

FORMULATION AND CHARACTERIZATION OF COPPER NANOPARTICLES GEL OF *ZIZIPHUS ROTUNDIFOLIA* FOR TREATMENT OF SKIN DISEASE

Satyam Kumar, Shubham Vishwakarma, Rajesh Singh Pawar, Jay Prakash Soni*

Truba Institute of Pharmacy, Karond Bypass Road, Gandhi Nagar, Bhopal 462038, MP, India.

*Corresponding Author's E mail: s.jayprakash.ind@gmail.com

Received 09 July 2026; Revised 15 July 2026; Accepted 13 July 2026, Available online 15 October 2026



Cite this article as: Kumar S, Vishwakarma S, Pawar RS and Soni JP. Formulation and Characterization of Copper Nanoparticles Gel of *Ziziphus Rotundifolia* for Treatment of Skin Disease. Asian Journal of Pharmaceutical Education and Research. 2026; 15(4): 83-99. <https://dx.doi.org/10.38164/AJPER/15.4.2026.83-99>

ABSTRACT

The present study aimed to develop and evaluate copper nanoparticle-based gel formulations containing ethanolic extract of *Ziziphus rotundifolia*. The plant extracts were evaluated for extractive yield and phytochemical constituents. The ethanolic extract showed a higher yield of 8.46% and contained alkaloids, carbohydrates, glycosides, phenols, tannins, flavonoids, proteins, and diterpenes. The total flavonoid and phenolic contents were 0.80 and 0.96 mg/100 mg of dried extract, respectively. Six copper nanoparticle formulations (F1–F6) were prepared using different CuSO_4 concentrations and extract-to- CuSO_4 ratios. The percentage yield ranged from $64.82 \pm 0.31\%$ to $84.67 \pm 0.16\%$, while flavonoid-associated content ranged from 0.612 ± 0.028 to 0.812 ± 0.016 mg QE/100 mg. Formulation F5 was selected as the optimized formulation, showing the highest yield and flavonoid content. F5 exhibited an average particle size of 230.32 nm and zeta potential of -40.25 mV. The optimized nanoparticles were incorporated into gel formulations GF1–GF3. The formulations showed satisfactory physical characteristics, spreadability, viscosity, pH, and homogeneity. The *in vitro* drug-release study showed progressive release, with GF3 exhibiting the highest cumulative release of 99.18% at 4 h. GF3 showed a better fit to the zero-order release model ($R^2 = 0.9160$). Antimicrobial evaluation demonstrated concentration-dependent activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with maximum inhibition zones of 17.36 ± 0.24 mm and 19.28 ± 0.22 mm, respectively, at 100 mg/mL. Overall, the developed copper nanoparticle gel demonstrated promising physicochemical, release, and antimicrobial properties for further topical evaluation.

Keywords: *Ziziphus rotundifolia*; Copper nanoparticles; CuSO_4 ; Ethanolic extract; Topical gel; Flavonoids; Drug release; Zero-order kinetics; Antimicrobial activity.

INTRODUCTION

Medicinal plants have been used traditionally for the management of various diseases and continue to serve as important sources of biologically active phytoconstituents¹. Plant-derived compounds such as flavonoids, phenolics, alkaloids, tannins, glycosides, and terpenoids are associated with several pharmacological properties, including antioxidant and antimicrobial activities².

However, the therapeutic application of many plant extracts may be limited by poor solubility, low stability, inadequate bioavailability, and difficulty in delivering active constituents effectively to the desired site³. Nanotechnology has emerged as a promising approach for improving the delivery and functional performance of bioactive compounds. Among different nanomaterials, copper nanoparticles (CuNPs) have attracted considerable attention because of their nanoscale properties, large surface area, physicochemical stability, and reported antimicrobial potential⁴.

