

Assessment of Protein and DNA Leakage analysis of Tannic acid-alginate nano formulation against wound pathogens

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Abstract: Tannic acid-alginate nanoformulations have shown promise as antimicrobial agents. This research is intended to synthesize them and assess their antimicrobial efficacy against a particular wound infection. Our main objectives were to extensively assay them and assess their antibacterial efficacy. Tannic acid-alginate nanoformulations are synthesized by using a biological synthesis approach with tannic acid and sodium alginate. The visual observation through the colour changes to conforming nanoformulation. Agar well diffusion, time-kill curve, protein leakage assay, and cytoplasmic analysis were among the various assays used to evaluate the antibacterial activity of the tannic acid-alginate nanoformulation against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*. Visual observation of tannic acid-alginate nanoformulation to form a brown colour, due to the presence of tannic acid-alginate. That nanoformulation revealed significant efficacy with *Pseudomonas aeruginosa* and *Escherichia coli* exhibiting higher zones of inhibition at 26mm and 31mm, respectively. While *Pseudomonas sp.*, *S. aureus*, demonstrated remarkable vulnerability to tannic acid-alginate, *E. coli* and *E. faecalis* exhibited 18 and 19mm inhibition, for a slower response. Tannic acid-alginate nanoformulation exhibited antibacterial strength against particular wound infections, suggesting its potential for use in wound care treatments. These discoveries aid in the creation of long-lasting antibiotic remedies that have consequences for both environmental sustainability and wellness. while these nanoformulations show promise in antibacterial, time-kill curve, protein leakage, as well as cytoplasmic leakage analysis, further investigation is needed to explore their clinical applications and risk assessment.

Keywords: Wound pathogen, Tannic acid, Alginate, DNA leakage, time-kill, Anti-bacterial agent

INTRODUCTION

Chronic wound infections are difficult to treat in clinical settings because their healing process is lengthy, and they are vulnerable to many bacterial invaders. Numerous illnesses have microorganisms as the primary cause (Jabeen, Prabhalakshmi, Dhanraj, & Ramasubburayan, 2025). A particular microorganism may reside in the human body, proliferate, colonize, and emit toxins (Palla, 2023). Skin is an important primary natural barrier in the human body that protects from pathogenic infestation and environmental assaults. That prevents infections from spreading to the inside tissues, and damage to it can result in wound formation. Bacterial pathogens infect these wounds, hindering the healing process and making wound treatment more resource-intensive (Su, Li, Liang, Zhang, & Li, 2021). Wound management is currently facing challenges in clinical settings due to the complexity of chronic wounds, the high cost of treatment, and controlling bacterial infections. This renders a need for multifunctional wound dressings that can support cell proliferation and decrease the risk of infection. Recent studies have focused on developing multifunctional wound dressings that facilitate wound healing as well as provide multiple therapeutic functions such as

antibacterial and antioxidant activity. Multifunctional wound dressings can also include therapeutic agents to heal the underlying disease that causes the formation of chronic wounds. The majority of aerobic and anaerobic microbes colonizing cutaneous wounds are mostly derived from mucosal surfaces, including the stomach and oral cavity (Akin, Akgul, Tasdurmazli, Abamor, Ozbek, Ozcelik, et al., 2024).

The number of individuals with chronic wounds has significantly increased recently due to the notable rise in chronic illnesses like diabetes, obesity, and vascular dysfunction. Burns, surgical incisions, contusions, trauma-related abrasions, etc., are the most common types of injuries. An organism's capacity for self-healing allows wounds to heal, depending on how severe the injury is, in three months. But if left unchecked, an acute infection can become chronic and linger for months or even years (Maitz, Merlino, Rizzo, McKew, & Maitz, 2023). An increasing number of people worldwide suffer from atopic dermatitis, an allergic inflammatory skin illness. Tannic acid has been shown to have a therapeutic impact on atopic dermatitis in a model of Nc/Nga mice through the inhibition of VEGF production, which in turn suppresses angiogenesis. Tannic acid can also reduce the expression of the related cytokines in this

animal, such as thymic stromal lymphopoietin, thymus, and activation-regulated chemokines (Kushwaha, Goswami, & Kim, 2022). In mice with Dfe-induced atopic dermatitis models, tannic acid treatment reduces clinical symptoms and successfully prevents acanthosis, hyperkeratosis, parakeratosis, and inflammatory cell infiltration (Chen, Tian, Yang, Tong, Jia, Zou, et al., 2019).

E. Coli and *Pseudomonas aeruginosa* were the next most common types of bacteria found, after *Staphylococcus aureus*. The intricate process of surgical infection pathophysiology involves the priming and pre-triggered host immune-inflammatory response to the pathogen, which is influenced by genetic predispositions and specifically tailored by the microorganisms' location, load, and virulence in surgical patients (Jing, Xiaolan, Yu, Feng, & Haifeng, 2022). According to microbiology, the main purposes of healthy, intact skin are to control the quantity of germs that live on its surface and prevent potential infections from proliferating and entering deeper tissue (Pauline, Akshita, Pavithra, Kannan, & Sivaperumal, 2025). The subcutaneous tissue exposed following a wound or other Loss of epidermal integrity creates a warm, moist, and nutrient-rich environment suitable for the colonization of bacteria and development. But the amount and diversity of bacteria present will depend on several factors, including the kind, location, depth, and quality of the wound; also, the amount of circulation of tissue and the efficiency of the host's immunological response and antimicrobial defences will have an impact (Pal, Sayana, Joshi, & Juyal, 2019).

Exposure of subcutaneous tissue to various microorganisms can result in contamination and colonization (Sivaperumal, Ganapathy, & Kamala, 2025). The proliferation of germs is more likely to occur when the immune system is repressed and tissue is weakened, such as through ischemia, hypoxia, or necrosis. In most cases, wound contamination originates from three primary sources: endogenous mucosal surfaces (particularly the gastrointestinal, oropharyngeal, and genitourinary tracts); resident skin flora (including diphtheroids, propionibacteria, micrococci, and *Staphylococcus epidermidis*); and environmental exposure (such as airborne microbes or those brought about by trauma) (Maitz, Merlino, Rizzo, McKew, & Maitz, 2023). In general, three biological processes are involved in wound healing. Proliferation normally lasts for two weeks after inflammation, which can last up to six days, and remodelling can last for up to two years (Čoma, Fröhlichová, Urban, Zajíček, Urban, Szabo, et al., 2021). Antibiotics are typically used to treat wound pathogens because they are effective and affordable, but recently, resistance to these drugs has increased significantly. As a result, we need to find an alternative to antibiotics to address this crisis, and tannic acid and alginate nano formulation may be a better option (Liu, Dong, Wang, Xu, Yang, Wu, et al., 2023). In place of antibiotics, nanoparticles are being utilized

more frequently to target bacteria, which helps treat bacterial infections. Examples of applications of nanoparticles include the use of antibacterial coatings on medical materials and implantable devices to stop infections. It promotes the healing of wounds; antibiotics are administered to treat diseases, antibacterial vaccinations are used to prevent bacterial infections, and microbial diagnostics are produced by detecting bacteria (Akin, et al., 2024).

