

Formulation and Characterization of Silver Nanoparticles gel Loaded with *Notonia grandiflora* Extract

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Abstract

The present study was aimed at the green synthesis, characterization, and evaluation of silver nanoparticles loaded gel using the ethanolic leaf extract of *Notonia grandiflora*. The leaves were extracted using petroleum ether and ethanol, and the ethanolic extract showed a higher percentage yield (8.50% w/w) compared to the petroleum ether extract (1.23% w/w). Preliminary phytochemical screening revealed the presence of glycosides, flavonoids, phenolic compounds, saponins, diterpenes, and carbohydrates. The total flavonoid and phenolic contents of the ethanolic extract were found to be 0.91 mg/100 mg and 0.62 mg/100 mg of dried extract, respectively. Silver nanoparticles were synthesized using different concentrations of silver nitrate and extract ratios. Among the prepared formulations, F3 exhibited the highest percentage yield ($79.12 \pm 0.18\%$) and entrapment efficiency (0.902 ± 0.019 mg/100 mg quercetin equivalent). The optimized formulation showed an average particle size of 215.45 nm and a zeta potential of -37.45 mV, indicating good stability. The nanoparticles were incorporated into Carbopol 940 gel and evaluated for physicochemical properties. The prepared gels exhibited acceptable appearance, homogeneity, spreadability, viscosity, extrudability, and skin-compatible pH. In vitro drug release studies demonstrated sustained release characteristics, with formulation F2 showing controlled drug release following zero-order kinetics ($R^2 = 0.9868$). Antimicrobial activity studies against *Staphylococcus aureus* and *Pseudomonas aeruginosa* revealed that the nanoparticle-loaded gel exhibited significantly higher zones of inhibition compared to the crude extract. The findings suggest that *Notonia grandiflora*-mediated silver nanoparticle gel possesses promising physicochemical and antimicrobial properties and may serve as a potential topical antimicrobial formulation.

Introduction

Nanotechnology has emerged as one of the most promising fields in pharmaceutical and biomedical sciences due to its ability to enhance the therapeutic efficacy, stability, and bioavailability of drugs. Among various nanomaterials, silver nanoparticles (AgNPs) have gained considerable attention because of their unique physicochemical properties,

including antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities ^[1]. Silver nanoparticles possess a large surface area-to-volume ratio, enabling enhanced interaction with biological systems and improved therapeutic performance. Consequently, AgNPs have found extensive applications in drug delivery systems,

cosmetics, diagnostics, and topical pharmaceutical formulations [2].

Conventional methods for the synthesis of silver nanoparticles often involve hazardous chemicals and energy-intensive processes that may pose environmental and biological risks. In contrast, green synthesis using plant extracts has emerged as an eco-friendly, cost-effective, and sustainable alternative [3].

Plant-mediated synthesis utilizes naturally occurring phytoconstituents such as flavonoids, phenolics, terpenoids, glycosides, and proteins as reducing and stabilizing agents, thereby eliminating the need for toxic chemicals. The resulting nanoparticles are generally biocompatible and exhibit enhanced biological activity due to the synergistic effect of plant-derived bioactive compounds [4].

Notonia grandiflora is a medicinal plant known to contain various phytochemical constituents including flavonoids, phenolic compounds, glycosides, diterpenes, and saponins, which are associated with several pharmacological activities [5].

The presence of these bioactive molecules makes the plant a suitable candidate for the green synthesis of silver nanoparticles. Furthermore, the phytoconstituents present in the extract can act as natural reducing and capping agents, contributing to the

formation of stable nanoparticles with potential therapeutic applications [6].

Topical drug delivery systems have gained increasing importance owing to their ability to deliver active agents directly to the site of action while minimizing systemic side effects. Gels are among the most widely used topical dosage forms because of their ease of application, non-greasy nature, patient acceptability, and ability to provide sustained drug release [7].

Incorporation of silver nanoparticles into a gel matrix can further enhance therapeutic efficacy by improving retention at the application site and ensuring controlled release of bioactive constituents [8].

Therefore, the present study was undertaken to formulate and characterize silver nanoparticles using the ethanolic extract of *Notonia grandiflora* leaves and to develop a nanoparticle-loaded gel formulation. The prepared nanoparticles were evaluated for percentage yield, entrapment efficiency, particle size, and zeta potential, while the developed gel was assessed for physicochemical properties, in vitro drug release behavior, and antimicrobial activity. The study aims to explore the potential of *Notonia grandiflora*-mediated silver nanoparticle gel as an effective topical antimicrobial formulation.

