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Original Article

# Topical Chitosan-Based Nanogel of *Filipendula ulmaria* (Meadowsweet) Extract against Bacterial Infections: Development, Characterization, Optimization and *in vitro* Studies

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## ABSTRACT

**Background:** Skin infections caused by bacterial pathogens pose a significant health concern, necessitating the exploration of innovative therapeutic approaches. This study aimed to investigate the potential therapeutic efficacy of *Filipendula ulmaria* extract-loaded chitosan nanoparticles against bacterial skin infections. **Materials and Methods:** The aerial part of the *Filipendula ulmaria* plant was extracted using the Soxhlet technique with water and methanol in a 70:30 v/v ratio. The resulting extract was utilized to formulate chitosan nanoparticles through the anionotropic gelation technique, which were subsequently incorporated into a gel matrix. The nanoparticles underwent comprehensive characterization for size, polydispersity index and entrapment efficiency percentage. Optimization was achieved using the Box-Behnken design, followed by *in vitro*-release testing. The nanogel underwent various analyses, including texture analysis, confocal laser scanning microscopy, dermatokinetic evaluation, stability assessment and antioxidant and antimicrobial activities. **Results:** The formulated nanoparticles exhibited a size of 100.5 nm, a polydispersity index of 0.2965 and an entrapment efficiency of 84.5%. The Korsmeyer-Peppas model exhibited the best fit with a linear correlation coefficient of $R^2$=0.95. Comparative analysis with the conventional drug (tetracycline) highlighted the superior dermatokinetic performance of the nanostructure on both epidermal and dermal skin layers, along with enhanced antioxidant scavenging activity. Minimum Inhibitory Concentrations (MIC) for inhibiting the growth of *B. subtilis* ranged from 9.07±0.53 to 16.88±0.51 and for *E. coli* ranged from 8.2±0.48 to 19.11±0.74, with the highest value observed for the 60 mg nanoformulation. **Conclusion:** The *Filipendula ulmaria* extract-loaded chitosan nanogel demonstrated potent efficacy against bacterial pathogens, suggesting its potential as a natural agent for the treatment of bacterial skin infections.

**Keywords:** Antibacterial, Chitosan, Dermatokinetics, *Filipendula ulmaria*, Nanogel, Nanoparticles.

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## INTRODUCTION

Acute or short-term infections caused by bacteria on the skin may lead to structural changes in the skin. These bacteria cause a diverse group of potentially fatal infections affecting the skin and the surrounding subcutaneous layer, including muscles and fascia. Bacterial skin infections, including cellulitis, infections on wounds, severe abscesses on the cutaneous layer and pyoderma, account for a great proportion.[1,2] These infections lead to lengthy hospitalizations, elevated mortality risk and worsening patients' outcomes, all of which place a heavy financial strain on the healthcare systems. These infections may be polymicrobial or monomicrobial, as they include organisms such as anaerobic, Gram-positive and Gram-negative bacteria.[3] People with medical histories who have had prior antibiotic therapy are frequently

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found to be additionally prone to polymicrobial infections. Individuals with these illnesses are probably not treated well, necessitating the use of anti-biotics.[1] Furthermore, the massive growth of MRSA (Methicillin-Resistant *Staphylococcus aureus*) bacteria and related infections cause the development of MDR (Multidrug-Resistant) pathogens, which has presented a serious issue for modern medical care strategies.[4] As a result, there has been an urge to formulate new categories of anti-biotics that have the potential for broad-spectrum activity.[5] However, despite the pro-found impact of bacterial skin diseases on the global disease burden, appears to be a lack of commensurate attention globally. With the aim of improving health systems and eliminating disparities, the Global Burden of Disease (GBD) database 2019 incorporates risk factors and other parameters and covers more than 200 countries and regions from 1990 to 2019.[6] There is a rising demand for naturally derived medicines containing phenolic components that have an antimicrobial effect and other pharmacological activities.[7-10] Plant-based antimicrobial drugs could be employed instead of synthetic chemicals to meet an individual's need for a secure and accessible medicine.[6] Chitosan-based nanomaterials could be utilized in various treatment strategies, notably oral and topical based therapy, to disperse bioactive constituents such as drugs or plant-based products. Chitosan nanoparticles are a promising and adaptable technique for addressing the biocompatibility, safety and integrity concerns of the majority of responsive drugs because they incorporate the properties of the polymer with expandable dimensions in addition to the possibility of surface modification based on personalized needs. Chitosan's specific physicochemical features allow for the effective encapsulation as well as controlled discharge of a wide variety of medicines, including its positive charge, hydrophilicity and biological compatibility. In addition, chitosan nanoparticles' efficiency in transporting medicinal substances to the target region may be improved by modifying their surfaces to permit specialized target as well as association with biological substances.[11] Nanocarriers are particles or systems on the nanometer scale that are engineered to encapsulate and transport biomolecules, medicines, or other therapeutic agents to their intended sites of action in the body. Using these nanocarriers results in better medication solubility, controlled release, greater stability and precise administration.[12]

Nanocarriers based on chitosan have showed impressive promise in a variety of settings, including medication delivery, gene therapy, tissue engineering and imaging.[13] These display a significantly wide range of anti-bacterial effects against Gram-negative and Gram-positive bacteria pathogens via distinct and innovative processes. The proportion of specific microbiological organisms, pH, temperatures and other parameters influence the action of antimicrobials.[14] The process of bactericidal effect by the nanoparticles is currently being researched, with the destruction of membranes and biofilms, oxidative stress and cytoplasm leakage as the mentioned mechanisms. These properties of the nanoparticles make them effective, viable and credible methods for treating moderate to profound infections caused by bacteria.[15] *Filipendula ulmaria* of the Rosaceae family is an herbaceous perennial that blooms in a whitish or cream color. It is also known as meadowsweet and has a slender and pinkish rhizome. The stems of the plant are 50-120 cm tall. It has been located throughout Asia and Europe in wetlands, damp places and meadows. The medicinal sections of the plant consist of leaves, rhizomes, blooms, or sometimes the whole herb.[16] These plant sections include various flavonoids such as spiraeoside, quercitrin, catechin, astragalin, rutin, hyperoside, kaempferol and quercetin, as well as other compounds like salicylate aglycons and tannins. Simultaneously, they comprise a range of phenolics such as caffeic acid, salicylic acid, gallic acid and ellagic acid that are closely connected to their effects as analgesic, astringent, antipyretic, diuretic and anti-rheumatic, among other uses.[17-21] Researchers have discovered that the floral extracts from plants have some exceptional attributes. Investigations have revealed that they possess anti-inflammatory,[22] antioxidant, anti-microbial,[17] anti-cancer,[23] and even anti-influenza properties.[19,20]

While several plants extracts, such as *Curcuma longa, Azadirachta indica* and *Camellia sinensis*, exhibit notable antibacterial and antioxidant properties, *Filipendula ulmaria* offers distinct advantages. Its rich composition of phenolic compounds, including salicylic acid, quercetin, gallic acid and hyperoside, contributes to its dual antibacterial and antioxidant effects. Additionally, the presence of tannins like rugosins and tellimagrandins sets it apart by enhancing membrane disruption in bacterial cells and modulating oxidative stress pathways. Unlike other plant extracts, *Filipendula ulmaria* has demonstrated synergistic effects when combined with nanocarrier systems, allowing for improved bioavailability, targeted delivery and prolonged therapeutic effects. These characteristics make *Filipendula ulmaria* a promising candidate for addressing bacterial skin infections, especially in an era of rising antibiotic resistance.[24-28]

The purpose of this research was to explore the prospect of *Filipendula ulmaria* plant extract-loaded chitosan nanogel as a new antibacterial agent against bacterial strains that commonly responsible for skin diseases. The novelty of this study lies in the innovative combination of *Filipendula ulmaria* extract with chitosan nanoparticles to develop a nanogel formulation for the treatment of bacterial skin infections. While *Filipendula ulmaria* is well-known for its antibacterial and antioxidant properties, its therapeutic application has been limited by poor bioavailability and stability in conventional formulations. The integration of chitosan nanoparticles addresses these limitations by enhancing the stability, targeted delivery and controlled release of the active compounds. Furthermore, the synergistic action between the plant extract's bioactive components and chitosan's inherent antimicrobial properties creates a dual-action therapeutic system. This formulation not only aims to combat bacterial

skin infections effectively but also reduces the risk of antibiotic resistance by offering a natural alternative to synthetic antibiotics. The study's novelty also lies in its comprehensive evaluation of dermatokinetic, antioxidant properties and antimicrobial efficacy, positioning this nanogel as a potential candidate for advanced skin infection therapies. Thus, the objective of this investigation is to aid in the advancement of possible alternative treatments for bacterial skin infections.[29,30]

## MATERIALS AND METHODS

### Materials

The reagents, solvents, deacylated chitosan with a molecular weight of 500,000 Da, Tween 20, Tween 80, phosphate buffer solution, ethanol, acetic acid, Mueller Hinton agar, rhodamine hydro-ethanolic solution, carbomer and distilled water and other chemicals were purchased from Sigma Merck, United States. The aerial part of *Filipendula ulmaria* was obtained from Universal Biotech, Delhi, India. A qualified botanist confirmed the species and a reference specimen was stored in the laboratory for future tests at the Department of Botany, Jamia Hamdard. The specimen number provided for the plant was BOT/DAC/2022/09. To evaluate the antibacterial efficacy, 2 distinct bacterial strains were utilized in this study. *Escherichia coli* (ATCC 11303), a Gram-negative bacterium, was obtained from Sigma Aldrich, Germany, in the form of lyophilized cells. On the other hand, *Bacillus subtilis* (ATCC 6633), a Gram-positive bacterium, was procured from the American Type Culture Collection (ATCC). These specific bacterial strains were selected for their relevance and well-established characteristics in antibacterial testing.

### Methods

A flowchart of experimental works that are embodied in the manuscript is presented in Supplementary Figure 1.