As wound dressings, hydrogels were previously functionalized with cationic polymers, metallic nanoparticles, and natural polyphenols. However, it was determined that metallic nanoparticles, such as silver nanoparticles (AgNPs), can cause cytotoxicity, while the toxicity of cationic polymers depends on different parameters, such as charge density and molecular weight (Rosa, Bonato, Mancuso, Martinelli, Okura, Malpass, et al., 2018).

Natural polyphenols are secondary metabolites of plants that consist of hydroxyl groups bonded to an aromatic carbon. A naturally occurring polyphenol that is a member of the tannin subgroup is tannic acid (TA). Because of its antibacterial and antioxidant qualities, it is widely utilized in biological applications. Bearing a high molecular weight, TA was previously used as a cross-linker in numerous studies as well. TA is considered a significant bioactive molecule for the creation of multipurpose wound dressings since additionally; it is additionally known to promote wound healing, vascularisation, and cell proliferation stages. Numerous investigations using hydrogels containing TA have demonstrated remarkable bioactivity as materials for wound healing (Wekwejt, Małek, Ronowska, Michno, Pałubicka, Zasada, et al., 2024).

Tannins, often known as tannic acid, are a class of naturally occurring organic chemical compounds. It is made up of molecules of glucose and gallic acid, which have strong biocompatibility, antibacterial, antioxidant, and anti-inflammatory properties (Guimarães, Costa, Madureira, Borges, Oliveira, Pintado, et al., 2023). Tannic acid helps heal burns by promoting skin regeneration, as proven. However, when it comes to wound applications, tannic acid, being a polyphenol, may have certain disadvantages. These include reduced biological performance at the wound site as a result of limited stability, poor bioavailability, and light sensitivity (Orlowski, Zmigrodzka, Tomaszewska, Ranhoszek-Soliwoda, Czupryn, Antos-Bielska, et al., 2018). Tannic acid is a good dressing for the first of three types of wounds. It is applied to adhesive plastered wounds that don't require stitching, as well as incised wounds (after sutures are placed).2. Roughly shaped, little wounds that recently emerged.3. Compound fractures with somewhat big wounds It is believed that tannic acid promotes faster wound healing through a variety of methods. 1) Reactive oxygen species and free radical scavenging 2) Promoting the wounds' contraction

3) an acceleration of the formation of capillary vessels and fibroblasts (Ali, Ullah, Ullah, Shakeel, Afsar, Husain, et al., 2024). Additionally, they cannot shield the site from microbial invasion, which results in the wound drying out, the accumulation of wound exudates on its surface, and inadequate gas permeability (Ali, et al., 2024).

Natural polysaccharides, including protein, proteoglycans, and alginates, are the raw materials with the greatest promise for burn and wound treatments. α -L-guluronate and α -D-mannuronate residues that are (1 $\rightarrow$ 4')-covalently bonded are present in variable amounts in the linear, unbranched natural polymer sodium alginate (Na-Alg-Alginate, also known as calcium alginate, calcium-sodium alginate, collagen alginate, or gelatin alginate, is a natural fiber dressing that is extremely absorbing. The source of alginate is algae. For wound dressing applications, Na-Alg is also used as a matrix material due to its demonstrated capacity to accelerate wound healing (Liu, et al., 2023). New technologies in chronic wound therapy, such as alginates, foams, hydrocolloids, and hydrogels, serve to keep the wound moist by reducing wound exudate, decreasing wound size, increasing vascular perfusion, and promoting granulation tissue (Wang, Hu, & Shao, 2017). The current research study discusses the preliminary in vitro studies evaluating the biosynthesised tannic acid-alginate nano formulation and assessing their antibacterial activities against wound pathogens. This research will help identify a more efficient and environmentally friendly strategy to combat the known risks posed by microbial populations.

MATERIALS AND METHODS

Preparation of Tannic Acid-Alginate Nano Formulations

The polyphenol tannic acid is well-known for its crosslinking and antioxidant qualities. After being weighed, 100 mg of tannic acid were incorporated with 10 mL of double-distilled water. Three hours were spent with the aqueous formulation in a magnetic stirrer. 100 mg of sodium alginate was dissolved in 10 mL of double-distilled water and carefully mixed. The resultant solution was shaken for 5 minutes at room temperature using an orbital shaker, then stirred continuously for 2 hours with a magnetic stirrer. 1 mL of sodium alginate solution and 1 mL of tannic acid were combined evenly at a pH of 7.5. Following that, 1 mL of 5% calcium chloride (CaCl₂) ions was used to cross-link the resulting nanoformulation, resulting in the production of stable soft materials. This was done with an orbital shaker for five minutes and then for two hours in that formulation solution using a magnetic stirrer. A 15-minute sonication was then applied to the mixer. When the tannic acid-alginate nanoformulation is formed, the visual colour shifts from dark brown. After that, the final formulation was subjected to further investigations.

Antimicrobial activity

The antibacterial characteristics of the tannic acid-assisted alginate nanocomposite were determined using the agar well diffusion technique. Mueller-Hinton agar was made and sterilized using an autoclave at 121°C for 15-20 minutes. The sterilized medium was then transferred to sterile Petri dishes and allowed to harden at room temperature. A uniform lawn of bacterial strains (*E. coli*, *S. aureus*, *E. faecalis*, and *Pseudomonas* sp.) was formed by spreading the bacterial suspension over the agar surface using sterile cotton swabs. The plates containing agar were punctured with a sterile polystyrene point to create nine-millimetre-wide wells. Tannic acid-assisted alginate particles at varying quantities (25, 50, and 100 μ g/mL) were subsequently added to the wells. Typically, an antibiotic (such as Bacteria-Amoxicillin or Fungi-Fluconazole) was utilized as a baseline. A full day was spent incubating the plates at 37 °C (Rajeshkumar, Tharani, Jeevitha, & Santhoshkumar, 2019; Rifaath, Rajeshkumar, Anandan, Munuswamy, Govindharaj, Shanmugam, et al., 2023; Roshan, Priyadharshini, Rajeshkumar, & Sinduja, 2021).

