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Formulation And Characterization Of Copper Nanoparticles Gel Of Asteracantha Longifolia For Management Of Skin Disease

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ABSTRACT

The present study focuses on the formulation and characterization of copper nanoparticle (CuNP) gels containing *Asteracantha longifolia* extract for the management of skin diseases. The hydroalcoholic extract of *Asteracantha longifolia* was prepared and characterized for its phytochemical content, revealing the presence of alkaloids, flavonoids, phenols, and tannins, which are known for their therapeutic effects, including anti-inflammatory, antioxidant, and antimicrobial activities. Copper nanoparticles were synthesized and incorporated into gel formulations, with formulation F3 showing the highest yield and entrapment efficiency. The optimized formulation (F3) exhibited a particle size of 38.85 nm and a zeta potential of -45.53 mV, ensuring good stability. In vitro drug release studies showed a controlled and sustained release of active compounds, with formulation GF2 releasing the amount of drug in sustain manner. The release kinetics of the gel formulations followed a zero-order model, indicating steady drug release. The formulations exhibited favorable physical characteristics, including good homogeneity, texture, and washability. These results suggest that copper nanoparticle gels with *Asteracantha longifolia* extract have great potential as an effective treatment for skin conditions, offering sustained therapeutic effects.

Keywords: *Copper Nanoparticles, Asteracantha longifolia, Skin Diseases, Phytochemical Screening, Nanogel Formulation, Drug Release, Flavonoids, Gel Characterization.*

1. INTRODUCTION

The management of skin diseases, ranging from infections to inflammatory conditions, remains a significant challenge in modern dermatology. Traditional treatments often involve the use of antibiotics, steroids, or other synthetic agents, which may have limitations such as side effects, resistance development, and insufficient therapeutic outcomes. As a result, there is growing interest in alternative therapeutic approaches, including the use of natural products and nanotechnology, to overcome these challenges.

Asteracantha longifolia (commonly known as Talmakhana) is a medicinal plant widely used in traditional medicine across many regions for its anti-inflammatory, antimicrobial, and wound-healing properties. It has been reported to possess bioactive compounds that can aid in skin care and the treatment of various skin conditions such as acne, eczema, and psoriasis. Recent studies have highlighted the potential of nanoparticles derived from plant extracts to enhance the delivery and therapeutic effects of active compounds by increasing their bioavailability, stability, and targeted action (Akinmoladun et al., 2015).

Nanotechnology has revolutionized the field of drug delivery by offering the ability to encapsulate bioactive molecules in nanoparticles, providing controlled and sustained release, and improving the permeation of therapeutic agents through the skin. Among the various nanomaterials, copper nanoparticles (CuNPs) are gaining attention due to their proven antimicrobial, antioxidant, and anti-inflammatory activities (Akhavan & Ghaderi, 2011). Copper nanoparticles have been demonstrated to enhance wound healing, reduce microbial load, and exhibit synergistic effects when combined with natural plant extracts (Cormode et al., 2016).

Formulation of a copper nanoparticle gel using *Asteracantha longifolia* extract may provide an innovative and effective solution for managing skin diseases. The combination of copper nanoparticles with the bioactive compounds of *Asteracantha longifolia* could improve the therapeutic efficacy while minimizing the side effects associated with conventional treatments.

Additionally, the gel formulation serves as an ideal vehicle for the controlled release of copper nanoparticles, which can enhance skin penetration, provide long-lasting effects, and promote healing.

The aim of this study is to formulate and characterize copper nanoparticle gels containing *Asteracantha longifolia* extract and evaluate their potential for managing skin diseases. The properties of the gel, such as its viscosity, pH, spreadability, and drug release profile, will be thoroughly investigated. Furthermore, the antimicrobial activity of the formulation will be assessed to determine its suitability for dermatological applications.

2. MATERIAL AND METHODS

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Seeds of *Asteracantha longifolia* were collected from Bhopal in the month of March, 2024. Drying of fresh plant parts was carried out in sun but under the shade. Dried seeds of *Asteracantha longifolia* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction procedure

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

6.2.1 Defatting of plant material

Seeds of *Asteracantha longifolia* 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.