Material and Methods

Material

Fresh leaves of *Notonia grandiflora* were collected and authenticated for the study. Silver nitrate (AgNO_3) was used for the synthesis of silver nanoparticles. Carbopol 940, polyethylene glycol 600 (PEG-600), methyl paraben, and triethanolamine were used for the preparation of the gel formulation. Petroleum ether and ethanol were employed as extraction solvents. Distilled water was used throughout the study for extraction, nanoparticle synthesis, and formulation preparation. All chemicals and reagents used were of analytical grade and procured from standard commercial suppliers.

Methods

Determination of percentage yield

The percentage yield of extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}}$$

Phytochemical screening

Phytochemical examinations were carried out for extract as per the standard methods [10].

Estimation of total flavonoids content

Extraction procedure

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs:

Defatting of plant materials

Leaves of *Notonia grandiflora* were shade dried at room temperature. 50 gram dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered leaves of *Notonia grandiflora* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C [9].

Determination of total flavonoids content was based on aluminium chloride method [11]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25 µg/ml were prepared in methanol. 10mg

of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% AlCl_3 methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method ^[11]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50 $\mu\text{g/ml}$ was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Biosynthesis of Silver nanoparticles

Silver nanoparticles (AgNPs) were synthesized using the leaf extract of *Notonia grandiflora* as a reducing and stabilizing agent. Initially, a 10 mM stock solution of AgNO_3 was prepared by dissolving the appropriate amount of silver nitrate powder in distilled water. Working solutions of 1 mM, 2 mM, and 3 mM were prepared from this stock solution. The AgNO_3 solutions were then mixed with the leaf extract at ratios of 1:1 and 1:2 (v/v) according to the concentrations listed in Table 1, with a final reaction volume of 50 mL per formulation. The reaction mixtures were protected from light by wrapping the flasks in aluminum foil and were heated in a water bath at 60°C for 5 hours to facilitate the reduction of silver ions to nanoparticles. During this process, a visible color change from pale yellow to brown indicated the formation of AgNPs. After completion of the reaction, the nanoparticles were cooled to room temperature and stored in a refrigerator to preserve their stability and prevent aggregation, making them ready for subsequent characterization and biological studies ^[12].

Table 1: Different formulation of silver nanoparticles

Formulation Code	Extract (mg)	AgNO_3 (mM)	Ratio
F1	500	1	1:1

F2	500	2	1:1
F3	500	3	1:1
F4	500	1	1:2
F5	500	2	1:2
F6	500	3	1:2

Characterization of synthesized silver nanoparticles formulations

Percentage yield

The silver nanoparticles, prepared with a size range of 200-300 nm, were gathered and quantified from various formulations. The calculated weight was then divided by

the total quantity of all non-volatile components utilized in the microsphere preparation^[13].

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \times 100$$

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of the drug associated with the formulations to the total mass of the drug. The entrapment efficiency was assessed using the dialysis method, where the silver nanoparticle-entrapped extract was separated from the free drug. For this purpose, the aforementioned formulations were loaded into dialysis bags, and the free drug was dialyzed for 24 hours in 50 ml of buffer at pH 1.2. The absorbance of the dialysate was measured against a blank buffer at pH 1.2, and the absorbance of the corresponding blank was measured under the same conditions. The

concentration of free flavonoids was determined based on the absorbance difference using a standard curve^[14]

Surface charge and vesicle size

The particle size, size distribution, and surface charge were determined using the Dynamic Light Scattering method (DLS) with a Malvern Zetamaster, ZEM 5002 instrument from Malvern, UK, at SAIF RGPV Bhopal^[15]. Zeta potential measurements for the silver nanoparticles were conducted based on the Helmholtz–Smoluchowsky equation derived from electrophoretic mobility. For zeta potential

measurement, a zetasizer was employed with field strength of 20 V/cm in a large bore measurement cell. Samples were appropriately diluted with 0.9% NaCl and adjusted to a conductivity of 50 μ S/cm.

Formulation development of silver nanoparticle gel

Gels containing *Notonia grandiflora* loaded silver nanoparticles were prepared according to the formulations listed in Table 6.4. Precise quantities of methyl paraben, polyethylene glycol 600, and the nanoparticle-loaded extract (500 mg) were dissolved in approximately 100 mL of distilled water in a beaker and stirred

vigorously using a mechanical stirrer or sonicator, following the method described by Raut et al. (2009) ^[16].

