



FORMULATION AND CHARACTERIZATION OF COPPER NANOPARTICLES OF  
*CURCUMA ZEDOARIA* FOR EFFECTIVE TOPICAL FUNGAL TREATMENT

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\*Article History:

Received: 02/01/2026

Revised: 29/01/2026

Accepted: 17/02/2026

ABSTRACT

Fungal infections of the skin are a widespread health concern requiring effective and safe topical therapies. The present study focuses on the formulation and characterization of copper nanoparticles using *Curcuma zedoaria* extract for enhanced antimicrobial and antifungal activity. The plant extract was prepared using petroleum ether and hydroalcoholic solvents, with the hydroalcoholic extract showing a higher yield (8.92% w/w). Phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, phenolics, saponins, and diterpenes, which play a vital role in nanoparticle synthesis and therapeutic activity. Copper nanoparticles were synthesized using a green synthesis approach and formulated into topical gel preparations (F1–F6). Among these, formulation F3 exhibited the highest percentage yield (80.62 ± 0.22%) and entrapment efficiency (0.728 ± 0.024 mg/100 mg). The optimized formulation showed an average particle size of 157.2 nm and a zeta potential of -40.26 mV, indicating good stability. The prepared gel formulations were evaluated for physical characteristics, spreadability, viscosity, pH, and drug content, where F3 demonstrated superior properties and compatibility with skin. In vitro drug release studies revealed that formulation F2 showed maximum cumulative drug release (98.78% at 4 hours) and followed zero-order kinetics, indicating controlled release behavior. Antimicrobial studies demonstrated that the copper nanoparticle gel exhibited significantly higher activity against *Streptococcus mutans* and *Escherichia coli* compared to the plain extract, confirming the enhanced efficacy of the nanoparticle system.

In conclusion, the study demonstrates that copper nanoparticles of *Curcuma zedoaria* can be successfully formulated into a stable and effective topical gel with improved antimicrobial activity. This approach offers a promising strategy for the treatment of fungal and microbial skin infections with enhanced therapeutic performance.

**Keywords:** *Curcuma zedoaria*, Copper nanoparticles, Green synthesis, Topical gel, Antifungal activity, Antimicrobial activity, Phytochemical screening, Drug release kinetics, Nanotechnology, Herbal formulation.

INTRODUCTION

Fungal infections of the skin represent a major global health concern, affecting a significant proportion of the population, particularly in

tropical and subtropical regions. Superficial fungal infections such as dermatophytosis, candidiasis, and aspergillosis are primarily caused by pathogens like *Trichophyton*,

*Candida albicans*, and *Aspergillus* species (Urban *et al.*, 2017).

These infections are often associated with symptoms such as itching, inflammation, redness, and scaling of the skin. Despite the availability of conventional antifungal agents such as azoles and polyenes, their prolonged use has led to challenges including drug resistance, toxicity, poor skin penetration, and recurrence of infections. Hence, there is a pressing need to develop safer and more effective topical antifungal therapies (Al *et al.*, 2021).

Nanotechnology has emerged as a revolutionary approach in pharmaceutical sciences, offering novel strategies for drug delivery and disease management. Nanoparticles exhibit unique physicochemical properties, including a high surface area-to-volume ratio, enhanced permeability, and improved bioavailability, which make them suitable for topical drug delivery systems (Atthapu *et al.*, 2026).

Metal nanoparticles, in particular, have demonstrated significant antimicrobial and antifungal activities due to their ability to disrupt microbial cell membranes, generate reactive oxygen species (ROS), and interfere with intracellular components. Among them, copper nanoparticles (CuNPs) have attracted considerable attention because of their potent antifungal activity, cost-effectiveness, and ease of synthesis (Nisar *et al.*, 2019).

The concept of green synthesis of nanoparticles using plant extracts has gained popularity as an eco-friendly and sustainable alternative to conventional chemical and physical methods. Plant-mediated synthesis utilizes naturally occurring phytochemicals such as flavonoids, phenolics, alkaloids, and

terpenoids as reducing and stabilizing agents, eliminating the use of toxic chemicals. This approach not only enhances the biocompatibility of nanoparticles but also improves their therapeutic efficacy through synergistic effects (Jha *et al.*, 2021).

