



Original Research Paper

# Rutin Incorporated Chitosan Nanocomposite Preparation and Antibacterial Activity Against Wound Pathogens: Cytoplasmic and Protein Leakage Investigation

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| Key Words                                                                                                      | Abstract                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
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| Antibacterial activity, Chitosan nanocomposite, Rutin incorporation, Protein leakage analysis, Wound pathogens | The rutin found in photosynthetic cells and chitosan, a fungal biopolymer, form environmentally friendly nanocomposites. Rutin chitosan nanocomposite was synthesized, and its antimicrobial capability was tested through the agar well diffusion assay and time kill curve analysis with wound pathogens like <i>E. faecalis</i> , <i>E. coli</i> , <i>Pseudomonas</i> sp., and <i>S. aureus</i> . Cytoplasmic and protein leakage analyses were performed using the cytoplasmic assay and Bradford assay. Amoxyrite was used as a standard antibiotic for comparison. In results agar well diffusion method showed that the rutin chitosan nanocomposite had a greater zone of inhibition than the control in <i>E. faecalis</i> and <i>E. coli</i> and was more effective at higher concentrations against <i>Pseudomonas</i> sp. and <i>S. aureus</i> . Time-kill curve analysis confirmed a decrease in living wound pathogen cells over time. Protein leakage analysis by the Bradford test showed higher optical density than the control at 20 µg/ml, 50 µg/ml, and 100 µg/ml but lower than the standard, with an increase in optical density at higher concentrations. Cytoplasmic leakage analysis indicated the release of cytoplasmic components from bacterial cells. Although the nanocomposite showed lower antimicrobial activity than the standard antibiotic, it significantly promoted cytoplasmic and protein leakage, highlighting its potential therapeutic applications. Rutin-chitosan nanocomposite demonstrated considerable antibacterial efficacy against <i>P. aeruginosa</i> , <i>E. coli</i> , <i>E. faecalis</i> , and <i>S. aureus</i> , confirmed by agar-well diffusion, time-kill curve, cytoplasmic leakage, and protein leakage assays. While its effects varied by concentration, it showed potential as an environmentally safe alternative to conventional antibiotics for therapeutic applications. |

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## Introduction

The majority of wound repair materials available today are passive and unable to react to the wound-causing microorganisms, which leads to a very low therapeutic outcome. To achieve the release of medications for a wound, it is important to build a wound dressing in response to the microorganisms [1]. Wound healing is a vital biological process that occurs in both humans and animals after tissue damage, tissue engineering [2]. It helps in the regeneration and restoration of damaged tissue [2]. Antiinflammatory cytokines are released by the body in response to an injury, by migrating defense cells to the site of damage and removing damaged tissue. One of the main causes that delays the wound healing process is bacteria so effective antibacterial agents are necessary for wound dressing. By choosing the appropriate loading quantities, processing methods, and nanofillers, useful nanocomposites can be produced [3]. Rutin offers a wide range of potential therapeutic applications. It is also known as sophorin, quercetin-3-O-rutinoside, and Ruto side. It is a flavonoid glycoside made up of the flavonol quercetin and the disaccharide rutinose ( $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranose). Rutin is a substance that is abundant in nature and may be found in plants, fruits, and vegetables. It is safe to eat on a daily basis [4]. But, because of their low bioavailability and poor solubility, they are not used more often even though they have a variety of biological functions [5].

Rutin and chitosan nanocomposite possess many medical purposes. When treating stomach infections, chitosan nanoparticles are another efficient medication delivery method [6]. Chitosan and rutin-based wound dressings draw excess fluid out of the wound while maintaining the moist environment needed for wound healing [7]. Because of their ability to change to a variety of wound forms and sizes, nanocomposite wound dressings can be shaped into films, hydrogels, or nanofibers [8]. Urinary tract infections (UTIs) are one of the most common kinds of kidney infections. Chitosan prevents bacteria from growing in the urinary tract. In an attempt to stop the formation of microbial biofilm in catheter-associated urinary tract infections (CAUTI), fungal biomasses are

created to deposit on the catheter surface with the help of chitosan [9]. It can also be used in respiratory tract infections by clearing the mucus and bacterial pathogens from the airways. Chitosan may function to prevent *P. aeruginosa*, a bacterium resistant to antibiotics, from producing pneumonia [10]. Chitosan interacts with collagen and extracellular matrix proteins, which helps in the formation of structured tissues. They promote factors necessary for wound healing and tissue regeneration. Angiogenesis and bone regeneration have been studied with chitosan hydrogels [11].

Assays for protein leakage and cytoplasmic leakage offer important insights into the cytotoxicity of substances. New and better nanoparticle features were synthesized based on sizes and shapes with potential use in the medical field [12]. The cytoplasmic and protein leakage can be analyze using these environmentally benign techniques, which are also economical, non-toxic, and dependable [13]. It is crucial to assess the safety profile of materials used in biomedical applications. Pharmacies, chemicals, and environmental pollutants can be evaluated for toxicity via cytoplasmic and protein leakage studies. For example, in animal studies, Mn toxicity is decreased by Fpn expression, which can be examined [14]. Secondary metabolites of plants are unique and are produced naturally as byproducts of regular metabolism; they have little role in the life cycle of the plant. One such metabolite that works against bacteria that are drug-sensitive and resistant is flavonoids [15]. But in human beings, rutin helps in membrane stabilization by interacting with cell membranes and adjusting their fluidity. For example, Rutin supports cell membranes in human beings by verifying the protective effect of rutin against thiram-induced damage in human erythrocytes using cytoplasmic and protein leakage analysis [16].

