RESEARCH PAPER

Study the Effect of Nano Chitosan Synthesized from Different Substances in Iran and Iraq on Escherichia Coli

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ABSTRACT

Chitosan is a non-toxic, high molecular weight, biodegradable polymer that closely resembles cellulose, a plant fiber. The main difference between chitosan and cellulose is the presence of an amine group (-NH2) in chitosan, as opposed to the hydroxyl group (-OH) found in cellulose. The aim of the study is the effect of nanochitosan production in Iran and Iraq on bacteria. Chitosan is derived from chitin, a substance found abundantly in the shells of shellfish such as crabs, lobsters, and shrimp. Extracting chitin from natural sources involves a process of demineralization and deproteinization. The purified chitin is then treated with concentrated alkaline compounds, such as sodium hydroxide, in a process known as deacetylation, resulting in chitosan. Chitosan exhibits enhanced antimicrobial properties when formed into nanoparticles and used as a coating material. Chitosan nanoparticles. These chitosan nanoparticle derivatives were evaluated for their antibacterial activity against Escherichia coli using methods such as well diffusion and microtiter method. The results showed the highest inhibition zone against Escherichia coli, the characteristics of nanochitosan, including its charge and size, ranged from 20-100 nm. The particle size of the nanochitosan was measured by (TEM) analyze the physical properties and morphology of nanochitosan. Microbiological investigations: Microbiological investigations were carried out to evaluate the antimicrobial properties of nanochitosan. Determination of MIC of nanochitosan was determined for some pathogenic bacteria, providing insights into their efficacy as antimicrobial agents. The results showed that among the nanostructures studied, the nanochitosan made from seashells from Iraq and Iran was the most effective. The results showed that the nanochitosan that appeared under the electron microscope and was characterized by its spherical shape had more antimicrobial properties compared to the nanochitosan that was characterized by its tubular shape due to its small size and its ability to penetrate bacterial cells easily.

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INTRODUCTION

Demineralization and deproteinization were carried out to obtain chitin from the shells, followed by deacetylation to obtain chitosan. Chitosan is a polymer derived from deacetylation of chitin. Chitin is naturally obtained from several sources which include cell wall of fungi, linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine. It is produced by treating the shells of the marine animals with the alkali sodium hydroxide. Chitosan is biocompartible and biodegradable and it enhances the transport

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of polar drugs across epithelial surface and as an antibacterial age[1]. Chitosan is can be easily biodegraded. Its residues are not toxic and can be easily eliminated and biodegraded by nature[2]. chitosan is the presence of a large number of hydroxyl and amino groups in its structure[3]. The combination of chitosan with other materials such as collagen can also increase the range of its features[4].And The specific use of the trypsin also induces the depigmentation, reducing the steps involved in the extraction of chitin[5].The two main advantages of chitosan over chitin is that in order to dissolve chitin, highly toxic solvents such as lithium chloride and dimethylacetamide are used whereas chitosan is readily dissolved in dilute acetic acid. Also, chitosan possesses free amino groups as active sites in many chemical reactions[6].The idea is to replace traditional raw materials with new ecofriendly materials which contribute to keeping a high production rate but also reducing its environmental impact and the costs. its low cost also makes it a good choice of material[7].Nanochitosans are considered natural bioactive materials with environmentallybeneficial physicochemical constituents. Nanochitosan may be produced by a variety of methods, including ionotropic gelatination of STPP and chitosan [8] Nanochitosan has a high effective potential in supplying drugs through nasal, gastrointestinal, and pulmonary pathways for the quick recovery of patients[9]Transmission electron microscopy (TEM) was used to observe the morphology of the nano-chitosan.MIC and MLC values of chitosan and nano- chitosan on some pathogenicbacteria were determinedby broth dilution method[10].