*Curcuma zedoaria* (Zingiberaceae), commonly known as white turmeric, is a medicinal plant widely used in traditional systems of medicine. The rhizome of *Curcuma zedoaria* is rich in bioactive compounds such as curcuminoids, sesquiterpenes, essential oils, and phenolic constituents, which contribute to its diverse pharmacological activities (Gharge *et al.*, 2021).

It has been reported to possess antimicrobial, antifungal, anti-inflammatory, antioxidant, and wound-healing properties. The antifungal activity of *Curcuma zedoaria* is mainly attributed to its ability to disrupt fungal cell membranes and inhibit fungal growth (Ghuman *et al.*, 2019).

The integration of *Curcuma zedoaria* extract with copper nanoparticles offers a promising approach for enhancing antifungal efficacy. In this system, plant phytoconstituents act as both reducing and capping agents in the synthesis of nanoparticles, resulting in stable and biologically active formulations. The combined effect of copper ions and plant-derived bioactive compounds leads to improved antifungal activity through multiple mechanisms, including oxidative stress induction, membrane damage, and inhibition of fungal metabolism (Vincent *et al.*, 2022).

Topical delivery of such nanoparticle-based formulations provides several advantages, including localized drug action, improved

penetration into the skin, reduced systemic side effects, and enhanced patient compliance. Therefore, the formulation and characterization of copper nanoparticles using *Curcuma zedoaria* for topical antifungal therapy represent a novel and effective strategy for managing fungal infections.

## MATERIALS AND METHODS

### Materials

The rhizomes of *Curcuma zedoaria* were procured from a local herbal supplier and authenticated. Copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) was used as a precursor for nanoparticle synthesis. Ethanol, methanol, petroleum ether, and other analytical-grade solvents were procured from standard suppliers. Carbopol 934 was used as a gelling agent, while propylene glycol was used as a penetration enhancer. Triethanolamine was used for pH adjustment. All chemicals and reagents used in the study were of analytical grade.

### Methods

#### Extraction procedure

##### Defatting of plant material

Rhizome of *Curcuma zedoaria* 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 rhizome of *Curcuma zedoaria* has been extracted with hydroalcoholic solvent (ethanol: water: 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007; Kokate, 1994).

### Determination of percentage yield

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

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

### Phytochemical screening

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

### Total flavonoids content estimation

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%  $\text{AlCl}_3$  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.

### Total Phenolic content estimation

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 10-50µg/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 using *Curcuma zedoaria* extract by a green synthesis method. For each formulation, the required concentration of copper sulfate (CuSO<sub>4</sub>) solution (1–3 mM) was prepared using double-distilled water. As per the formulation design, 80 mL of CuSO<sub>4</sub> solution was mixed with 20 mL of *Curcuma zedoaria* extract to obtain the desired extract-to-metal salt ratio (1:1 or 1:2), as shown in Table 6.1.

The reaction mixture was stirred thoroughly and kept undisturbed at room temperature for 24 hours. During this period, a gradual change in the color of the solution was observed, indicating the reduction of Cu<sup>2+</sup> ions and the formation of copper nanoparticles. After completion of the reaction, the mixture was centrifuged at 10,000 rpm for 15 minutes to separate the nanoparticles.

The obtained pellet was washed repeatedly with double-distilled water to remove unreacted copper ions and residual biological materials. The purified copper nanoparticles were then redispersed in double-distilled water and stored for further characterization studies (Hariprasad *et al.*, 2016).

**Table 1: Different formulation of copper nanoparticles**

| Formulation Code | <i>Curcuma Zedoaria</i> Extract (mg) | CuSO <sub>4</sub> (mM) | Ratio |
|------------------|--------------------------------------|------------------------|-------|
| F1               | 100                                  | 1                      | 1:1   |
| F2               | 100                                  | 2                      | 1:1   |
| F3               | 100                                  | 3                      | 1:1   |
| F4               | 100                                  | 1                      | 1:2   |
| F5               | 100                                  | 2                      | 1:2   |
| F6               | 100                                  | 3                      | 1:2   |

### Characterization of synthesized copper nanoparticles formulations

#### 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).

% Yield

$$= \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}}$$

#### 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  $\mu$ S/cm.

### Formulation development of copper nanoparticle gel

Precise quantities of methyl paraben, glycerin, polyethylene glycol, and Copper nanoparticle of *Curcuma zedoaria* 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)                               | F1     | F 2   | F3    |
|--|--------|-------|-------|
| Copper nanoparticle of <i>Curcuma zedoaria</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 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:**  $S = m \times l \times t$  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 AlCl<sub>3</sub> 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.