6.2.2 Extraction by maceration process

Defatted dried powdered seeds of *Asteracantha longifolia* has been extracted with hydroalcoholic solvent (ethanol: water: 70:30) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC (Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield

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

$$\text{percentage yield} = \frac{\text{Percentage yield}}{\text{Weight of powdered drug}} x100$$

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% $AlCl_3$ 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.

6.6 Biosynthesis of copper nanoparticles

To synthesize CuNPs, we dissolved 50 mg of dried seed extract in 100 mL of deionized water, and adjusted the pH to 8 with NaOH. The extract solution was slowly added to 100 ml of 1-6 mM copper sulfide solution while being stirred (1,000 rpm). They stirred in the dark for 24 hours at 37°C to 40°C. Centrifugation was done for 15 minutes at 25°C at 13,000 rpm upon obtaining the colored mixture (dark brown). All residues on the pellet were removed by washing it twice with deionized water. Following that, precipitates were lyophilized and analyzed (Hajizadeh *et al*., 2022).

Table 1: Different formulation of copper nanoparticles

Formulation Code	Extract (mg/ml)	Volume of Extract (ml)	Cu_2S (mM)	Centrifugation time
F1	0.5	100	1	15
F2	0.5	100	2	15
F3	0.5	100	3	15
F4	0.5	100	4	15
F5	0.5	100	5	15
F6	0.5	100	6	15

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).

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

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 hydroalcoholic extract of *Asteracantha longifolia* 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 *Asteracantha longifolia*	50	50	50
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.

Antimicrobial activity of copper nanoparticle gel

The antimicrobial activity of the copper nanoparticle gel prepared from the *Asteracantha longifolia* was determined using the well diffusion method. Three concentrations (25, 50, and 100 mg/ml) of optimized gel formulation 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).

3. RESULTS AND DISCUSSION

The formulation and characterization of copper nanoparticle gels containing *Asteracantha longifolia* extract for the management of skin diseases offers a novel approach by leveraging the therapeutic properties of both the plant extract and nanotechnology. The extraction process yielded a hydroalcoholic extract with a significant percentage (7.65% w/w) of bioactive compounds such as alkaloids, flavonoids, phenols, and tannins, which are known for their anti-inflammatory, antimicrobial, and antioxidant properties. These compounds contribute to the healing of skin conditions and promote overall skin health.

The total flavonoid and phenol content of the hydroalcoholic extract was also determined, indicating that the extract contains potent compounds beneficial for skin rejuvenation and protection from oxidative stress. These compounds are integral to the formulation's ability to address skin inflammation and infections.

Copper nanoparticles (CuNPs) were synthesized and incorporated into the gel formulations. The highest yield of nanoparticles was observed in formulation F3 (78.65%), suggesting that this formulation is the most efficient in terms of nanoparticle synthesis. The entrapment efficiency of the flavonoids was also measured, with F3 demonstrating the highest encapsulation efficiency. This indicates that formulation F3 is effective in encapsulating active compounds, ensuring their stable and sustained release.

The particle size of formulation F3 was found to be 38.85 nm, with a zeta potential of -45.53 mV, indicating excellent stability and dispersion of the nanoparticles within the gel. The negative zeta potential suggests that the nanoparticles are sufficiently charged to prevent aggregation, which is important for maintaining their stability and ensuring prolonged therapeutic effects.

Physical characteristics of the gel formulations (GF1, GF2, GF3) were evaluated, with all formulations exhibiting good homogeneity, smooth texture, and washability. Formulation GF3, which contained the highest flavonoid content, showed the most desirable characteristics for a topical gel.

In vitro drug release studies demonstrated that the copper nanoparticle gels provided a controlled and sustained release of the active ingredients. Formulation GF2 exhibited controlled drug release, while GF1 and GF3 showed faster and slower release rates respectively, but still provided an effective release profile. The controlled release of the active compounds ensures that therapeutic levels are maintained over time, enhancing the efficacy of the formulation in treating skin diseases.