MATERIALS AND METHODS

Materials MIC of Chitosan Nanoparticles

Experience One focuses on the determination of the Minimum Inhibitory Concentration (MIC) of Chitosan Nanoparticles, a critical aspect in understanding their antimicrobial properties. Chitosan, derived from crustacean shells, has garnered significant attention for its potential as an antimicrobial agent due to its biocompatibility and biodegradability. This experiment aims to assess the effectiveness of chitosan nanoparticles in inhibiting the growth of Escherichia coli (E. coli), a common bacterial pathogen. The MIC represents the most reduced concentration of an antimicrobial specialist required to repress the unmistakable development of a microorganism. By deciding the MIC of chitosan nanoparticles against E. coli, we will discover their strength and potential application in combating bacterial infections. This segment subtle elements the exploratory methods utilized to assess the MIC of chitosan nanoparticles, counting the planning of bacterial societies, the detailing of chitosan nanoparticle arrangements, and the assurance of MIC values through standardized strategies. Through efficient experimentation and examination, we point to contribute important experiences into the antimicrobial adequacy of chitosan nanoparticles, clearing the way for their utilization in different biomedical and natural applications [11].

Materials

Eosin Methylene Blue Agar (EMB Agar) for selective growth of E. coli, Brain Heart Infusion (BHI) Agar for active growth of E. coli, BHI Broth 1X and 2X for bacterial culture, Bacterial suspension. Preparation antibiotic: Chitosan nanoparticles mixed with Oxacillin sodium salt, Tetrazolium reagent, Plate Elisa, Pipettes (10µl & 100µl).

Extraction of Chitosan

Commonplace era of chitosan from shellfish shells for the foremost portion comprises of three principal steps: demineralization, deproteinization, and deacetylation. The shells were demineralized by ceaseless blending with 5% HCl at a extent of 1:15 (w/v, shell to course of action) for 36 h at room temperature. The demineralized shells were treated with a 5% NaOH course of action at a shell to course of action extent of 1:10 (w/v) at 90-95°C for 6h. The deproteinized shells were filtered and washed with deionized water until the NaOH was completely ousted, and after that dried overnight in an broiler at 55-60°C. The shells were filtered and washed with deionized water until they have to be be fair-minded. At that point, the deacetylation of chitosan was carried out by hydrolysis with 80% NaOH at a extent of 1:20 (w/v, chitin to dissolvable) at 90-95 °C for 5 h. This thing was washed with deionized water until it ought to be fair and dried overnight at 55-60°C. Inside the arranging of chitosan arrangements,1.0%(w/v)chitosan was scattered in 1.0% (v/v) acidic destructive course of action as shown in Fig. 1 [12].

Planning of chitosan nanoparticles

Within the show work, the chitosan nanoparticles were synthesized from the chitosan

utilizing sodium tripolyphosphate as a crosslinking operator by ionotropic gelation method. Initially in arrange to make the homoge- neous chitosan arrangement, around 1.5 g of chitosan broken up in 200 ml of 2% acidic corrosive arrangement was kept beneath attractive blending handle for approximately 20 min. At that point to the over arranged chitosan arrangement, 0.8 g of sodium tripolyphosphate broken down in 107 ml of conductivity water was included drop astute and blended well for almost 30 min to reach equilibrium.A smooth colored emulsion like appearance of chitosan nanoparticles was shaped upon the ionic cross connecting between the sodium tripoly- phosphate and chitosan arrangement. After coming to harmony, The suspension was shaped in over said conditions. The nanoparticles were isolated by centrifugation at 20,000 rpm for 30 min, solidify- dried and put away at 5 ± 3°C (Fig. 1) [13].

Media used for selective the E. coli (Eosin methylene blue agar)

Preparation the media

Suspend 3.5 grams of EMB Agar in 1000 mls of distilled water,heat to dissolve the medium completely,idspense and sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes "Avoid overheating",cool to 50 °C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue color) and to suspend the flocculent precipitate ,pour into Petridishes[14].

Preparation the brain heart inffusion BHI agar

Suspend 3.5 grams of BHI agar in 100 ml of distilled water, heat to dissolve the medium completely,dispense and sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes"Avoid overheat",cool to 50 °C and shake the medium in order to oxidize the methylene blue (i.e. to restore its blue color) and to suspend the flocculent precipitate[15].