Carbopol 940 was then gradually added to the mixture under continuous stirring to ensure uniform dispersion. The solution was neutralized by the slow addition of triethanolamine with constant stirring, leading to gel formation. The final gel formulations F1, F2, and F3 contained increasing amounts of Carbopol 940 (250 mg, 500 mg, and 750 mg, respectively), while other ingredients were kept constant, producing gels of varying consistency suitable for further evaluation.

Table 2: Formulation of gel

Ingredients (mg)	F1	F 2	F3
<i>Notonia grandiflora</i> loaded silver nanoparticles optimized formulation	500	500	500
Carbopol 940	250	500	750
Polyethylene Glycol 600	0.2	0.2	0.2
Methyl Paraben	0.08	0.08	0.08
Triethanolamine	1.0	1.0	1.0
Distilled Water	100 ml	100ml	100ml

Evaluation of gel

Appearance and Consistency: The physical appearance and texture of gel formulations were visually inspected, and observations were recorded in table.

Washability: Formulations were applied to the skin and manually assessed for ease and degree of washing with water ^[17].

Extrudability Determination: Gel formulations were filled into aluminum collapsible tubes, sealed, and pressed to

extrude the material. Extrudability of the formulation was noted^[18].

Determination of Spreadability:

Spreadability, a crucial factor for gel formulations, was evaluated using a specially designed apparatus. Two glass slides (6x2) were chosen, and the gel formulation to be tested was placed between them over a length of 6 cm. The time taken for the slides to separate under the application of a 20-gram load was recorded. The experiment was repeated six times for each formulation, and average was calculated^[19].

Method: Two glass slides were selected, and the gel formulation was placed over one slide. The second slide was placed over the formulation, sandwiching it over a length of 6 cm. A 20-gram weight was applied, forming a thin layer. The time taken for the slides to separate under the weight was recorded.

$$S=m \times l t$$

Where, S = Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams), l = length of the glass slide (6 cm), t = time taken in seconds.

Viscosity: The viscosity of the gel was determined using a Brookfield digital viscometer with spindle no. 6 at 10 rpm and

at a room temperature of 25-30°C. Measurements were taken after allowing the gel samples to settle for more than 30 minutes^[20].

Drug Content: The drug content was measured by dissolving 1g of gel in methanol in a 10 ml volumetric flask. A mixture of 3 ml of stock solution and 1 ml AlCl₃ solution (2%) was vortexed, and the color production was allowed to stand at 40°C for 30 minutes. Absorbance was measured at 420 nm using a spectrophotometer^[21].

Determination of pH: The pH of the gels was measured using a digital pH meter. One gram of gel was dissolved in 25 ml of purified water, and the electrode was dipped into the gel solution until a steady reading was obtained. pH measurements were repeated twice for each formulation^[22].

In vitro Diffusion Profile (In vitro Permeation in Rat Skin): In vitro diffusion experiments were conducted using Franz diffusion cells. Rat abdominal skin was used as the membrane for dialysis, tied to the diffusion cell. Isotonic phosphate buffer solution (pH 7.4) served as the substrate for receptors. A weighed quantity of the formulation equivalent to 1g of gel was applied to the rat skin, and aliquots were

withdrawn at different time intervals, measured at 295 nm. The total percent release was calculated for each time period, and the diffusion media were replaced with fresh medium after each withdrawal [23].

Results and Discussion

The present investigation was aimed at the green synthesis of silver nanoparticles using the ethanolic leaf extract of *Notonia grandiflora* and their incorporation into a topical gel formulation for evaluation of physicochemical and antimicrobial properties.

The extraction of *Notonia grandiflora* leaves using different solvents revealed that the ethanolic extract produced a substantially higher yield (8.50% w/w) compared to the petroleum ether extract (1.23% w/w), as shown in Table 3. The higher yield obtained with ethanol indicates the presence of a greater amount of polar phytoconstituents, which are readily extracted by polar solvents. Therefore, the ethanolic extract was selected for further formulation and evaluation studies.

Preliminary phytochemical screening of the ethanolic extract demonstrated the presence of glycosides, flavonoids, phenolic compounds, saponins, diterpenes, and carbohydrates, whereas alkaloids, proteins, and tannins were absent (Table 4). The

presence of flavonoids and phenolic compounds is particularly significant, as these phytoconstituents are known to possess reducing and stabilizing properties that facilitate the biosynthesis of silver nanoparticles. Additionally, these compounds contribute to antioxidant and antimicrobial activities.

