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Research **Article**

SYNTHESIS OF A CHITOSAN BASED HYDRO GEL FROM SCALES OF LABEO ROHITO FOR EFFECTIVE WOUND HEALING

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ABSTRACT
The present work illustrates a met hod to synthesize an organic hydro gel that is superior, cheaper, bio-compatible and enables faster wound healing. Chitosan-based hydro gel was synthesized with acetic acid as the solvent medium and glutaraldehyde as the cross linker. The prepared hydro gel was characterized and tested using FTIR (Fourier Transform Infrared Analysis), SEM (scanning electron microscopy), also swelling test, and antibacterial activity was observed. The results of the present study demonstrate that the prepared hydro gel showed antibacterial activity with effective swell ability and structural and surface characteristics. Based on the characterization studies, it is concluded that the synthesized hydro gel had shown better characteristics and can be used as a promising material for wound healing applications.

Keywords: Hydrogel, Chitosan, Chitosan-based hydrogel, Anti microbial activity, wound healing.

INTRODUCTION

Wound healing is a normal biological process in the human body and is achieved through four precisely and highly programmed phases: homeostasis, inflammation, proliferation, and remodeling. So many wound dressing processes are taken based on different purposes. Several different kinds of wound dressing have certain important limitations like the addition of antimicrobial agents. Such antimicrobial agents have cytotoxic effects, leading to delayed wound healing. Also, the wound dressings stick onto the surface of the wound and injure the newly formed epithelium (Neha et al.,2019). The development of resistance among the microbial flora to the existing synthetic antimicrobial agents creates a serious risk to public health. This state has led to a re-assessment of the therapeutic use of traditional mediation, such as fish scales, Potato peel powder (pp), and orange peel powder (OP) are natural polymers been used for enabling superior wound healing. Especially, the pps and ops have various medicinal and nutritional value, and they are the source of phenolic compounds, flavonoids, glycol alkaloids, vitamins, and minerals like potassium. The glycol alkaloids present in pps and ops are toxic to microorganisms and these glycol alkaloids have beneficial properties, such as antipyretic, anti-inflammatory, and antimicrobial activities against pathogenic microbes (Ahmadi et al., 2015). In an attempt to overcome the limitations of wound dressings, the use of hydro gel and a three-dimensional macromolecular network of polymers in wound healing is investigated. The networks of the hydrogel can extensively swell up in water since water is the major constituent of the human body system, and a hydro gel, which can absorb large amounts of water, is considered to have huge potential when it is applied for

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pharmaceutical purposes (Billiet et al.,2012) The good biocompatibility of hydro gel emanates from their higher water content (over 70%). Hydro gel are hydrophilic three-dimensional networks that are chemically cross-linked or physically entangled with excellent water swelling capacity. On a molecular level, water in a hydro gel is either bonding to polar hydrophilic groups as a bond or is filling the space between the network chains, pores, or voids as free water. Hydro gel is either natural or synthetic cross-linked polymers used in a variety of biomedical fields. It consists of a matrix of insoluble polymers with about 96% water content. These hydro gel can donate water to the wound site and thus help in maintaining a moist environment, which helps in faster wound healing. These hydro gel are prepared using natural polymer such as Chitosan obtained from Rohu fish scales. Chitosan, a natural polymer derived from chitin, is a linear polysaccharide and hydrophilic (Sheng et al.,2019). Chitosan-based hydro gel has been demonstrated to promote wound healing at different wound healing stages and also can alleviate the factors against wound healing (such as excessive inflammation and chronic wound infection). The unique biological properties of a Chitosan-based hydro gel enable it to serve as both a wound dressing and as a drug delivery system (DDS) to deliver antibacterial agents, growth factors, stem cells, and so on, which could further accelerate wound healing. The Chitosan-based hydro gel has shown biocompatibility, biodegradability, antimicrobial properties, and non-toxicity (Kumari et al., 2014;Sacco et al.,2018) Potato-peel(pp) and orange peel(op), an industry waste material, inexpensive and valuable base material is used for the extraction of valuable products, such as natural antioxidants, dietary fibers, and biopolymers, due to the presence of primary and secondary metabolites, such as starch, proteins, polyphenols, lignin's, and small quantities of lipids. The chemical compositions of the pp are water, protein, Lipids, carbohydrates, starch and dietary fiber. The present work aims to incorporate the properties of pp and op in wound care with Chitosan producing a hydro gel which is superior, cheaper, biocompatible, and to enable faster healing.(Pantange et al.,1996; Terzioğlu et al.,2021)

MATERIALS AND METHODS:

Sample collection:

Fresh rohu fish scales were obtained from the local market in Coimbatore. The fish scales were washed with clean water and rinsed several times in sterile distilled water. The scale was sun-dried and crushed into pieces 10g of rohu fish scales were weighed and used for chitin/Chitosan extraction. The potato was obtained from the local market in Coimbatore. The potato was washed with clean water and rinsed several times in sterile distilled water. The skin was peeled off and dried and then powdered by using a mechanical grinder and crushed into a fine powder using a mortar and pestle 0.1 g of the powdered sample was weighed and used for Hydro gel synthesization. (Neha et al.,2019). The orange was obtained from the local market in Coimbatore. The orange was washed with clean water and rinsed several times in sterile distilled water. The skin was peeled off and dried and then powdered by using a mechanical grinder and crushed into a fine powder using a mortar and pestle. 0.1 g of the powdered sample was weighed and used for Hydro gel synthesization (Manjunath et al.,2015). The following are the chemicals required for the synthesis and testing of hydro gel: prepared Chitosan powder, glacial acetic acid (99.5% pure), glutaraldehyde (25%wt)(Pandharipande et al.,2018)

Synthesis of chitin and Chitosan:

Chitosan was prepared according to the modified protocol of (Rajagopal et al.,2019).The process of preparation of the hydrogel has been filed for patenting, Patent application no: 202241023801A-BIOCOMPATIBLE.