Chitosan interacts with bacterial cell membranes through electrostatic interactions, hydrogen bonding, and hydrophobic interactions. This interaction can disrupt the permeability of the bacterial membrane, resulting in leakage of cytoplasmic contents. This is seen in *h pylori* by targeting its cell membrane, causing leakage of the contents and promoting a successful treatment by

its property of adhering to the mucus of the stomach [17]. According to previous studies, cytoplasmic leakage is indicated by elevated cytoplasmic enzyme levels in the supernatant. Protein leakage is indicated by elevated protein levels in the supernatant. The chitosan rutin nanocomposite has increased cytoplasmic and protein leakage [18]. For example, increased amounts of lactate dehydrogenase or certain proteins in the extracellular medium due to bacteria could sometimes indicate cell death. Chitosan is employed as a tumour marker for rectal and colon cancer [19]. It has been demonstrated that rutin shields DNA from oxidative stress and genotoxic substances from various microorganisms. Rutin lessens DNA damage by primarily protecting it from the mutations of bacteria and also by preventing the damage of DNA strands, by scavenging free radicals, and by promoting DNA repair mechanisms. Rutin and chitosan under normal conditions showed no discernible cytotoxicity, even at high dosages [20]. As a result,

it can also be used in bone degeneration as it maintains cell integrity in human beings [21]. *Enterococcus faecalis* is one of the primary bacteria that cause recurrent infections in the urinary tract, surgical wounds, dental root canals, and heart valves [21]. DNA isomerase IV may be inhibited by rutin, which also exhibits strong antibacterial activity against *E. coli* [22]. Chitosan interacts with bacterial cell membranes through electrostatic interactions, hydrogen bonding, and hydrophobic interactions. This interaction can disrupt the permeability of the bacterial membrane, resulting in leakage of cytoplasmic contents in bacteria like *S. aureus*, pathogenicity [23].

## MATERIALS AND METHODS:

### Preparation of Rutin-Chitosan Nanoparticle

The 100 mg of rutin was dissolved in 1 mL of distilled water, similarly, 500 mg of chitosan was dissolved with 49 mL of distilled water and 1 mL of glacial acetic acid. 1 mL of rutin was added to 4 mL of chitosan to create the nanocomposite (Fig 1).

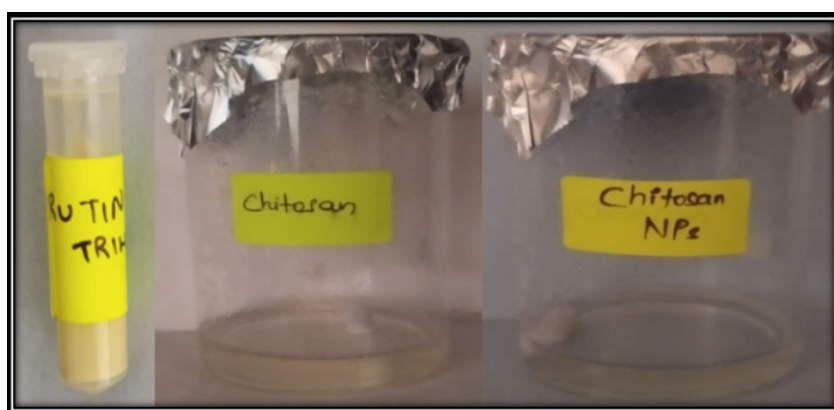


**Fig. 1. Schematic representation of Rutin-Chitosan Nanoparticles preparation**

### Antimicrobial activity:

The antimicrobial activity of rutin-incorporated chitosan nanocomposite formulation against wound pathogens, including *Enterococcus faecalis*, *E. coli*, *Pseudomonas sp.*, and *Staphylococcus aureus*. Mueller-Hinton Agar was utilized for this activity to determine the zone of inhibition. Muller-Hinton agar culture dishes are made and sterilized at 121 degrees Celsius for 15 minutes. The medium was placed on sterilized plates and left unobstructed until it solidified. The test organisms were swabbed after the wells were cut with 9mm sanitized polystyrene tips. Gels were diluted to various

quantities (25  $\mu$ L, 50  $\mu$ L, and 100  $\mu$ L) before loading into wells. An identical method was used with conventional wound healing gel. The plates were incubated at 37 degrees Celsius for 24 hours. The zones of inhibition were determined following the incubation [24].