Time kills curve assay

In the time-kill assay, synthesized nanoparticles exhibited concentration-dependent antibacterial activity against several wound pathogens (*Pseudomonas* spp., *Staphylococcus aureus*, *E. coli*, and *E. faecalis*) as compared to the control. When compared to the control condition, all nanoparticle concentrations (25, 50, and 100 micrograms) showed a significant reduction in *Staphylococcus aureus*. More precisely, at the higher concentration of 100 micrograms, there was a noticeable bactericidal effect. *Pseudomonas* species showed a noticeable decrease in growth as compared to the control at a higher dosage of 100 micrograms. *E. Coli* and *E. faecalis* did, however, exhibit comparatively low resistance to the taken nanocomposite at the maximum dose of 100 micrograms as compared to the control.

To evaluate the bactericidal activity of the Nanocomposite, a time-kill curve assay is performed, and its parallelism with the growth of different wound pathogens is analysed, and the results are plotted in a graph (Munusamy & Shanmugam, 2023; Sankar, Shanmugam, Anandan, & Jayasree, 2024; Tharani, Rajeshkumar, Al-Ghanim, Nicoletti, Sachivkina, & Govindarajan, 2023).

Protein Leakage Analysis or Bradford Assay:

The investigation of protein leakage using the Bradford test, cells, bacteria from *Escherichia coli*, *Pseudomonas* sp, and *Staphylococcus aureus*, were exposed to varying doses of tannic acid-aided alginate for 24 to 48 hours. There were three different concentrations: 25, 50, and 100 μ g/ml were taken. An antibiotic (such as Bacteria-Amoxicillin or Fungi-Fluconazole) was usually used as a standard. The supernatant phase was separated from the bacterial culture by centrifuging it for 10 minutes at 3000 rpm following treatment, and it was eventually collected. 200 μ L of supernatant was needed for each sample, and

it was subsequently transferred to 96-well ELISA plates. In a dark environment, incubate for 10 minutes after adding 50 μ L of Bradford reagent. At 595 nm, the optical density (OD) of the sample was determined (Dharmaraj, Krishnamoorthy, Rajendran, Karuppiah, Jeyaraman, & Ethiraj, 2021; Munusamy & Shanmugam, 2023; Shanmugam, Munusamy, Jayakodi, Al-Ghanim, Nicoletti, Sachivkina, et al., 2023).

Cytoplasmic DNA leakage assay:

Bacterial cells treated with nanoparticles (NPs) release their cytoplasmic constituents, such as DNA and protein. As a result, we tried to determine the cytoplasmic

leakage of bacterial cells after they were treated with tannic acid-assisted alginate components in this work. The amount of DNA was estimated by cultivating 10 mL of UTI pathogen microbiological broth in MHA broth for the full night in an incubator. The culture was collected the following day using centrifugation for 10 minutes at 5000 rpm. After washing, the pellet was reconstituted in a 1X PBS buffer (pH 7.2). The bacterial cell density was increased to 1×10^5 cells/mL. After adding tannic acid-assisted alginate to different aliquots of cell cultures, they were (Khater, Kulkarni, Khater, Gholap, & Patil, 2020).

RESULTS AND OBSERVATIONS:

Visual Observation–

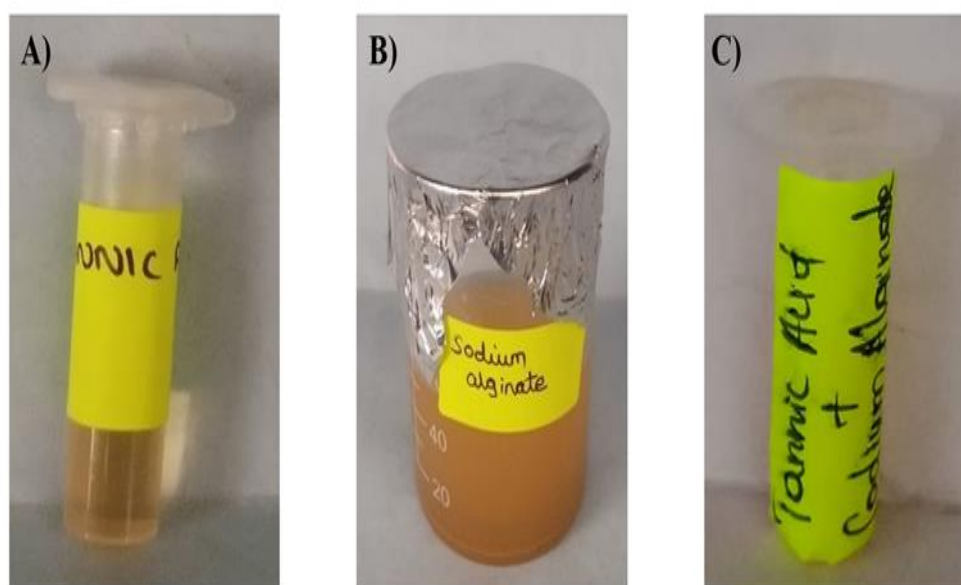


Figure 1: Visual observation of images of Tannic acid-alginate nano formulation
A) Tannic acid, B) Sodium alginate solution, C) Tannic acid-alginate nano formulation

Antimicrobial Activity of Tannic Acid-Alginate

The antimicrobial activity was evaluated, and the developed tannic acid-alginate nano formulation was used by using the agar well diffusion method. The nano formulation shows the highest zone of inhibition of 26 mm with a concentration of 100 μ g/mL of tannic acid-alginate against *Pseudomonas* sp. In comparison, 9 mm was the zone of inhibition for the control. The nano formulation developed a zone of inhibition of 31 mm at a concentration of 100 μ g/mL against *S. aureus*, while the control achieved a zone of inhibition of 9 mm. The zone of inhibition of nano formulation 100 μ g/mL against *E. coli* was 18 mm, and the control was 9 mm. At three distinct concentrations, the nano formulation showed a 19 mm zone of inhibition against *E. faecalis*. exhibited a lower zone of inhibition of 18 mm against *E. coli* at the same concentration and a higher zone of inhibition of 31 mm against *S. aureus* (Figure 2).