HYDROGEL AND A METHOD FOR ITS SYNTHESIS

Preparation of Chitosan from fish scales was performed by comprising of demineralization, decolourisation and deacetylation. Raw fish scales were washed thoroughly with water, dried in oven and soaked in 1%HCL solution for 36 hours. It was then washed dried in oven and kept in 2N NaOH solution for 36 hours for demineralization. Fish scales were then kept in potassium permanganate solution (having composition 1g of oxalic acid in 100ml distilled water) for 1 hour for the process of decolourization of the experimental sample. These processes resulted in chitin as the product which was further treated with 50%w/v NaOH (50 gram of NaOH in 100 ml distilled water) for the process of deacetylation resulting in Chitosan as the end product (Rajagopal et al, 2019).

Preparation of Chitosan-based Hydro gel:

One gram of prepared Chitosan was weighed and suspended in 20.0 ml each of 2% acetic acid at room temperature for 24 hours with constant stirring (450rpm) to obtain pale yellow gelatinous Chitosan solutions

(cs). The ppp and opp of 0.1 g was dissolved in 5ml each of 2%aqueous acetic acid by continuous stirring for 5 hours (650 rpm) to obtain viscous brown Chitosan/ppp/opp solutions. 1ml of aqueous glutaraldehyde (1%) solutions was supplemented to samples of clear brown cs and heat stirred for about 5-10 minutes to form cross-linked Chitosan-based hydro gels. The obtained hydro gels was dried under freeze-drier at -50°c for 24 hours followed by freezing at 90°c for 5 hours for further analysis (Neha et al.,2019).

Evaluation of Chitosan-based Hydrogel:

FTIR (Fourier Transform Infrared Spectroscopy) analysis: The nature of chemical bonds or functional groups present in Chitosan and Chitosan-based hydro gel are identified through the FTIR. The gel sample was loaded in FTIR spectroscope with a wavelength of 4000-400 cm-1. The spectrophotometer was operated in the attenuated total reflection (ATR) mode (El-Hefian et al.,2010; Ganji et al.,2007)

SEM (Scanning Electron Microscopy) analysis: The hydro gel texture was examined by field emission scanning electron microscopy (FESEM) to ensure that it retains its structure or not. The dried hydro gel was cut to expose its inner structure for SEM studies. The freeze-dried sample was imaged using FEI quanta200F operating at a voltage of 30 KV (Neha et al., 2019).

Swelling study: The swelling study of the test sample was done at room temperature with distilled water of pH value 6.5-8.5 and the effect of time on the percentage swell ability was calculated at varying periods of 10,20,30,40,50,60 minutes at a constant pH. The hydro gel was then drawn from solutions at varying time intervals and their wet weight was measured after first blotting with a Whatsman No. 1 filter paper followed by blowing with a stream of air to eliminate the surface water and weighed right away (Neha et al.,2019).

The percentage of swelling (S) of cross-linked Chitosan-based hydro gels were studied by using Equation.

$$S = (Ws-Wd)/Wd \times 100$$

Where,

Wd–dry weight of the sample in grams, Ws –wet (swollen) weight of the sample at time t.

Antibacterial study (Agar well diffusion test):

The antibacterial activity of hydro gel was evaluated by using the agar diffusion method against the gram-negative *(Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli)* and gram-positive *(Bacillus subtilis)* bacteria. The antibacterial activity of Chitosan solution, Chitosan-based hydro gel, Antibiotic, and Chitosan-based hydro gel + antibiotic was measured using the agar diffusion method. Briefly, the bacterial suspension was added and spread out nutrient agar media surface. Then the samples were added to the suspension culture. The dishes were incubated upside down at 37°c, overnight. Zones of inhibition were evaluated by measuring the diameter of the bacterial growth inhibition zone around the membrane. The samples were performed with triplicates for each bacterial strain. The positive control was a commercial antibiotic, tetramycine which is a commercially available antibiotic. The negative sample was the hydro gel which was prepared by Chitosan–based hydro gel.

Results and Discussion:

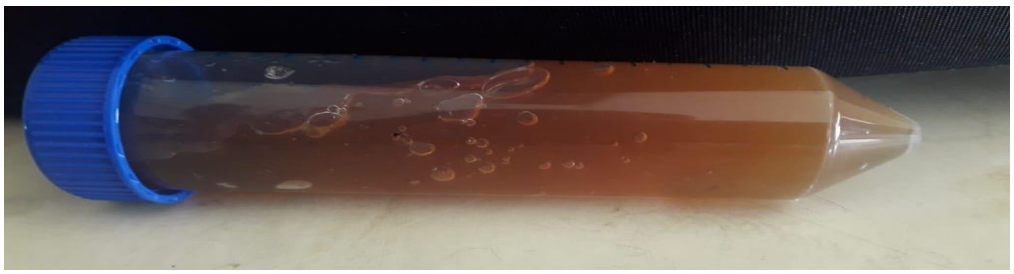
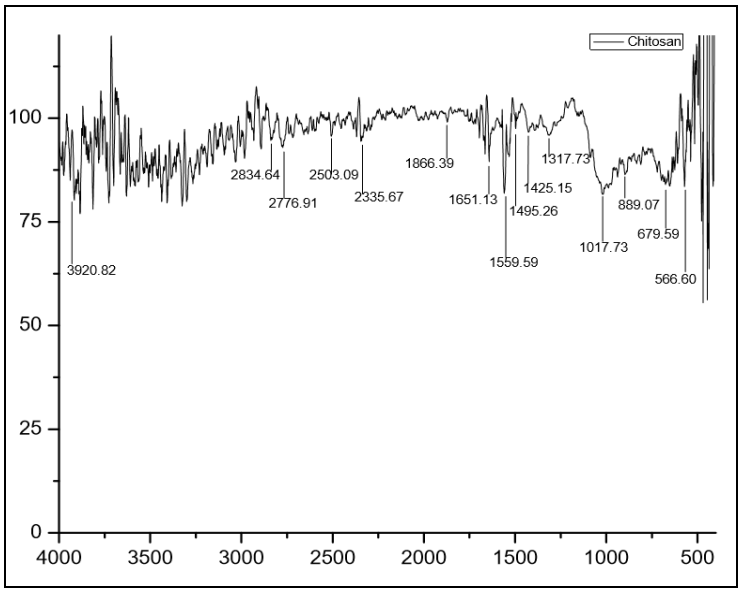