### Extraction

The aerial parts of *Filipendula ulmaria* were left to dry under shade, then crushed into powder and kept at 4°C in airtight bags. The Soxhlet process was used for extraction, using distilled water as the solution of solvents and the water and ethanol in 70:30 v/v ratios in a round bottom flask. The crushed powder of *Filipendula ulmaria* was put into the Soxhlet extractor. The solvents were then warmed until they started evaporating and moved across to the condenser. The residues then dripped into the reservoir. The ethanol was removed using a rotary evaporator and the concentrated plant extract was collected.[31]

### Preparation of *Filipendula ulmaria* chitosan nanoparticles and gel

The *Filipendula ulmaria* extract-loaded chitosan nanoparticles were prepared using the ionotropic gelation method. Chitosan was first dissolved in a 1% diluted acetic acid solution and stirred continuously for 12 hr to form a homogenous mixture, followed by the addition of Tween 80. Separately, the *Filipendula ulmaria* extract was accurately weighed and dissolved in a methanol-water mixture (1:1 ratio) and gradually added drop by drop into the chitosan solution. This mixture was stirred at 600 rpm for 120 min. Sodium tripolyphosphate was then introduced into the solution at a controlled rate of 1 mL/hr while maintaining the stirring speed at 600 rpm, facilitating the formation of nanosized particles. The successful formation of nanoparticles was confirmed by the solution became turbid.[32-34]

The gel was prepared by precisely weighing carbomer (1% w/w) to achieve appropriate consistency along with viscosity, followed by a gradual addition of distilled water while stirring the mixture. NaOH solution was incorporated for gel formation to maintain the optimal pH and adjust the dispersion of carbomer. The prepared nanoparticles were then slowly introduced into the carbomer dispersion while stirring the mixture continuously. Once an appropriate amount of nanoformulation was distributed within the gel matrix by constant stirring, the pH of the gel was adjusted again using NaOH solution. The homogeneous formulation of nanogel was achieved and then meticulously transferred for storage in glass containers.[33,35]

### Characterization of *Filipendula ulmaria* chitosan nanoparticles

#### *Size of particles and determination of Polydispersity Index (PDI)*

Scanning was performed using the light of a dynamic laser to evaluate the particle size of the nanoparticles and their PDI at 25°C on the Malvern Nano Zetasizer, UK. The sample was dispersed in Milli-Q water with a concentration of 1 mg/mL. The Refractive Index (RI) of the material was measured to be 1.46, while the RI of the dispersant was 1.36. In order to maintain the precision of the experiment, the test was repeated three times, with the sample being diluted in distilled water and filtered through a 0.45 µM membrane before each measurement.[36,37]

### Entrapment efficiency (%)

The percentage efficiency of entrapment was measured through ultrafiltration method of the *Filipendula ulmaria* chitosan nanoparticles by utilizing filter tubes for centrifuging having a molecular weight of 10 kDa. Furthermore, 2 mL of *Filipendula ulmaria* chitosan nanoparticles were placed within centrifugal filter tube and underwent centrifugation at 10,000 rpm at 25°C for an hour.[38] Salicylic acid being the major constituent was analyzed and the following equation was utilized to determine the values.

$$EE\ (\%)=(Qint-Qsup.)/Qint.\times 100$$

$Q_{sup}$ is the quantity of *salicylic acid* evaluated within the supernatant, while $Q_{int}$ is the quantity of *salicylic acid* within the chitosan-loaded nanoparticles.

## Optimization of *Filipendula ulmaria* chitosan nanoparticles

Design-Expert Version 11 software was employed to optimize the nanoparticles using Box Behnken design for three factors. The independent variables taken for the analysis were X1: amount of chitosan, X2: amount of *Filipendula ulmaria* extract and X3: amount of Tween 20, while the dependent variables taken were Y1: particle size and Y2: entrapment efficiency. These variables were evaluated at low, high and medium variations to obtain the most relative composition of these independent variables. Several runs of the formulation was done to observe the effect of variables having three points in the center and having different equations of polynomials to get the surface plots of response. Quadratic and linear models were provided by the equations. However, the model containing the quadratic formula produced the most significant effects on these variables.

## *In vitro* drug release study

The dialysis bag technique was used to carry out the *in vitro* drug release studies. The dialysis bag with molecular cut-off 12000 to 14000 Daltons was formerly treated and filled with *Filipendula ulmaria* chitosan nanoparticles and sealed. The filled bag was then put into a solution of 250 mL of pH 7.4 Phosphate buffered saline (pH 7.4) and 25% methanol. The medium was stirred at 300 rpm on a thermostatic control magnetic stirrer at room temperature. The amount of salicylic acid (major constituent of the extract) was analyzed and the results were reported as the % drug release.[39]

## Texture Profile Analysis (TPA)

The texture of the prepared gel was evaluated using TA-XT Plus texture analyzer by Stable Micro System, United Kingdom. The experiment involved a straightforward compression, where the prepared nanoformulation was positioned in a 50 mL glass container. The aim was to assess the firmness, uniformity and cohesion work of chitosan nanoliposomal gel. Throughout the investigation, specific parameters were configured for the texture analyzer. The probe moved a distance of 10.00 mM, with a testing speed of 5.00 mM/s. The upper probe contacted the sample, encountering an auto-trigger force of 5.0 g before being withdrawn at a post-test speed of 5.00 mM/s. To process and analyze the data, Texture Exponent 32 software was employed to measure the force necessary to separate the probe from the prepared gel.[40]

## Confocal Laser Scanning Microscopy (CLSM)

The prepared nanoformulation underwent scanning through a confocal laser to examine the depth of penetration of nanoformulation. The prepared nanoformulation was dyed with rhodamine red B dye. At the same time, the control had rhodamine

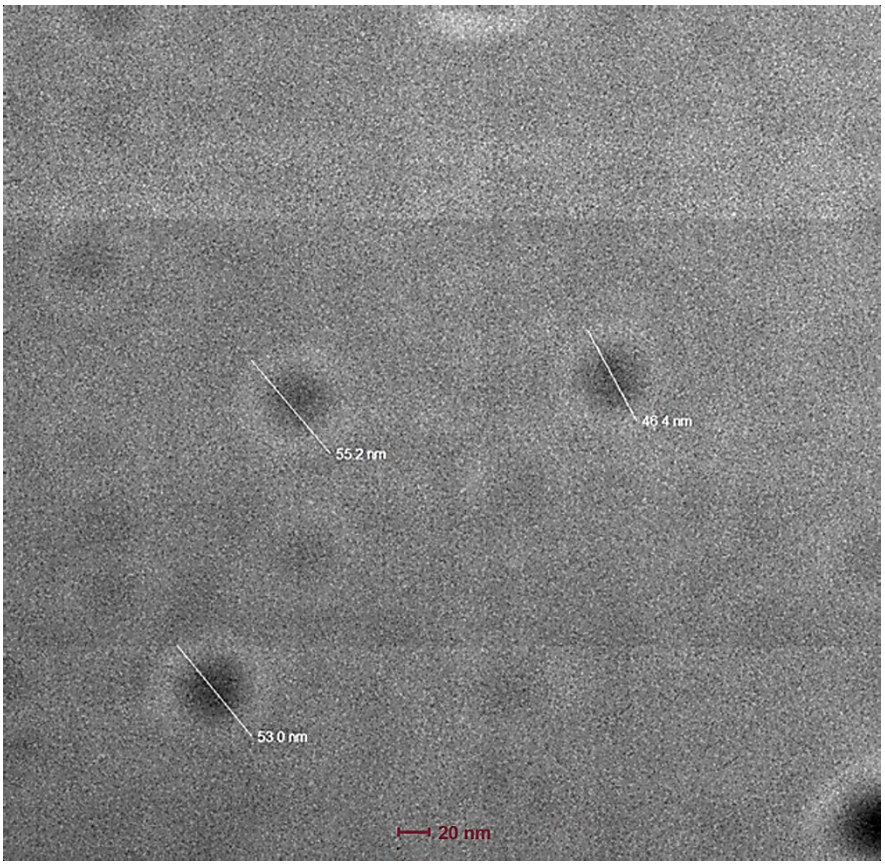


**Figure 1:** TEM of *Filipendula ulmaria* chitosan nanoparticles.

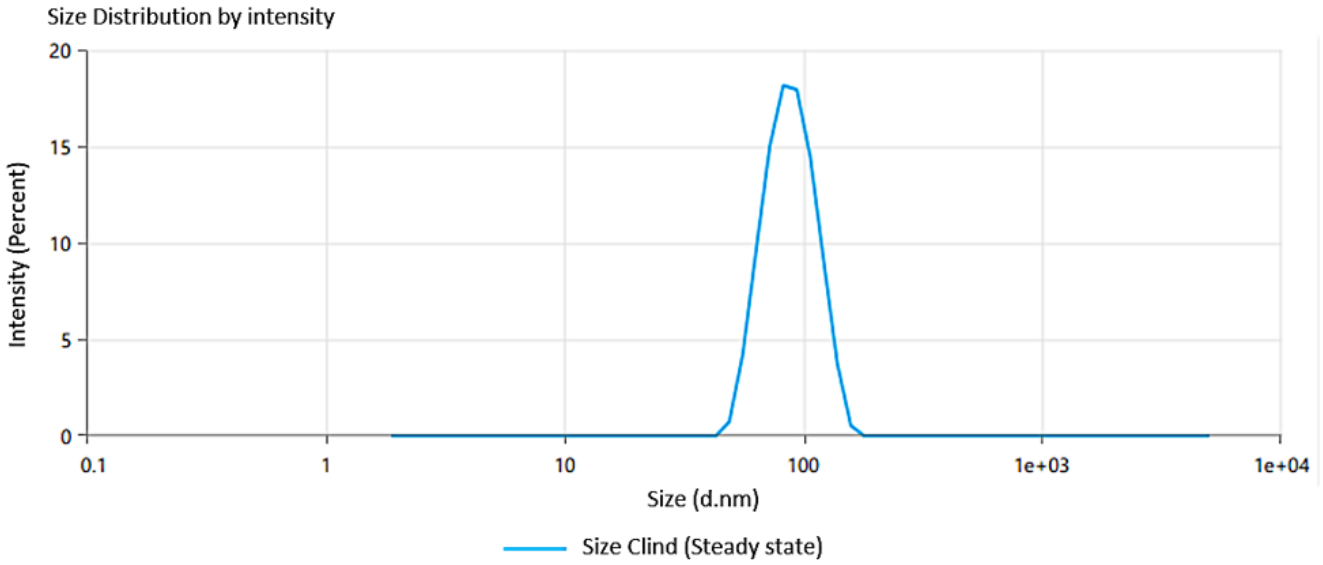


**Figure 2:** *Filipendula ulmaria* chitosan nanoparticle size.

**Table 1: Optimization of *Filipendula ulmaria* chitosan nanoparticles with variables utilized in BBD.**

| Factors | Levels used | | |
|---|---|---|---|
| **Independent variables** | **Low** | **Medium** | **High** |
| X1-Chitosan | 0.05 | 0.15 | 0.2 |
| X2-*Filipendula ulmaria* extract | 25 | 50 | 75 |
| X3-Tween 20 | 0.25 | 0.50 | 0.75 |
| Dependent variables | Measurements used | | |
| Y1-Particle size (nm) | 100.15 nm | | |
| Y2-Entrapment efficiency (%) | 84.5% | | |

