RESEARCH PAPER

FORMULATION AND CHARACTERIZATION OF *SMILAX ZEYLANICA*-MEDIATED COPPER NANOPARTICLE-LOADED HYDROGEL FOR ANTIMICROBIAL EFFECT

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Abstract
The present study aimed to prepare and evaluate copper nanoparticles using ethanolic extract of Smilax zeylanica and incorporate them into topical gel formulations for potential antimicrobial applications. Six nanoparticle formulations (F1–F6) were prepared using different copper sulfate concentrations and extract-to-copper sulfate ratios. The ethanolic extract exhibited a percentage yield of 6.25% w/w and contained several phytoconstituents, including flavonoids, phenolic compounds, tannins, glycosides, and alkaloids. Among the prepared nanoparticle formulations, F5 demonstrated the highest percentage yield of 86.74 ± 0.18% and the highest reported flavonoid content/entrapment efficiency value of 0.675 ± 0.033. The optimized nanoparticle formulation exhibited an average particle size of 240.36 nm and a zeta potential of −37.65 mV. Three gel formulations (GF1–GF3) were prepared using different concentrations of Carbopol 940 and evaluated for physical characteristics, viscosity, spreadability, pH, and in-vitro release. GF3 exhibited the highest viscosity of 4756 ± 11 cP and cumulative release of 97.65 ± 0.98% at 4 hours. The antimicrobial study demonstrated that GF3 produced inhibition zones of 17.36 ± 0.29 mm against Staphylococcus aureus and 19.25 ± 0.31 mm against Pseudomonas aeruginosa at 100 mg/mL. These inhibition zones were greater than those observed with the plant extract alone at the same concentration. The findings suggest that Smilax zeylanica-mediated copper nanoparticles may be promising candidates for developing topical antimicrobial gel formulations. Further studies are required to establish their safety, stability, and therapeutic efficacy.

Keywords: Smilax zeylanica, Copper nanoparticles, Green synthesis, Herbal gel, Carbopol 940, Antimicrobial activity, Staphylococcus aureus, Pseudomonas aeruginosa.
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Introduction

Medicinal plants have long been recognized as important sources of bioactive compounds and continue to play a significant role in the development of novel pharmaceutical and healthcare products. Plant-derived phytoconstituents, including flavonoids, phenolic compounds, tannins, alkaloids, and terpenoids, possess diverse biological activities, including antioxidant and antimicrobial properties (Dar et al., 2023). In recent years, the integration of traditional herbal medicines with nanotechnology has attracted considerable attention because nanoscale delivery systems may improve the stability, dispersion, and biological performance of plant-derived compounds (Li et al., 2025).

Smilax zeylanica is a medicinal plant belonging to the family Smilacaceae and is used in traditional systems of medicine. The plant contains various phytoconstituents that may contribute to its pharmacological potential. Previous studies have reported analgesic, antioxidant, and antibacterial activities associated with *S. zeylanica* (Hossain et al., 2013). The presence of phenolic compounds and flavonoids in plant extracts is particularly relevant because these compounds may contribute to various biological activities. However, the therapeutic potential of plant extracts can be influenced by factors such as the concentration of active constituents, stability, and interaction with the biological environment. Therefore, developing suitable formulation approaches for plant-derived materials is important for exploring their pharmaceutical applications (Sabarisenthil and Kalaichelvan, 2017; Dhanya Shree et al., 2018).

Green synthesis of metal nanoparticles using plant extracts has emerged as an alternative approach to conventional chemical synthesis. Plant-derived phytochemicals can participate in the reduction of metal ions and may also contribute to nanoparticle stabilization. This approach has gained attention because it can utilize naturally occurring compounds during nanoparticle formation and has potential applications in biomedical research (Singh et al., 2023). Copper nanoparticles have attracted interest because of their potential antimicrobial properties and

applications in pharmaceutical and biomedical research. Their biological performance may depend on characteristics such as particle size, surface charge, synthesis conditions, and formulation composition, making systematic characterization essential (Maliki et al., 2022).