#### Antimicrobial activity of copper nanoparticle gel

#### Media preparation; Composition of nutrient agar media

|                 |            |
|-----------------|------------|
| Peptone         | - 0.5      |
| Beef extract    | - 0.5      |
| Agar            | - 1.5 gms. |
| Distilled water | - 100 ml.  |

The preparation of agar medium involved dissolving it in distilled water and boiling the mixture in a conical flask. Dry ingredients were added to the flask, and the medium was completely dissolved by heating. The

medium-filled flask was then sterilized in an autoclave at 121°C and 15 lbs/inch<sup>2</sup> pressure for 15 minutes. After sterilization, the medium was poured (20 ml/plate) into sterile petri dishes, left at room temperature to solidify, and incubated overnight at 37°C to ensure sterility. The plates were dried at 50°C for 30 minutes before use. Microbial cultures used in the study were obtained in lyophilized form. Aseptic techniques were employed to inoculate lyophilized cultures in sterile nutrient broth, followed by incubation at 37°C for 24 hours. After incubation, the growth was observed in the form of turbidity. These broth cultures were then inoculated onto nutrient agar plates with a loop full of microbes and further incubated for 24 hours at 37°C to obtain pure cultures, stored as stocks for future research. The antimicrobial activity of the copper nanoparticle gel was determined using the well diffusion method. Three concentrations (25, 50, and 100 mg/ml) of extracted phytochemical were used. Wells containing antibiotics were placed on the agar surface immediately after inoculation with the test organism. Undiluted overnight broth cultures were avoided as inoculums. The plates were then incubated at 37°C for 24 hours and examined for clear zones of inhibition around the wells with specific concentrations of the drug, following the standard procedure by Bauer *et al.* (1966).

#### RESULTS AND DISCUSSION

The present study focused on the formulation and characterization of copper nanoparticles of *Curcuma zedoaria* and their incorporation into a topical gel for enhanced antifungal and antimicrobial activity. The results obtained from extraction, phytochemical screening, nanoparticle formulation, characterization,

and biological evaluation provide significant insights into the effectiveness of the developed system.

The percentage yield of extracts revealed that the hydroalcoholic extract (8.92% w/w) showed a significantly higher yield compared to the petroleum ether extract (1.37% w/w). This indicates that hydroalcoholic solvents are more efficient in extracting polar and semi-polar phytoconstituents such as flavonoids, phenolics, and saponins. These compounds are known for their antimicrobial and antioxidant properties, which play a crucial role in therapeutic efficacy.

Phytochemical screening of the hydroalcoholic extract confirmed the presence of important bioactive constituents including flavonoids, phenolics, saponins, and diterpenes, while alkaloids, proteins, and tannins were mostly absent. The presence of flavonoids and phenolic compounds was further supported by quantitative estimation, showing total flavonoid content of 0.92 mg/100 mg and total phenolic content of 0.87 mg/100 mg. These phytoconstituents are known to act as reducing and stabilizing agents during the green synthesis of nanoparticles and also contribute to antimicrobial activity.

The preparation of copper nanoparticles showed satisfactory percentage yield across all formulations, with formulation F3 exhibiting the highest yield ( $80.62 \pm 0.22\%$ ). This suggests optimized conditions for nanoparticle synthesis in F3. Similarly, the entrapment efficiency was also highest in F3 ( $0.728 \pm 0.024$  mg/100 mg), indicating better incorporation of bioactive constituents within the nanoparticle matrix. This enhanced

entrapment may lead to improved stability and sustained release of active compounds.

Characterization studies revealed that the optimized formulation F3 exhibited an average particle size of 157.2 nm, which lies within the desirable nanorange, facilitating better skin penetration and enhanced therapeutic effect. The zeta potential value of -40.26 mV indicates good stability of the nanoparticle system due to sufficient electrostatic repulsion preventing aggregation. The physical evaluation of gel formulations demonstrated that F3 possessed superior characteristics in terms of color uniformity, homogeneity, texture, washability, and extrudability compared to other formulations. These properties are essential for patient compliance and effective topical application. The spreadability and viscosity studies indicated that F3 had moderate viscosity ( $3185 \pm 14$  cp) and acceptable spreadability, ensuring ease of application and proper distribution over the skin surface. The pH of all formulations was found to be near skin pH (6.8–6.95), indicating compatibility and minimal risk of irritation.