Figure 1. Chitosan-based Hydrogel

FTIR analysis:

Table 1a.Interpretation of spectrogram



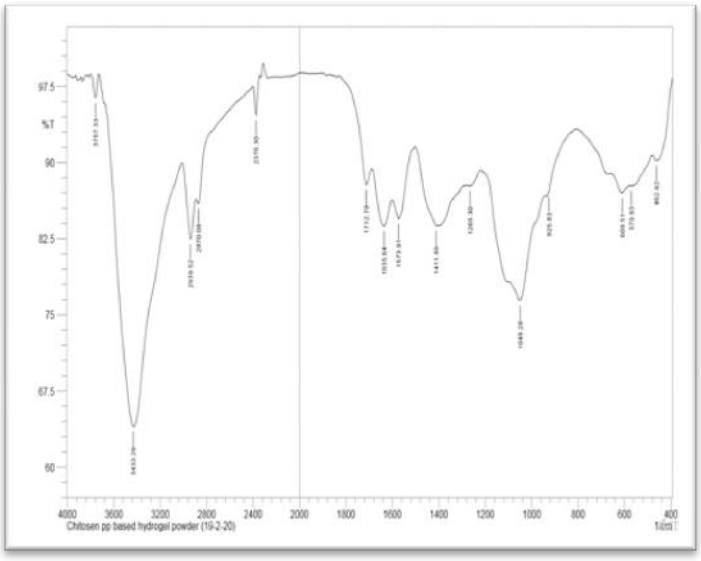
Peaks value	Functional group	Description
3500-3940	**C-H O-H -NH**	Stretch and medium stretch
2834	**C-H**	Stretch and medium
2776	**-C=C-**	Symmetrical alkynes
2335	**O=C=O**	Stretch and strong
1866	**C=O**	Stretch and strong
1556	**N-O**	Stretch and strong
889	**=C-H**	Bending and strong
679	**C=C**	Bending and Strong

Figure 2a. FTIR image of Chitosan Powder,

X axis- Frequency (cm-1), Y axis- Transmittance (%)

The product obtained was analyzed by FTIR in which the presence of functional groups of Chitosan has been ascertained such as C-H (aldehyde group), O-H (hydroxyl group), -NH (amine group), -C=C-(hydroxyl group), C=O (carbonyl group), =C-H (aldehyde group). The Figure 2a.Spectrogram peaks reveal that the following functional groups are Chitosan powder.

Table 1b. Interpretation of Spectrogram



Peaks value	Functional group	Description
3757	C-N	Weak and bend
3433	OH	Broad and Stretch
2939	-C-H	Weak and Stretch
2376-2870	C=C	Weak and bend
1712	C=O	Strong
1573-1635	-C=C-	Medium & Stretch
1049	C-F	Strong
609	C-Br	Strong

Figure 2b.Dried Chitosan based hydro gel,

X axis- Frequency (cm-1), Y axis- Transmittance (%)

SEM analysis:

The Figure 2b.Representative FTIR spectrum of the cross-linked hydro gel shows a broad, stretch peak at 3433 revealing the O-H (alcohol and phenol). The peaks at 2870.08 and 2376.30 reveal the C=C Weak and bend (Alkynes). The peaks at 1635.64 and 1573.91 reveal the –C=C-(Alkenes). The peaks at 2376.30 and 1712.79 are allowed for the stretching vibration of the amino group of Chitosan added. The peak around 1411.89 is attributed to the presence of polysaccharide content of the Chitosan. The peaks at 1049 correspond to the C-O (Tertiary alcohols) stretching, which corresponds to the efficient cross-linking of Chitosan hydro gel with glutaraldehyde that formed at the amino group of Chitosan (Akakuru,Isiuku.,2017)

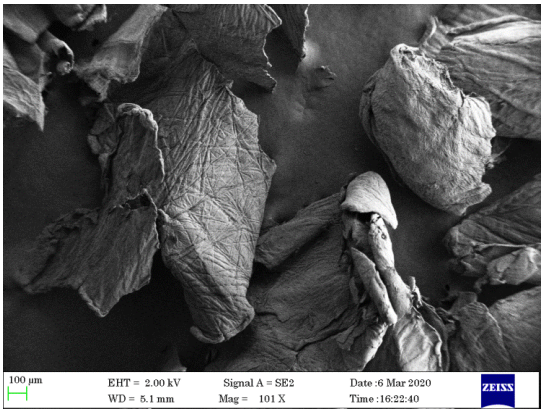


Figure 3a. SEM image of Chitosan Powder

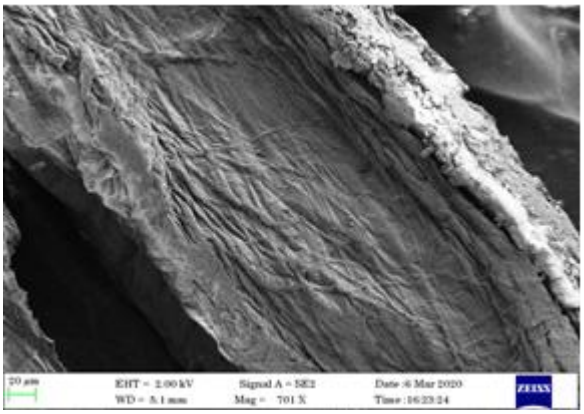


Figure 3b. SEM image of Chitosan Powder

Figure 3a. & 3b. Shows the SEM photographs of prepared chitin and Chitosan from fish scales. Both samples exhibited soft and thick surface morphology under electron microscopic examination at 50 X magnification.