Quantitative estimation of bioactive constituents revealed that the ethanolic extract contained total flavonoid content of 0.91 mg/100 mg and total phenolic content of 0.62 mg/100 mg of dried extract (Table 5). These findings further support the suitability of the extract for nanoparticle synthesis and biological applications.

Silver nanoparticles were prepared using different concentrations of silver nitrate and extract-to-metal ion ratios (Table 1). The prepared formulations (F1–F6) exhibited percentage yields ranging from $66.12 \pm 0.27\%$ to $79.12 \pm 0.18\%$, while entrapment efficiency values ranged from 0.736 ± 0.017 to 0.902 ± 0.019 mg/100 mg quercetin equivalent (Table 6). Among all formulations, F3 showed the highest percentage yield ($79.12 \pm 0.18\%$) and maximum entrapment efficiency (0.902 ± 0.019), indicating superior nanoparticle formation and efficient incorporation of phytoconstituents.

The optimized formulation F3 was further characterized for particle size and zeta potential. The average particle size was found to be 215.45 nm with a zeta potential of -37.45 mV (Table 7). The particle size distribution graph (Figure 1) confirmed the formation of nanoparticles within the desired nanometric range. The high negative zeta potential indicates good colloidal stability due to strong electrostatic repulsion between particles, thereby minimizing aggregation. Furthermore, the vesicle size distribution profile illustrated in Figure 2 demonstrated uniform particle dispersion throughout the formulation.

The optimized silver nanoparticles were incorporated into Carbopol-based gel formulations using varying concentrations of polymer as presented in Table 2. Evaluation of the prepared gels demonstrated satisfactory physical characteristics (Table 8). All formulations were homogeneous, smooth in texture, free from clogging, and exhibited acceptable washability and extrudability. Among the formulations, F3 showed superior physical properties, indicating better formulation stability and consistency.

The spreadability, viscosity, flavonoid content, and pH of the prepared gels were evaluated and are summarized in Table 9.

Spreadability values ranged from 10.80 ± 0.95 to 14.20 ± 0.45 g·cm/sec, while viscosity values ranged from 4200 ± 25 to 5800 ± 20 cP. Formulation F3 exhibited the highest spreadability (14.20 ± 0.45 g·cm/sec) and viscosity (5800 ± 20 cP), suggesting better retention at the site of application while maintaining ease of spreading. The pH values of all formulations ranged from 6.81 ± 0.02 to 6.95 ± 0.03 , which are within the physiological skin pH range and are therefore considered suitable for topical administration without causing irritation.

The in vitro drug release study demonstrated sustained release behavior from the prepared gel formulations (Table 10). Formulation F1 showed the fastest release profile, reaching 99.45% cumulative drug release within 4 hours, whereas F3 exhibited a comparatively slower release pattern (89.98% at 4 hours). Formulation F2 provided a balanced and controlled release profile, achieving 99.05% drug release at 4 hours. The observed release pattern may be attributed to differences in polymer concentration and gel viscosity, which influence the diffusion of active constituents from the gel matrix.

To understand the mechanism of release, kinetic analysis was performed for formulation F2. The regression coefficient

for the zero-order model ($R^2 = 0.9868$) was significantly higher than that of the first-order model ($R^2 = 0.8259$), as shown in Table 11. These findings suggest that drug release from the gel followed zero-order kinetics, indicating a concentration-independent release pattern that is desirable for maintaining prolonged therapeutic efficacy.

The antimicrobial activity of the ethanolic extract and nanoparticle-loaded gel formulation was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Table 12). Both the extract and F2 gel formulation exhibited concentration-dependent antimicrobial activity. However, the nanoparticle-loaded gel demonstrated significantly greater zones of inhibition

compared to the crude extract at all tested concentrations. Against *S. aureus*, the F2 gel produced inhibition zones of 11.0 ± 0.20 mm, 13.0 ± 0.50 mm, and 14.5 ± 0.35 mm at concentrations of 25, 50, and 100 mg/mL, respectively, whereas the extract produced zones of 8.0 ± 0.45 mm, 10.2 ± 0.40 mm, and 12.0 ± 0.42 mm. Similarly, against *P. aeruginosa*, the F2 gel exhibited inhibition zones of 12.0 ± 0.35 mm, 14.0 ± 0.70 mm, and 15.8 ± 0.30 mm compared to 6.0 ± 0.50 mm, 8.0 ± 0.60 mm, and 10.2 ± 0.40 mm for the extract. The enhanced antimicrobial efficacy of the gel may be attributed to the synergistic action of silver nanoparticles and bioactive phytoconstituents, which improve microbial membrane disruption and cellular damage.