Topical gel formulations are widely used for the localized delivery of therapeutic agents because they are generally easy to apply, spread readily over the skin, and provide a suitable vehicle for incorporating active ingredients (Patil et al., 2019). Carbopol 940 is a commonly used gelling agent that helps produce formulations with appropriate viscosity, consistency, and spreadability. Other excipients, such as polyethylene glycol 600, methyl paraben, and triethanolamine, may be incorporated to support formulation characteristics, preservation, and pH adjustment. Incorporating plant-mediated copper nanoparticles into a gel base may offer a practical approach for topical application. However, the resulting formulations require evaluation of their physical properties, pH, viscosity, spreadability, in-vitro release, and antimicrobial activity.

The present study aimed to prepare copper nanoparticles using an ethanolic extract of *Smilax zeylanica* and incorporate the optimized nanoparticle formulation into Carbopol 940-based topical gels. The prepared nanoparticles were evaluated for percentage yield, reported flavonoid content or entrapment efficiency, particle size, and zeta potential. The developed gel formulations were assessed for physical characteristics, homogeneity, consistency, spreadability, viscosity, pH, and in-vitro release behavior. Furthermore, antimicrobial activity was investigated against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The study was designed to investigate the formulation characteristics and preliminary antimicrobial potential of *S. zeylanica*-mediated copper nanoparticles for possible development as topical antimicrobial gel formulations.

Material and Methods

Material

The materials used in the present study included Smilax zeylanica plant material, copper sulfate (HiMedia Laboratories Pvt. Ltd., Mumbai, India), Carbopol 940 (Lubrizol Corporation, USA), polyethylene glycol 600 (Loba Chemie Pvt. Ltd., Mumbai, India), methyl paraben (HiMedia Laboratories Pvt. Ltd., Mumbai, India), triethanolamine (Loba Chemie Pvt. Ltd., Mumbai, India), ethanol (Merck Life Science Pvt. Ltd., India), and distilled water prepared in the laboratory. Manufacturer details should be verified against the actual suppliers of the materials used.

Methods

Extraction procedure

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

Defatting of plant materials

Roots of *Smilax zeylanica* 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 of *Smilax zeylanica* has been extracted with ethanol solvent using maceration process for 24 hrs, filtered and dried using vacuum evaporator at 40ºC (Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield

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

Percentage yield = Weight of Extract/ Weight of powdered drug x 100

Phytochemical screening

Phytochemical examinations were carried out for the extracts as per the standard methods (Audu *et al.,* 2007).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Gaur Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/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% $AlCl_3$ 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 (Gaur Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5-25µg/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 copper nanoparticles

Copper nanoparticles were synthesized by a green synthesis method using hydroalcoholic extract of *Smilax zeylanica* as a reducing and stabilizing agent. The formulations were prepared using different concentrations of $CuSO_4$ (1, 2, and 3 mM) and two extract:$CuSO_4$ ratios (1:1 and 1:2), as presented in Table 6.4. For the preparation of the formulations, 100 mg of dried *Smilax zeylanica* extract was accurately weighed and dispersed in the required quantity of distilled water. Fresh aqueous solutions of $CuSO_4$ at concentrations of 1, 2, and 3 mM were prepared separately. For formulations F1–F3, 100 mg of *Smilax zeylanica* extract was mixed with 100 mL of 1, 2, and 3 mM $CuSO_4$ solution, respectively, maintaining an extract:$CuSO_4$ ratio of 1:1. For formulations F4–F6, 100 mg of extract was mixed with 200 mL of 1, 2, and 3 mM $CuSO_4$ solution, respectively, maintaining an extract:$CuSO_4$ ratio of 1:2. The reaction mixtures were continuously stirred using a magnetic stirrer at 600 rpm for 60 min at room temperature (25 ± 2°C). The formation of copper nanoparticles was indicated by a gradual change in the color of the reaction mixture from light green to dark brown, suggesting reduction of copper ions and formation of nanoparticles. The reaction mixtures were subsequently incubated for 24 h at room temperature to facilitate completion of the reduction process. The synthesized copper nanoparticles were separated by centrifugation at 15,000 rpm for 20 min. The obtained pellet was collected and washed three times with distilled water, followed by washing with ethanol to remove unreacted constituents and other impurities. The purified nanoparticles were dried in a hot-air oven at 45°C until constant weight was obtained. The dried copper nanoparticles were collected, stored in airtight amber-colored containers, and used for further characterization (Hariprasad *et al.,* 2016).

