitosan were examined under SEM. The morphology was found to be in the form of flakes and with rough surfaces.

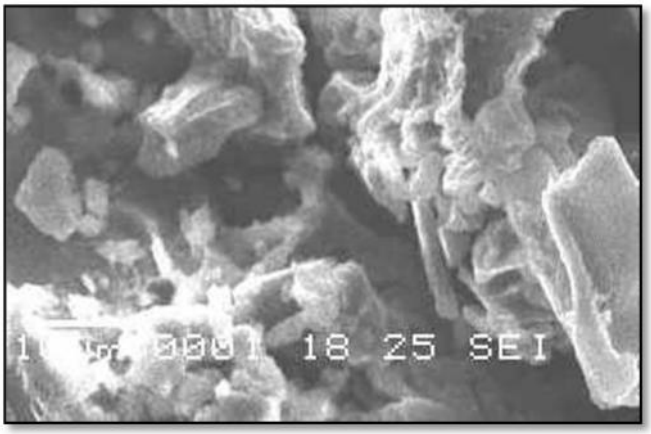

Fig.3 Analysis of surface topology by Scanning Electron Microscopy of fungal chitosan

D. UV Spectrometry Analysis of Drug bound fungal Chitosan

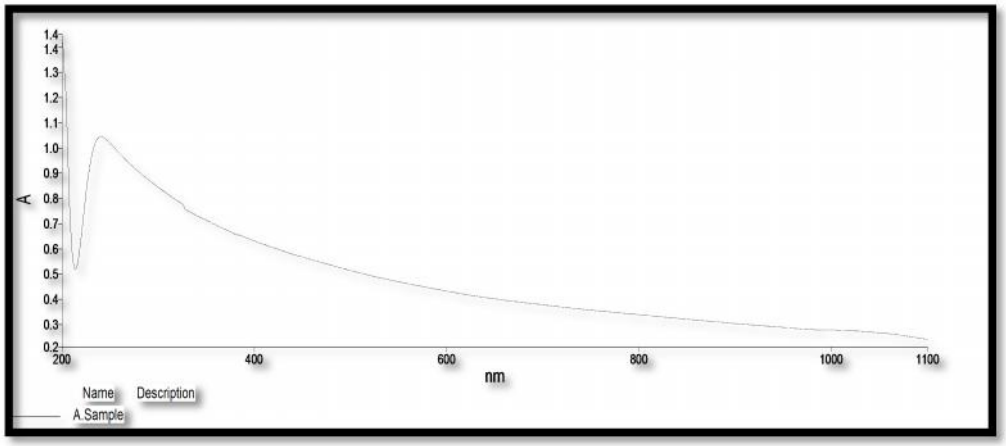

Fig. 4(A) UV – Visible Spectrophotometer for drug-tetracycline alone

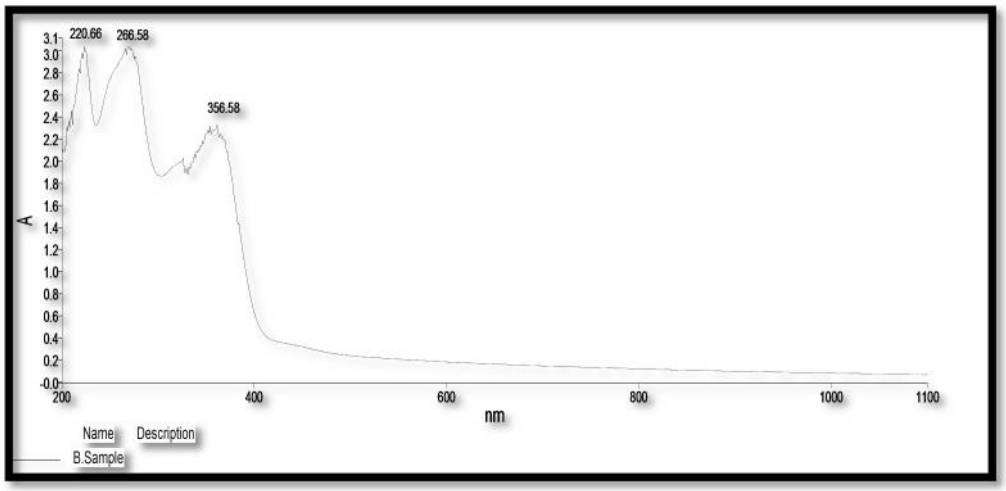

Fig.4 (B) Drug- tetracycline bound fungal chitosan.

