Pharmacogn. Res., 2026; 18(2):404-414.
https://www.phcogres.com

Original Article

Green Synthesis of Zinc Oxide Nanoparticles Using *Withania somnifera* and *Centella asiatica* for Topical Herbal Skincare Applications

Nimisha Jain*, Akash Nayaka, Sarah Priyanka, Madhan M

Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bangalore, Karnataka, INDIA.

ABSTRACT

Background: Growing interest in herbal-based cosmetic products has encouraged the use of environmentally friendly nanotechnology for topical skincare applications. Zinc Oxide (ZnO) nanoparticles are widely used in skin formulations because of their antimicrobial, Ultraviolet (UV) protective, and anti-inflammatory properties; however, many conventional synthesis methods rely on hazardous chemicals. **Materials and Methods:** In the present study, ZnO nanoparticles were prepared using a green synthesis approach with aqueous extracts of *Withania somnifera* (Ashwagandha) and *Centella asiatica* (Gotu Kola), two medicinal plants known for their antioxidant, antibacterial, and wound-healing properties. The nanoparticles were synthesized by reacting zinc acetate dihydrate with the plant extracts, followed by controlled alkaline precipitation using sodium hydroxide. Characterization using UV-visible spectroscopy, Fourier-Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), particle size analysis, and zeta potential measurements confirmed successful nanoparticle formation, surface stability, and phytochemical capping. **Results:** High-Performance Liquid Chromatography (HPLC) analysis showed a maximum withanolide entrapment efficiency of 62.89%. *In vitro* release studies demonstrated a sustained release of bioactive compounds over an 8-hr period, supporting prolonged topical action. The prepared ZnO nanoparticles were incorporated into two topical cream formulations: a stearic acid-based emulsion and a beeswax-paraffin base. Both formulations showed desirable physicochemical characteristics, including skin-compatible pH (5.18-5.70), moderate viscosity (25,000-40,000 cps), good homogeneity, smooth texture, and stability under accelerated conditions. Antimicrobial testing against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans* revealed greater inhibitory activity for ZnO nanoparticle-loaded creams compared to formulations containing only the herbal extracts. **Conclusion:** Overall, the study demonstrates that combining green-synthesized ZnO nanoparticles with herbal bioactives offers a stable and promising approach for developing sustainable, nanotechnology-based skincare formulations.

Keywords: *Centella asiatica*, Green synthesis, Topical cream, *Withania somnifera*, Zinc oxide nanoparticles.

Correspondence:
Dr. Nimisha Jain
Department of Pharmaceutics,
Krupanidhi College of Pharmacy,
Bangalore, Karnataka, INDIA.
Email: nimijain@gmail.com

Received: 22-12-2025;
Revised: 19-01-2026;
Accepted: 09-03-2026.

INTRODUCTION

The skin is the largest organ of the human body and plays a vital role as the first line of defense against environmental pollutants, Ultraviolet (UV) radiation, and microbial pathogens. Continuous exposure to these external stressors can impair skin function, leading to infections, inflammation, acne, and premature aging. As a result, maintaining skin health has become a major focus within both the pharmaceutical and cosmetic industries. Topical delivery systems, particularly creams, are widely preferred due to their ease of application, ability to deliver active ingredients locally, and improved patient compliance. In addition to providing hydration and occlusion, creams serve as effective carriers for therapeutic agents such as antioxidants and antimicrobial compounds (James and Dubery, 2009; Mishra *et al*., 2000).

Recent advances in nanotechnology have significantly influenced the development of modern skincare formulations by enhancing the stability, penetration, and bioavailability of active ingredients. Among various nanomaterials, Zinc Oxide (ZnO) nanoparticles have attracted considerable attention because of their broad-spectrum antimicrobial activity, effective UV protection, anti-inflammatory properties, and favorable biocompatibility. However, conventional synthesis methods for ZnO nanoparticles often involve toxic chemicals and high energy requirements, raising concerns regarding environmental safety and potential skin toxicity. In contrast, green synthesis approaches using

DOI: 10.5530/pres.20260001

plant extracts offer a sustainable and environmentally friendly alternative. Phytochemicals present in plant extracts act as natural reducing and stabilizing agents, while also contributing additional biological functionality to the nanoparticles (Raghupathi *et al.*, 2011; Shukla *et al.*, 1999).