Table 1: Different Formulations of Copper Nanoparticles

Formulation Code	***Smilax zeylanica* Extract (mg)**	**$CuSO_4$ Concentration (mM)**	**Extract:$CuSO_4$ Ratio**	**Amount of $CuSO_4$ Solution**
F1	100	1	1:1	100 mL of 1 mM $CuSO_4$
F2	100	2	1:1	100 mL of 2 mM $CuSO_4$
F3	100	3	1:1	100 mL of 3 mM $CuSO_4$
F4	100	1	1:2	200 mL of 1 mM $CuSO_4$
F5	100	2	1:2	200 mL of 2 mM $CuSO_4$
F6	100	3	1:2	200 mL of 3 mM $CuSO_4$

Characterization of synthesized copper nanoparticles formulations

UV–Visible Spectroscopic Analysis

The synthesized CuNPs were analyzed using a UV–Visible spectrophotometer to confirm nanoparticle formation through surface plasmon resonance (SPR).

Approximately 1 mL of the nanoparticle suspension was diluted with distilled water, and the absorbance spectrum was recorded in the wavelength range of 200–800 nm against distilled water as the blank. The characteristic absorption peak corresponding to copper nanoparticles confirmed successful synthesis.

Percentage yield

The copper 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 (Saranyaadevi *et al.,* 2014).

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

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 (Moniri *et al.,* 2017). The entrapment efficiency was assessed using the dialysis method, where the copper 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.

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. Zeta potential measurements for the copper nanoparticles were conducted based on the Helmholtz–Smoluchowsky equation derived from electrophoretic mobility (Usha *et al.,* 2017). 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 µS/cm.

Formulation development of copper nanoparticle gel

Precise quantities of methyl paraben, glycerin, polyethylene glycol, and Copper nanoparticle of *Smilax zeylanica* were dissolved in approximately 100 ml of water in a beaker. The mixture was vigorously stirred using a mechanical stirrer or sonicator, following the standard method (Pawar *et al.,* 2017).

Subsequently, Carbopol 940 was gradually introduced into the beaker containing the aforementioned liquid while maintaining continuous stirring. The solution was neutralized by slowly adding a triethanolamine solution, stirring constantly, until the gel formation occurred.

Table 2: Formulation of gel

Ingredients (mg)	GF1	GF 2	GF3
Copper nanoparticle of *Smilax zeylanica*	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.

Washability

Formulations were applied to the skin and manually assessed for ease and degree of washing with water (Pawar *et al.,* 2017).

Extrudability Determination

Gel formulations were filled into aluminum collapsible tubes, sealed, and pressed to extrude the material. Extrudability of the formulation was noted.

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 the average was calculated. 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 (Ren *et al.,* 2009).

Spreadability Formula: $S=m\times lt$

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.

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 $AlCl_3$ 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 (Maqusood *et al.,* 2014).

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.

***In vitro* diffusion profile**

In vitro diffusion experiments were conducted using Franz diffusion cells. Egg membrane 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 membrane, 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.

Results and Discussion

The present study focused on the preparation and evaluation of copper nanoparticles using ethanolic extract of Smilax zeylanica and their incorporation into a gel formulation. Six nanoparticle formulations (F1–F6) were prepared using different copper sulfate concentrations and extract-to-copper sulfate ratios, as shown in Table 1. Three gel formulations (GF1–GF3) were subsequently prepared using different concentrations of Carbopol 940, while the quantities of copper nanoparticles and other excipients were maintained constant (Table 2).

The percentage yield of the ethanolic extract of Smilax zeylanica was found to be 6.25% w/w (Table 3). Phytochemical screening revealed the presence of alkaloids, glycosides, phenolic compounds, tannins, flavonoids, proteins, and diterpenes in selected tests, whereas saponins were absent (Table 4). The total flavonoid and total phenolic contents were 0.68 and 0.92 mg/100 mg of dried extract, respectively (Table 5). These phytoconstituents may contribute to the biological properties of the extract and potentially participate in the formation and stabilization of copper nanoparticles.