Preparation BHI broth 1X

Suspend 1 grams of BHI broth in 25ml of distilled water , heat to dissolve the medium completely,dispense and sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes " Avoid overheating" ,cooled and saved in glass. [16].

Preparation BHI broth 2X

Suspend 2 grams of BHI broth in 25 ml of distilled water,heat to dissolve the medium completely,dispense and sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes "Avoid overheating",cooled and saved in glass [16].

Preparation the samples by McFarland method

Prepare tubes Added normal saline all tubes Addad E.coli by loop to all tubes in different concentration and mix the bacterial suspension and become ready [17].

Tetrazolium chloride reagrnt (TTC)

Tetrazolium chloride is a redox indicator that can differentiate between living and dead tissues based on the reduction of TTC to 2,3,5-triphenylformazan, which is red, by dehydrogenases present in the living tissues. This results in living tissues staining a degree of red, while dead tissues remain unstained.It is a white crystalline powder, soluble in water, ethanol and

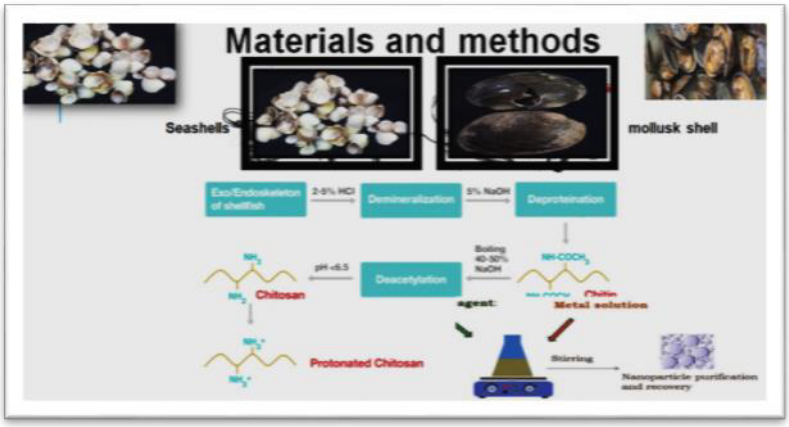

Fig. 1. Schematic illustration of different steps of Chitosan Nanoparticles synthesis.

acetone but insoluble in ether[18,24].

Tetrazolium salts serve as substrates for active cellular dehydrogenases and reductases. In the presence of NADH/NADPH, these salts are reduced to formazan products and produce strong, distinct colors. Since these reactions take place in cells actively producing NAD(P)H, tetrazolium substrates have gained widespread use as a means of distinguishing living, metabolically active cells through staining and through cell viability assays [25].

Preparation antibiotic

Chitosan nanoparticles mix with Oxacillin sodium salt -95% (TLC) [19, 20].

The method works on an ELISA plate.

1. Dispense 100µl of Brain Heart Infusion (BHI) Broth 2X into all wells of a microplate, except for wells 11 (control positive) and 12 (control negative)

2. Dispense 100µl of BHI Broth 1X into wells 11 and 12.

3. Add 100µl of the antibiotic solution to well 1.

4. Perform serial dilutions by transferring 100µl from well 1 to well 2, and continue diluting up to well 10. Discard the last 100µl from well 10.

5. Add 10µl of bacterial *E.coli* suspension to each well

Incubate the microplate for 24 hours at 37°C. After incubation, add 10µl of Tetrazolium reagent to each well. Incubate the microplate for an additional 2 hours. Finally, read the results, which will indicate the presence or absence of bacterial growth based on the color change induced by the Tetrazolium reagent [21].