solution alone and was treated for 6 hr using an extracted and washed part of the mouse intestine using distilled water. The prepared nanoformulation was analyzed using a confocal laser scanning microscope equipped with an argon-ion laser operating at a wavelength of 488 nm, with fluorescence emission detected at 532 nm. Both the prepared formulation and the control were analyzed under the CLSM.

## Dermatokinetic studies

*Filipendula ulmaria* chitosan nanogels were topically tested through Franz cells for diffusion by administering the prepared formulation on rat skin and examining the changes occurring during different timelines.[41] The removed skin was taken from Wistar rats and stored at -20°C until it was required. Before the analysis, the skin was further defrosted, warmed up and any existing hair was shaved off using a razor. The skin's stratum corneum was then oriented towards the donor section and put into the Franz Diffusion cell. Phosphate Buffered Saline (PBS) with a pH of 7.2 was poured in the receptor part and the produced nano formulation was set in the donor portion. The apparatus was continually agitated to maintain the room temperature for the experiment. The formulations were then taken from their allocated sections at predetermined intervals (0.5, 1, 2, 4, 8, 12 and 24 hr). The whole skin was then removed from the Franz cell and washed three times to remove any remaining formulation.[42,43] The cleansed skin was then immersed in warm water at 60°C for half a minute to help separate the dermal and epidermal layers of skin. HPLC was used to determine the composition of samples based on the amount of salicylic acid that entered the skin and the amount that remained on it.

## Stability activity

*Filipendula ulmaria* chitosan nanogel were tested for 30 days at normal temperature. Also, the prepared formulation was preserved for 3months under 31±2°C and 41±2°C temperatures with a 59±6% humidity range according to the International Council of Harmonization,[44] guidelines for stability testing.[44] A previous study was referred to examine the PDI, particle size and EE% and repeatability was assured by conducting these experiments thrice.[45]

## Antioxidant activity

The effect of *Filipendula ulmaria* chitosan nanogel to produce antioxidant activity was examined with the technique of DPPH (2, 2-diphenyl-1-picrylhydrazyl), having a free radical method. The experiment was carried out after the DPPH solution of methanol (3 mL) and the sample (0.5 mL) to be tested were mixed at an ambient temperature with the solution of DPPH turning from the color of violet to a plain colourless mixture indicating the electron diversion and the presence of antioxidants.[46] The following sample mixture was kept for 60 min for the reaction to occur in a darkened room. It was observed that the sample had the ability of hydrogen donation as the alterations in color reflected the potential of the antioxidants present within them. Furthermore, a blank sample (3.3 mL) was taken and methanol (0.3 mL) was mixed. The sample for control, having a solution of DPPH (0.3 mL) and methanol (3.5 mL), was also prepared, tested and compared at an absorbance level of 517 nm using a spectrophotometer.

## Anti-microbial activity

*Filipendula ulmaria* chitosan nanogel were assessed for their potential antimicrobial effect by the disc diffusion technique to evaluate the prepared formulation's antibacterial effect. The effects of the nanoformulation were assessed on 2 strains of bacterial species (i.e., *Escherichia coli* and *Bacillus subtilis*) after leaving them to grow in a medium of Mueller-Hinton agar for a night at room temperature. Sterilized saline water (5 mL) was used to harvest the bacteria through a spectrophotometer. At 580 nm, the cell count observed was $10^7$ CFU/mL to examine the bactericidal effect; the prepared nanoformulation was tested in different doses (20 mg/mL, 40 mg/mL and 60 mg/mL) by dissolving them in ethanol (2.5 mL). Furthermore, the 0.22 mM filter of Millipore was utilized for purifying and loading the sterilized disc to acquire 10 mg per disc concentration. Finally, the medium containing the suspension of bacteria was put onto agar media of Mueller-Hinton (10 mL) within the Petri dishes. Discs containing the prepared nanoformulation and Ampicillin as a standard control from the separate sterilized paper for filtration were put in the Mueller-Hinton agar plates. These plates were refrigerated for 120 min at 5°C before incubating at 35°C for a day.[47]

## The minimum inhibitory concentration of nanoparticles and $IC_{50}$ calculation

After suppressing microbial growth, the evaluation of Minimum Inhibitory Concentration (MIC) was conducted. The MIC was determined using different concentrations, which were prepared by dissolving 50 mg of the formulated nanoformulation in ethanol (2.5 mL) and passing it through a Millipore filter onto 8 mM clean discs. Mueller-Hinton agar was used as the medium to culture the bacterial strains on Petri dishes. The samples with their respective concentrations were collected from the filter paper on the discs. The plates were then incubated at room temperature for a day, following 2 hr refrigeration at 4°C. The inhibition zones

**Table 2: Optimization of *Filipendula ulmaria* chitosan nanoparticles.**

| | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 |
|---|---|---|---|---|---|
| Run | A: chitosan | B: *Filipendula ulmaria* extract | C: tween 20 | particle size | EE |
| | mg | mg | mg | Nm | % |
| 1 | 0.125 | 50 | 0.5 | 100.5 | 87.62 |
| 2 | 0.125 | 25 | 0.75 | 150.51 | 79.73 |
| 3 | 0.125 | 50 | 0.5 | 90.32 | 86.87 |
| 4 | 0.2 | 25 | 0.5 | 110.52 | 79.67 |
| 5 | 0.2 | 50 | 0.25 | 97.41 | 74.71 |
| 6 | 0.125 | 75 | 0.75 | 195.65 | 82.86 |
| 7 | 0.05 | 75 | 0.5 | 160.63 | 76.83 |
| 8 | 0.125 | 50 | 0.5 | 90.62 | 85.78 |
| 9 | 0.2 | 75 | 0.5 | 155.71 | 66.79 |
| 10 | 0.125 | 50 | 0.5 | 90.65 | 83.33 |
| 11 | 0.05 | 50 | 0.25 | 120.53 | 65.66 |
| 12 | 0.05 | 25 | 0.5 | 120.79 | 60.65 |
| 13 | 0.2 | 50 | 0.75 | 145.71 | 72.35 |
| 14 | 0.125 | 50 | 0.5 | 95.91 | 85.76 |
| 15 | 0.125 | 75 | 0.25 | 140.79 | 78.65 |
| 16 | 0.05 | 50 | 0.75 | 159.65 | 73.82 |
| 17 | 0.125 | 25 | 0.25 | 113.65 | 81.76 |

were subsequently observed using Vernier calipers.[47] The range of concentrations that was involved in the analysis was further used to calculate the $IC_{50}$ through the usage of statistical software.

### Statistical Analysis

Version 9.0 of the GraphPad Prism program was used for statistical analysis. Tukey's multiple comparisons test was used to identify substantial distinctions among each group using one-way Analysis of Variance (ANOVA). The findings are presented as mean±Standard Deviation (SD) and statistical significance was set at 0.05. To ensure the reproducibility and validity of the results, each experiment was carried out in triplicate and the findings were then analyzed employing the proper statistical procedures.

## RESULTS

### Characterization of nanoparticle morphological structure

The shapes of the nanoparticles stood out over the lighter-colored background as partially dark grey. They were noted to be scattered across the sample and to have dimensions that ranged from 46.4 nm to 55.2 nm, with an average size of 53.0 nm in Figure 1.

### Optimization of *Filipendula ulmaria* chitosan nanoparticles

The results acquired using various independent and dependent factors were presented in the response plots of the surface in three-dimensional graphs. The findings of a Box Behnken design, which was employed to examine the interactions between three factors (chitosan, *Filipendula ulmaria extract* and Tween 20), on 2 response variables (particle size and EE %), are shown in Tables 1 and 2. For each of the 17 runs, the standard deviation, run number and values for the three variables are displayed in the table. The particle size in nm and the EE% are shown in the final 2 columns for each run. Chitosan levels ranged from 0.05 to 0.2 mg, *Filipendula ulmaria* extract values ranged from 25 to 75 mg and tween 20 values ranged from 0.25 to 0.75 mg. Particle size (nm) and EE% (%) values were used to calculate the responses. The particle size and EE% following the evaluation are shown in Figures 3a and 3b.

Particle size: 93.60-6.53A +19.66B+22.39C+1.34AB+2.30AC+4.50BC +11.99A2+31.32B2+25.23C2

Entrapment efficiency: 85.87+2.07A+0.4150B+0.9975C-7.26AB-2.63AC+1.56BC-12.00A2-2.89B2-2.24C2

Supplementary Figure 2A depicts the changes observed in particle size. It was observed that the particle size increased with a decrease in *Filipendula ulmaria* extract, chitosan and Tween 20, while it drastically increased when Tween 20 and *Filipendula ulmaria* extract together were assessed. The lighter green portion of graph indicated the rise in particle size, while the darker blue portion indicated the decrease of other variables.