The biological activity of copper nanoparticles may be influenced by their particle size, surface characteristics, and interaction with microbial cells. Incorporation of plant extracts during nanoparticle preparation may additionally provide phytoconstituents that can participate in the reduction and stabilization of nanoparticles, offering an eco-friendly approach to nanoparticle development⁵.

Ziziphus rotundifolia is a medicinal plant belonging to the family Rhamnaceae and is traditionally associated with various therapeutic applications. The plant contains diverse secondary metabolites, including flavonoids, phenolic compounds, alkaloids, tannins, glycosides, and other constituents that may contribute to its biological activities⁶.

The presence of phenolic and flavonoid compounds is particularly important because these constituents may exhibit antioxidant and antimicrobial effects. Nevertheless, systematic development of *Ziziphus rotundifolia*-based nanostructured topical formulations requires appropriate optimization and characterization⁷.

Topical gel formulations provide several advantages, including ease of application, patient acceptability, localized drug delivery, and the ability to maintain the formulation at the site of application⁸. Carbopol 940 is widely used as a gelling agent because it can produce gels with suitable viscosity and consistency at relatively low concentrations. Incorporating optimized copper nanoparticles into a Carbopol-based gel may combine the beneficial properties of the plant extract, copper nanoparticles, and topical delivery system⁹.

Therefore, the present research was designed to develop and characterize copper nanoparticle-based gel formulations containing ethanolic extract of *Ziziphus rotundifolia*. The study included extraction and phytochemical evaluation of the plant material, preparation and selection of an optimized copper nanoparticle formulation, physicochemical characterization of the nanoparticles, incorporation into topical gels, evaluation of physical properties, *in vitro* drug-release studies, release-kinetic analysis, and antimicrobial evaluation against selected bacterial strains. The developed formulation was subsequently assessed for its potential as a promising topical antimicrobial delivery system.

MATERIAL AND METHODS

Material

The study utilized ethanolic extract of *Ziziphus rotundifolia*, copper sulphate (CuSO_4), Carbopol 940, polyethylene glycol 600 (PEG 600), methyl paraben, triethanolamine, distilled water, and other analytical-grade laboratory reagents. Copper sulphate, Carbopol 940, PEG 600, methyl paraben, and triethanolamine were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India, while solvents and other chemicals were of analytical grade and obtained from standard laboratory suppliers.

Methods

Extraction procedure

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

Defatting of plant materials

Leaves of *Ziziphus rotundifolia* 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 *Ziziphus rotundifolia* has been extracted with ethanol solvent using maceration process for 24 hrs, filtered and dried using vacuum evaporator at 40°C^{10-11} .

Determination of percentage yield

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

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening

Phytochemical examinations were carried out for the extracts as per the standard methods¹².

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method¹³. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25 $\mu\text{g/ml}$ 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 method¹⁴. 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 copper nanoparticles

Copper nanoparticles were synthesized by the green synthesis method using the hydroalcoholic extract of *Ziziphus rotundifolia* as the reducing and stabilizing agent. Briefly, 500 mg of the dried plant extract was dissolved in 50 mL of distilled water and filtered through Whatman No. 1 filter paper. Copper sulfate (CuSO_4) solutions of 1, 2, and 3 mM were prepared separately in distilled water. The plant extract was then mixed with the CuSO_4 solution according to the formulation composition (Table 6.1) using extract: CuSO_4 ratios of 1:1 and 1:2.

The reaction mixture was stirred continuously on a magnetic stirrer at 600 rpm for 60 minutes at room temperature ($25 \pm 2^\circ\text{C}$). Formation of copper nanoparticles was indicated by a gradual color change of the reaction mixture from light green to dark brown. The mixture was further incubated for 24 hours to ensure complete reduction of Cu^{2+} ions.

The synthesized copper nanoparticles were separated by centrifugation at 15,000 rpm for 20 minutes. The obtained pellet was washed three times with distilled water followed by ethanol to remove unreacted constituents and impurities. The purified nanoparticles were dried in a hot-air oven at 45°C until a constant weight was obtained, collected, and stored in airtight amber-colored containers for further characterization¹².