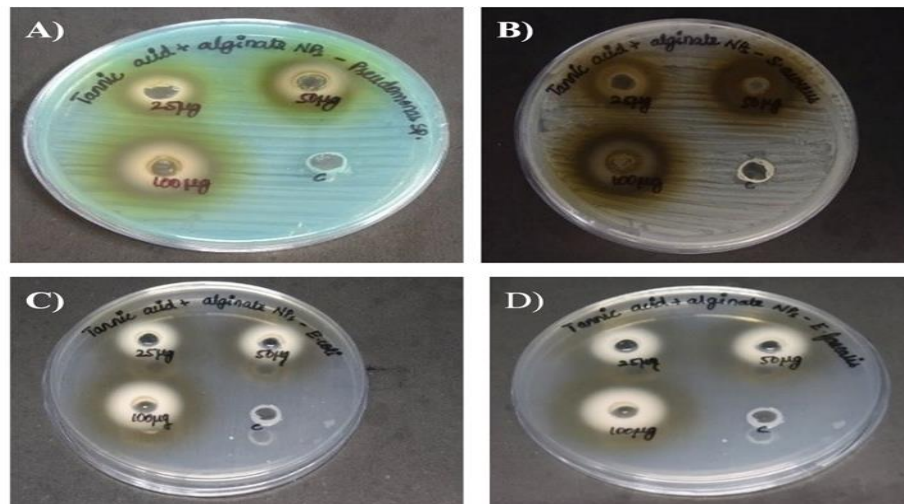


Figure 2: Antibacterial activity of agar well diffusion methods by using tannic acid-alginate nano formulation against wound pathogens. A) *Pseudomonas* sp, B) *S. aureus*, C) *E. coli*, D) *E. faecalis*

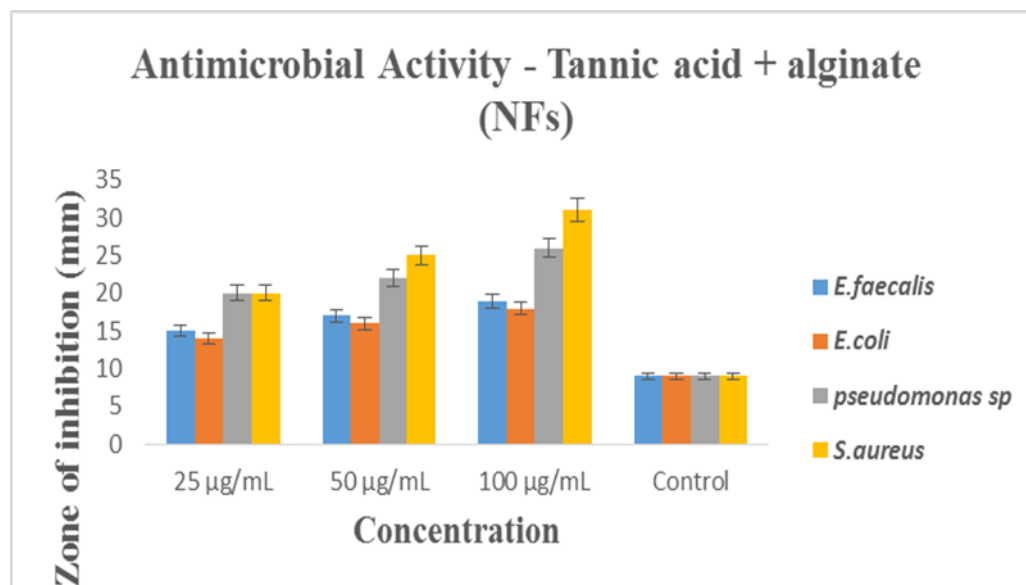


Figure 3: Graphical representation of antimicrobial activity of tannic acid + alginate against different wound pathogens, are *E. faecalis*, *E. coli*, *Pseudomonas* sp., and *S. aureus* Time-kill curve assay

The time-kill curve assessment of nano formulation of tannic acid-alginate against the four pathogens, *Pseudomonas* sp, *S. aureus*, *E. coli*, and *E. faecalis*, when compared to the control group, showed concentration-dependent bactericidal properties. At every concentration of

tannic acid alginate nano formulation (25µg, 50µg, 100µg), there was an exhibit reduction in *Pseudomonas* sp, and *S. aureus* count in comparison with the standard group for the duration of the assay (Figures A and B) demonstrated a notable decrease in *Pseudomonas* sp. and *S. aureus* counts over the first two hours at the higher dose of 100µg, suggesting intense bacterial activity in (figure C) similarly to *Pseudomonas* sp, and *S. aureus*, nano formulation of tannic acid-alginate exhibit the dose-dependent bactericidal effect against *E. coli*. In all concentrations of tannic acid-alginate in comparison to the standard and control groups, *E. coli* levels were decreased. At the maximum dose (100µg), there was a considerable drop in *E. coli* count during the first hour, indicating strong antibacterial effectiveness. Tannic acid-alginate similarly demonstrated concentration-dependent antibacterial activities against *E. faecalis* (Figure D); however, the effects were more gradual. The decrease in *E. faecalis* counts was less noticeable even at the highest concentration (100µg), suggesting that it is less susceptible to nano formulation than the other three bacteria.

Nonetheless, a noticeable reduction in *E. coli* and *E. faecalis* counts was observed over time when compared to the standard and control groups. These findings collectively emphasise the concentration-dependent antibacterial properties of tannic acid-

alginate against the tested wound pathogens. While *Pseudomonas* sp, while *E. coli* and *E. faecalis* responded more slowly to tannic acid-alginate, whereas *S. aureus* showed a quick responsiveness. Tannic acid-alginate significantly reduced bacterial counts when demonstrated their potential as compared to the standard and control group antibacterial agents for wound infections.