On the other hand, Supplementary Figure 2B provides the result obtained with respect to EE%. It was observed that EE% increased with a decrease in *Filipendula ulmaria* extract, Tween 20 and an increase in chitosan. The red portion indicated the increase in EE%, while the light blue portion indicated the decrease of other variables.

Figure 2 illustrates the size of the created nanoparticles. The material under examination had an absorbance of 0.001 nm, 1.074 cP dispersion viscosities and a dispersant dielectric constant of 25.1 at 25°C. The z-average was 100.5 nm, with a PDI of 0.2965 and intercepts of 0.8724. The peak intensity area was measured and reported as 100%, with an average particle size of 100.5 nm.

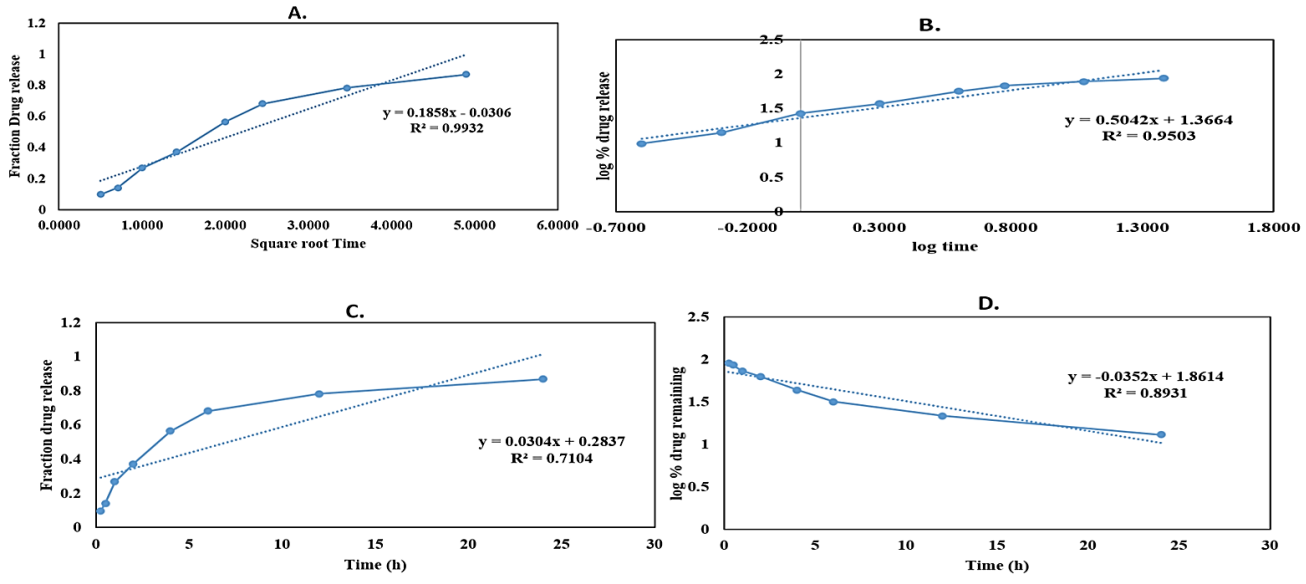


**Figure 3:** *In vitro* release activity by; A. Higuchi model, B. Korsmeyer Peppas model, C. Zero-order model, D. First-order model.

**Table 3: Dermatokinetic study chart (Epidermis).**

| Formulation on Epidermis | $T_{max}$ (hr) | $C_{max}$ (µg/mL) | AUC (mg·h/L) | Ke (h-1) |
|---|---|---|---|---|
| *Filipendula ulmaria* chitosan nanogel (Formulation). | 1.5 | 130.404 | 613.114 | 0.08992 |
| Tetracycline (Conventional medicine). | 1.5 | 50.0781 | 226.22 | 0.1307 |
| Formulation on Dermis | | | | |
| *Filipendula ulmaria* chitosan nanogel (Formulation). | 2 | 114.869 | 520.589 | 0.10171 |
| Tetracycline (Conventional medicine). | 2 | 54.5559 | 226.791 | 0.10777 |

## *In vitro* release activity

The *in vitro* release activity of the synthesized *Filipendula ulmaria* chitosan nanoparticles and conventional formulations was evaluated as depicted in Figure 3. The evaluation involved assessing the formulations at various time intervals: 0, 0.25, 0.5, 1, 2, 4, 6 and 12 hr. Four different models were employed to determine the optimal activity exhibited by the formulated nanoparticles. As per the wavelength of maximum absorption determined, we found salicylic acid to be the most significant constituent that was assessed from the extract in the nanoparticles with a wavelength of 297 nm.

Figure 3a presents the Higuchi analysis model with y=0.1858x-0.306 and $R^2$=0.9932, showing a gradual rise of *in vitro* release. Figure 3b depicts the Korsmeyer-Peppas model of analysis with y=0.5042x+1.3664 and $R^2$=0.9503. This model provides a steady rise and shows an almost linear graph. The zero-order release model is plotted in Figure 3c, which also shows a similar result comparable to the Higuchi model with y=0.0304x+0.2837 and $R^2$=0.7104. However, the first-order release model shown in Figure 3d produced a gradual decline in the release findings with y=-0.0319x+1.9609 and $R^2$=0.8931. With a linear graph and a progressive increase in the *in vitro* release activity, the Korsmeyer-Peppas model outperformed all other evaluated models in terms of outcomes as it increased with time.[48]

## Texture analysis

The test commenced with a pre-test speed of 1.50 mM/sec, followed by the main test speed set at 2.00 mM/sec and concluded with a post-test speed of 2.00 mM/sec. The variable number was established at 5.0 g, a parameter related to the test setup. The target mode was designated as distance, with a specific target distance of 20.000 mM to be reached during the test and a strain of 10.0% was applied to determine the deformation capacity. The trigger type was configured as Auto (Force), automatically initiating the test when the specified Trigger Force of 10.0 g was achieved. The A/BE-d35 probe, which is the back extrusion rig equipped with a 35 mM disc, was employed for the test. Data were collected at a high resolution, with a rate of 250 points per second. The texture analysis was conducted by CIF, denoting the responsible entity for performing this analysis.

The prepared nanogel underwent testing as depicted in Supplementary Figure 3, yielding the following recorded values for its characteristics: firmness (49.37 g), consistency (421.94 g.sec), cohesiveness (-29.80 g) and work of cohesion (-232.46 g.sec). The force-time curve derived from the experiment visually represented the gel's hardness, as indicated by the peak positive force and its adhesiveness and as indicated by the area under the negative force peak.

## Dermatokinetic studies

By plotting the graphs shown in Figures 4a and 4b, the differences between the created nano-formulation and conventional formulation (neomycin) were identified. Table 3 displays the findings of the dermatokinetic investigation of two formulations, the conventional medication and the *Filipendula ulmaria* chitosan nanogel. The *Filipendula ulmaria* chitosan nanogel demonstrated a longer $T_{max}$ of 1.5 hr compared to conventional medicine for the epidermis layer. As compared to the conventional drug's $C_{max}$ of 50.0781 µg/mL, the $C_{max}$ of the *Filipendula ulmaria* chitosan nanogel was longer at 130.404 mg/mL. As compared to the conventional drug's AUC value of 226.22 mg·h/L, the chitosan nanogel from *Filipendula ulmaria* had a higher AUC value of 613.114 mg/mL. The *Filipendula ulmaria* chitosan nanogel, however, had a lower Ke value, suggesting a slower rate of removal from the epidermal layer. The *Filipendula ulmaria* chitosan nanogel demonstrated a longer $T_{max}$ of 2 hr compared to conventional medicine for the dermis layer. The $C_{max}$ of the *Filipendula ulmaria* chitosan nanogel was also greater, peaking up at 114.869 mg/mL as opposed to 54.5559 mg/mL for conventional medicine. AUC for the nanogel was 520.589 mg/mL as opposed to 226.791 mg/mL for conventional medicine. The conventional drug's Ke value was marginally greater, suggesting a little greater rate of elimination from dermis layer.

## CLSM analysis

In Supplementary Figure 4, it is evident that the formulated nano-formulation demonstrated a sustained brightness throughout the subsequent scans, indicating their stable presence. On the other hand, the suspension appeared darker, suggesting a decrease in concentration or dispersion of the particles over time. These observations signify the effectiveness of the formulation

in achieving improved suspension penetration. The CLSM images revealed uniform distribution of nanoparticles within the epidermis and dermis layers, confirming deep penetration of the formulation. The sustained brightness indicates that the formulation successfully retained its nanoparticulate structure and did not experience aggregation or settling. This study suggests that *Filipendula ulmaria* chitosan nanogel has the potential to be an effective alternative for treating bacterial skin infections.

## Antioxidant activity

At 517 nm wavelength, the reduction of DPPH property by the antioxidants was examined as it reduced and produced the colorless hue in the mixture from violet. *Filipendula ulmaria* chitosan nanogel presented a slightly higher antioxidant effect than the standard control (ascorbic acid) (Supplementary Figure 5). The percentage values obtained were 18, 21.1, 37.3, 60.2 and 77.9 for *Filipendula ulmaria* nanogel and the standard presented with 17.7, 19.8, 33.1, 57.3 and 70.1 It was thus determined that the nanoformulation did not alter the entrapment potential of the drug encapsulated.