### Time-kill curve Assay:

The bactericidal characteristics and concentration-dependent relationship between rutin chitosan-based nanocomposite and the net development rate of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *E.*

*faecalis* were evaluated using a time-kill curve assay at regular intervals. Time-kill curve study was done after the four-wound infection-causing agents were grown in Mueller-Hinton Broth augmented with varying concentrations of rutin chitosan nanocomposite (25, 50, and 100 µg/ml). As a standard, an antibiotic (such as Amoxyrite) was employed. To ensure that all infections had entered an ideal early-to-mid logarithmic phase, growth curves were constructed before the test after a four-hour pre-incubation period in an antimicrobial-free medium. Each pathogen was produced as a 0.5 McFarland of an inoculum in Sterile saline with phosphate buffer. These samples were obtained from cultures that were recently cultivated for 18-20 hours at 37°C on Mueller-Hinton agar plates. Subsequently, 15 mL of antimicrobial-free Mueller-Hinton Broth medium, warmed up to 37°C, was used to dilute 30 µL of the inoculum. The resulting mixture was then equally distributed over each well of a 96-well ELISA plate, using 90 µL. Ten microliters of rutin-based chitosan nanocomposite, available in five different concentrations, were added to each well holding 90 µL, and incubated beforehand with wound pathogens, along with the untreated control. A time-kill curve experiment was used to assess the microbiological characteristics and influence of concentration connections between the facilitated rutin nanocomposite and eventual growth rate across scheduled periods. After cultivation of both oral pathogens in Muller-Hinton broth enhanced with varied quantities of rutin nanocomposites, a time-kill curve study was undertaken. Prior to the experiment, growth charts were done following a four-hour pre-incubation duration in a medium having no antimicrobial substances in order to ensure every infectious agent had attained a steady early-to-mid log phase. The quantity of dead cells was then determined at periodic times using a 600 nm wavelength [24, 25].

#### Cytoplasmic leakage analysis:

Cytoplasmic components, including DNA and protein, are liberated from bacterial cells when they are treated with nanoparticles. Therefore, we have attempted to assess in this work the cytoplasmic leakage of bacterial cells following treatment with rutin chitosan nanocomposite. Microbial broth

containing *E. faecalis*, *E. coli*, *Pseudomonas*, and *S. aureus* (10 mL) was cultivated in an incubator and left overnight.

#### Protein leakage analysis:

To estimate the amount of DNA. The culture was extracted the next day using centrifugation for 10 minutes at 5000 rpm. After being cleaned, the pellet was again suspended in 1X PBS buffer (pH 7.2). A bacterial cell density of  $1 \times 10^5$  cells per millilitre was maintained. Following treatment with rutin chitosan nanocomposite, various aliquots of cell suspensions were incubated for three and five hours at room temperature. As a control, the bacterial culture without the rutin chitosan nanocomposite was used. As a standard, an antibiotic (such as Amoxyrite) was employed. Following a 10-minute centrifugation at 5000 rpm in an incubator, the luminescence of the supernatant was detected at 260 nm [26].

The inquiry into protein leakage employed the Bradford test. For 24 to 48 hours, bacterial cells from *S. aureus*, *P. aeruginosa*, *E. coli*, and *E. faecalis* were exposed to different concentrations of rutin chitosan nanocomposite. The corresponding values were 25, 50, and 100 µg/mL. As a standard, an antibiotic (such as Amoxyrite) was employed. After treatment, the bacterial culture was centrifuged for 10 minutes at 3000 rpm to separate the uppermost phase, which was subsequently retrieved. Each sample required 200 µL of supernatant, which was then transferred to 96-well ELISA plates. These were incubated for 10 minutes in a dark setting with 50 µL of Bradford reagent added. At 595 nm, the optical density (OD) of the sample was determined [25, 27, 28].

## RESULTS

#### Antimicrobial activity:

The agar well diffusion method was used to assess the antibacterial capacity of the rutin-incorporated chitosan nanocomposite formulation towards the subject wound pathogens (Fig. 2). The graph represents the developed formulation's antibacterial activity in opposition to the tested pathogens. At a dose of 100 µL, the formulation displayed the highest antibacterial efficacy against *C. albicans* (12 mm), *S. aureus* (32 mm), and *S. mutans* (22 mm), but no inhibition for *E. faecalis*.

However, in comparison to the normal amoxyrite, the produced formulation displayed somewhat less

antibacterial efficacy, other than in *S. aureus* (Fig 2).

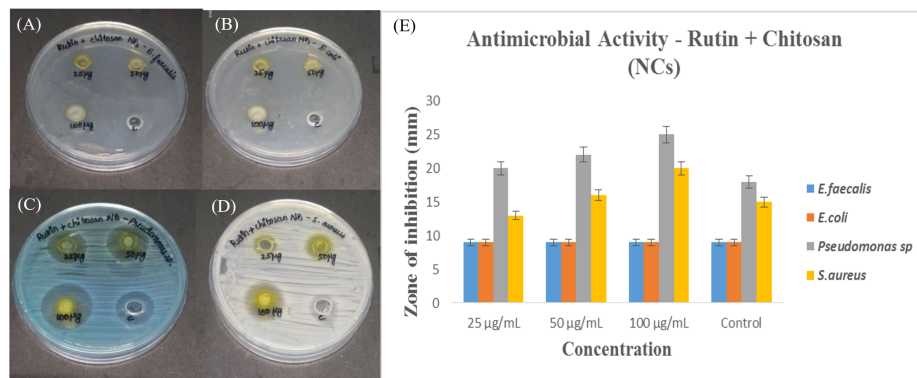


**Fig. 2.** Antimicrobial activity of rutin-incorporated chitosan nanocomposite formulation against wound pathogens; *E. faecalis*, *E. coli*, *Pseudomonas sp*, and *S. aureus*.