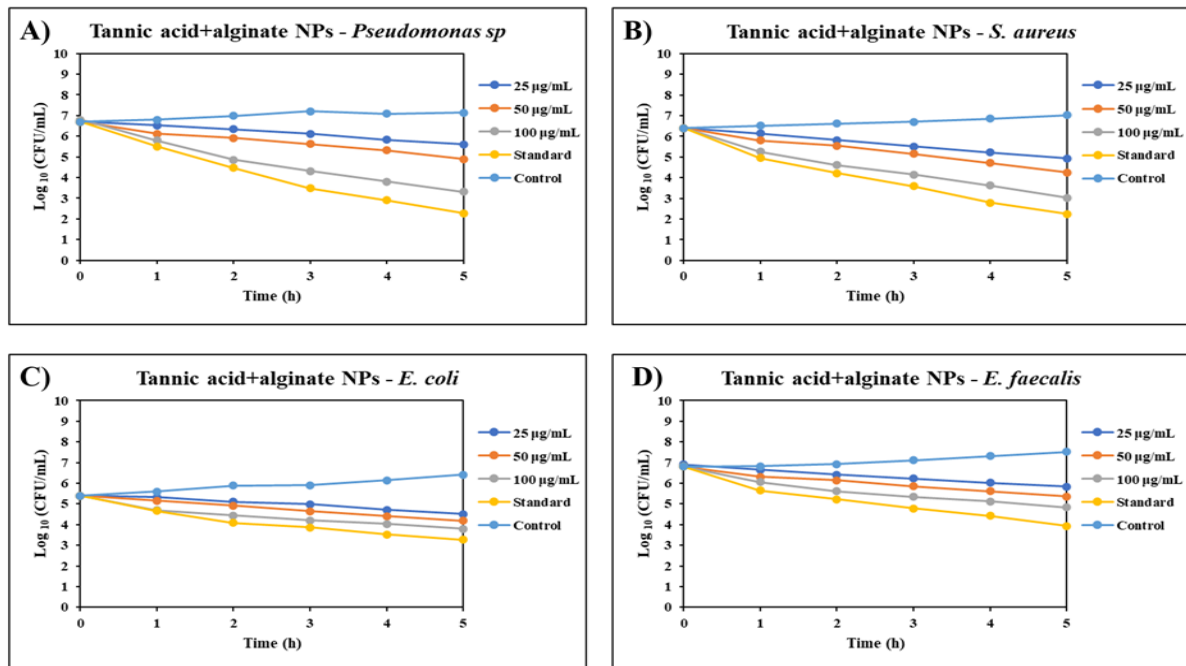


Figure 4 Antimicrobial activity assessed using Time kill curve assay of synthesized Tannic acid-alginate nano formulation against wound pathogens A) *Pseudomonas* sp, B) *S. aureus*, C) *E. coli*, D) *E. faecalis*

Protein leakage assay

The protein leakage assay was conducted to evaluate the antibacterial activity of tannic acid-alginate at different concentrations (25µL, 50µL, 100µL), against four bacterial strains, *E. faecalis*, *E. coli*, *Pseudomonas* sp, and *S. aureus*. The results of the protein leakage assay demonstrated that the Tannic acid-alginate nanoformulation exhibited Antibacterial properties towards *E. faecalis*, *E. coli*, *Pseudomonas* sp, and *S. aureus* at all tested doses. Particularly, the amount of protein leaks was discovered to be dependent upon concentration, with higher concentrations resulting in greater protein leakage from bacterial cells. Accordingly, at the concentrations of 25µL, 50µL, and 100µL, the antibacterial activity of tannic acid-alginate nano formulation against *E. faecalis*, *E. coli*, *Pseudomonas* sp, and *S. aureus* was comparable to the standard and control. (Figure 5) This shows that the tannic acid-alginate Efficiency is comparable to the standards. Amoxicillin induces protein leaks and prevents the formation of bacteria, especially when *S. aureus*, *Pseudomonas* sp, and *E. faecalis*. Overall, the findings of the protein leakage assay show efficacy against *E. faecalis*, *E. coli*, *Pseudomonas* sp, and *S. aureus*. Furthermore, the nano formulation showed encouraging antibacterial activity on par with the prescription medication.

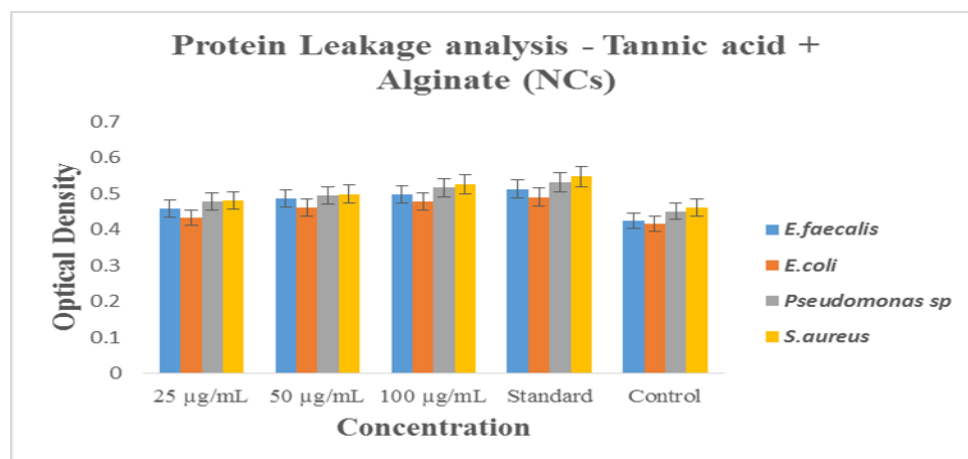


Figure 5 Graphical Representation of protein leakage assay or Bradford assay of tannic acid-alginate nano formulation against wound pathogens (*E. faecalis*, *E. coli*, *Pseudomonas* sp, *S. aureus*).

Cytoplasmic leakage assay

The antibacterial effect was evaluated using the cytoplasmic leakage assay of tannic acid-alginate nano formulation at different concentrations, 25µL, 50µL, and 100µL, against four pathogens, such as *E. faecalis*, *E. coli*, *Pseudomonas*, and *S. aureus*. The standard and control used in the investigation were the antibiotic drug Amoxicillin. Nevertheless, it was discovered that the cytoplasmic leakage was concentration-dependent, with more substantial effects occurring at higher doses. The cytoplasmic leakage results (Figure 6) revealed *E. faecalis*, *E. coli*, *Pseudomonas* sp, and *S. aureus*, which demonstrated more cytoplasmic leak than the control and standard at every tested dose. The increased cytoplasmic leakage suggests that the tannic acid-alginate has a strong antibacterial impact on this particular strain of bacteria.