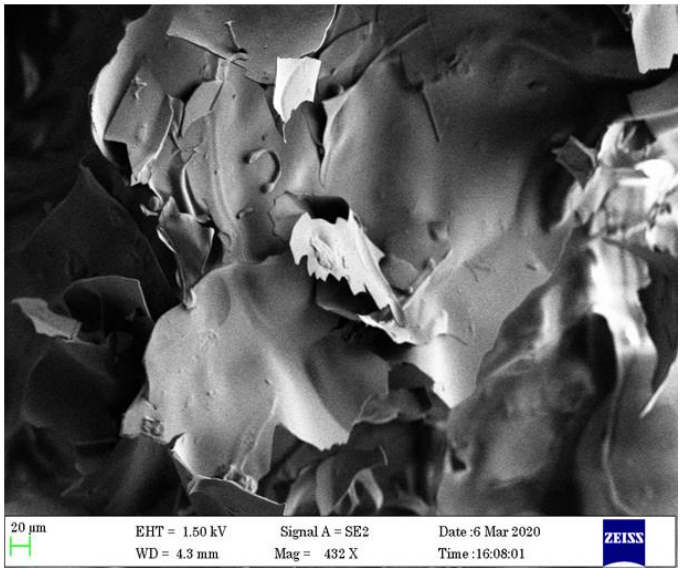


Figure 3c. Chitosan-based Hydro gel

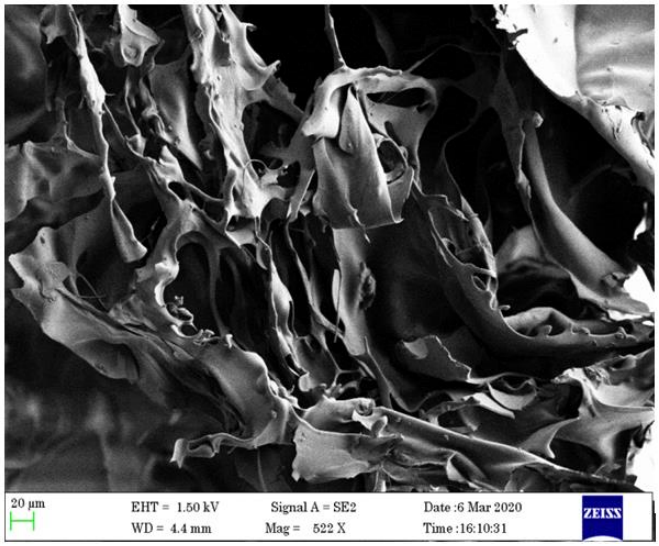


Figure 3d. Chitosan-based hydro gel

Figure 3c. & 3d. Shows the SEM photographs of prepared Chitosan–based hydro gel. Both samples exhibited expansion and stiffness surface morphology under electron microscopic examination at 50 x magnification.

Swelling study:

Swelling kinetics and time-dependent swelling properties of Chitosan hydrogels in de-ionized water were tested. The results revealed that the percentage swelling of the cross-linked Chitosan-based hydro gel increases as time increases, but near equilibrium was achieved after 50 minutes. This property is due to the presence of hydroxyl groups in the Chitosan which is responsible for its hydrophilic nature and the flexible property of the matrix. The swell ability of Chitosan-based hydrogel is commonly affected by three aspects. The hydrophilic nature of Chitosan is due to the presence of hydroxyl (-OH) groups in its side chain. The presence of amino (-NH2) groups, which get protonated in water, predominately in acidic medium. Elasticity of the Chitosan polymeric matrix, which allows easy diffusion of the sample(Rohindra et al,2004).