#### Time Kill Curve analysis:

The decreased optical density, the stronger the antibacterial activity. The results revealed that the studied microorganisms were totally suppressed by

Rutin + Chitosan NPs. At 5 hours, *E. coli* showed maximum growth inhibition with 0.2 optical density at a concentration of 100 µL. *S. aureus* exhibited 0.1 optical density at 100 µL concentration, as shown in (Fig. 3).

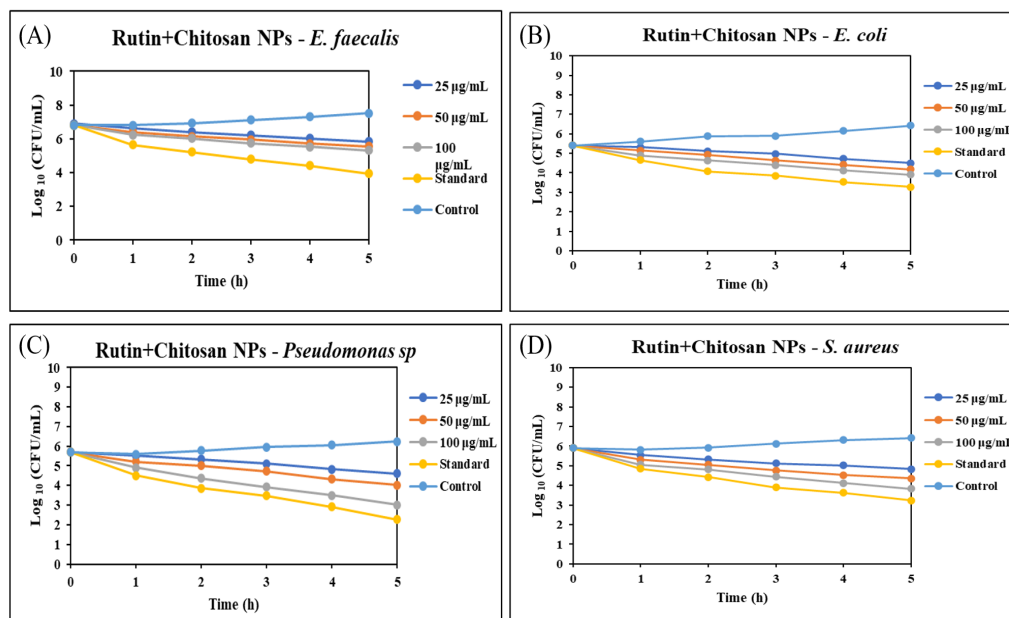


**Fig. 3.** A time-kill curve assay was performed in MHB media, and Optical density values were used to assess the antibacterial activity of Rutin + Chitosan NPs against *E. faecalis*, *E. coli*, *Pseudomonas sp.*, and *S. aureus*.

#### Protein leakage analysis

The *E. faecalis* at 25, 50 and 100 µg/mL showed optical densities of 0.354, 0.371, 0.397, respectively, while the standard showed the optical density of 0.412. And the control showed an optical density of 0.325. *E. coli* at 25, 50 and 100 µg/mL

shows optical density of 0.368, 0.389, 0.406, respectively, while the standard showed optical density of 0.425 and the control showed optical density of 0.341. *Pseudomonas sp* at 25, 50 and 100 µg/mL shows optical density of 0.425, 0.456, 0.487, respectively. The standard had an optical

density of 0.49. And the control revealed an optical density of 0.411. *S. aureus* at 25, 50 and 100  $\mu\text{L}$  showed optical density of 0.388, 0.416, 0.432 respectively. In contrast, the standard had an optical density of 0.456. And the control revealed an optical density of 0.371 (Fig 4). It shows that the optical density of the protein leaked is more than

the control at all concentrations and less than the standard at all concentrations. However, the difference in leakage between the composite and standard is less at higher concentrations, indicating that the protein leakage increases with concentration.

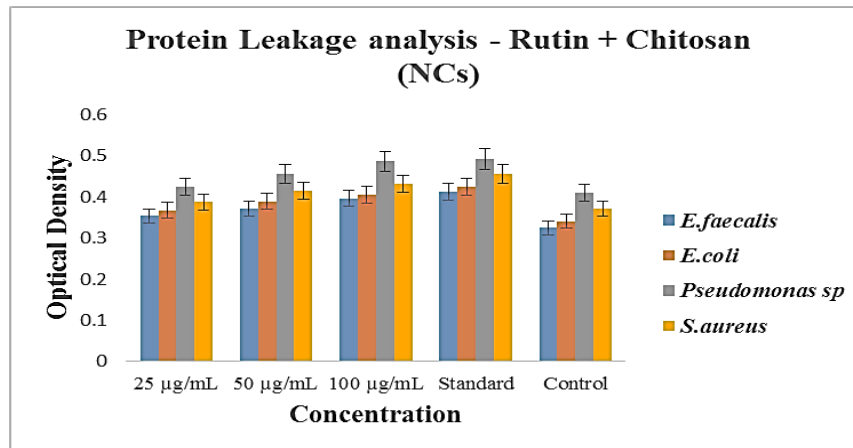


**Fig. 4. Protein leakage analysis with rutin and chitosan at different concentrations was taken against pathogens; *E. faecalis*, *E. coli*, *Pseudomonas sp* and *S. aureus*.**