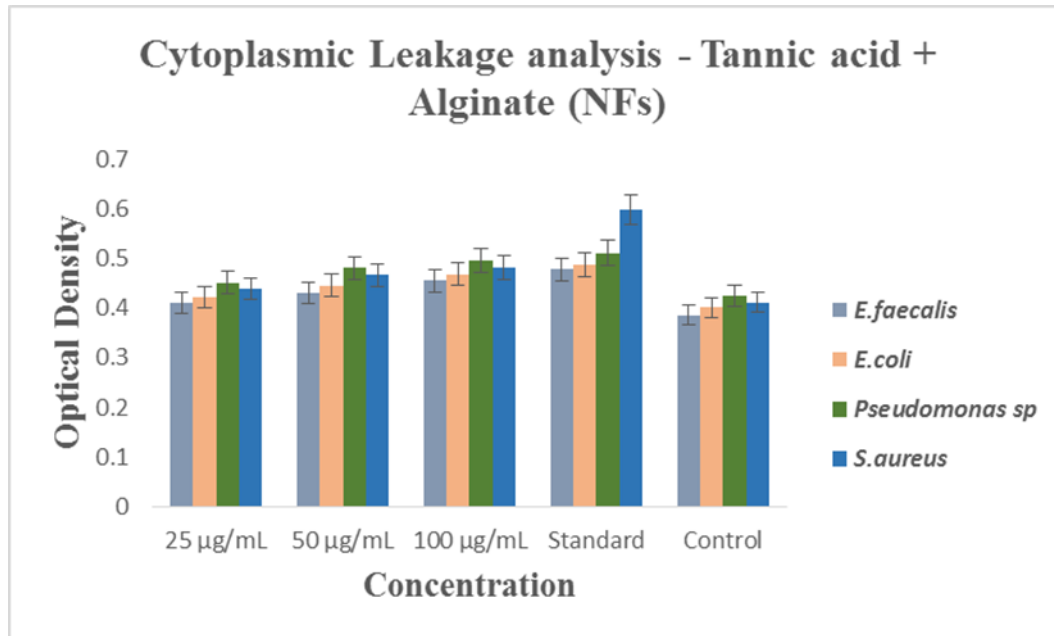


Figure 6 Graphical representation of cytoplasmic leakage analysis of Tannic acid alginate nano formulation, which was tested against wound pathogens *E. faecalis*, *E. coli*, *S. aureus*, and *Pseudomonas* sp. The assessment of protein and DNA leakage analysis of tannic acid-alginate nano formulation against wound pathogens provides valuable insights into its potential efficacy as a wound care therapy.

DISCUSSION

The current study aimed to investigate and compare the potential antibacterial properties against wound pathogens of tannic acid-alginate nano formulation. The research included the synthesis of tannic acid-alginate nanoformulation, confirmed by visual observation (Figure 1A, B, C), and subsequently, in vitro antibacterial activity against wound pathogens, as well as protein and cytoplasmic leakage assays. In recent years, Biofilm has been linked to the majority of non-healing wounds and wound infections, which are believed to occur in approximately 78% of chronic wounds. By stimulating the production of nitric oxide, inflammatory mediators, and free radicals, biofilm in a chronic wound can slow down the healing process and prolong the inflammatory state of the wound for an extended period. Numerous bandages for wounds have been devised to prevent infection and promote recovery (Srichaiyapol, Maddocks, Thammawithan, Daduang, Klaynongsruang, & Patramanon, 2022).

As previously reported, the creation of affordable, Biocompatible hydrogel dressings that are low-swelling, antimicrobial, and antioxidant is essential for speeding

up wound healing. The D- (+)-gluconic acid δ -lactone/ CaCO_3 combination was used to create a versatile alginate hydrogel dressing. The inclusion of hyaluronic acid and tannic acid (TA) gave the hydrogel antioxidant (ROS-scavenging), hemostatic, and wound-healing characteristics (Ma, Fu, Meng, Li, Wang, Shao, et al., 2024). The structure of the cell walls of *S. aureus* and *E. coli* explains their differing antibacterial activities. In contrast to *E. coli*, *S. aureus* TA penetrates the bacterial cell wall more quickly and disrupts the internal membrane structure due to its thick peptidoglycan layer. The inhibitory zone diameters did not significantly alter when the TA concentration rose. A 12 mm inhibitory zone was thought to be a reliable sign of potent antibacterial action (Tuna, Arisoy, Oktay Başçömez, & Baydemir Peşint, 2025).

The previous study demonstrated that biopolymers of tannic acid-alginate and a combination of chitosan incorporation with scaffold assembly enhanced biocompatibility and advancement of medication delivery technologies for wound healing. The biopolymer of the scaffold acceleration and remodels tissue, complemented by released tannic acid at the early

stage of wound healing. The increased frequency of big blood arteries and inflammatory cells, alginate-RGD-tannic acid, enhanced tissue remodelling in the tissue (Mndlovu, du Toit, Kumar, & Choonara, 2023). Early observations regarding sodium alginate-tannic acid antibacterial properties against *S. aureus* and *E. coli* were investigated utilizing the agar diffusion through disks method. Furthermore, sodium alginate films devoid of tannic acid exhibit minimal antibacterial action against *E. coli* and *S. aureus*. Then, tannic acid incorporated sodium alginate showed enhanced inhibition against *E. coli* and *S. aureus*. The bacterial cell membrane damage caused by TA was the reason for its inhibitory action, which ultimately resulted in killing germs by causing internal components to flow (H. Li, Liu, Sun, & Lv, 2022). Previously, the findings show that biosynthesized AgNPs have a significant impact on bacterial cells by causing oxidative stress and altering protein contents. AgNPs' negative charge is what causes the harm to the bacterial cell wall membrane, which results in cell lysis. It was noted that the concentration of *P. aeruginosa* revealed a clear relationship with the quantity of cellular protein released by AgNPs made with TCF, indicating that TCF-AgNPs can be employed as potent antimicrobial agents (Tharani, Rajeshkumar, Al-Ghanim, Nicoletti, Sachivkina, & Govindarajan, 2023).

Previously, reports have shown that Cu-PTA nanoparticles are made to be microenvironment-responsive. They have a hierarchical influence on the healing process of infected wounds and are strong (dually crosslinked), antibacterial, antioxidative, and anti-inflammatory (Dongying Li, Li, Wang, Wang, & Teng, 2023). In this early study, we used electrospinning and fibre breakage-recombination to create Excellent mechanical properties, exceptional antibacterial efficacy against drug-resistant microorganisms, and favourable biocompatibility characterize this antibacterial hydrogel wound dressing. Hydrogel production involves both chemical and physical crosslinking (Dangwei Li, Dong, Liu, Lin, Yang, Shi, et al., 2024).