UV Spectrometer analysis indicates the absorption of the drug - tetracycline at 220 nm [12]. There was a shift observed at 355 nm (Fig.4B). The results indicates (266.58 and 356.58) that the obtained fungal chitosan was bound with tetracycline. So it is comfirmed that the drug tetracycline has been bound with the fungal chitosan.

D. FeSEM of Fungal Chitosan Based Nanocomposite Film Bind with Tetracycline

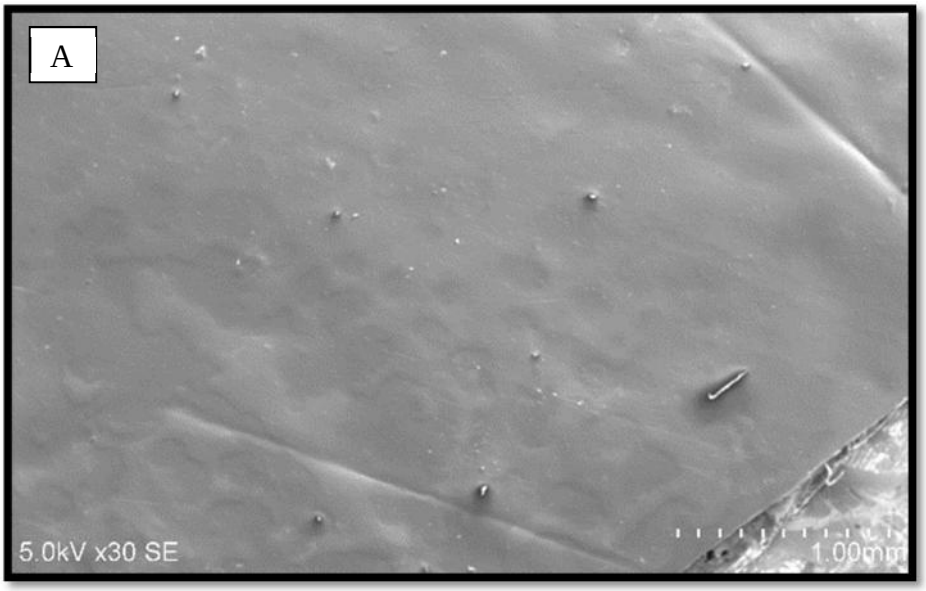

Fig. 5 (A) Nanocomposite film at lower magnification