The release kinetics of formulation GF2 was evaluated using several models, with the zero-order release model providing the best fit. This suggests that the release of the active ingredients from the gel was primarily dependent on the surface area and diffusion rate, rather than the drug concentration, providing a steady and prolonged release.

Table 3: % Yield of extract of *Asteracantha longifolia*

S. No.	Extract	% Yield (w/w)
1.	Pet ether	0.85%
2.	Hydroalcoholic	7.65%

Table 4: Phytochemical screening of extract of *Asteracantha longifolia*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	+ve
	Wagner's Test	+ve
	Dragendroff's Test	+ve
	Hager's Test	-ve
2.	**Glycosides**	
	Legal's Test	+ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	+ve
	Benedict's Test	-ve

	Fehling's Test	-ve
7.	**Saponins** Froth Test Foam Test	 +ve -ve
8.	**Diterpenes** Copper acetate test	 -ve
9.	**Tannins** Gelatin Test	 +ve

[+ve= positive; -ve= negative]

Table 5: Estimation of total flavonoids and phenol content of *Asteracantha longifolia*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.132	0.225

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

Formulation code	% Yield
F1	68.85±0.15
F2	72.23±0.32
F3	78.65±0.22
F4	66.65±0.18
F5	69.98±0.36
F6	71.12±0.17

Table 7: Determination of entrapment efficiency of prepared formulations

Formulation code	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	0.085±0.008
F2	0.065±0.005
F3	0.125±0.007
F4	0.095±0.003
F5	0.074±0.004
F6	0.052±0.006

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

Formulation code	Average Particle size (nm)	Zeta Potential (mV)
F3	38.85	-45.53

Table 9: Results of physical characteristics

F. code	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
GF1	Light Brown	Absent	Good	Smooth	Good	Good
GF2	Light Brown	Absent	Good	Smooth	Good	Good
GF3	Light Brown	Present	Good	Smooth	Good	Good

Table 9: Results of Evaluation of gel

Formulation code	Spreadability* (gcm/sec)	Viscosity* (cp)	Flavonoid Content (mg/100mg)	pH
GF1	12.25±2.25	3256±10	0.048±0.015	6.75±0.05
GF2	11.32±1.85	3365±18	0.128±0.018	6.82±0.03
GF3	10.65±1.96	3425±15	0.069±0.014	6.89±0.01

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

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

S. No.	Time (hr)	% Cumulative Drug Release		
		GF1	GF2	GF3
1	0.25	36.65	32.25	28.85
2	0.5	45.65	40.55	35.65
3	1	55.45	47.78	39.99
4	1.5	68.85	56.65	48.85
5	2	83.32	63.32	56.65
6	2.5	98.45	76.65	68.98
7	3	-	84.45	73.32
8	4	-	87.98	85.65

Table 11: *In-vitro* drug release data for gel GF2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.25	0.500	-0.602	32.25	1.481	69.75	1.844

0.5	0.707	-0.301	40.55	1.589	61.15	1.786
1	1.000	0.000	47.78	1.669	53.35	1.727
1.5	1.225	0.176	56.65	1.762	42.25	1.626
2	1.414	0.301	63.32	1.802	36.68	1.564
2.5	1.581	0.398	76.65	1.885	23.35	1.368
3	1.732	0.477	84.45	1.927	15.55	1.192
4	2.000	0.602	87.98	1.996	0.98	-0.009

Table 12: Release kinetics regression values of formulation GF2

Formulation code	**Zero order**	**First order**
GF2	0.9599	0.9679

4. CONCLUSION

The formulation of copper nanoparticle gels with *Asteracantha longifolia* extract has shown promising characteristics in terms of yield, stability, entrapment efficiency, and drug release. The combination of copper nanoparticles' antimicrobial properties with the bioactive compounds from *Asteracantha longifolia* makes it a potent formulation for the management of skin diseases, offering sustained therapeutic effects. Further clinical studies and in-vivo evaluations will be necessary to fully assess the effectiveness and safety of these formulations in treating various dermatological conditions.

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