#### Cytoplasmic leakage analysis

The *E. faecalis* at 25 $\mu\text{g/mL}$ , 50 $\mu\text{g/mL}$ , 100 $\mu\text{g/mL}$  shows optical density of 0.324, 0.348, 0.367, respectively, while the standard showed optical density of 0.389, and the control showed an optical density of 0.311. *E. coli* at 25  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$ , and 100  $\mu\text{g/mL}$  shows optical density of 0.351, 0.369, and 0.381, respectively, while the standard showed optical density of 0.394, and the control showed optical density of 0.326. *Pseudomonas sp* at 25 $\mu\text{g/mL}$ , 50 $\mu\text{g/mL}$  and 100 $\mu\text{g/mL}$  shows optical densities of 0.418, 0.435, and 0.466, respectively,

whereas the standard had an optical density of 0.478. And the control revealed an optical density of 0.401. *S. aureus* at 25  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  shows optical densities of 0.406, 0.427, and 0.454, respectively, while the standard showed the optical density of 0.468. The control showed an optical density of 0.387. It shows that the optical density of the cytoplasmic content leaked is more than the control at all concentrations and less than the standard at all concentrations. It shows that the rutin chitosan nanocomposite shows better cytoplasmic leakage than the control, but not the standard (Fig 5).

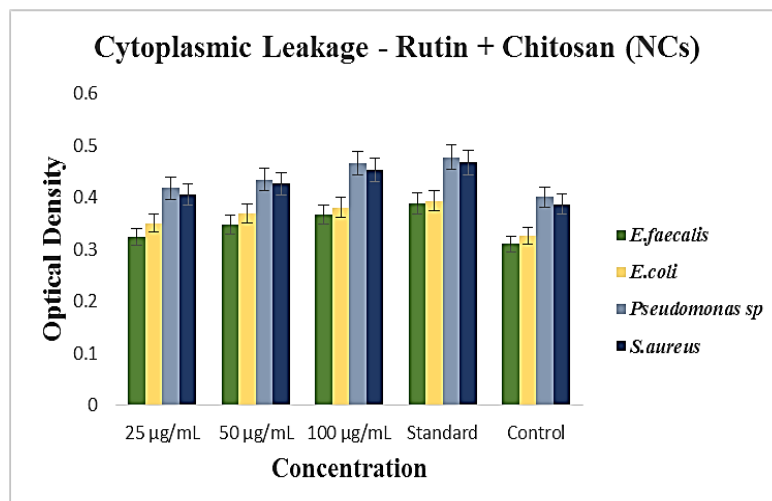


**Fig. 5. Cytoplasmic leakage analysis with rutin and chitosan at different concentrations was taken against pathogens; *E. faecalis*, *E. coli*, *Pseudomonas sp.*, and *S. aureus*.**

In our study, rutin was incorporated with chitosan to check their antibacterial activities compared to the control. Rutin and chitosan both possess a lot of unique characteristics, and their combination gives significantly higher antibacterial activities. They have different mechanisms against gram-positive and gram-negative bacteria. At 100 µg/ml concentration, they showed the maximum antibacterial activity compared to 25 µg/ml and 50 µg/ml. Rutin and chitosan in combination with other components showed great antimicrobial activities, which are already quoted in lots of previous studies. Rutin was incorporated with chitosan to analyze the cytoplasmic and protein leakage of *E. faecalis*, *E. coli*, *Pseudomonas*, and *S. aureus*. compared to the control and standard. Protein leakage analysis is used to determine the denaturation of proteins present in the cytoplasm of bacteria that showed a significant optical density than the control. Similarly, cytoplasmic leakage analysis is used to determine the cytoplasmic contents that come out of the cell. The optical density showed promising results. This analysis is helpful in determining the antimicrobial activity. There are lots of previous studies that can justify our results.