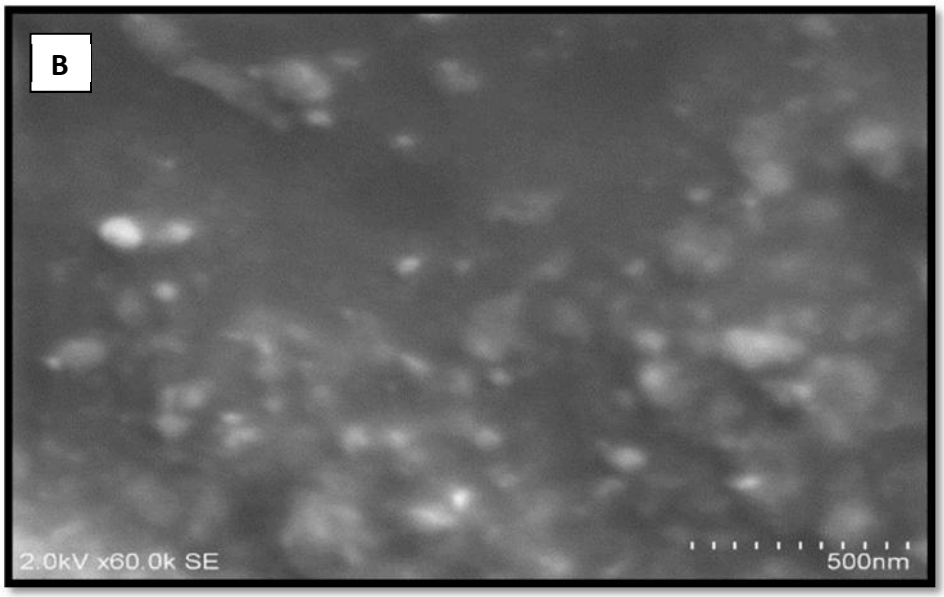

Fig. 5 (B) Nanoparticles approximately ranging from 10-100nm at higher magnification

E. Atomic force microscopy

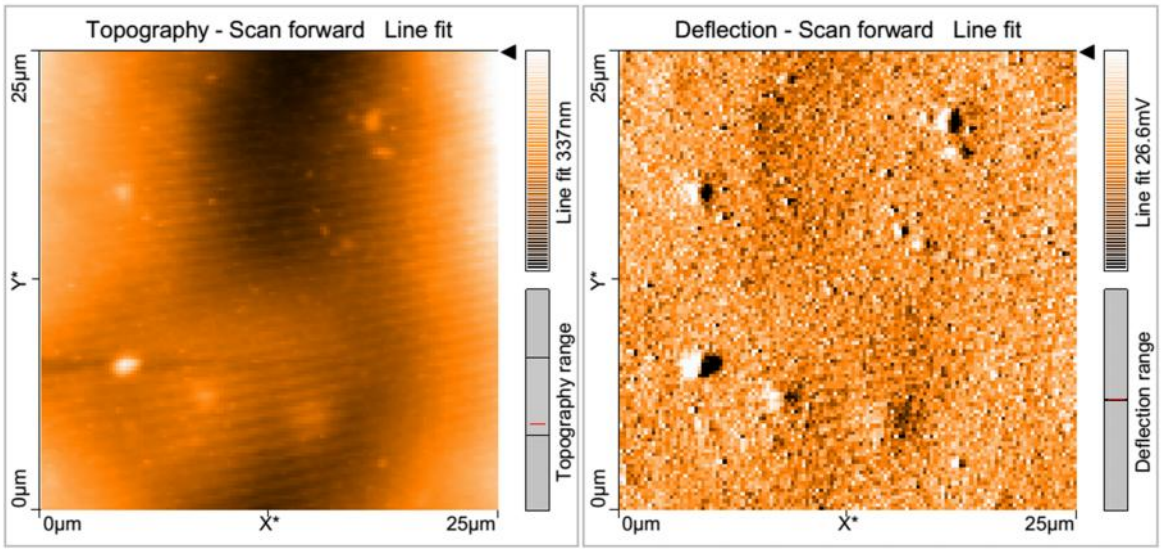

Fig. 6 (A) Presence of nanoparticles on the surface nanocomposite films

When the surface morphology of the nanocomposite was probed with AFM, the nanoparticles are observed on the surface which denotes the size ranges from 100nm to 250nm approximately and also indicates that these particles are doped on the surface of the films.