Experience Two: Burning the Rabbit's Ear

Experience Two delves into a crucial aspect of our research, focusing on the controlled burning of a rabbit's ear to observe the effects and subsequent healing process. This experiment was

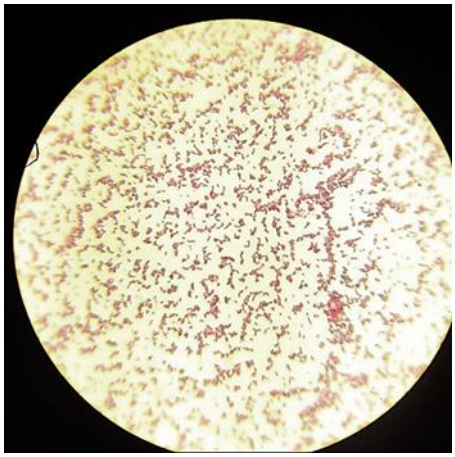

Fig. 2. Test for identifying *Escherichia coli* Gram stain test.

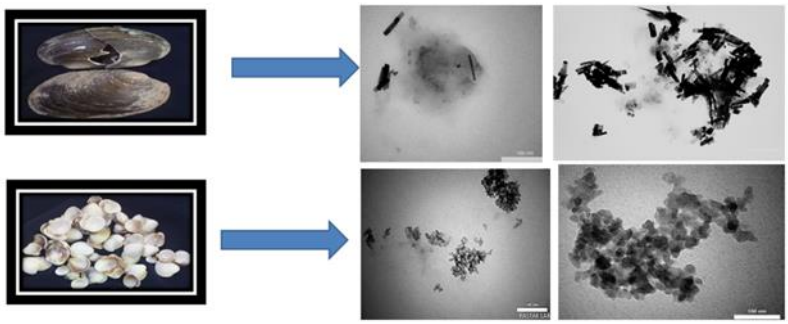

Fig. 3. TEM image of Chitosan Nanoparticles colloidal nanoparticles.

conducted to understand the response of rabbit tissue to superficial burns and assess the efficacy of Nano Chitosan Cream in promoting healing. By meticulously documenting the progression of the burn and its treatment over a span of five days, we aimed to gain insights into potential therapeutic interventions for burn injuries. This section provides a detailed account of the methodology employed and the observations made during the course of the experiment (Fig. 2) [22].

Materials include: Rabbits, Flame of fire, hair removal machine, marker, Nano Chitosan Cream

The methods was done as the following: Hair Removal: Utilizing a specialized hair removal machine, the hair on the rabbit's ear was carefully removed to ensure a clear surface for the subsequent procedure

Burn Area Determination: The burn area on the rabbit's ear was delineated with precision using a marker to clearly demarcate the region of interest.

Controlled Burn: Employing a flame of fire, the rabbit's ear was subjected to a controlled burning process to induce a superficial burn.

Application of Nano Chitosan Cream: Following the superficial burn, Nano Chitosan Cream was administered to the affected area to initiate the treatment process

Observation of Burn Healing: Daily monitoring of the burn site was conducted to assess the progression of healing. On the first day, initial observations were made to track the response to treatment.

Daily Treatment Regimen: Over the subsequent days, the burn site was washed with normal saline and treated with Nano Chitosan Cream. This regimen was repeated on the second and third days to facilitate healing.

Continuation of Treatment: On the fourth day, the treatment regimen was maintained with washing using normal saline and application of Nano Chitosan Cream to promote further healing.

Final Treatment: At the conclusion of the experiment on the fifth day, the burn site was washed with normal saline and treated once again with Nano Chitosan Cream to ensure comprehensive care.

RESULTS AND DISCUSSION

In microscopy, the shape of Escherichia coli (E. coli) bacteria can vary depending on their growth conditions and stage of growth. Generally, E. coli is described as a rod-shaped bacterium, meaning it has a cylindrical shape with rounded ends. However, there can be variations in the length and width of these rods

Under a microscope, E. coli cells typically appear as elongated structures with a uniform width along their length. They may sometimes appear slightly curved or bent, especially if they are actively growing or dividing.

Here's a general description of what you might see when observing E. coli under a microscope:

Rod-shaped cells with a length typically ranging from 1 to 3 micrometers.

The width of E. coli cells is usually around 0.5 to 1 micrometer.

Cells may appear singly or in chains, depending on their growth stage and conditions.

Some staining techniques, such as Gram staining, can provide additional visual characteristics of E. coli, such as its Gram-negative cell wall.