## Minimum inhibition concentration

The antibacterial effect of *Filipendula ulmaria* chitosan nanogel and Ampicillin was evaluated through the disc diffusion method. Two strains of bacteria, *Escherichia coli* and *Bacillus subtilis* were tested using different doses of the formulation. The outcomes demonstrated that both bacterial strains' growth was significantly inhibited by the nanogel. Table 4 presents the antimicrobial activity analysis of several formulations towards two bacterial strains, *E. coli* and *B. subtilis*. SAL 7, SAL 5, SAL 9 and SAL 10 are formulations with varying amounts of plant extract. The inhibition zone, which is the clear region surrounding the disc where no bacterial growth is visible, is assessed for each formulation for both strains of bacteria. The inhibitory zone for both strains of bacteria expands as the dose of the plant extract increases from 10 mg/mL to 60 mg/mL. SAL 10, at the maximum

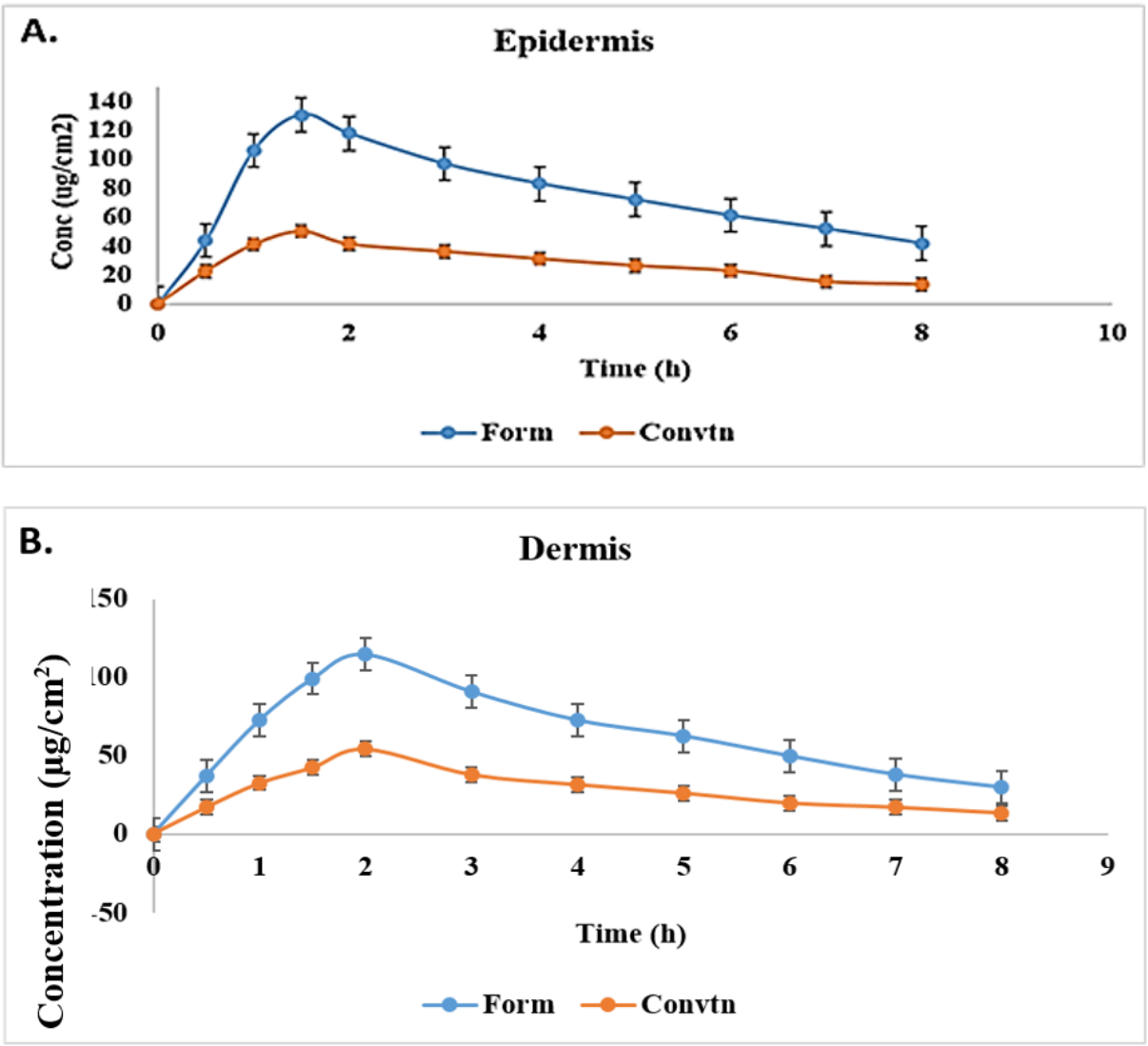


**Figure 4:** Dermatokinetic activity of formulations on; A. Epidermis, B. Dermis.

**Table 4: MIC and inhibition zone of the *Filipendula ulmaria* chitosan nanogel and ampicillin.**

| Dose of formulations | Concentration (mg/mL) | Minimum Inhibitory concentration | | Inhibition zone | |
|---|---|---|---|---|---|
| | | *B. subtilis* | *E. coli* | *E. coli* | *B. subtilis* |
| SAL 7 (Plant extract) | 10 | 9.07±0.53 | 8.2±0.48 | 11.5±0.5 | 10.2±0.4 |
| SAL 5 | 20 | 13.59±0.87 | 14.10±0.23 | 12.8±0.4 | 11.5±0.3 |
| SAL 9 | 40 | 14.98±0.61 | 15.12±0.3 | 13.5±0.3 | 12.2±0.4 |
| SAL 10 | 60 | 16.88±0.51 | 19.11±0.74 | 17±0.6 | 15.3±0.2 |
| Ampicillin | 250 | 17.6±0.24 | 19.3±0.48 | 21.8±0.8 | 20.5±0.7 |

dose of 60 mg/mL, had an approximate inhibitory zone of 17±0.6 mM against *E. coli* and 15.3±0.2 mM against *B. subtilis*.

In contrast, the positive control, ampicillin (250 mg/mL), exhibits the highest inhibitory zone of 21.8 0.8 mM against *E. coli* and 20.5 0.7 mM against *B. subtilis*. In summary, the findings indicate that varied doses of the plant extract had antimicrobial action against the investigated bacterial strains, with higher doses exhibiting greater effects.

The findings presented in Table 4 showed that SAL 7, SAL 5, SAL 9 and SAL 10 formulations were evaluated against the 2 bacterial strains at doses of 10, 20, 40 and 60 mg/mL. The MIC readings for each of the formulations for each bacterial strain are subsequently calculated. The findings reveal that as formulation dosage rises, MIC values drop, suggesting increased antibacterial action. SAL 10 demonstrated the greatest bactericidal effect of all formulations, with MIC values of 16.88 0.51 and 19.11 0.74 against *B. subtilis* and *E. coli*, accordingly, at a dose of 60 mg/mL. The plant extract (SAL 7) showed antibacterial action as well, however, the MIC values were greater than in the other formulations. In the experiment, ampicillin, a frequently administered antibiotic, was employed as a positive control and it had the lowest MIC values towards both bacterial strains when compared to the tested formulations.

Supplementary Figure 6 provides images of bacterial strains treated with the formulation and drug and the changes seen within them. The median Inhibitory Concentration ($IC_{50}$) of *Filipendula ulmaria* chitosan nanogel remained at 58.51±1.23 mg/mL. The $IC_{50}$ value of the plant extract was 74.5±0.97 while the standard drug produced an $IC_{50}$ value of 53.9±0.76.

## Stability analysis

The stability of the *Filipendula ulmaria* chitosan nanogel was evaluated over for three months. The nanogel showed no change in appearance and nanoparticle size during the course of the experiment (Supplementary Table 1). The drug content was found to be 88.14±0.12% at the beginning of the experiment and remained stable over three months with values of 88.3±0.2%, 88.16±0.2% and 88.14±0.11% being recorded at months 1, 2 and 3, respectively (Supplementary Table 1). These results suggest that the nanogel is stable over time and can be used as a potential candidate for antimicrobial applications.

# DISCUSSION

Previous studies have indicated that plant extracts and their effects on bacteria are caused by the contents of phenols, terpenoids and alkaloids. These components induce membrane lysis of cell walls in bacteria. Also, due to the lysis, proton efflux occurs, stopping the amino acid synthesis within the bacteria.[49] Similarly, another research attributed the impact of extracts to their characteristic of being hydrophobic as it creates an interaction with bacterial cell membrane proteins resulting in structural and permeability changes along with lysis.[50] Earlier studies have shown that the herb is indeed very rich in its phytoconstituents having phenolic chemicals such as gallic and salicylic acids and flavonoid chemicals like spiraeoside, catechins, quercetin and hyperoside. Tannins such as rugosins and tellimagrandins were also observed to be present in *Filipendula ulmaria*.[51-53] Chitosan nanoparticles have also shown the ability to produce a considerable antibacterial effect when tested against *Escherichia coli, Staphylococcus aureus, Staphylococcus typhimurium* and *Staphylococcus choleraesuis.* It was also observed that chitosan nanoparticles provided a strong affinity against the cells of bacteria as they impact quantum size. The chitosan nanoparticles tend to have a stronger antibacterial effect in comparison to the usual chitosan because of their locations of the negatively charged poly-cationic site upon the cells of bacteria.[54,55] As a result, greater density of surface charge is observed upon the poly-cations that interact with the bacterial cell through the chitosan nanoparticle. Since the chitosan nanoparticles have the potency of providing greater surface areas, can firmly adsorb within the cell surface of bacteria to rupture its membrane, resulting in leakage of internal constituents and bacterial cell death.[54] The antimicrobial activity produced could be attributed to increased penetration within the cell membranes of bacteria along with the substantial depolarizing effect of membranes through chitosan.[56] Previous studies have mentioned the importance of the extracts of *Filipendula ulmaria* and blackberry leaves to have the highest antimicrobial effects in comparison to other herbs.[57,58] The antioxidant supplement *Filipendula ulmaria* extract was investigated for its ability to reduce systemic toxicity brought on by Calcium Phosphate (CaP) nanoparticles. The results demonstrated that CaP did not alter the concentrations of liver enzymes while increasing Low-Density Lipoproteins (LDL), serum calcium, High Density Lipoproteins