Previously, we investigated the antibacterial and antibiofilm properties of silver nanoparticles (AgNPs) were examined using tannic acid (TA) and sodium alginate (Na-Alg) as stabilising and reducing agents, respectively, in the green manufacturing process. The creation of biologically viable AgNPs-loaded TA/Na-Alg complexes using an easy, economical, and environmentally friendly method could be appropriate for the development of a new *S. aureus* defence strategy (Tian, Hu, Chen, Liu, Xue, & Han, 2022). Overall, biosynthesized using tannic acid-alginate nano formulation shows promise for several uses, particularly the treatment of wounds. Their potential to treat diseases linked to wounds is demonstrated by their concentration-dependent antibacterial, time-kill curve assay, protein leakage, and cytoplasmic leakage properties against wound pathogens.

Limitations

The present investigation on the production and bioactivity of the tannic acid-alginate nano formulation yields interesting results; yet, it has several drawbacks. It is primarily concerned with a limited range of biological activity in vitro, lacks in vivo confirmation, fails to analyse biocompatibility and toxicology adequately, and ignores long-term stability and environmental factors. Additional studies might involve larger biological activity examination, in vivo tests, thorough cytotoxicity evaluations, and long-term stability investigations, as well as considerations for flexibility, ecological impact, and compliance with regulations.

CONCLUSION

The present study promised to synthesise tannic acid-alginate nano formulation, and antibacterial activity was studied, and it was found to be effective against wound pathogens due to its antibacterial activity. Antibacterial and time-kill curve assay, protein, and cytoplasmic leakage assay. Tannic acid-alginate nano formulation exhibits exceptional antibacterial properties. These qualities have great promise for reducing the impacts of inflammation and oxidative stress, two factors that are essential to the intricate wound-healing process, as well as for tackling the urgent problem of wound infections. Conclusions from the research offer a strong basis for the ongoing research on tannic acid-alginate produced for medicinal purposes, with an emphasis on wound care and infection control. These nanoparticles offer a viable path for the creation of novel and efficient therapies targeted at improving wound healing and lowering the incidence of infections in the field of medicine by utilizing their strong antibacterial properties and superior safety profile.

CRedit authorship contribution statement

Banupriya Palani: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. Karthikeyan Shanmugam: Validation, Methodology, and editing with review of the manuscript, Data curation, and Visualization. Rajeshkumar Shanmugam: Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualisation. Santhoshkumar Jayakodi: Investigation, Validation, Review and editing.

Declaration of Competing Interest

The authors declare no financial or personal interests that could have influenced the work presented in this study

Data availability

No data availability.

Ethical Statement

Not applicable

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REFERENCES

RESEARCH ARTICLE

1. Akin, B., Akgul, B., Tasdurmazli, S., Abamor, E. S., Ozbek, T., Ozcelik, B., Su, E., & Ozmen, M. M. (2024). Tannic Acid Incorporated Antibacterial Polyethylene Glycol-Based Hydrogel Sponges for Management of Wound Infections. *Macromolecular Bioscience*, 24(8), 2400101.
2. Ali, M., Ullah, S., Ullah, S., Shakeel, M., Afsar, T., Husain, F. M., Amor, H., & Razak, S. (2024). Innovative biopolymers composite-based thin film for wound healing applications. *Scientific Reports*, 14(1), 27415.
3. Chen, Y., Tian, L., Yang, F., Tong, W., Jia, R., Zou, Y., Yin, L., Li, L., He, C., & Liang, X. (2019). Tannic acid accelerates cutaneous wound healing in rats via activation of the ERK 1/2 signaling pathways. *Advances in wound care*, 8(7), 341-354.
4. Čoma, M., Fröhlichová, L., Urban, L., Zajíček, R., Urban, T., Szabo, P., Novák, Š., Fetisso, V., Dvořánková, B., & Smetana Jr, K. (2021). Molecular changes underlying hypertrophic scarring following burns involve specific deregulations at all wound healing stages (inflammation, proliferation and maturation). *International Journal of Molecular Sciences*, 22(2), 897.
5. Dharmaraj, D., Krishnamoorthy, M., Rajendran, K., Karuppiyah, K., Jeyaraman, R., & Ethiraj, K. (2021). Protein leakage induced marine antibiofouling activity of biosynthesized zinc oxide nanoparticles. *Journal of Cluster Science*, 32(3), 643-650.
6. Guimarães, I., Costa, R., Madureira, S., Borges, S., Oliveira, A. L., Pintado, M., & Baptista-Silva, S. (2023). Tannic acid tailored-made microsystems for wound infection. *International Journal of Molecular Sciences*, 24(5), 4826.
7. Jabeen, N., Prabhalakshmi, K., Dhanraj, G., & Ramasubburayan, R. (2025). Biosynthesis of titanium dioxide nanoparticles using *Sargassum tenerimum* as reductant and deciphering its antibiofilm role against cariogenic *Candida albicans*. *Microbial Pathogenesis*, 202, 107452.
8. Jing, W., Xiaolan, C., Yu, C., Feng, Q., & Haifeng, Y. (2022). Pharmacological effects and mechanisms of tannic acid. *Biomedicine & Pharmacotherapy*, 154, 113561.
9. Khater, M. S., Kulkarni, G. R., Khater, S. S., Gholap, H., & Patil, R. (2020). Study to elucidate effect of titanium dioxide nanoparticles on bacterial membrane potential and membrane permeability. *Materials Research Express*, 7(3), 035005.
10. Kushwaha, A., Goswami, L., & Kim, B. S. (2022). Nanomaterial-based therapy for wound healing. *Nanomaterials*, 12(4), 618.
11. Li, D., Dong, X., Liu, X., Lin, H., Yang, D., Shi, X., Chen, C., Tao, F., Jiang, L., & Deng, H. (2024). Cellulose nanofibers embedded chitosan/tannin hydrogel with high antibacterial activity and hemostatic ability for drug-resistant bacterial infected wound healing. *Carbohydrate Polymers*, 329, 121687.
12. Li, D., Li, J., Wang, S., Wang, Q., & Teng, W. (2023). Dually crosslinked copper-poly (tannic acid) nanoparticles with microenvironment-responsiveness for infected wound treatment. *Advanced healthcare materials*, 12(17), 2203063.
13. Li, H., Liu, C., Sun, J., & Lv, S. (2022). Bioactive edible sodium alginate films incorporated with tannic acid as antimicrobial and antioxidative food packaging. *Foods*, 11(19), 3044.
14. Liu, C., Dong, S., Wang, X., Xu, H., Yang, X., Wu, S., Jiang, X., Kan, M., & Xu, C. (2023). Research progress of polyphenols in nanoformulations for antibacterial application. *Materials Today Bio*, 21, 100729.
15. Ma, X., Fu, X., Meng, J., Li, H., Wang, F., Shao, H., Liu, Y., Liu, F., Zhang, D., & Chi, B. (2024). A low-swelling alginate hydrogel with antibacterial hemostatic and radical scavenging properties for open wound healing. *Biomedical Materials*, 19(6), 065010.
16. Maitz, J., Merlino, J., Rizzo, S., McKew, G., & Maitz, P. (2023). Burn wound infections microbiome and novel approaches using therapeutic microorganisms in burn wound infection control. *Advanced Drug Delivery Reviews*, 196, 114769.
17. Mndlovu, H., du Toit, L. C., Kumar, P., & Choonara, Y. E. (2023). Tannic acid-loaded chitosan-RGD-alginate scaffolds for wound healing and skin regeneration. *Biomedical Materials*, 18(4), 045009.
18. Munusamy, T., & Shanmugam, R. (2023). Green synthesis of copper oxide nanoparticles synthesized by *Terminalia chebula* dried fruit extract: characterization and antibacterial action. *Cureus*, 15(12).
19. Orłowski, P., Zmigrodzka, M., Tomaszewska, E., Ranaszek-Soliwoda, K., Czupryn, M., Antos-Bielska, M., Szemraj, J., Celichowski, G., Grobelny, J., & Krzyzowska, M. (2018). Tannic acid-modified silver nanoparticles for wound healing: the importance of size. *International journal of nanomedicine*, 991-1007.
20. Pal, S., Sayana, A., Joshi, A., & Juyal, D. (2019). *Staphylococcus aureus*: A predominant cause of surgical site infections in a rural healthcare setup of Uttarakhand. *Journal of Family Medicine and Primary Care*, 8(11), 3600-3606.
21. Palla, P. (2023). CHARACTERIZATION AND ANTIMICROBIAL SENSITIVITY PATTERNS OF BACTERIAL PATHOGENS IN CHRONIC WOUND INFECTIONS. *Int J Acad Med Pharm*, 5(6), 1542-1546.
22. Pauline, C., Akshita, Pavithra, T., Kannan, K., & Sivaperumal, P. (2025). Characterization and biological activity of silver nanoparticles from (*Rhizophora Mucronata*) mangrove extract. *Nano Life*, 15(03), 2450018.
23. Rajeshkumar, S., Tharani, M., Jeevitha, M., & Santhoshkumar, J. (2019). Anticariogenic Activity