In vitro drug release studies showed that formulation F2 exhibited the highest cumulative drug release (98.78% at 4 hours), followed closely by F1 and F3. The faster release from F2 may be attributed to its lower viscosity and better diffusion characteristics. Release kinetics analysis indicated that F2 followed zero-order kinetics ( $R^2 = 0.9325$ ), suggesting a controlled and sustained drug release profile, which is desirable for topical formulations.

The antimicrobial activity results demonstrated that the copper nanoparticle gel (F2) exhibited significantly enhanced activity

compared to the plain extract against both *Streptococcus mutans* and *Escherichia coli*. The increase in the zone of inhibition with increasing concentration confirms dose-dependent activity. Notably, the nanoparticle formulation showed superior antimicrobial efficacy, which can be attributed to the synergistic effect of copper nanoparticles and phytoconstituents of *Curcuma zedoaria*. The nanoparticles enhance penetration into microbial cells, disrupt cell membranes, and

induce oxidative stress, leading to improved antimicrobial action.

The study confirms that the formulation of copper nanoparticles using *Curcuma zedoaria* extract significantly enhances the physicochemical properties, drug release behavior, and antimicrobial activity. Among all formulations, F3 was found to be optimal in terms of nanoparticle characteristics and gel properties, while F2 showed superior drug release and antimicrobial performance.

**Table 3: % Yield of extract of *Curcuma zedoaria***

| S. No. | Extract        | % Yield (w/w) |
|--------|----------------|---------------|
| 1.     | Pet ether      | 1.37          |
| 2.     | Hydroalcoholic | 8.92          |

**Table 4: Phytochemical screening of extract of *Curcuma zedoaria***

| S. No. | Constituents  | Hydroalcoholic extract   |
|--------|---|--------------------------|
| 1.     | <b>Alkaloids</b><br>Mayer's Test<br>Wagner's Test<br>Dragendroff's Test<br>Hager's Test | -ve<br>-ve<br>-ve<br>+ve |
| 2.     | <b>Glycosides</b><br>Legal's Test   | -ve                      |
| 3.     | <b>Flavonoids</b><br>Lead acetate<br>Alkaline test                                      | +ve<br>+ve               |
| 4.     | <b>Phenol</b><br>Ferric chloride test   | +ve                      |
| 5.     | <b>Proteins</b><br>Xanthoproteic test   | -ve                      |
| 6.     | <b>Carbohydrates</b><br>Molisch's Test<br>Benedict's Test<br>Fehling's Test             | -ve<br>-ve<br>+ve        |
| 7.     | <b>Saponins</b><br>Froth Test<br>Foam Test  | +ve<br>+ve               |
| 8.     | <b>Diterpenes</b><br>Copper acetate test  | +ve                      |
| 9.     | <b>Tannins</b><br>Gelatin Test  | -ve                      |

[+ve= positive; -ve= negative]

**Table 5: Estimation of total flavonoids and phenol content of *Curcuma zedoaria***

| S. No. | Extract        | Total flavonoids content (mg/ 100 mg of dried extract) | Total phenol content (mg/ 100 mg of dried extract) |
|--------|----------------|--|--|
| 1.     | Hydroalcoholic | 0.92   | 0.87   |

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

| Formulation Code | % Yield      |
|------------------|--------------|
| F1               | 66.12 ± 0.28 |
| F2               | 71.45 ± 0.30 |
| F3               | 80.62 ± 0.22 |
| F4               | 72.08 ± 0.25 |
| F5               | 70.14 ± 0.20 |
| F6               | 68.36 ± 0.24 |

**Table 7: Determination of entrapment efficiency of prepared formulations**

| Formulation code | Entrapment efficiency (Flavonoid mg/100mg quercetin equivalent) |
|------------------|---|
| F1               | 0.662 ± 0.028   |
| F2               | 0.694 ± 0.030   |
| F3               | 0.728 ± 0.024   |
| F4               | 0.658 ± 0.031   |
| F5               | 0.635 ± 0.034   |
| F6               | 0.682 ± 0.027   |