Protein leakage in bacterial infections may be caused by disruptions or damage to the bacterial cell membrane. This leakage may cause the bacteria to perform less well, which may result in cell death. Because of its antioxidant qualities, rutin may have an impact on the integrity of the bacterial membrane or the bacterial cell wall, which could indirectly cause protein leakage. In a previous research study, it was seen that the incorporation of chitosan with amino acids by cross-linking, blending, and grafting showed potential wound-healing processes. Chitosan incorporated with ficin shows increased efficacy against *Staphylococci* [28]. In another study, rutin hydrate and rutin with amikacin combination showed antibacterial activities against strains of *S.aureus*, *Klebsiella Pneumonia*, and *Acinetobacter Baumannii* [29]. The uses of rutin and chitosan very similar to our study have been quoted by other studies as well. A flavonoid-rich extract of *Glycyrrhiza glabra* can be

used in gastrointestinal, metabolic, and psychological disorders involving bacteria [30]. In another research, Rutin ant virulence mechanisms included downregulating certain genes as well as having an effect on the synthesis of *P. aeruginosa* and disrupting the permeability of membranes [31-36]. There are other research studies similar to our study that have successfully shown the cytoplasmic and protein leakage analysis of chitosan rutin nanocomposite. The nanoparticles-laden contact lens has enhanced antibiotic efficacy against vancomycin, which is used in the treatment of bacterial keratitis. It is generally inactive against gram-negative bacteria, but with nanoparticles, it act against *Proteus mirabilis* and *Pseudomonas aeruginosa* [32,37-40]. In another study, because of their effective cytotoxic effects, biogenic routes, particularly the plant-based manufacture of selenium nanoparticles (SeNPs), are safe, environmentally benign, and thought to be among the finest methods for treating cancer [33,41-42]. Right now, the blueprint for nanoengineering focuses on customizing the surface structure of nanoparticles in combination with biopolymers derived from secondary metabolites of plants. By improving their surface properties at the nanoscale, the' area of applicability is expanded [43].

## CONCLUSION

In summary, the synthesized rutin chitosan nanocomposite demonstrated significant antibacterial activity against the investigated wound pathogens *P. aeruginosa*, *E. coli*, *E. faecalis*, and *S. aureus*. This is confirmed by the results of both agar-well diffusion and time-kill curve investigations. According to this study, environmentally safe rutin chitosan nanoparticles may offer a possible substitute for conventional synthetic medications that may have negative side effects in a variety of therapeutic applications. The cytoplasmic and protein leakage analysis was done with the help of rutin chitosan nanocomposite for *E. faecalis*, *E. coli*, *Pseudomonas*, and *S. aureus*. This was confirmed with the cytoplasmic assay and Bradford assay. At different concentrations, the gram-positive and gram-negative bacteria showed significant cytoplasmic and protein leakage, but less

than the standard antibiotic in some cases. The results confirmed that rutin chitosan nanocomposite can be used in further studies of microbial activities in the field of medicine in the future.

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### Ethics approval and consent to participate

All the data was available from public databases, and there is no need for ethics approval and consent.

### Credit authorship contribution Statement:

SJ: Writing-Review & editing, Writing Original Draft, VR: Methodology, Data curation, Visualization, SD: Drew the figures and pictures, Conceptualization. PE: Helped in writing the manuscript and revised it. SJ: Validation and editing with review of the manuscript. RS: Designed protocols for all the experiments, supervised, proofread, analyzed the data, and revised the reviewer's comments, investigation, and Conceptualization, AS & AP: Conceptualization Validation, Review. All authors reviewed and discussed the results, provided comments on the manuscript, and approved its final published version.

### Consent for publication

Not applicable.

### Conflict of interest

The authors state no conflict of interest

### Declaration of competing interest

The authors declare that they have no conflict of interest.

## References

- [1] Zhu S, Zhao B, Li M, Wang H, Zhu J, Li Q, et al. Microenvironment responsive nanocomposite hydrogel with NIR photothermal therapy, vascularization and anti-inflammation for diabetic infected wound healing. *Bioactive materials* 2023;26:306-20.
- [2] Vimalraj, S., Saravanan, S. Tooth-derived stem cells integrated biomaterials for bone and dental tissue engineering. *Cell Tissue Res*, 2023; 394, 245–255.
- [3] Li X, Xu W, Xin Y, Yuan J, Ji Y, Chu S, et al. Supramolecular polymer nanocomposites for biomedical applications. *Polymers* 2021;13(4):513.
- [4] Hu T, Yuan X, Ye R, Zhou H, Lin J, Zhang C, Brown adipose tissue activation by rutin ameliorates polycystic ovary syndrome in rat. *The Journal of Nutritional Biochemistry* 2017;47:21-8.
- [5] Samsonowicz M, Kamińska I, Kalinowska M, Lewandowski W. Alkali metal salts of rutin–Synthesis, spectroscopic (FT-IR, FT-Raman, UV–VIS), antioxidant and antimicrobial studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 2015;151:926-38.
- [6] Moosazadeh Moghaddam M, Bolouri S, Golmohammadi R, Fasihi-Ramandi M, Heiat M, Mirnejad R. Targeted delivery of a short antimicrobial peptide (CM11) against *Helicobacter pylori* gastric infection using concanavalin A-coated chitosan nanoparticles. *Journal of Materials Science: Materials in Medicine* 2023;34(9):44.
- [7] Rajeshkumar S, Roy A, Santhoshkumar J, Lakshmi T. Herbal Formulation Mediated Synthesis of Silver Nanoparticles and Its Antifungal Activity Against *Candida albicans*. *Indian Journal of Public Health Research & Development* 2019;10(11).
- [8] Shivakumar P, Gupta MS, Jayakumar R, Gowda DV. Prospection of chitosan and its derivatives in wound healing: Proof of patent analysis (2010–2020). *International Journal of Biological Macromolecules* 2021;184:701-12.
- [9] Bharti S, Paliya BS, Nayaka S, Kumar R. Deposition of *Daldivia starbaeckii* (ELF) functionalized silver nanoparticles on urinary catheter tube using chitosan polymer to prevent microbial biofilms formation during UTI infection. *Archives of Microbiology* 2023;205(8):277.
- [10] Senthil, Rethinam. "Formation of bone tissue apatite on starch-based nanofiber-capped nanohydroxyapatite and reduced graphene oxide: a preliminary study." *Oral and Maxillofacial Surgery* 29.1 (2024): 6.
- [11] Chen Y, Sheng W, Lin J, Fang C, Deng J, Zhang P, et al. Magnesium oxide nanoparticle coordinated phosphate-functionalized chitosan injectable hydrogel for osteogenesis and angiogenesis in bone regeneration. *ACS Applied Materials & Interfaces* 2022;14(6):7592-608.