Withania somnifera (Ashwagandha) and *Centella asiatica* (Gotu Kola) are well-recognized medicinal plants widely used in traditional medicine systems. Ashwagandha contains withanolides, which exhibit notable antioxidant, anti-inflammatory, and antimicrobial activities (Zhang *et al.*, 2007). Similarly, *C. asiatica* is rich in triterpenoids such as asiaticoside and madecassoside, compounds known to promote wound healing, enhance collagen synthesis, and support skin regeneration. Beyond their therapeutic benefits, these plant extracts play a key role in the green synthesis process by facilitating nanoparticle formation and stabilization, thereby enhancing the biological performance of the final formulation (Babayevska *et al.*, 2022; Sharma *et al.*, 2020).

The antimicrobial activity of ZnO nanoparticles is attributed to multiple mechanisms, including the generation of Reactive Oxygen Species (ROS), disruption of microbial cell membranes, and the release of Zn^{2+} ions that interfere with essential enzymatic functions. These properties make ZnO nanoparticles particularly suitable for topical applications aimed at preventing infections, supporting wound healing, and protecting the skin barrier. When combined with herbal extracts, ZnO nanoparticles offer a synergistic approach that enhances formulation performance while aligning with the increasing demand for natural, safe, and sustainable skincare solutions (Padalia *et al.*, 2015).

The present study focuses on the green synthesis of ZnO nanoparticles using extracts of *Withania somnifera* and *Centella asiatica*, followed by their incorporation into an oil-in-water cream formulation. The developed formulations were evaluated for physicochemical properties, antimicrobial activity, spreadability, and stability. This work highlights a promising herbal nanotechnology-based approach for the development of effective and sustainable topical skincare formulations.

MATERIALS AND METHODS

Materials

Withania somnifera and *Centella asiatica* extracts were kindly provided by Kapiva Ayurveda (Bangalore, India). Sodium hydroxide pellets and zinc acetate were procured from NR Chemicals, Bangalore. Stearic acid, cetyl alcohol, glycerin, liquid paraffin, beeswax, borax, triethanolamine, and methyl paraben were of analytical grade and used as received without further purification.

Pre-formulation Studies

Pre-formulation studies were conducted to evaluate the organoleptic properties, solubility, and pH of *Withania somnifera* (Ashwagandha) and *Centella asiatica* extracts. These assessments were performed to evaluate the suitability of the extracts for green synthesis of zinc oxide nanoparticles and their incorporation into topical cream formulations.

Method Development for HPLC Analysis

A High-Performance Liquid Chromatography (HPLC) method was developed for the quantitative estimation of withanolides using a C18 column (4.6 × 250 mm) maintained at 27°C. The mobile phase consisted of phosphate buffer (prepared by dissolving 0.14 g of KH_2PO_4 and adding 1 mL of Orthophosphoric Acid (OPA) to 1000 mL of distilled water) and acetonitrile. Methanol was used as the diluent. The sample solution was prepared by dissolving 50 mg of *Withania somnifera* extract in methanol, while the standard solution was prepared using 5 mg of withanolide, followed by sonication and mild heating to ensure complete dissolution. Chromatographic analysis was carried out at a flow rate of 1.5 mL/min with an injection volume of 20 µL, and detection was performed at 227 nm.

Formula for Assay

$$\%\text{Assay} = \left(\frac{\text{sample area}}{\text{standard area}} \times \frac{\text{standard weight}}{\text{dilution}} \times \frac{\text{sample dilution}}{\text{sample weight}}\right) \times \text{potency}$$

Formulation of Zinc Oxide Nanoparticles

Zinc Oxide (ZnO) nanoparticles were initially synthesized by mixing 100 mL of a 0.1 M zinc acetate solution with varying amounts of *Centella asiatica* and *Withania somnifera* extracts. The reaction mixture was subjected to controlled alkaline precipitation by the dropwise addition of sodium hydroxide solution until a pH of 8 was achieved. The mixtures were continuously stirred at room temperature for 3 hr and subsequently centrifuged at 3000 rpm to separate the formed nanoparticles. The resulting light yellow precipitate was collected and dried at 60°C for 4-6 hr. Various formulation parameters, including plant extract ratios, sodium hydroxide concentration, and drying time, were refined to achieve maximum nanoparticle yield (Iqbal *et al.*, 2019; Singh *et al.*, 2018; Singh *et al.*, 2016).