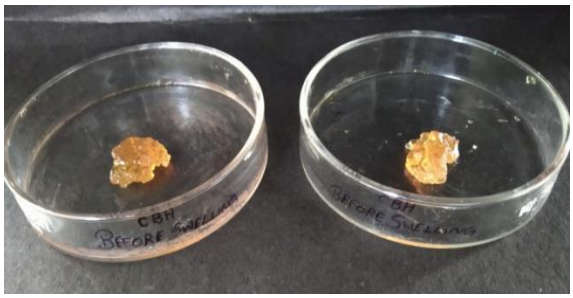


Figure 4a. Before swell ability

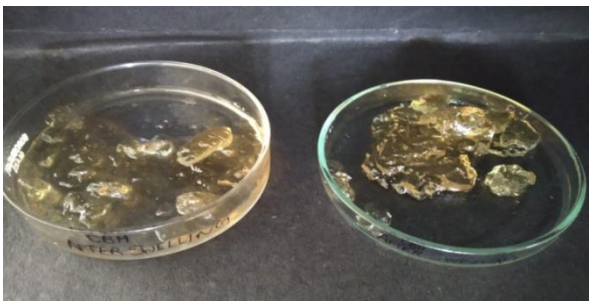


Figure 4b. After swell ability

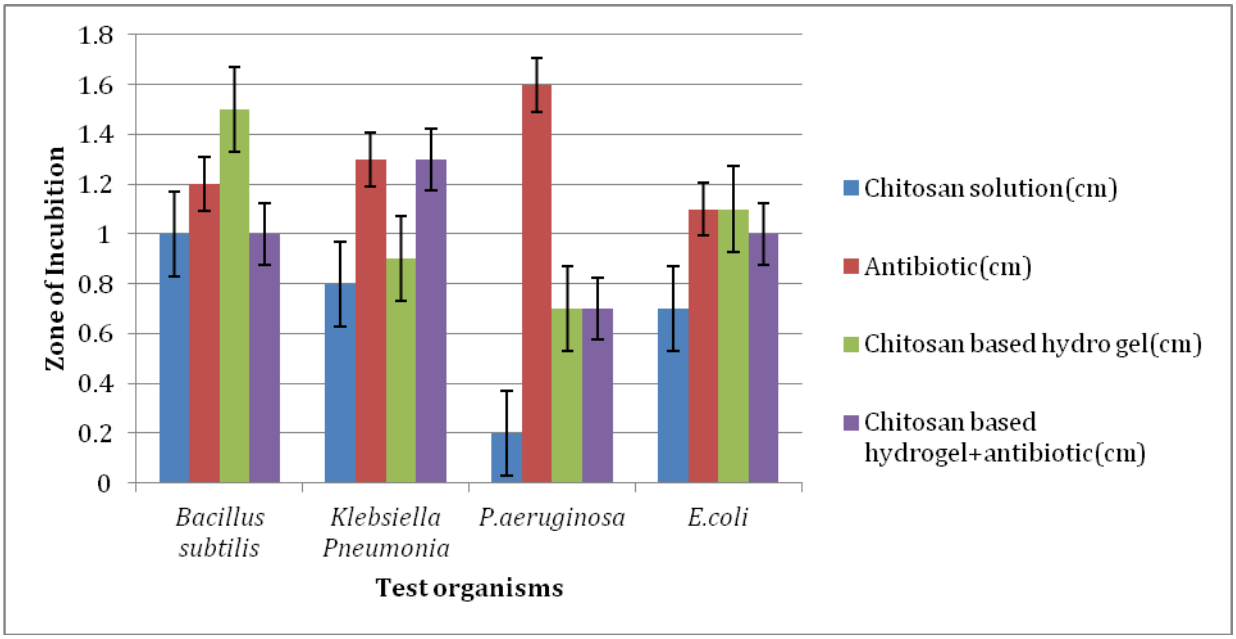


Figure 5. Antimicrobial activity of four different microbial strains

P.aeruginosa-Pseudomonas aeruginosa, E.coli-Escherichia coli

The antibacterial activity of four organisms *Bacillus subtillus, Klebsiella Pneumonia, P.aeruginosa, Escherichia coli* were studied. A clear zone of inhibition adjacent to hydro gel indicates antibacterial activity. Higher antibacterial activity of the hydro gel is observed against *Bacillus subtilis*, whereas lower activity is observed against *Pseudomonas aeruginosa*.

A significantly higher growth inhibitory effect on the (*Bacillus subtilis*) gram-positive bacteria was evident when compared to the gram-negative bacteria. This is because gram-positive bacteria as far more susceptible to the water deficit caused by the fluid uptake than the gram-negative bacteria. Gram-positive and gram-negative bacteria investigated the antimicrobial property of PP against different bacteria, the results indicated that the antimicrobial properties of PP and op are species-dependent and also had noteworthy effects against *Escherichia coli*, *Pseudomonas aeruginosa* when compared with the standard antibiotic, Tetramycin. The interaction between the positive charge of Chitosan with negatively charged microbial cell membranes was responsible for its antimicrobial properties and it was well established also it results in the osmotic imbalance at the same time PPP and OPP demonstrate a synergistic effect which harms the cell membrane to direct the overall growth of the microbe, may be the probable mechanism of action.

The antimicrobial activity and the exhibition of significant zone of inhibition by the Chitosan-based hydrogel may be due to the presence of Chitosan, whose antibacterial property is attributed to its cationic nature, or the PP and OP content which is rich in flavonoids, phenolic compounds and the glycol alkaloids which are toxic to the phyto-pathogens and helps in healing wounds ultimately. Therefore, the results obtained in the present study

indicated that the hydro gel formulations exhibited good antibacterial activity against the various gram-positive and negative strains.