(HDL) and triglycerides concentrations and lowering those of testosterone as well as LH. Additionally, the associated expression levels of Bax and Bcl-2 switched to proapoptotic activity, while the levels of oxidative stress indicators in the liver, kidney and testicle were significantly changed. The infusion of *Filipendula ulmaria* extract, nevertheless, significantly reduced the majority of the adverse reactions brought on by nanosized CaP, indicating its promise as a form of therapy to reduce overall toxicity.[59] Furthermore, *Filipendula ulmaria* has been implied as a beneficial herb that may help in reducing neurotoxicity occurring due to intake of calcium phosphates through expression of certain receptors and neurotrophins in the prefrontal cortex of rats by producing antioxidant effects.[29]

The current study provides some novel findings after combining chitosan nanoparticles with the *Filipendula ulmaria* plant extract. The results also suggest that *Filipendula ulmaria* chitosan nanogel could be used as a broad-spectrum antibacterial treatment, since it could be effective against both gram-positive and gram-negative bacterial strains and has the essential dermatokinetic effects needed for any drug to be used on the skin. On the other hand, combining both constituents resulted in a better formulation that could be applied topically for the possible treatment of bacterial infections. Since the nanoformulation was prepared to keep in mind all the essential parameters required for topical use, the newly developed nanoformulation is expected to possess enhanced activity against bacterial infections, better absorption and ease of application. In addition to its antibacterial efficacy, the *Filipendula ulmaria* chitosan nanogel demonstrated significant antioxidant activity, as evidenced by its superior DPPH radical scavenging potential compared to the standard ascorbic acid control. This antioxidant activity plays a crucial role in wound healing and bacterial infection management by reducing oxidative stress, which is often exacerbated in infected tissues. The antioxidant properties of the nanogel can complement its bactericidal effect by preventing oxidative damage to skin tissues, thus creating a more favourable microenvironment for tissue regeneration and repair. The presence of phenolic compounds, such as salicylic acid, quercetin and gallic acid, in the *Filipendula ulmaria* extract likely contributes to this observed antioxidant effect. Furthermore, oxidative stress is known to impair immune response and delay wound healing. By mitigating oxidative damage, the nanogel not only suppresses bacterial proliferation but also promotes faster recovery of damaged skin tissues. The dual-action mechanism of the nanogel-combining antioxidant and antibacterial properties-underscores its therapeutic potential as an effective treatment for bacterial skin infections.

This study, however, has several limitations. Firstly *E. coli* and *B. subtilis*, were the only two bacterial strains included in the study, may not be generically prevalent in skin diseases. Additional reliable results may arise from more research including a larger variety of bacterial strains and other microbes that might play a part in skin diseases. Secondly, the experiment was carried out *in vitro* using agar diffusion tests, which might not precisely represent how nanoparticles behave in human skin. Future studies will focus on evaluating the efficacy of the *Filipendula ulmaria* chitosan nanogel in animal models to assess its *in vivo* antibacterial and antioxidant performance. Such studies will also explore its pharmacokinetic profile, safety and potential for reducing oxidative stress in infected tissue. Further research may include testing against a broader spectrum of bacterial pathogens and optimizing the formulation for enhanced skin permeability and stability. Further *in vivo* studies, including animal model testing, are necessary to confirm the clinical relevance and safety of this formulation in treating bacterial skin infections. Future studies could further investigate the mechanisms between antioxidant and antibacterial pathways to optimize the formulation for enhanced therapeutic outcomes.

## CONCLUSION

The skin contains several strains of bacteria and only a limited number of antimicrobials are effective against skin infections. Newer and more efficient antibiotics are constantly in demand to treat and be safer against various bacterial skin diseases. Furthermore, *Filipendula ulmaria* chitosan nanogel could be a potential natural candidate that can possibly be used in place of other chemically synthesized antibiotics. The prepared nanoformulation was found to be effective against bacterial growth and had an optimal size, potent entrapment efficiency and a polydispersity index. Similarly, it produced better performance in comparison to the standard drug during the *in vitro* release studies, dermatokinetic analysis and antioxidant and antimicrobial tests against *Escherichia coli* and *Bacillus subtilis*, as they comprised of Gram-positive and Gram-negative characteristics of bacteria. The developed nanoformulation was observed to be stable in all weather conditions and could be stored for longer durations as applicable. Also, since newly prepared nano formulations were found to have more advantages than standard drug administration techniques, the retention time of the drug, drug targeting and bioavailability, adequate *in vivo* studies can be performed to obtain confirmatory results.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**FU:** *Filipendula ulmaria*; **MIC:** Minimum inhibitory concentrations; **MRSA:** Methicillin-resistant *Staphylococcus aureus*; **MDR:** Multidrug-resistant; **GBD:** Global Burden of

Disease; **ATCC:** American Type Culture Collection; **PDI:** Polydispersity index; **EE:** Entrapment Efficiency; **CLSM:** Confocal Laser Scanning Microscopy; **PBS** Phosphate buffered saline; **HPLC:** High performance liquid chromatography; **TPA:** Texture profile analysis; **DPPH 2:** 2-diphenyl-1-picrylhydrazyl; **$IC_{50}$:** Half-maximal inhibitory concentration; **SD:** Standard deviation; **ANOVA:** Analysis of variance; **TEM:** Transmission electron microscopy; **BBD:** Box Behnken design; **AUC:** Area Under the Curve; **$T_{max}$:** Time to peak drug concentration; **$C_{max}$:** Maximum Plasma Concentration/Peak Plasma Concentration; **Ke:** Elimination rate constant; **CaP:** Calcium phosphate; **LDL:** Low-density lipoproteins; **HDL:** High density lipoproteins; **LH:** Luteinizing hormone.

## SUMMARY

This study explores the therapeutic potential of *Filipendula ulmaria* extract-loaded chitosan nanoparticles for treating bacterial skin infections. The extract was obtained from the plant's aerial parts using the Soxhlet technique and formulated into chitosan nanoparticles via anionotropic gelation. These nanoparticles were incorporated into a gel and characterized for size, polydispersity and entrapment efficiency, with optimization achieved using the Box-Behnken design. The nanogel demonstrated enhanced antioxidant and antimicrobial activities, showing superior dermatokinetic performance compared to tetracycline. Minimum Inhibitory Concentrations (MIC) against *Bacillus subtilis* and *Escherichia coli* confirmed its antibacterial efficacy. Overall, the results suggest *F. ulmaria* nanogel's potential as an effective natural agent for treating bacterial skin infections.

## INSTITUTIONAL REVIEW BOARD STATEMENT

All animal-handling procedures, along with sample collection and disposal, were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines, under the supervision of the Faculty of Veterinary Medicine, University of Sadat City, Egypt, with approval number VUSC-031-1-23.

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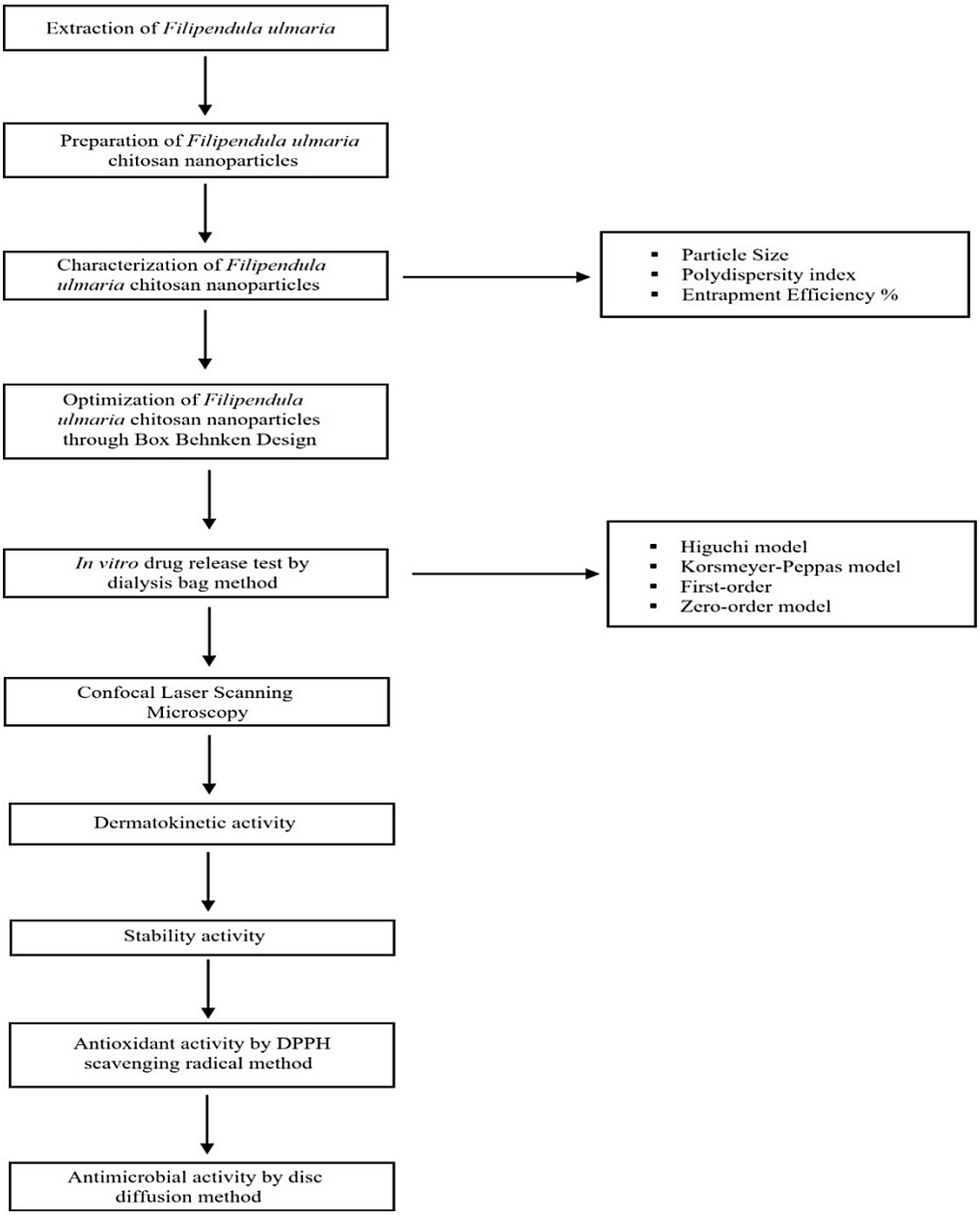
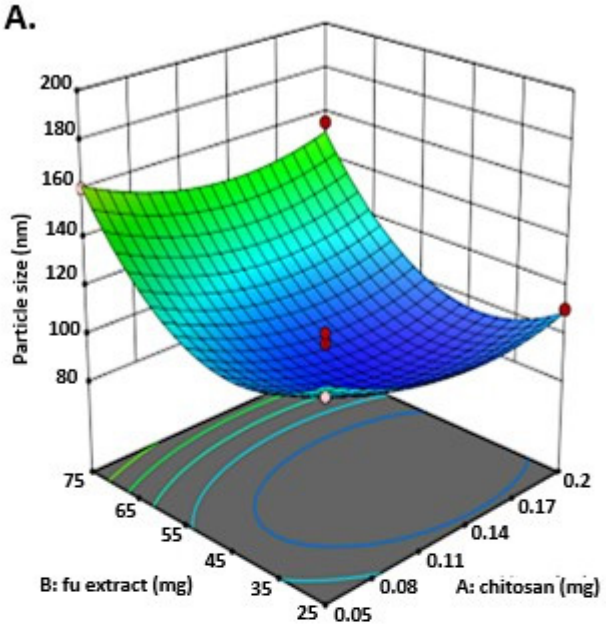
**Supplementary Table 1: Stability of *Filipendula ulmaria* chitosan nanogel at different time points.**