- of Fresh Aloe Vera Gel Mediated Copper Oxide Nanoparticles. *Indian Journal of Public Health Research & Development*, 10(11).
24. Rifaath, M., Rajeshkumar, S., Anandan, J., Munuswamy, T., Govindharaj, S., Shanmugam, R., Jayasree, A., Munusamy, T., & Sulochana, G. (2023). Preparation of herbal nano-formulation-assisted mouth paint using titanium dioxide nanoparticles and its biomedical applications. *Cureus*, 15(11).
 25. Rosa, J. M., Bonato, L. B., Mancuso, C. B., Martinelli, L., Okura, M. H., Malpass, G. R. P., & Granato, A. C. (2018). Antimicrobial wound dressing films containing essential oils and oleoresins of pepper encapsulated in sodium alginate films. *Ciência Rural*, 48(03), e20170740.
 26. Roshan, A., Priyadharshini, R., Rajeshkumar, S., & Sinduja, P. (2021). Preparation of mouth wash using *Musa sapientum* mediated silver nanoparticles and its antimicrobial activity. *Journal of Pharmaceutical Research International*, 33(64A), 177-185.
 27. Sankar, H. N., Shanmugam, R., Anandan, J., & Jayasree, A. (2024). Green synthesis of *Euphorbia tirucalli*-mediated titanium dioxide nanoparticles against wound pathogens. *Cureus*, 16(2).
 28. Shanmugam, R., Munusamy, T., Jayakodi, S., Al-Ghanim, K. A., Nicoletti, M., Sachivkina, N., & Govindarajan, M. (2023). Probiotic-bacteria (*Lactobacillus fermentum*)-wrapped zinc oxide nanoparticles: biosynthesis, characterization, and antibacterial activity. *Fermentation*, 9(5), 413.
 29. Sivaperumal, P., Ganapathy, D., & Kamala, K. (2025). Evaluating the efficacy of doripenem against *Staphylococcus aureus* in vancomycin-resistant strains. *Microbial Pathogenesis*, 202, 107449.
 30. Srichaiyapol, O., Maddocks, S. E., Thammawithan, S., Daduang, S., Klaynongsruang, S., & Patramanon, R. (2022). TA-AgNPs/Alginate Hydrogel and Its potential application as a promising antibiofilm material against polymicrobial wound biofilms using a unique biofilm flow model. *Microorganisms*, 10(11), 2279.
 31. Su, J., Li, J., Liang, J., Zhang, K., & Li, J. (2021). Hydrogel preparation methods and biomaterials for wound dressing. *Life*, 11(10), 1016.
 32. Tharani, M., Rajeshkumar, S., Al-Ghanim, K. A., Nicoletti, M., Sachivkina, N., & Govindarajan, M. (2023). *Terminalia chebula*-assisted silver nanoparticles: biological potential, synthesis, characterization, and ecotoxicity. *Biomedicines*, 11(5), 1472.
 33. Tian, S., Hu, Y., Chen, X., Liu, C., Xue, Y., & Han, B. (2022). Green synthesis of silver nanoparticles using sodium alginate and tannic acid: Characterization and anti-*S. aureus* activity. *International Journal of Biological Macromolecules*, 195, 515-522.
 34. Tuna, B., Arisoy, P., Oktay Başeğmez, H. İ., & Baydemir Peşint, G. (2025). Advancing wound healing: controlled release of tannic acid via epitope imprinted antimicrobial spongy cover material. *World Journal of Microbiology and Biotechnology*, 41(2), 1-15.
 35. Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *International journal of nanomedicine*, 1227-1249.
 36. Wekwejt, M., Małek, M., Ronowska, A., Michno, A., Pałubicka, A., Zasada, L., Klimek, A., & Kaczmarek-Szczepańska, B. (2024). Hyaluronic acid/tannic acid films for wound healing application. *International Journal of Biological Macromolecules*, 254, 128101.