Table 3: % Yield of leaves extract of *Notonia grandiflora*

S. No.	Extract	% Yield (w/w)
1.	Pet ether	1.23
2.	Ethanolic	8.50

Table 4: Phytochemical screening of extract of leaves of *Notonia grandiflora*

S. No.	Constituents	Ethanolic extract
1.	Alkaloids	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	Glycosides	
	Legal's Test	+ve

3.	Flavonoids Lead acetate Alkaline reagent test	+ve -ve
4.	Phenol Ferric chloride test	+ve
5.	Proteins Xanthoproteic test	-ve
6.	Carbohydrates Molisch's Test Benedict's Test Fehling's Test	-ve -ve +ve
7.	Saponins Froth Test Foam Test	+ve -ve
8.	Diterpenes Copper acetate test	+ve
9.	Tannins Gelatin Test	-ve

Table 5: Estimation of total flavonoids and phenol content of leaves of *Notonia grandiflora*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.91	0.62

Table 6: Determination of % yield of prepared silver nanoparticles formulations

S. No.	Formulation Code	% Yield	Entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
1	F1	66.12 ± 0.27	0.855±0.015
2	F2	70.45 ± 0.30	0.736±0.020
3	F3	79.12 ± 0.18	0.902±0.019
4	F4	71.05 ± 0.25	0.765±0.016

5	F5	69.30 ± 0.20	0.882 ± 0.014
6	F6	68.55 ± 0.22	0.736 ± 0.017

*Average of three determinations (n=3 $\pm$ SD)

Table 7: Characterization of average particle size and zeta potential of optimized formulation F3

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F3	215.45	- 37.45 mV

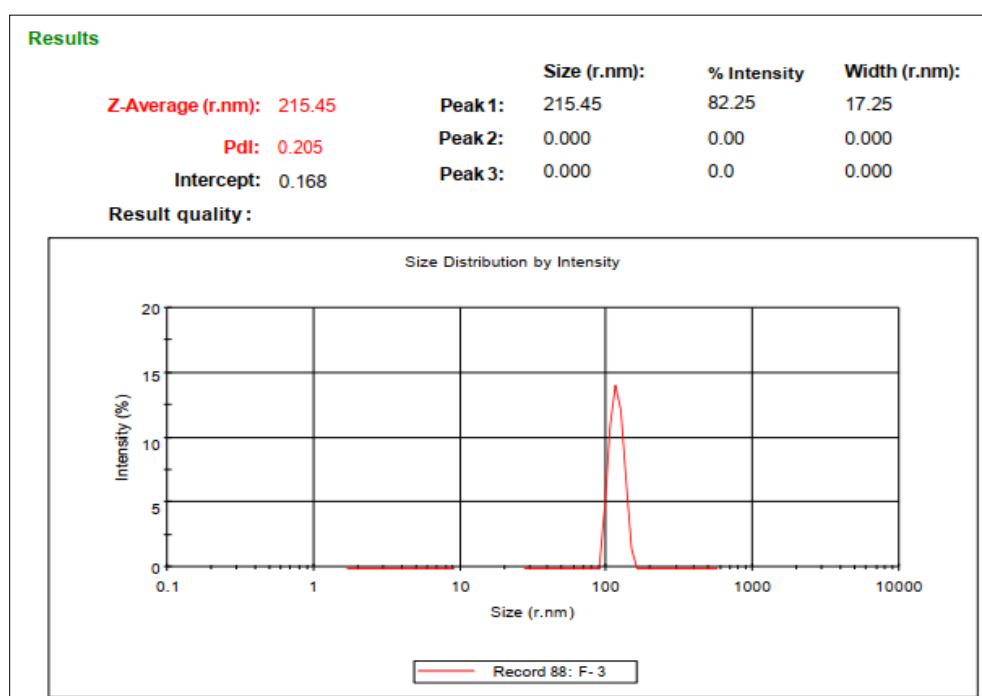
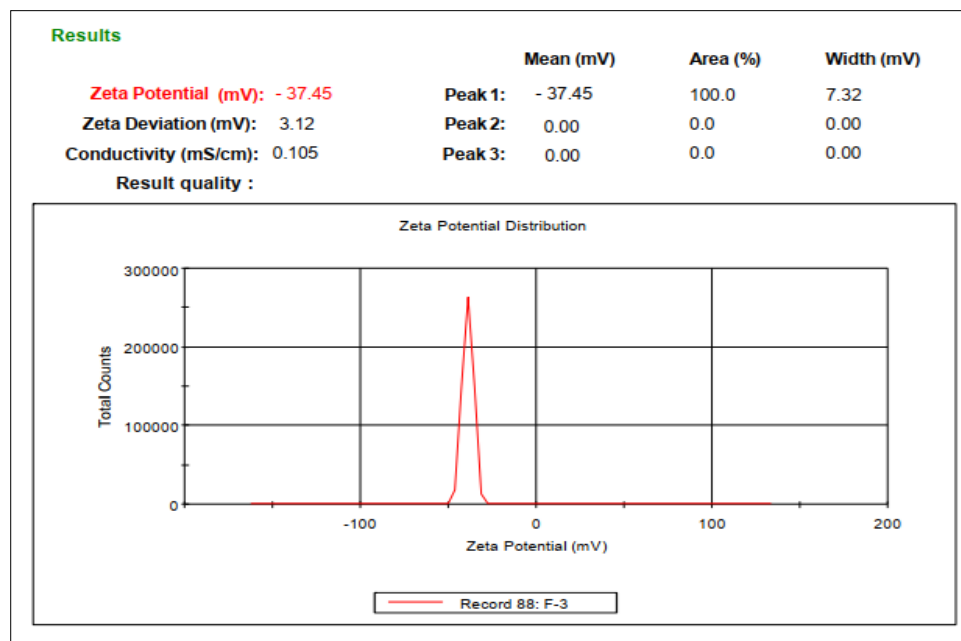