- [12] Nath N, Chilkoti A. Label free colorimetric biosensing using nanoparticles. *Journal of Fluorescence* 2004;14:377-89.
- [13] Vimalraj S, Sekaran S. RUNX Family as a Promising Biomarker and a Therapeutic Target in Bone Cancers: A Review on Its Molecular Mechanism(s) behind Tumorigenesis. *Cancers*. 2023; 15(12):3247.
- [14] Yin Z, Jiang H, Lee ESY, Ni M, Erikson KM, Milatovic D, et al. Ferroportin is a manganese-responsive protein that decreases manganese cytotoxicity and accumulation. *Journal of neurochemistry* 2010;112(5):1190-8.
- [15] Amin MU, Khurram M, Khattak B, Khan J. Antibiotic additive and synergistic action of rutin, morin and quercetin against methicillin resistant *Staphylococcus aureus*. *BMC complementary and alternative medicine* 2015;15:1-12.
- [16] Salam S, Arif A, Sharma M, Mahmood R. Protective effect of rutin against thiram-induced cytotoxicity and oxidative damage in human erythrocytes. *Pesticide Biochemistry and Physiology* 2023;189:105294.
- [17] Arif M, Sharaf M, Samreen, Dong Q, Wang L, Chi Z, et al. Bacteria-targeting chitosan/carbon dots nanocomposite with membrane disruptive properties improve eradication rate of *Helicobacter pylori*. *Journal of Biomaterials Science, Polymer Edition* 2021;32(18):2423-47.
- [18] Maru V, Shetty O, Dixit U, Warke VG, Khande M, Mane S. SHED' s Response to Various Pulpotomy Materials: Cytotoxicity and Gene Expression Analysis. *Journal of Clinical Pediatric Dentistry* 2021;45(6):406-13.
- [19] Sadreddini S, Safaralizadeh R, Baradaran B, Aghebati-Maleki L, Hosseinpour-Feizi MA, Shanehbandi D, et al. Chitosan nanoparticles as a dual drug/siRNA delivery system for treatment of colorectal cancer. *Immunology letters* 2017;181:79-86.
- [20] Nassiri-Asl M, Ghorbani A, Salehisar S, Asadpour E, Sadeghnia HR. Effect of rutin on oxidative DNA damage in PC12 neurons cultured in nutrients deprivation condition. *Iranian journal of basic medical sciences* 2020;23(3):390.
- [21] Xu C, Lei C, Meng L, Wang C, Song Y. Chitosan as a barrier membrane material in periodontal tissue regeneration. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2012;100(5):1435-43.
- [22] Motallebi M, Khorsandi K, Sepahy AA, Chamani E, Hosseinzadeh R. Effect of rutin as flavonoid compound on photodynamic inactivation against *P. aeruginosa* and *S. aureus*. *Photodiagnosis and Photodynamic Therapy* 2020;32:102074.
- [23] Kannan, Kamala, et al. "Marine archaeal extracellular polymeric substances from *Halococcus* AMS12, their characterization, and biological properties." *Journal of Marine Science and Engineering* 10.11 (2022): 1788.
- [24] Sankar HN, Shanmugam R, Anandan J. Green synthesis of *Euphorbia tirucalli*-mediated titanium dioxide nanoparticles against wound pathogens. *Cureus* 2024;16(2).
- [25] Munusamy T, Shanmugam R. Green synthesis of copper oxide nanoparticles synthesized by *Terminalia chebula* dried fruit extract: characterization and antibacterial action. *Cureus* 2023;15(12).
- [26] Adams CP, Walker KA, Obare SO, Docherty KM. Size-dependent antimicrobial effects of novel palladium nanoparticles. *PloS one* 2014;9(1):e85981.
- [27] Tharani M, Rajeshkumar S, Al-Ghanim KA, Nicoletti M, Sachivkina N, Govindarajan M. *Terminalia chebula*-assisted silver nanoparticles: biological potential, synthesis, characterization, and ecotoxicity. *Biomedicines* 2023;11(5):1472.
- [28] Inbakandan D, Kumar C, Abraham LS, Kirubakaran R, Venkatesan R, Khan SA. Silver nanoparticles with anti microfouling effect: a study against marine biofilm forming bacteria. *Colloids and Surfaces B: Biointerfaces* 2013;111:636-43.
- [29] Torkaman S, Rahmani H, Ashori A, Najafi SHM. Modification of chitosan using amino acids for wound healing purposes: A review. *Carbohydrate polymers* 2021;258:117675.
- [30] Murugan SK, Bethapudi B, Raghunandhakumar S, Purusothaman D, et al. A flavonoid rich standardized extract of *Glycyrrhiza glabra* protects intestinal epithelial barrier function and regulates the tight-junction proteins expression. *BMC complementary medicine and therapies* 2022;22(1):38.
- [31] Ayyadurai, T., Mohan, P. K., Annamalai, A., Kamaraj, C., & Waheeb, M. Q. (2026). Biogenic silver nanoparticles synthesis from *Guilandina bonduc* L. seed kernel extract with antibacterial, antioxidant and anticancer potential against ovarian Cancer cells. *Results in Chemistry*, 103246.
- [32] Jancyhelan, J., Thirumurugan, A., Alagumanian, S., & Vignesh, K. (2026). Eco-friendly biosynthesis of silver nanoparticles using marine algal extract and their biomedical applications. *Results in Chemistry*, 103606.
- [33] Sakthivel V, Ilanthiraya S, Jeevanantham G, Aravindhan T, Priya K, Thirumurugan A, Larvicidal