Remember that the actual appearance of E. coli cells under a microscope can be influenced by factors such as the staining method used, the age of the culture, and the specific strain of E. coli being observed (Fig. 2) [23].

The images labeled 1 and 2 depict the results of TEM (Transmission Electron Microscopy) used in the production of nanochitosan from sources in Iraq and Iran. Image 1 showcases the nanotubular

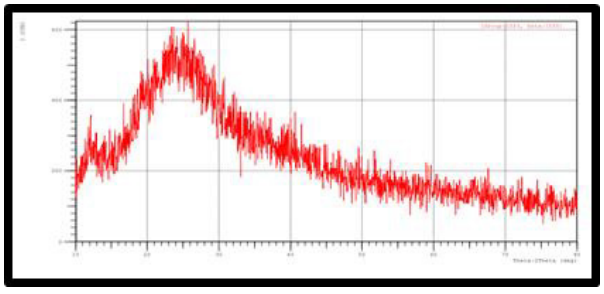

Fig. 4. X-ray diffraction pattern of Chitosan Nanoparticles.

shape obtained from oyster shells in Lake Dukan, Iraq, while image 2 displays the nanospherical shape obtained from oyster shells in the Caspian Lake, Iran (Fig. 3).

Nanoparticles The crystalline phase of the X-ray diffraction pattern of Chitosan Nanopart was confirmed by recording the XRD patterns from angles 25° as shown in Fig. 4.

Eosin-methylene blue agar is selective for gram-negative bacteria against gram-positive bacteria. In addition, EMB agar is useful in isolation and differentiation of the various gram-negative bacilli and enteric bacilli, generally known as coliforms and fecal coliforms respectively EMB agar inoculated with Escherichia coli (a gram-negative bacterium) demonstrating growth with green-metallic sheen colonies (for details see the Atlas page) (Fig. 5) [41].

Brain Heart Infusion (BHI) agar, a type of growth medium used in microbiology. BHI agar is a nutrient-rich medium commonly used for the cultivation of a wide variety of microorganisms, including bacteria and fungi. It contains beef heart infusion, peptones, and other nutrients that support the growth of many organisms (Fig. 6).

The MIC values were evaluated for two groups of antibacterial materials (nanoparticle chitosan manufactured in seashells of Iraq, nano-chitosan manufactured in seashells in Iran).The MIC values of nano-finished chitosan from Iraqi shells were calculated in the range of 0.05-15 µg/ml. The MIC values of nanoparticle chitosan manufactured from Iranian shells were calculated in the range of 0.05-7.5 µg/ml. The bactericidal effect of nano-chitosan manufactured from Iranian shells was stronger than that of nano-chitosan manufactured from Iraqi shells, because the shape, size, and concentrations of different nanomaterials have a

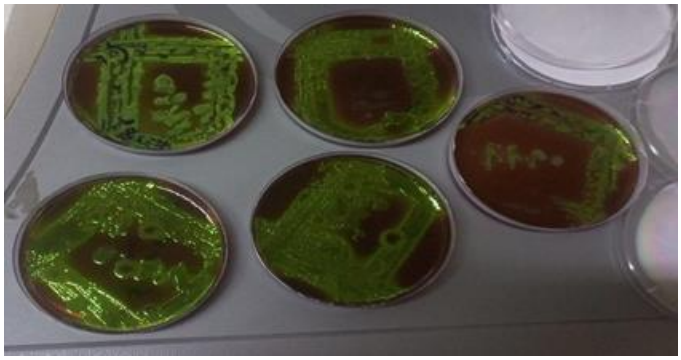

Fig .5. Shape E.coli in EMB agar.

Fig .6. Brain heart infusion agar in plate.

role in the bactericidal effect on bacteria. Through our study, we found that the spherical shape and smaller size of nano-chitosan manufactured from Iran shells has a stronger effect, and this is due to the reason: Because the spherical shape has a better ability to penetrate the wall of bacteria than the tube shape that we found in the nano-chitosan manufactured from the shells of Iraq, and the smaller size also has the ability to penetrate better as shown in Fig. 7.