Table 1: Different formulation of copper nanoparticles

F. Code	<i>Ziziphus rotundifolia</i> Extract (mg)	CuSO_4 Concentration (mM)	Extract: CuSO_4 Ratio (mg:mL)	Amount of CuSO_4 Solution
F1	500	1 mM	1:1	500 mg extract : 500 mL of 1 mM CuSO_4 solution*
F2	500	2 mM	1:1	500 mg extract : 500 mL of 2 mM CuSO_4 solution*
F3	500	3 mM	1:1	500 mg extract : 500 mL of 3 mM CuSO_4 solution*
F4	500	1 mM	1:2	500 mg extract : 1000 mL of 1 mM CuSO_4 solution*

F5	500	2 mM	1:2	500 mg extract : 1000 mL of 2 mM CuSO ₄ solution*
F6	500	3 mM	1:2	500 mg extract : 1000 mL of 3 mM CuSO ₄ solution*

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 preparation¹³.

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

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 drug¹⁴. 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 mobility¹⁵. 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 leaves of *Ziziphus rotundifolia* 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 method¹⁶.

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 <i>Ziziphus rotundifolia</i>	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 water¹⁷.

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 recorded¹⁸.

Spreadability Formula: $S = m \times l / t$ 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.

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 spectrophotometer¹⁹.

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.

Antimicrobial Activity by Agar Well Diffusion Method

The antimicrobial activity of the copper nanoparticle gel was evaluated by the agar well diffusion method. Sterile nutrient agar plates were inoculated with the test microorganisms, and wells were prepared using a sterile cork borer. Gel formulations at 25, 50, and 100 mg/mL were introduced into the wells, while a standard antibiotic was used as the positive control. The plates were incubated at 37°C for 24 h, and the zone of inhibition (mm) was measured to determine the antimicrobial activity²⁰⁻²¹.

RESULTS AND DISCUSSION

The different formulations of copper nanoparticles prepared using *Ziziphus rotundifolia* extract are presented in Table 1. In all formulations, 500 mg of *Ziziphus rotundifolia* extract was used, while the concentration of CuSO₄ was varied between 1, 2, and 3 mM. The extract-to-CuSO₄ ratio was varied between 1:1 and 1:2, allowing the influence of precursor concentration and extract proportion on nanoparticle formation to be investigated. Formulations F1, F2, and F3 were prepared using a 1:1 extract-to-CuSO₄ ratio, whereas F4, F5, and F6 employed 1:2 ratio. The CuSO₄ concentration was progressively varied from 1 to 3 mM within each ratio. This formulation strategy provided different experimental conditions for evaluating the effect of precursor concentration and extract-to-metal salt ratio on the characteristics of the resulting copper nanoparticles.

The composition of the copper nanoparticle gel formulations GF1, GF2, and GF3 is presented in Table 2. Each formulation contained 500 mg of copper nanoparticles prepared from *Ziziphus rotundifolia*, along with polyethylene glycol 600, methyl paraben, triethanolamine, and distilled water. The major formulation variable was the concentration of Carbopol 940, which was increased from 250 mg in GF1 to 500 mg in GF2 and 750 mg in GF3. The concentration of the other excipients was maintained constant. This variation in Carbopol 940 was intended to produce gels with different viscosity and consistency characteristics and thereby influence their spreadability and drug-release behavior. The resulting formulations were subsequently evaluated for their physical, physicochemical, release, and antimicrobial properties.

The percentage yield of the different extracts of *Ziziphus rotundifolia* is presented in Table 3. The petroleum ether extract showed a yield of 1.25% (w/w), whereas the ethanolic extract showed a considerably higher yield of 8.46% (w/w). The higher yield obtained with ethanol indicates that ethanol extracted a greater quantity of soluble constituents from the plant material under the conditions employed. Therefore, the ethanolic extract was selected for further phytochemical and quantitative investigations.