Figures and Tables

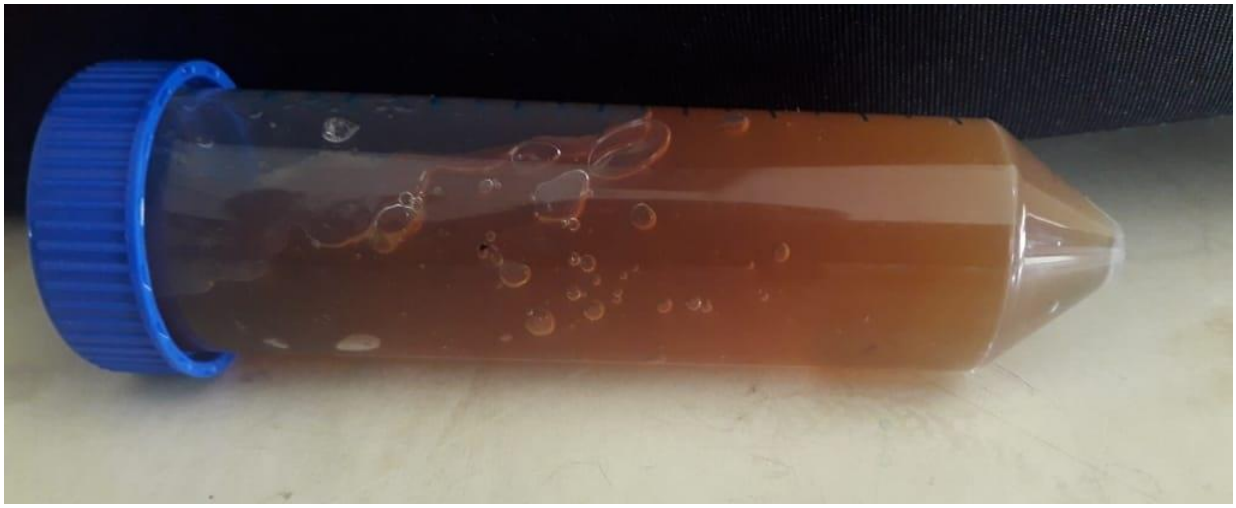


Figure 1. Chitosan-based Hydrogel

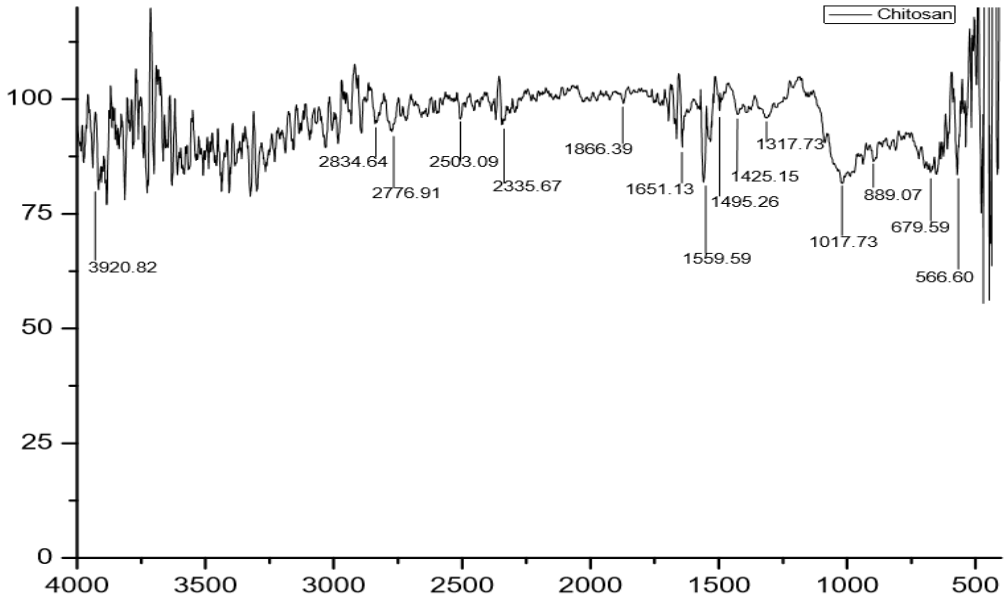


Figure 2a. FTIR image of Chitosan Powder

Table 1a. Interpretation of spectrogram

S.No	Peaks value	Functional group	Description
1	3500-3940	C-H O-H -NH	Stretch and medium stretch
2	2834	C-H	Stretch and medium
3	2776	-C=C-	Symmetrical alkynes
4	2335	O=C=O	Stretch and strong
5	1866	C=O	Stretch and strong
6	1556	N-O	Stretch and strong
7	889	=C-H	Bending and strong
8	679	C=C	Bending and Strong

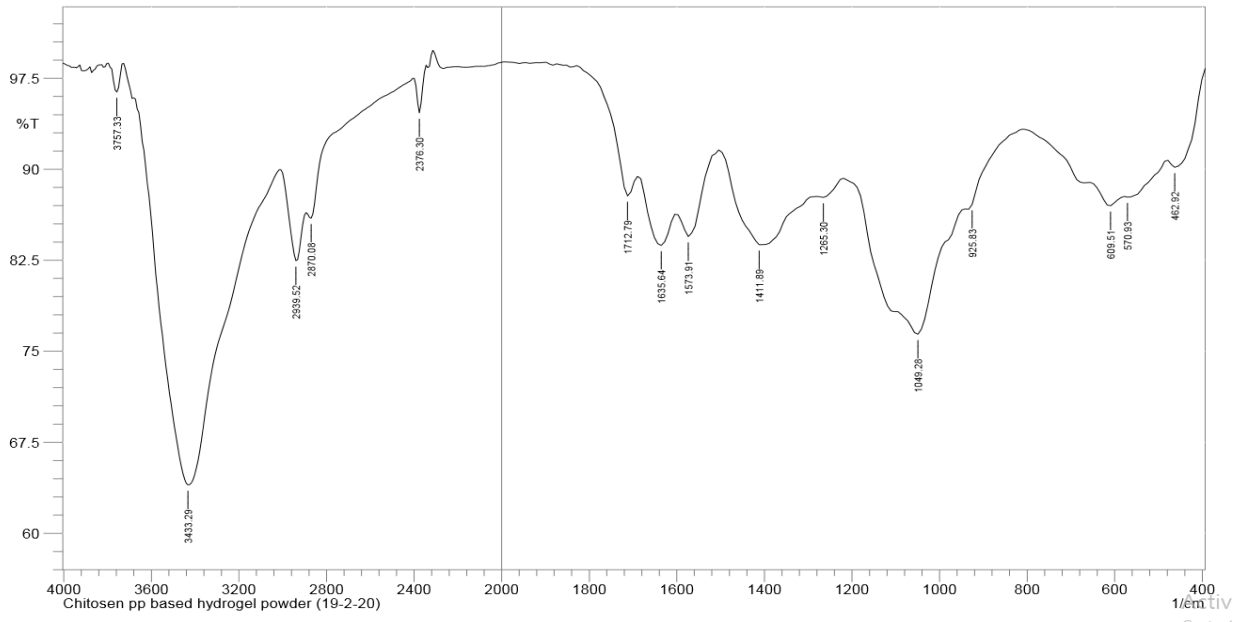


Figure2b. Dried Chitosan based hydro gel X axis- Frequency (cm-1), Y axis- Transmittance (%)

Table 1b. Interpretation of spectrogram

S.No	Peaks value	Functional group	Description
1	**3757**	**C-N**	Weak and bend
2	**3433**	**OH**	Broad and Stretch
3	**2939**	**-C-H**	Weak and Stretch
4	**2376-2870**	**C=C**	Weak and bend
5	**1712**	**C=O**	Strong
6	**1573-1635**	**-C=C-**	Medium and Stretch
7	**1049**	**C-F**	Strong
	609	**C-Br**	Strong