Burns are known to be slow-healing wounds. It has long been believed that proper treatment and care of burns can speed up the healing process and prevent chronic infection. Chitosan nanoparticles derived from seashells and containing natural cellulose are biocompatible and biodegradable. Under sterile conditions and general anesthesia, a circular burn with a diameter of 1 cm with full skin thickness was created on the ear of a rabbit. The day of the burn was considered as day zero. The rabbit was treated topically with a solution of chitosan nanoparticles. Within 4 to 7 days, the burns on the rabbits began to heal after daily treatment. The wound was cleaned with chloroform before applying the chitosan nanoparticles. After treatment, we observed that the burns gradually healed, hair began to grow in the burned area, and the wounds were removed. There was a significant increase. Based on these results, it can be concluded that chitosan nanoparticles have a positive effect on angiogenesis. Further research on nanomaterials can be conducted to achieve progress in wound healing (Fig. 8).

The effect of nanochitosan made from shell sources from different countries was studied, and it was found that the shape of nanochitosan made from seashells from Iran had a stronger effect on negative bacteria than the effect of nanochitosan made from seashells from Iraq. On bacteria. The more the shape tends to be spherical, the easier and better it is to penetrate the bacterial wall, as well as the size. Since it is chitosan made from Iranian shells, it tends to be spherical in shape and its size is smaller. Therefore, its penetration into the bacterial wall was much better and its antibacterial effect was better than the tubular shape we obtained. from chitosan made from Iraqi shells. Note that nanochitosan particles are biocompatible and biodegradable. In addition to hydrolysis, the decomposition of chitosan may have a positive effect on the healing of burns in the microvascular system. Therefore, in this research, nanochitosan was used to treat burns, as it was found that the healing of burns with the presence of nanochitosan is better and faster due to the use of the nanochitosan fiber matrix as an alternative to the skin structure, as it works to enhance adhesion, increase the production of new

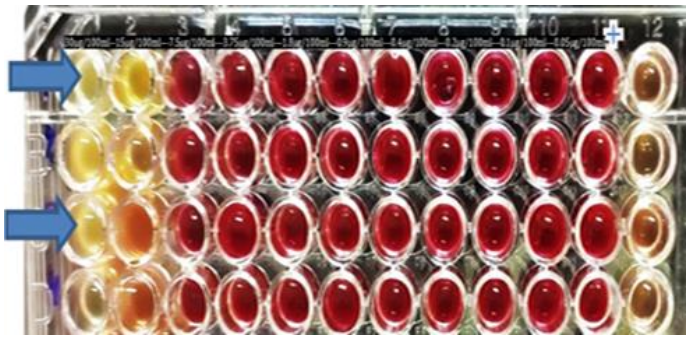

Fig. 7. The MIC of chitosan nanoparticles.

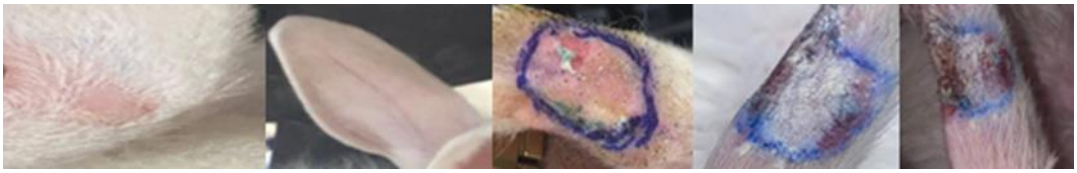

Fig. 8. The stages of recovery after an injury or surgery for a rabbit.

cells, and increase the healing process. Therefore, we recommend using nanochitosan made from seashells as an ointment to treat wounds and as materials to rejuvenate the skin and restore its appearance.

CONCLUSION

The results of this study show that an ointment for treating burns can be manufactured from environmentally friendly materials, because nanosized gitosan, which contains cellulose fibers that contribute to skin restoration, was manufactured from sea oyster shells from different countries, in addition to being an antibacterial.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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