Figure 1: Graph of average particle size of formulation F3

**Figure 2: Graph of Average vesicle size formulation F3****Table 8: Results of physical characteristics**

Formulation Code	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
F1	Light Brown	Absent	Very Good	Smooth	Excellent	Good
F2	Dark Brown	Absent	Good	Smooth	Good	Very Good
F3	Brown	Absent	Very Good	Smooth	Excellent	Very Good

Table 9: Results of spreadability of gel

Formulation code	Spreadability* (gcm/sec)	Viscosity* (cP)	Flavonoid Content (mg/100mg)	pH
F1	12.50±1.15	4200±25	0.855	6.86±0.04
F2	10.80±0.95	5100±15	0.901	6.81±0.02
F3	14.20±0.45	5800±20	0.896	6.95±0.03

*Average of three determinations (n=3 ±SD)

Table 10: *In vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release
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		F1	F2	F3
1	0.25	31.15	28.85	26.65
2	0.5	43.25	36.65	33.32
3	1	55.65	43.32	41.12
4	1.5	63.32	55.58	54.65
5	2	78.98	66.69	63.32
6	2.5	98.85	78.85	72.25
7	3	99.12	86.65	79.95
8	4	99.45	99.05	89.98

Table 11: Release kinetics regression values of formulation F2

Formulation code	Zero order	First order
F2	0.9868	0.8259

Table 12: Antimicrobial activity of Extract and F2 Gel formulation against selected microbes

S. No.	Microbes	Extract			F2 Gel formulation		
		Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
	Concentration	25 mg/mL	50 mg/mL	100 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL
1	<i>S. aureus</i>	8.0 ± 0.45	10.2 ± 0.40	12.0 ± 0.42	11.0 ± 0.20	13.0 ± 0.50	14.5 ± 0.35
2	<i>P. aeruginosa</i>	6 ± 0.50	8.0 ± 0.60	10.2 ± 0.40	12.0 ± 0.35	14.0 ± 0.70	15.8 ± 0.30

Conclusion

The present study demonstrated the successful green synthesis of silver nanoparticles using the ethanolic leaf extract of *Notonia grandiflora*. The optimized nanoparticle formulation showed desirable particle size, good stability, and high

entrapment efficiency. The nanoparticle-loaded gel exhibited satisfactory physicochemical properties, controlled drug release, and enhanced antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These findings

suggest that the developed silver nanoparticle gel has potential as an effective topical antimicrobial formulation and warrants further in vivo and clinical investigations.

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