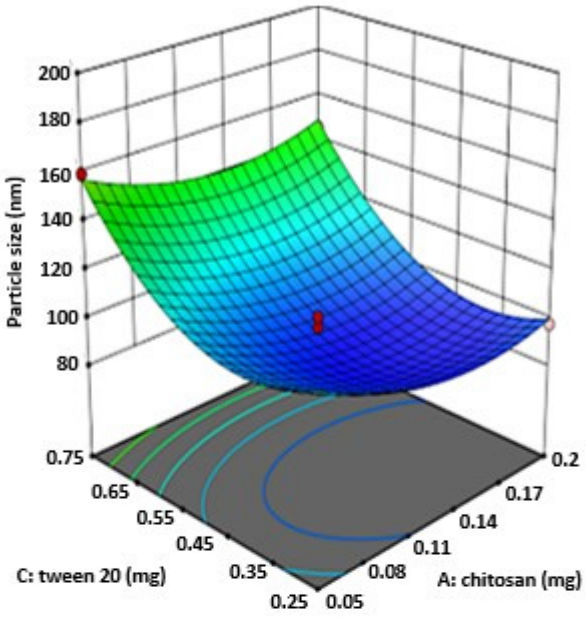
| Experiment parameters | Month | | | |
|---|---|---|---|---|
| | Initial | 1 | 2 | 3 |
| Appearance | No change in appearance | | | |
| Particle size | No change in particle size | | | |
| Drug content (%) | 88.14±0.12 | 88.3±0.2 | 88.16±0.2 | 88.14±0.11 |

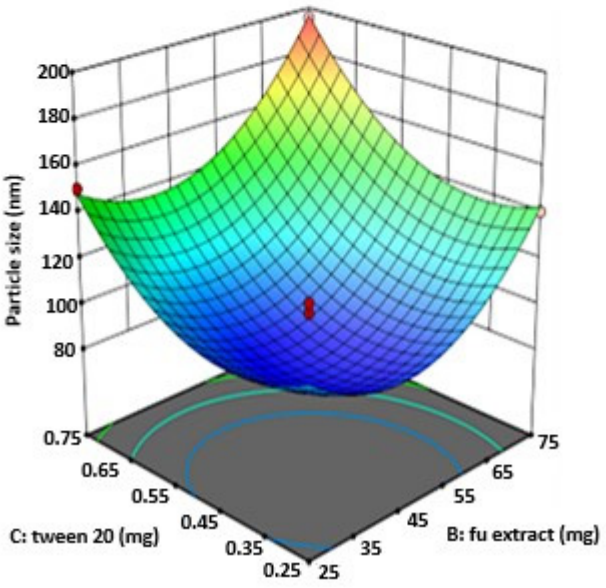


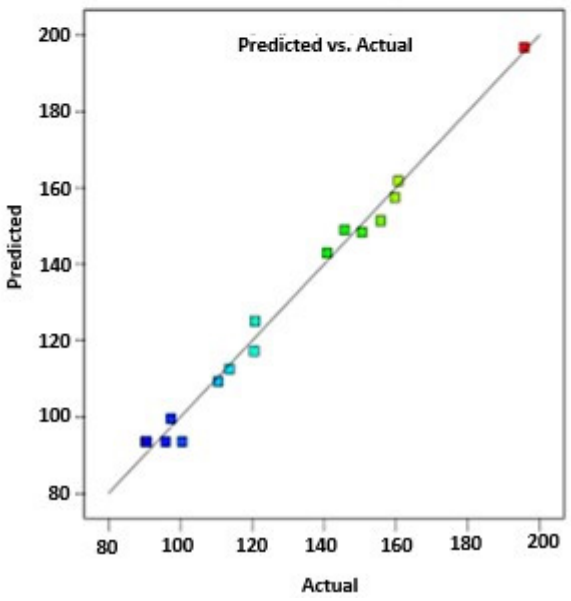

**Supplementary Figure 1:** Graphical representation of methodology.

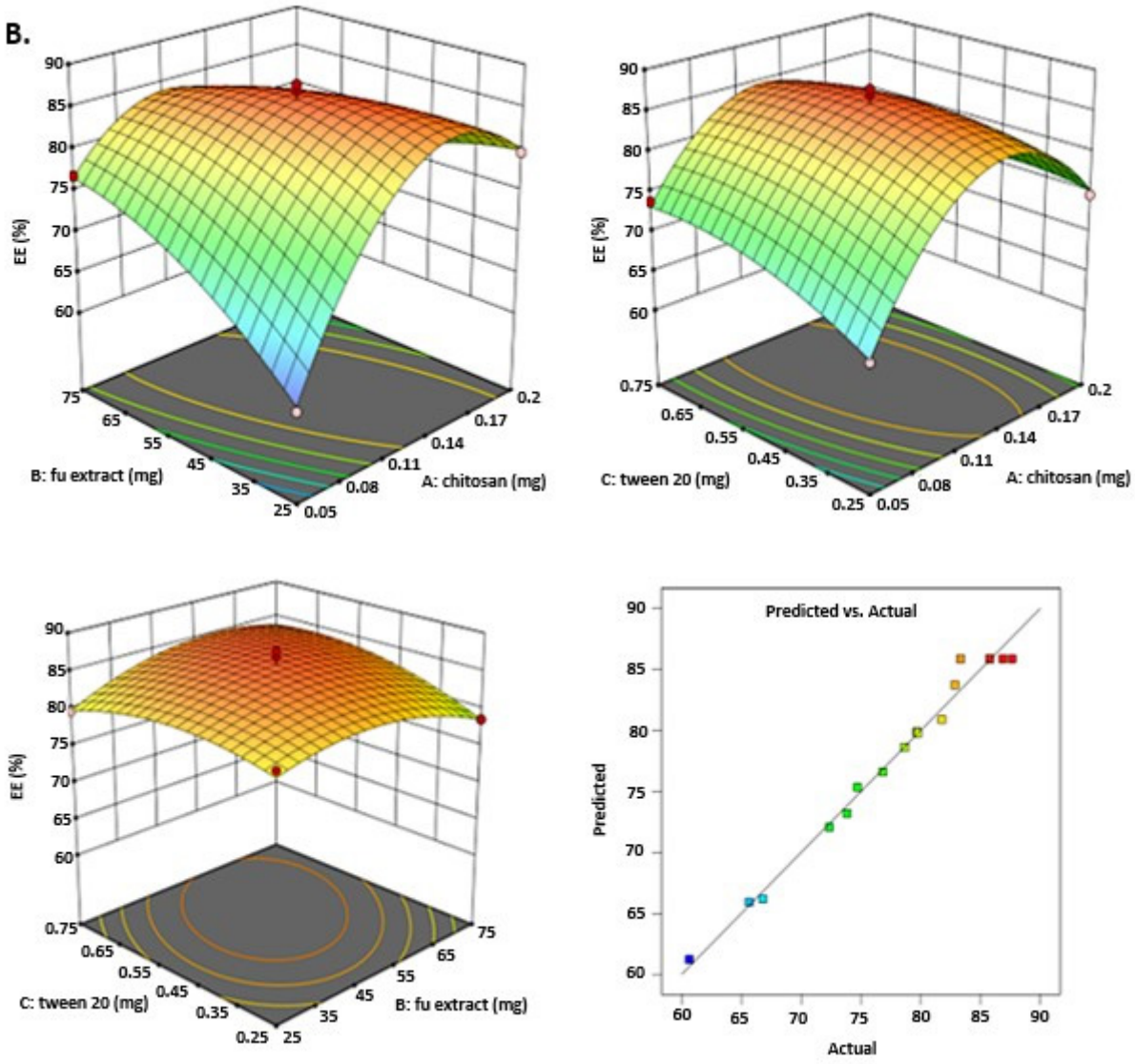
A.