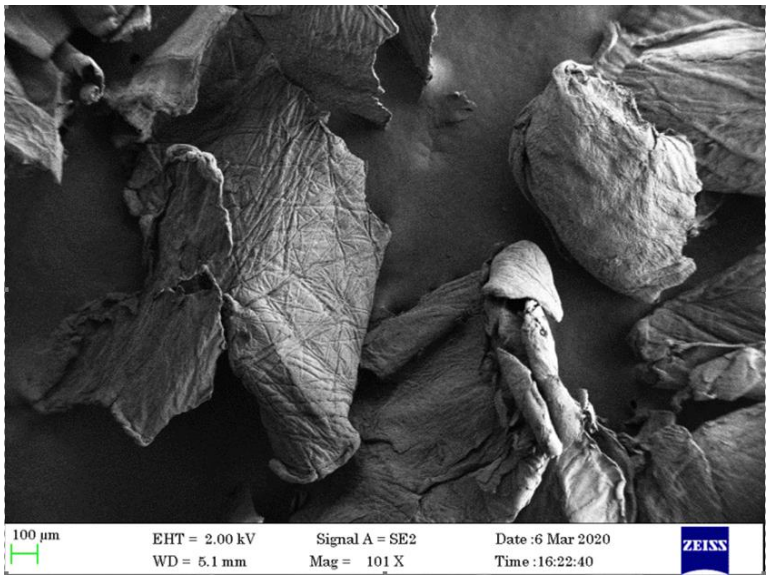


Figure 3a. SEM image of Chitosan Powder

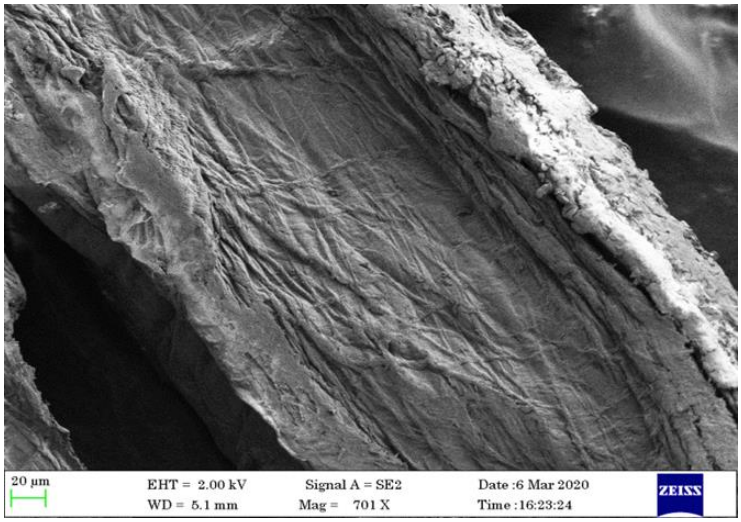


Figure 3b. SEM image of Chitosan Powder

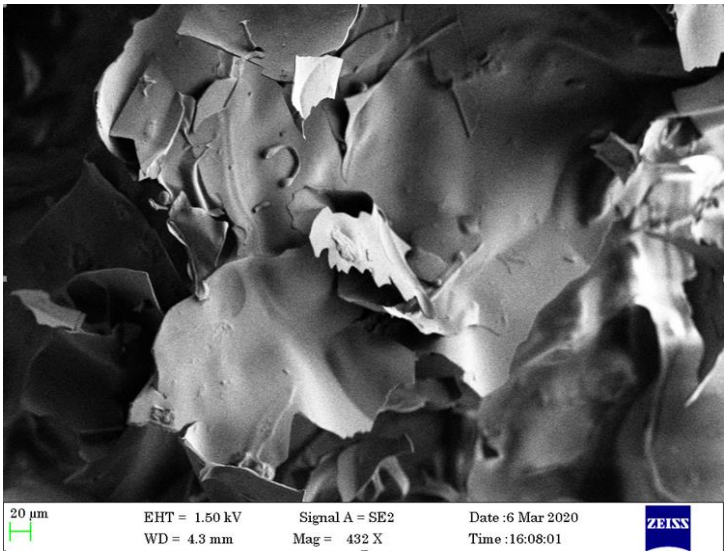


Figure 3c. Chitosan-based Hydro ge

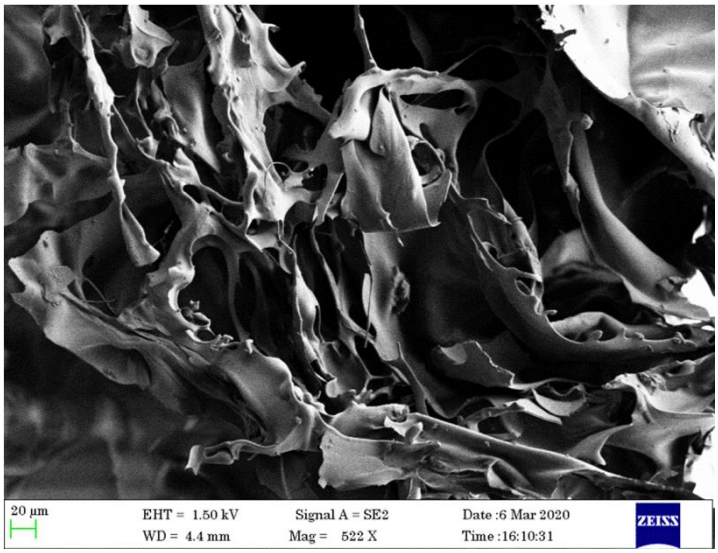


Figure 3d. Chitosan-based Hydro gel

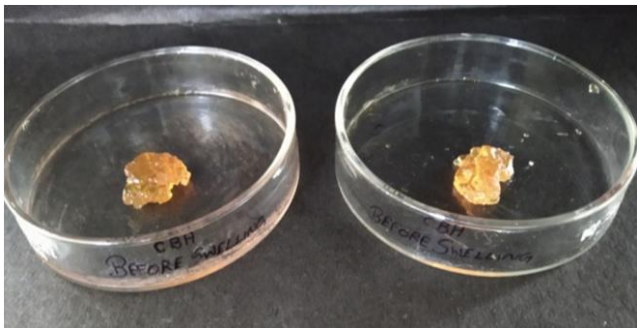


Figure 4a. before swell ability

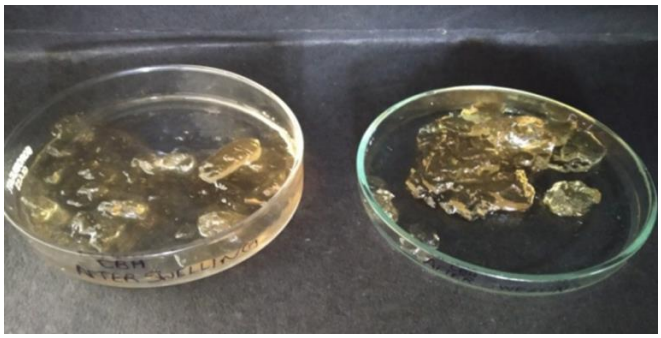


Figure 4b. after swell ability

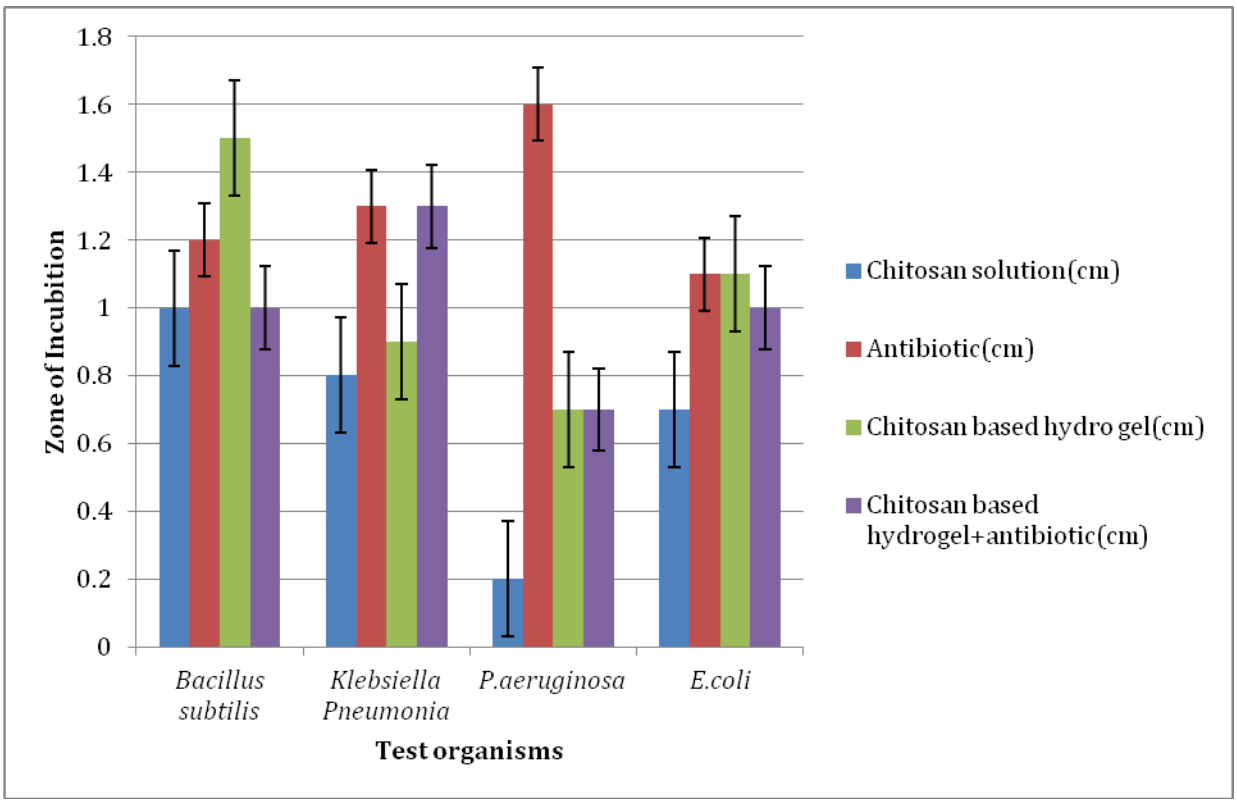


Figure 5. Antimicrobial activity of four different microbial strains

P.aeruginosa-Pseudomonas aeruginosa, E.coli- Escherichia coli

Conclusion

The chitosan-based hydro gel has been demonstrated to promote wound healing at different wound healing stages and also can alleviate the factors against wound healing. For that hydro gel synthesization, Chitosan, a natural polymer, Potato-peel(pp) and orange peel(op), an industry waste material, the inexpensive and valuable base material is used for the extraction of valuable products, such as natural antioxidants, dietary fibers, and biopolymers were selected to perform gel with a cross-linker as glutaraldehyde to determine the hydro gel. FTIR Interpretation of the spectrogram is used to analyze the presence of functional groups. Here, Chitosan has been ascertained such as C-H (aldehyde group), O-H (hydroxyl group),-NH(amine group),-C=C- (hydroxyl group), C=O(carbonyl group),=C-H(aldehyde group). The resulting spectrogram peaks reveal that the following functional groups are Chitosan powder. SEM is used to analyze the complete morphology structure of the sample given. Here, are SEM photographs of prepared chitin and Chitosan from fish scales. Both samples exhibited rough and thick surface morphology under electron microscopic examination at 50 X magnification. Then the SEM photographs of the prepared Chitosan-based hydro gel. Both samples exhibited rough and thick surface morphology under electron microscopic examination at 50 x magnification In the hemagglutination test, Auto agglutination was not observed in the RBCs treated with the drug (hydro gel). A normal settling pattern was seen. The antibacterial activity of hydro gel was evaluated by using the agar diffusion method against the gram-negative (*Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli)* and gram-positive *(Bacillus subtilis*) bacteria. So finally, the present work aims to incorporate the properties of Chitosan-based hydro gel in wound care have shown biocompatibility, biodegradability, antimicrobial properties, and non-toxicity and enable faster healing.

Acknowledgement

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