- Activity Of Marine Macroalgae: A Comprehensive Review. *Int J Drug Deliv Technol.* 2026;16(4s): 908-917; DOI: 10.25258/ijddt.16.4s.106
- [34] Ayyadurai, T., Ravi, S., & Annamalai, A. (2026). Steroidogenic Enzyme Dysregulation in Polycystic Ovary Syndrome: Mechanistic Insights and Emerging Therapeutic Strategies. *The Journal of Steroid Biochemistry and Molecular Biology*, 107088.
- [35] Anbazhagan, V., Natrajan, A., Arul, J. et al. Strategic development of a buffalo farming framework and optimization of the foraging behaviour using mathematical models. *Trop Anim Health Prod* 58, 424 (2026). <https://doi.org/10.1007/s11250-026-05211-6>
- [36] Ivanov M, Novović K, Malešević M, Dinić M, Stojković D, Jovčić B, et al. Polyphenols as inhibitors of antibiotic resistant bacteria—Mechanisms underlying rutin interference with bacterial virulence. *Pharmaceuticals* 2022;15(3):385.
- [37] Tong WY, Tan W-N, Azizi MAK, Leong CR, El Azab IH, Lim JW, et al. Nanoparticle-laden contact lens for controlled release of vancomycin with enhanced antibiotic efficacy. *Chemosphere* 2023;338:139492.
- [38] Meda SSR, Joseph NM, Lakshmikanthan M, Ayyadurai T, Muthu S. Comparative Efficacy of Mandelic Acid Peel versus Topical Kojic Acid 2% in the Treatment of Periorbital Melanosis. *Int J Drug Deliv Technol.* 2026;16(15s): 118-123. DOI: 10.25258/ijddt.16.15s.14.
- [39] Meda SSR, Srinivasan VR, Lakshmikanthan M, Ayyadurai T, Muthu S. Topical Prostaglandin Analogues in Androgenetic Alopecia: A Systematic Review of Clinical Evidence. *Int J Drug Deliv Technol.* 2026;16(15s): 108-117. DOI: 10.25258/ijddt.16.15s.13.
- [40] Ayyadurai, T., Moola, A.K., Mohan, P.K., Thiruppathi, S.K., Shanmugam, A. and Bollipo Diana, R.K., 2022. Ameliorative effects of *Guilandina bonduc* L. aqueous seed extract on letrozole induced polycystic ovary syndrome in female wistar albino rats. *Advances in Traditional Medicine*, 22(4), pp.885-903.
- [41] Jeyaram, M., Jeyaram, Y., Ayyadurai, T., Gurusamy, M. (2026). Exploration of Streptomycetes as Biocontrol Agents Against Plant Pathogens. In: Kaur, T., Kumar, H., Manhas, R.K. (eds) *Streptomycetes: Biological Candidates for Sustainable Agriculture*. Springer, Singapore. [https://doi.org/10.1007/978-981-95-5803-2\\_6](https://doi.org/10.1007/978-981-95-5803-2_6)
- [42] Mi X-J, Choi HS, Perumalsamy H, Shanmugam R, Thangavelu L, Balusamy SR, et al. Biosynthesis and cytotoxic effect of silymarin-functionalized selenium nanoparticles induced autophagy mediated cellular apoptosis via downregulation of PI3K/Akt/mTOR pathway in gastric cancer. *Phytomedicine* 2022;99:154014.
- [43] Fu X, Rehman U, Wei L, Chen Z-S, Abourehab MA, Kesharwani P, et al. Silver-dendrimer nanocomposite as emerging therapeutics in anti-bacteria and beyond. *Drug Resistance Updates* 2023;68:100935.