Particle size (nm)
200
180
160
140
120
100
80
75
65
55
45
35
25
B: fu extract (mg)
0.05
0.08
0.11
0.14
0.17
0.2
A: chitosan (mg)

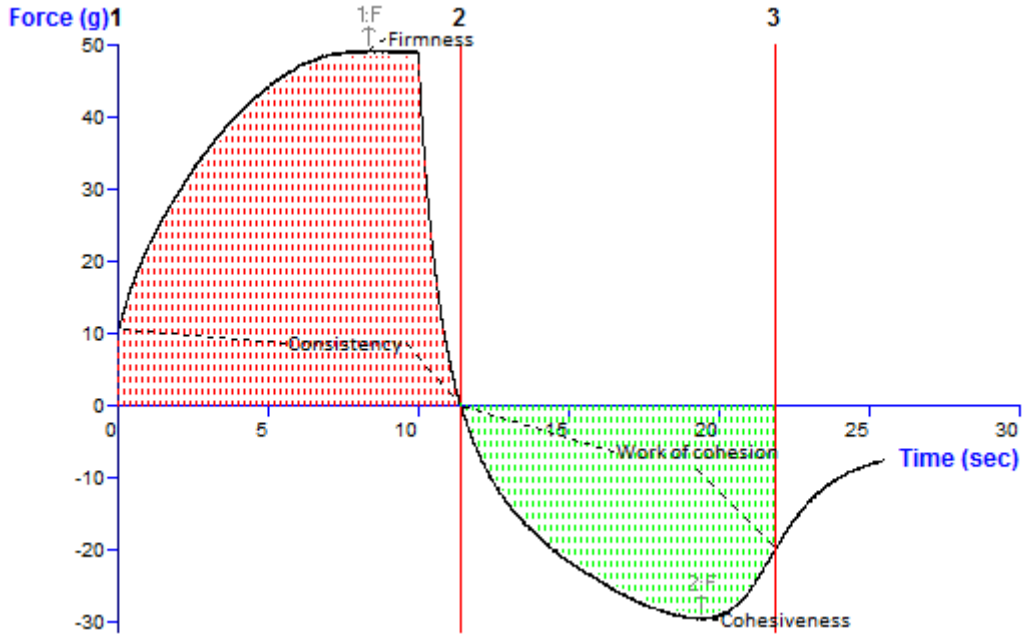

Particle size (nm)
200
180
160
140
120
100
80
0.75
0.65
0.55
0.45
0.35
0.25
C: tween 20 (mg)
0.05
0.08
0.11
0.14
0.17
0.2
A: chitosan (mg)


Particle size (nm)
200
180
160
140
120
100
80
0.75
0.65
0.55
0.45
0.35
0.25
C: tween 20 (mg)
25
35
45
55
65
75
B: fu extract (mg)

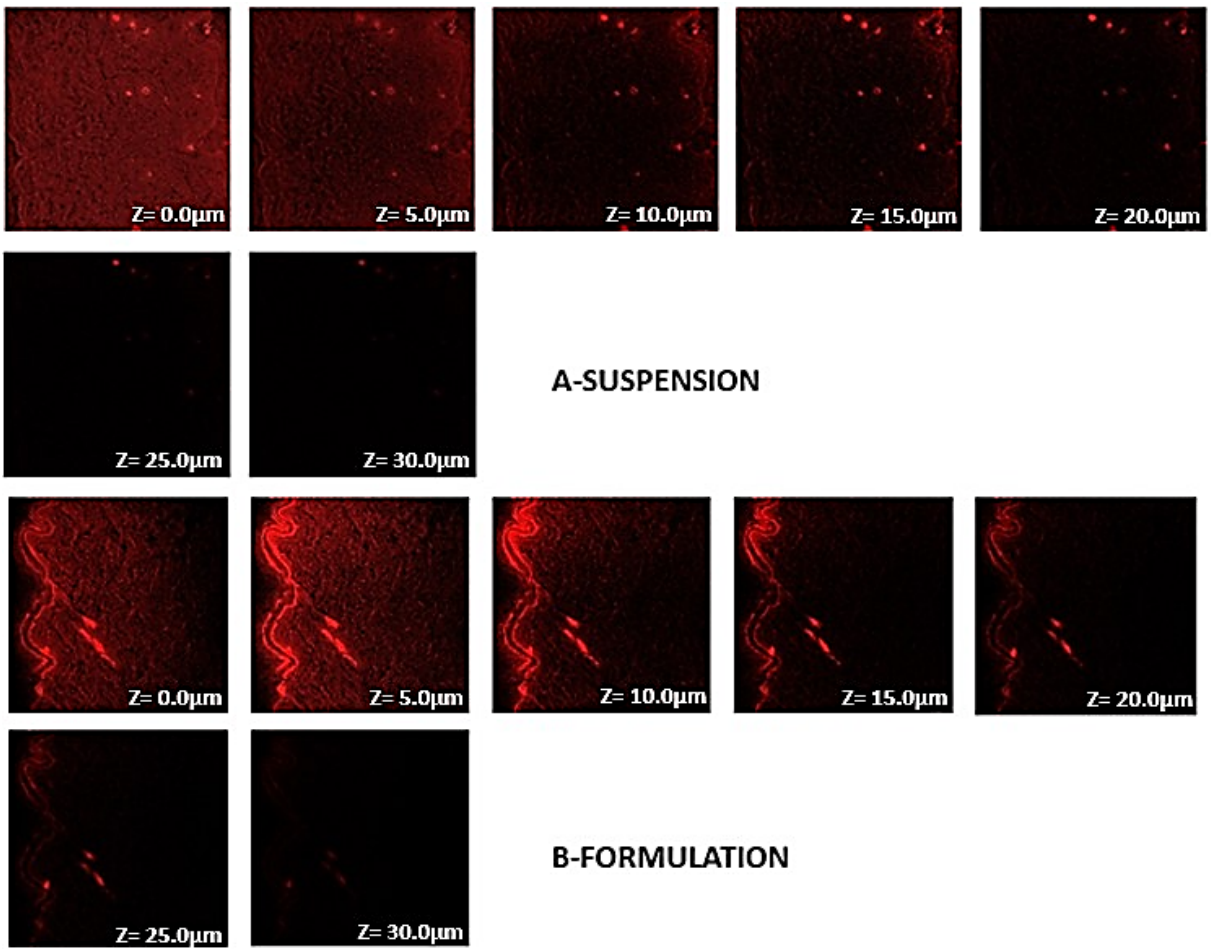

Predicted vs. Actual
Predicted
200
180
160
140
120
100
80
80
100
120
140
160
180
200
Actual



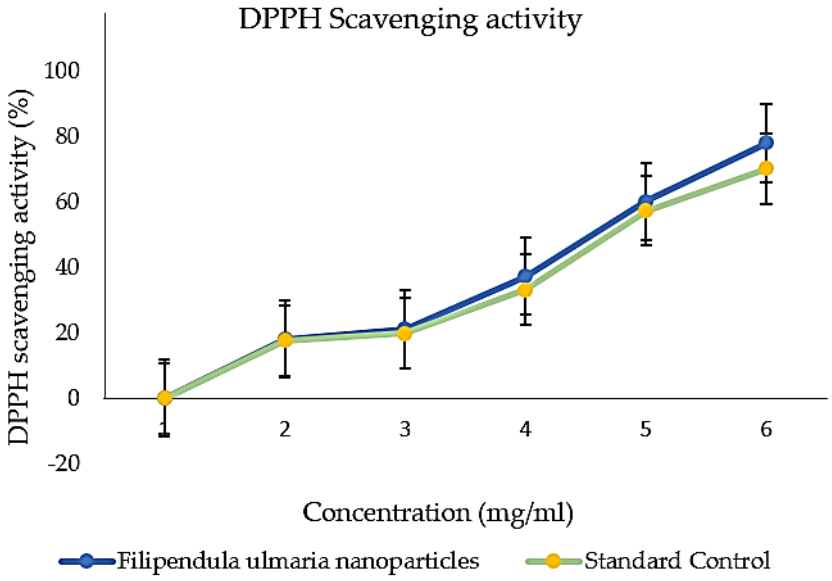

**Supplementary Figure 2:** Three-dimensional surface response graphs of *Filipendula ulmaria* chitosan nanoparticles. (A) Effect of independent variables (chitosan concentration, *Filipendula ulmaria* extract concentration and Tween 20 concentration) on particle size. (B) Effect of the same independent variables on Entrapment Efficiency (EE%).



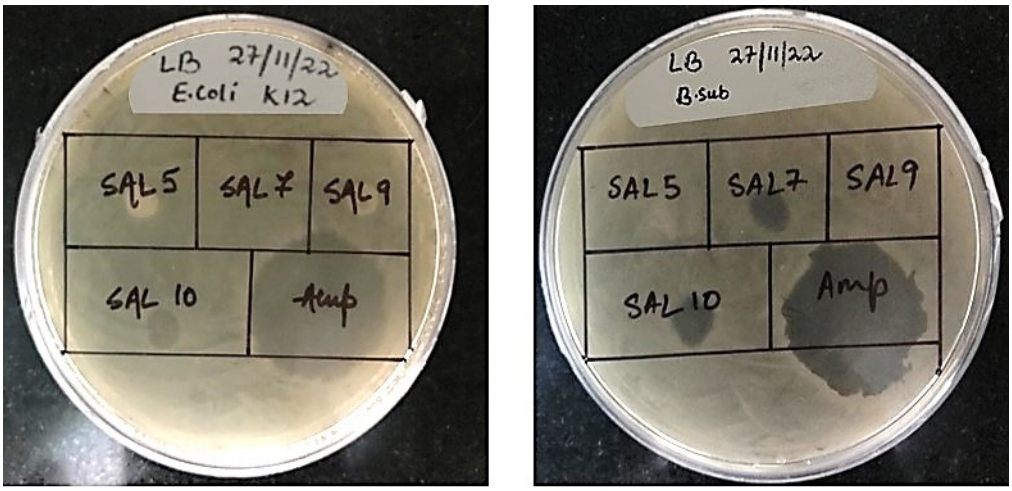

**Supplementary Figure 3:** Texture analysis of *Filipendula ulmaria* chitosan nanogel.




**Supplementary Figure 4:** CLSM images of a perpendicular cross-section of rat skin surface.




**Supplementary Figure 5:** DPPH scavenging activity of *Filipendula ulmaria* nanoparticles against the standard control.




**Supplementary Figure 6:** Inhibition of bacterial growth caused by *Filipendula ulmaria* chitosan nanogel and Ampicillin on; a) *Escherichia coli*, b) *Bacillus subtilis*.