The results of preliminary phytochemical screening of the ethanolic extract are presented in Table 4. The extract showed positive reactions for alkaloids, carbohydrates, glycosides, phenols, tannins, flavonoids, proteins, and diterpenes, whereas saponins were not detected. The presence of several classes of phytoconstituents indicates that the ethanolic extract contains a diverse range of biologically relevant compounds. These constituents may contribute to the biological properties of the extract and may also participate in the formation or stabilization of copper nanoparticles.

The quantitative estimation of total flavonoid and phenolic contents is presented in Table 5. The ethanolic extract contained 0.80 mg/100 mg of dried extract of total flavonoids and 0.96 mg/100 mg of dried extract of total phenolic compounds. The presence of these compounds supports the phytochemical screening results and indicates that the extract contains phenolic and flavonoid constituents that may contribute to its antioxidant and other biological activities.

The percentage yield of the prepared copper nanoparticle formulations is presented in Table 6. The yield ranged from $64.82 \pm 0.31\%$ for F1 to $84.67 \pm 0.16\%$ for F5. F5 exhibited the highest yield, followed by F6, while F1 showed the lowest yield. The higher yield obtained with F5 suggests comparatively efficient formation and recovery of copper nanoparticles under its formulation conditions. The relatively low standard deviations indicate good repeatability of the yield determination. The results demonstrate that changes in CuSO_4 concentration and extract-to- CuSO_4 ratio influenced the nanoparticle yield.

The entrapment efficiency/flavonoid-associated content of the prepared formulations is presented in Table 7. The values ranged from 0.612 ± 0.028 to 0.812 ± 0.016 mg/100 mg quercetin equivalent. F5 showed the highest value (0.812 ± 0.016), whereas F1 showed the lowest (0.612 ± 0.028). The progressive improvement in the measured flavonoid-associated content up to F5 indicates comparatively greater incorporation or association of extract constituents with the nanoparticle system. Considering the percentage yield and flavonoid content together, F5 was selected as the optimized copper nanoparticle formulation.

The particle size and zeta potential of optimized F5 are presented in Table 8. F5 exhibited an average particle size of 230.32 nm and a zeta potential of -40.25 mV. The particle-size result confirms the formation of particles in the nanoscale/submicron range. The relatively high negative zeta potential indicates substantial surface charge and electrostatic repulsion among particles, which may help reduce aggregation and support dispersion stability.

The average particle-size characteristics of F5 are graphically represented in Figure 1, while the vesicle-size profile is represented in Figure 2. Thus, Table 8 and Figures 1–2 collectively provide the characterization profile of the optimized nanoparticle formulation.

The physical characteristics of GF1, GF2, and GF3 are presented in Table 9. GF1, GF2, and GF3 showed light brown, brown, and dark brown coloration, respectively. All formulations exhibited a uniform appearance, absence of clogging, smooth texture, and absence of grittiness and phase separation. Homogeneity was rated as good for GF1 and GF2 and excellent for GF3. Similarly, consistency was rated as good for GF1 and GF2 and excellent for GF3. These findings indicate that the prepared gels possessed satisfactory physical characteristics and were free from visible signs of instability.

The results of spreadability, viscosity, flavonoid content, and pH are presented in Table 10. Spreadability values were 8.25 ± 0.22 , 7.68 ± 0.18 , and 6.92 ± 0.15 g·cm/sec for GF1, GF2, and GF3, respectively. GF1 therefore demonstrated the highest spreadability, whereas GF3 exhibited the lowest. In contrast, viscosity increased from 3525 ± 18 cP in GF1 to 4878 ± 12 cP in GF3. This inverse relationship between viscosity and spreadability suggests that the higher Carbopol 940 concentration used in GF3 resulted in a more viscous gel with comparatively reduced spreading ability.