The nanoparticle yield ranged from 62.48 ± 0.34% to 86.74 ± 0.18%, with formulation F5 exhibiting the highest yield. F5, prepared using 2 mM copper sulfate at an extract-to-copper sulfate ratio of 1:2, also showed the highest reported flavonoid content/entrapment efficiency value of 0.675 ± 0.033 (Tables 6 and 7). Characterization of F5 revealed an average particle size of 240.36 nm and a zeta potential of −37.65 mV (Table 8). The negative zeta potential suggests potentially favourable electrostatic repulsion between particles, which may help limit aggregation; however, stability should be confirmed through appropriate storage and particle-size measurements.

The physical evaluation of the copper nanoparticle gel formulations indicated uniform appearance, smooth texture, absence of clogging and grittiness, and no visible phase separation (Table 9). GF3 exhibited the highest viscosity (4756 ± 11 cP) and lowest spreadability (7.58 ± 0.19 g·cm/s), whereas GF1 showed the lowest viscosity (3482 ± 16 cP) and highest spreadability (10.12 ± 0.24 g·cm/s). GF2 demonstrated intermediate viscosity and spreadability, with a pH of 6.78 ± 0.05 (Table 10). These findings suggest that increasing Carbopol 940 concentration increased gel viscosity and reduced spreadability, highlighting the importance of balancing consistency and ease of application.

The in-vitro release study demonstrated progressive release from all three gel formulations (Table 11). At 4 hours, cumulative release reached 83.32 ± 0.96% for GF1, 88.46 ± 0.91% for GF2, and 97.65 ± 0.98% for GF3. GF3 exhibited the highest cumulative release at the final time point. The reported regression coefficients for GF3 were 0.9794 for the zero-order model and 0.9755 for the first-order model (Table 12), suggesting a slightly better fit to the zero-order model among these two models. Antimicrobial testing showed concentration-dependent inhibition against Staphylococcus aureus and Pseudomonas aeruginosa. The copper nanoparticle gel GF3 produced inhibition zones of 17.36 ± 0.29 mm and 19.25 ± 0.31 mm, respectively, at 100 mg/mL, exceeding those reported for the plant extract alone (Table 13). Overall, the findings indicate the potential of Smilax zeylanica-mediated copper nanoparticles for incorporation into antimicrobial gel formulations. Further characterization, safety testing, and validation of the biological **activity are required before drawing conclusions about clinical suitability.**

Table 3: % Yield of extract of *Smilax zeylanica*

S. No.	Extract	% Yield (w/w)
1.	Ethanolic	6.25

Table 4: Phytochemical screening of extract of *Smilax zeylanica*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	+ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	+ve
3.	**Glycosides**	
	Modified Borntrager's Test	+ve
	Legal's Test	+ve
4.	**Saponins**	
	Froth Test	-ve
5.	**Phenol**	
	Ferric chloride test	+ve
6.	**Tannins**	
	Gelatin Test	+ve
7.	**Flavonoids**	
	Lead acetate test	+ve
	Alkaline test	+ve
8.	**Proteins**	
	Xanthoproteic test	+ve
9.	**Diterpenes**	
	Copper acetate test	+ve

[+ve= positive; -ve= negative]

Table 5: Estimation of total flavonoids and phenol content of *Smilax zeylanica*

S. No.	Extract	Total flavonoids content	Total phenol content
		(mg/ 100 mg of dried extract)	
1.	Ethanolic	0.68	0.92

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

Formulation Code	$CuSO_4$ Concentration	Extract:$CuSO_4$ Ratio	% Yield
F1	1 mM	1:1	62.48 ± 0.34
F2	2 mM	1:1	70.36 ± 0.29
F3	3 mM	1:1	76.52 ± 0.26
F4	1 mM	1:2	74.68 ± 0.23
F5	2 mM	1:2	86.74 ± 0.18
F6	3 mM	1:2	81.56 ± 0.21

Table 7: Determination of entrapment efficiency of prepared formulations

Formulation Code	$CuSO_4$ Concentration	Extract:$CuSO_4$ Ratio	Flavonoid Content / Entrapment Efficiency*
F1	1 mM	1:1	0.558±0.025
F2	2 mM	1:1	0.585±0.036
F3	3 mM	1:1	0.598±0.0

			45
F4	1 mM	1:2	0.596±0.021
F5	2 mM	1:2	0.675±0.033
F6	3 mM	1:2	0.603±0.014

Table 8: Characterization of average particle size and zeta potential of optimized formulation F5