G. Antibacterial Properties of Blended Film

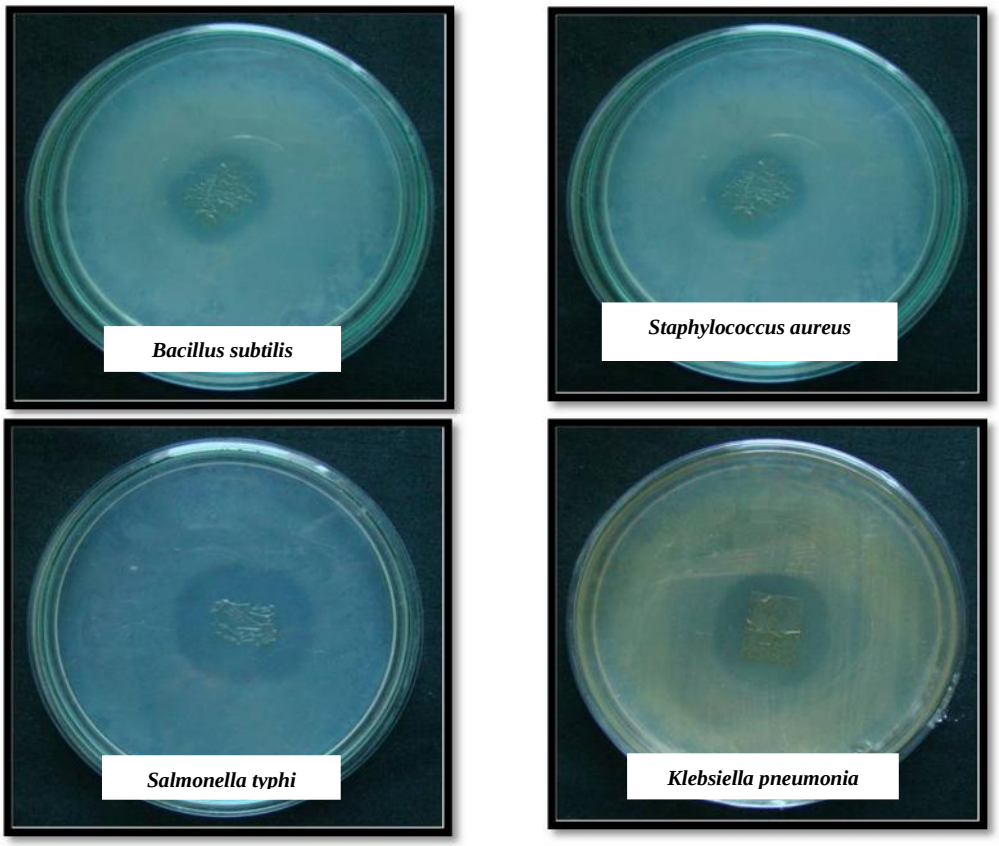

Fig. 7 Antibacterial activity of nanocomposite film against Gram positive and Gram negative bacteria

The nanocomposite films were subjected to antibacterial activity against Gram positive and Gram negative bacteria. The fig.7 shows good antibacterial activity against both Gram positive bacteria and Gram negative bacteria. However the maximum activity was observed in Gram negative bacteria viz., *Salmionella typhi* and *Klebsiella pneumonia* while comparatively minimal activity was observed in Gram positive bacteria viz., *Bacillus subtitlis* and *Staphylococcus aureus.* The antimicrobial activity is mostly attributed to its cationic nature (positively charged), where many of the many of the microbial cell components are negatively charged such as cell wall, DNA, and RNA. Chitosan could interact with microbial cells on their surface, which leads to the increasing of cell wall permeability and intracellular components leakage, or inside the cell, which inhibits the cellular synthesis of DNA and RNA. [13]. The antimicrobial activity of chitosan has been pointed out as one of its most promising properties [9]. Chitosan and its derivatives or oligomers has been recognized and is considered to be one of the most important antimicrobial properties, corresponding directly to their possible biological applications [23].

IV.CONCLUSION

Chitosan has desired properties for safe use as a pharmaceutical excipient which accelerates research activities worldwide on nanoparticles as drug delivery vehicles. Chitosan features many advantages viz., biocompatible, biodegradable, mucoadhesive and other unique biological properties. The presence of the functional group in chitosan possess unique properties. Overall, these chitosan tetracycline bound nanocomposite films can be suggested for wound healing.

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