The flavonoid content increased from 0.628 ± 0.014 mg QE/100 mg gel in GF1 to 0.815 ± 0.013 mg QE/100 mg gel in GF3. The pH values ranged from 6.62 ± 0.03 to 6.91 ± 0.02 . Overall, the formulations demonstrated consistent physicochemical characteristics, with GF3 showing the highest viscosity and flavonoid content.

The *in vitro* drug-release profiles of GF1, GF2, and GF3 are presented in Table 11. All formulations showed a progressive increase in cumulative drug release with increasing time. At 4 h, GF1, GF2, and GF3 exhibited cumulative drug-release values of 89.76%, 95.85%, and 99.18%, respectively. GF3 showed the highest release throughout the study period and achieved 99.18% release at 4 h.

The higher release observed with GF3, despite its greater viscosity, indicates that the formulation composition and nanoparticle characteristics influenced the release behavior. The progressive release pattern from all three formulations demonstrates effective release of the incorporated constituents during the experimental period.

The release-kinetic regression values of GF3 are presented in Table 12. GF3 showed an R^2 value of 0.9160 for the zero-order model and 0.7470 for the first-order model. The higher R^2 value for the zero-order model indicates that the release data fitted the zero-order model more closely than the first-order model. Thus, the release profile of GF3 can be described more appropriately by zero-order kinetics among the models evaluated.

The antimicrobial activity of *Ziziphus rotundifolia* extract and copper nanoparticle gel GF3 against selected microorganisms is presented in Table 13. The ethanolic extract demonstrated concentration-dependent activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For *S. aureus*, the zone of inhibition increased from 8.25 ± 0.42 mm at 25 mg/mL to 13.18 ± 0.45 mm at 100 mg/mL. Against *P. aeruginosa*, the corresponding inhibition zones increased from 9.42 ± 0.36 mm to 14.26 ± 0.37 mm.

The copper nanoparticle gel GF3 demonstrated greater antimicrobial activity than the extract alone. Against *S. aureus*, the inhibition zone increased from 11.48 ± 0.31 mm at 25 mg/mL to 17.36 ± 0.24 mm at 100 mg/mL. Against *P. aeruginosa*, the inhibition zone increased from 12.86 ± 0.29 mm to 19.28 ± 0.22 mm. The maximum activity was observed at 100 mg/mL, particularly against *P. aeruginosa*. The enhanced activity of the nanoparticle gel compared with the extract alone suggests that incorporation into the copper nanoparticle-based gel system improved the antimicrobial response under the tested conditions.

Table 3: % Yield of extract of *Ziziphus rotundifolia*

S. No.	Extract	% Yield (w/w)
1.	Pet ether	1.25
2.	Ethanolic	8.46

Table 4: Phytochemical screening of extract of *Ziziphus rotundifolia*

S. No.	Constituents	Ethanol extract
1.	Alkaloids	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendorff'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	+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 *Ziziphus rotundifolia*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanol extract	0.80	0.96

Table 6: Determination of % yield of prepared coppernanoparticles formulations

Formulation Code	% Yield
F1	64.82 ± 0.31
F2	71.46 ± 0.28
F3	75.18 ± 0.22
F4	78.94 ± 0.19
F5	84.67 ± 0.16
F6	80.35 ± 0.21

Table 7: Determination of entrapment efficiency of prepared formulations

Formulation Code	Percentage Entrapment Efficiency (Flavonoid mg/100 mg Quercetin Equivalent)
F1	0.612 ± 0.028
F2	0.658 ± 0.024
F3	0.701 ± 0.021
F4	0.735 ± 0.019
F5	0.812 ± 0.016
F6	0.768 ± 0.022