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F5	240.36	- 37.65 mV

Table 9: Results of physical characteristics

F. Code	Colour	Appearance	Clogging	Homogeneity	Texture	Consistency	Grittiness	Phase Separation
GF1	Light Brown	Uniform	Absent	Good	Smooth	Good	Absent	Absent
GF2	Brown	Uniform	Absent	Very Good	Smooth	Very Good	Absent	Absent
GF3	Dark Brown	Uniform	Absent	Excellent	Smooth	Excellent	Absent	Absent

Table 10: Spreadability of Copper Nanoparticle Gel Formulations

Formulation Code	Spreadability (g·cm/sec)*	Viscosity (cP)*	Flavonoid Content (mg Quercetin Equivalent/100 mg Gel)*	pH*
GF1	10.12 ± 0.24	3482 ± 16	0.592±0.025	6.25± 0.08
GF2	8.74 ± 0.21	4218 ± 14	0.605±0.036	6.78± 0.05
GF3	7.58 ± 0.19	4756 ± 11	0.585±0.028	6.35± 0.03

Values are expressed as Mean ± SD (n = 3).

Table 11: *In vitro* drug release profile of copper nanoparticle gel formulations

S. No.	Time (h)	GF1 (%)	GF2 (%)	GF3 (%)
1	0.25	16.63 ± 0.42	14.85 ± 0.36	21.32 ± 0.48
2	0.5	25.65 ± 0.51	24.72 ± 0.44	35.65 ± 0.56
3	1.0	34.65 ± 0.63	33.58 ± 0.52	47.74 ± 0.64
4	1.5	48.85 ± 0.72	46.82 ± 0.65	58.98 ± 0.71
5	2.0	56.65 ± 0.81	55.46 ± 0.73	76.63 ± 0.83
6	2.5	70.32 ± 0.85	68.74 ± 0.79	86.65 ± 0.91
7	3.0	79.98 ± 0.92	78.52 ± 0.86	90.32 ± 0.95
8	4.0	83.32 ± 0.96	88.46 ± 0.91	97.65 ± 0.98

Table 12: Release kinetics regression values of formulation GF2

Formulation code	Zero order	First order
GF3	0.9794	0.9755

Table 13: Antimicrobial activity against selected microbes

S. No.	Test Sample	Microorganism	25 mg/mL (mm)	50 mg/mL (mm)	100 mg/mL (mm)
1	*Smilax zeylanica* Extract	*Staphylococcus aureus*	7.42 ± 0.28	9.86 ± 0.31	12.47 ± 0.36
2	*Smilax zeylanica* Extract	*Pseudomonas aeruginosa*	8.36 ± 0.27	11.15 ± 0.34	13.68 ± 0.39
3	Copper Nanoparti	*Staphylococcus aureus*	11.24 ±	14.58 ±	17.36 ±

	cle Gel (GF3)		0.26	0.33	0.29
4	Copper Nanoparti cle Gel (GF3)	*Pseudomon as aeruginosa*	12.56 ± 0.24	15.84 ± 0.28	19.25 ± 0.31

Conclusion

The present study successfully demonstrated the preparation of copper nanoparticles using ethanolic extract of Smilax zeylanica and their incorporation into Carbopol 940-based gel formulations. Among the nanoparticle formulations, F5 exhibited the highest percentage yield (86.74 ± 0.18%), with an average particle size of 240.36 nm and a zeta potential of −37.65 mV. Among the gel formulations, GF3 showed the highest viscosity (4756 ± 11 cP) and cumulative release (97.65 ± 0.98%) at 4 hours. The antimicrobial evaluation demonstrated that GF3 produced greater inhibition against Staphylococcus aureus and Pseudomonas aeruginosa than the plant extract alone at the tested concentrations. The gel formulations also exhibited satisfactory physical characteristics, including uniform appearance, smooth texture, and absence of visible phase separation. These findings suggest that Smilax zeylanica-mediated copper nanoparticles have potential for further development into topical antimicrobial gel formulations. However, additional studies on nanoparticle characterization, formulation stability, skin irritation, cytotoxicity, and antimicrobial efficacy are necessary to establish their safety and suitability for topical application.

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