**Table 8: Characterization of particle size and zeta potential of optimized formulation F3**

| Formulation code | Average Particle size (nm) | Zeta Potential (mV) |
|------------------|----------------------------|---------------------|
| F3               | 157.2                      | - 40.26mV           |

**Table 9: Results of physical characteristics**

| Formulation Code | Colour      | Clogging | Homogeneity | Texture | Washability | Extrudability |
|------------------|-------------|----------|-------------|---------|-------------|---------------|
| F1               | Dark brown  | Absent   | Fair        | Smooth  | Good        | Fair          |
| F2               | Brown       | Absent   | Good        | Smooth  | Good        | Good          |
| F3               | Light brown | Absent   | Excellent   | Smooth  | Excellent   | Excellent     |

**Table 10: Results of spreadability of gel**

| Formulation Code | Spreadability* ( $g \cdot cm/sec$ ) | Viscosity* ( $cp$ ) | Flavonoid Content (mg/100 mg) | pH              |
|------------------|-------------------------------------|---------------------|-------------------------------|-----------------|
| F1               | $11.08 \pm 0.35$                    | $3480 \pm 12$       | $0.628 \pm 0.016$             | $6.85 \pm 0.02$ |
| F2               | $9.92 \pm 0.28$                     | $3325 \pm 16$       | $0.768 \pm 0.020$             | $6.80 \pm 0.01$ |
| F3               | $7.12 \pm 0.18$                     | $3185 \pm 14$       | $0.642 \pm 0.015$             | $6.95 \pm 0.02$ |

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

**Table 11: *In vitro* drug release study of prepared gel formulation**

| S. No. | Time (hr) | % Cumulative Drug Release |       |       |
|--------|-----------|---------------------------|-------|-------|
|        |           | F1                        | F2    | F3    |
| 1      | 0.25      | 34.45                     | 31.15 | 29.98 |
| 2      | 0.5       | 46.65                     | 43.32 | 30.25 |
| 3      | 1         | 59.98                     | 55.45 | 38.85 |
| 4      | 1.5       | 66.32                     | 64.45 | 46.65 |
| 5      | 2         | 86.65                     | 76.65 | 59.96 |
| 6      | 2.5       | 94.65                     | 88.85 | 69.95 |
| 7      | 3         | 98.12                     | 96.32 | 76.65 |
| 8      | 4         | 98.85                     | 98.78 | 86.65 |

**Table 12: Release kinetics regression values of formulation F2**

| Formulation code | Zero order | First order |
|------------------|------------|-------------|
| F2               | 0.9325     | 0.7451      |

**Table 13: Antimicrobial activity against selected microbes**

| S. No. | Microbes         | Zone of Inhibition (mm) |                 |                 | Zone of Inhibition (mm)       |                 |                 |
|--------|------------------|-------------------------|-----------------|-----------------|-------------------------------|-----------------|-----------------|
|        |                  | Extract                 |                 |                 | Copper Nanoparticles Gel (F2) |                 |                 |
|        |                  | 25 mg/ml                | 50 mg/ml        | 100 mg/ml       | 25 mg/ml                      | 50 mg/ml        | 100 mg/ml       |
| 1      | <i>S. mutans</i> | $7.8 \pm 0.45$          | $10.1 \pm 0.40$ | $11.8 \pm 0.42$ | $9.9 \pm 0.20$                | $12.0 \pm 0.60$ | $13.5 \pm 0.55$ |
| 2      | <i>E. coli</i>   | $10.0 \pm 0.90$         | $12.0 \pm 0.85$ | $13.2 \pm 0.50$ | $10.8 \pm 0.50$               | $12.8 \pm 0.80$ | $15.5 \pm 0.30$ |

## CONCLUSION

The study successfully developed copper nanoparticles of *Curcuma zedoaria* using a green synthesis approach and incorporated them into a topical gel. The hydroalcoholic

extract showed good phytochemical content, which aided in nanoparticle formation and stability. Among the formulations, F3 exhibited optimal characteristics, while F2 showed maximum drug release and antimicrobial activity. The nanoparticle gel

demonstrated enhanced stability, controlled drug release, and improved antimicrobial efficacy, indicating its potential as an effective topical treatment for fungal infections.

#### DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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