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

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F5	230.32	- 40.25 mV

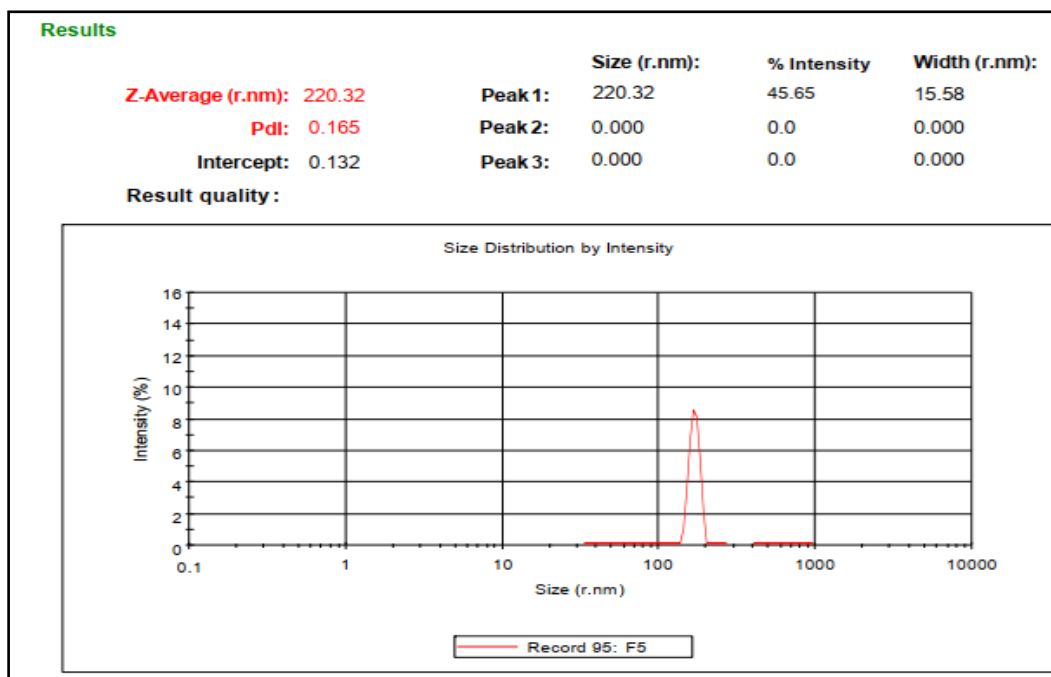


Figure 1: Graph of average particle size of formulation F5

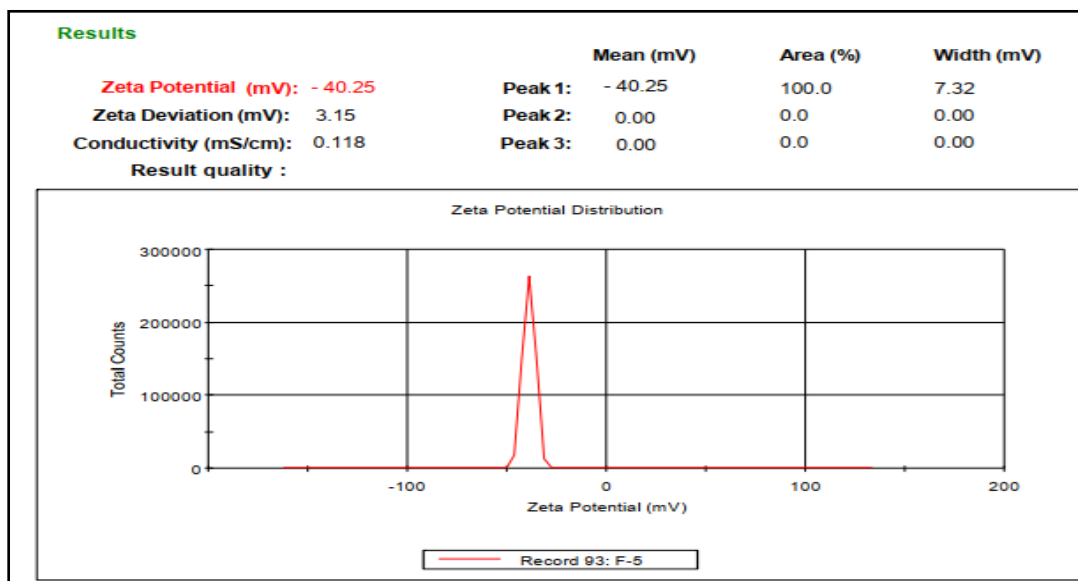


Figure 2: Graph of Average vesicle size formulation F5

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	Good	Smooth	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	8.25 ± 0.22	3525 ± 18	0.628 ± 0.014	6.62 ± 0.03
GF2	7.68 ± 0.18	4185 ± 15	0.742 ± 0.017	6.78 ± 0.02
GF3	6.92 ± 0.15	4878 ± 12	0.815 ± 0.013	6.91 ± 0.02

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

Table 11: *In vitro* Drug Release Profile of Copper Nanoparticle Gel Formulations

S. No.	Time (h)	% Cumulative drug release		
		GF1	GF2	GF3
1	0.25	18.65	22.84	26.95
2	0.50	28.72	34.65	40.82
3	1.00	39.58	47.92	54.68
4	1.50	50.24	59.85	66.72
5	2.00	61.48	71.68	78.95
6	2.50	72.36	82.45	89.68
7	3.00	81.94	91.36	94.82
8	4.00	89.76	95.85	99.18

Table 12: Release kinetics regression values of formulation GF3

Formulation code	Zero order	First order
GF3	0.9160	0.7470

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	<i>Ziziphus rotundifolia</i> Extract	<i>Staphylococcus aureus</i>	8.25 ± 0.42	10.62 ± 0.38	13.18 ± 0.45
2	<i>Ziziphus rotundifolia</i> Extract	<i>Pseudomonas aeruginosa</i>	9.42 ± 0.36	11.95 ± 0.41	14.26 ± 0.37

3	Copper Nanoparticle Gel (GF3)	<i>Staphylococcus aureus</i>	11.48 ± 0.31	14.22 ± 0.28	17.36 ± 0.24
4	Copper Nanoparticle Gel (GF3)	<i>Pseudomonas aeruginosa</i>	12.86 ± 0.29	15.84 ± 0.26	19.28 ± 0.22

CONCLUSION

The present study successfully demonstrated the development of a copper nanoparticle-based topical gel containing ethanolic extract of *Ziziphus rotundifolia*. The ethanolic extract showed better extractive yield and contained several important phytoconstituents, particularly phenolic and flavonoid compounds. Among the prepared copper nanoparticle formulations, F5 exhibited the highest percentage yield and flavonoid-associated content and was therefore selected as the optimized formulation. The optimized nanoparticles showed a particle size of 230.32 nm with a zeta potential of -40.25 mV, indicating satisfactory nanoscale characteristics and dispersion stability. The optimized nanoparticles were successfully incorporated into Carbopol 940-based gel formulations. Among the prepared gels, GF3 demonstrated excellent physical characteristics, favorable viscosity and pH, and the highest flavonoid content. It also exhibited 99.18% cumulative drug release at 4 h and showed a better fit to the zero-order release model. Furthermore, GF3 demonstrated concentration-dependent antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, indicating enhanced antimicrobial potential compared with the plant extract alone. The findings suggest that *Ziziphus rotundifolia* copper nanoparticle gel possesses promising physicochemical, drug-release, and antimicrobial properties and may serve as a potential topical formulation. Further studies, including detailed stability, safety, and in vivo evaluation, are recommended to establish its therapeutic applicability.

References

1. Latif R, Nawaz T. Medicinal plants and human health: a comprehensive review of bioactive compounds, therapeutic effects, and applications. *Phytochem Rev.* 2026;25(3):2299-342.
2. Elshafie HS, Camele I, Mohamed AA. A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *Int J Mol Sci.* 2023;24(4):3266.

3. Bonifácio BV, da Silva PB, Ramos MA, Negri KM, Bauab TM, Chorilli M. Nanotechnology-based drug delivery systems and herbal medicines: a review. *Int J Nanomedicine*. 2014;9:1-5.
4. Patel P, Garala K, Singh S, Prajapati BG, Chittasupho C. Lipid-based nanoparticles in delivering bioactive compounds for improving therapeutic efficacy. *Pharmaceuticals (Basel)*. 2024;17(3):329.
5. Singh H, Desimone MF, Pandya S, Jasani S, George N, Adnan M, et al. Revisiting the green synthesis of nanoparticles: uncovering influences of plant extracts as reducing agents for enhanced synthesis efficiency and its biomedical applications. *Int J Nanomedicine*. 2023;18:4727-50.
6. Sakna ST, Maghraby YR, Abdelfattah MS, Farag MA. Phytochemical diversity and pharmacological effects of triterpenes from genus *Ziziphus*: a comprehensive review. *Phytochem Rev*. 2023;22(6):1611-36.
7. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines (Basel)*. 2018;5(3):93.
8. Singh Malik D, Mital N, Kaur G. Topical drug delivery systems: a patent review. *Expert Opin Ther Pat*. 2016;26(2):213-28.
9. Pahlevi MR, Maulida IH, Supriadi D, Pratama R. Formulation of Moringa leaf ethanol extract gel (*Moringa oleifera* L.) using Carbopol 940 as a gelling agent and antioxidant activity. *Trop J Nat Prod Res*. 2026;10(2):72-84.
10. Mukherjee PK. Quality Control of Herbal Drugs. 2nd ed. New Delhi: Business Horizons; 2007. p. 2-14.
11. Kokate CK. Practical Pharmacognosy. 4th ed. Mumbai: VallabhPrakashan; 1994. p. 112-120.
12. Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of *Lophiralanceolata* (Ochnaceae). *Life Sci J*. 2007;4(4):75-79.
13. Mishra AG, Singh R, Patil N, Parkhe G. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superba* seed extract. *Asian J Pharm Educ Res*. 2017;6(2):12-17.
14. Hariprasad S, et al. Green synthesis of copper nanoparticles from medicinal plants. *Indo Am J Pharm Sci*. 2016;3(8):831-835.
15. Saranyaadevi K, Subha V, Ernest Ravindran RS, Renganathan S. Synthesis and characterization of copper nanoparticle using *Capparis zeylanica* leaf extract. *Int J ChemTech Res*. 2014;6(10):4533-4541.

16. Moniri S, Ghoranneviss M, Hantehzadeh MR, AsadiAsadabad M. Synthesis and optical characterization of copper nanoparticles prepared by laser ablation. *Bull Mater Sci.* 2017;40(1):37-43.
17. Usha S, Ramappa KT, Hiregoudar S, Vasanthkumar GD, Aswathanarayana DS. Biosynthesis and characterization of copper nanoparticles from Tulasi (*Ocimum sanctum* L.) leaves. *Int J Curr Microbiol Appl Sci.* 2017;6(11):2219-28.
18. Pawar D, Shahista S, Deshmukh S, Rohini K. Green synthesis of copper nanoparticles using *Gloriosa superba* leaf extract. *Int J Pharm Pharm Res.* 2017:203-209.
19. Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. *Int J Antimicrob Agents.* 2009;33(6):587-90.
20. Maqsood A, Hisham A, Alhadlaq MAS. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. *J Nanomater.* 2014;2014:4.
21. Aguilar-Pérez D, Vargas Coronado R, Cervantes JM, et al. Antibacterial activity of a glass ionomer cement doped with copper nanoparticles. *Dent Mater J.* 2020;39(3):389-396.