Identification of ZnO Nanoparticles

The formation of ZnO nanoparticles was confirmed using UV-Visible spectrophotometry. A measured quantity of the dried nanoparticles was dispersed in deionized water and sonicated for 10 min to obtain a uniform suspension. The sample was scanned over a wavelength range of 200-800 nm using deionized water as the blank. The characteristic absorption peak corresponding to ZnO nanoparticles was recorded to confirm nanoparticle formation.

Zinc Oxide Nanoparticle Formulation

Based on preliminary studies, ZnO nanoparticles were synthesized using a 0.5 M zinc acetate solution. This solution was prepared by dissolving 27.44 g of zinc acetate dihydrate in 200 mL of distilled water and adjusting the final volume to 250 mL. A 2 M sodium hydroxide solution was prepared by dissolving 8 g of sodium hydroxide pellets in 70-80 mL of distilled water and making up the volume to 100 mL. For nanoparticle synthesis, 2.5 mL of the 0.5 M zinc acetate solution was mixed with 1, 1.5, or 2 g of each plant extract and stirred at room temperature for 2 hr. The sodium hydroxide solution was then added dropwise until the reaction mixture reached pH 8. The mixture was further stirred for 1 hr, during which a light yellow precipitate formed, indicating nanoparticle formation. The precipitate was collected by centrifugation at 3000 rpm, transferred to a Petri dish, and dried at 60°C for 6 hr.

Drug Loading Efficiency of ZnO Nanoparticles

Drug loading efficiency was defined as the ratio of withanolides associated with the ZnO nanoparticles to the total amount of withanolides used during formulation. The quantities of free (unloaded) and entrapped (loaded) withanolides were determined using High-Performance Liquid Chromatography (HPLC), a sensitive and selective analytical technique. Higher loading efficiency indicates effective incorporation of bioactive compounds into the nanoparticles, enabling reduced nanoparticle dosage while maintaining adequate therapeutic levels.

Formula for loading efficiency

$$\%\ \text{drug loaded} = \frac{\text{Practical }\%}{\text{Theoritical }\%} \times 100$$

In vitro Drug Release Studies of ZnO Nanoparticles

The *in vitro* release of withanolides from ZnO nanoparticles with high loading capacity was studied using the membrane diffusion method. The extract-loaded ZnO nanoparticles were placed in a dialysis membrane and immersed in 100 mL of Phosphate-Buffered Saline (PBS, pH 7.4) containing 10% v/v methanol as the receptor medium to maintain sink conditions. The receptor medium was maintained at 37°C under continuous stirring using a magnetic stirrer. At predetermined time intervals, 5 mL aliquots were withdrawn and replaced with an equal volume of fresh receptor medium to maintain constant volume. The collected samples were analyzed for withanolide content using High-Performance Liquid Chromatography (HPLC).

$$\%\ \text{drug released}(t) = \frac{\%\text{content present in the medium}}{\%\text{content present in formulation}} \times 100$$

FTIR STUDIES

Fourier-Transform Infrared (FTIR) spectroscopy was employed to analyze the chemical characteristics of ZnO nanoparticles and their compatibility with excipients used in the skincare cream formulation. FTIR spectra of the ZnO nanoparticles and ZnO nanoparticles incorporated with excipients were recorded using a Bruker ATR Alpha spectrophotometer at an ambient temperature of 25.0±0.5°C. The samples were placed directly on a Zinc Selenide (ZnSe) crystal plate, and spectra were recorded over the wavenumber range of 4000-400 cm^{-1}.

Particle Size Analysis and Zeta Potential Measurement

The average particle size of the prepared ZnO nanoparticles was determined using the Dynamic Light Scattering (DLS) technique. A small volume of the nanoparticle dispersion was diluted with double-distilled water to minimize multiple scattering effects. The diluted sample was transferred to a clean cuvette and analyzed using a particle size analyzer (Horiba SZ-100) at 25°C. The mean particle diameter was recorded in nanometers (nm), and the Polydispersity Index (PDI) was determined to assess the uniformity of particle size distribution. The surface charge of the ZnO nanoparticles was measured by zeta potential analysis based on Electrophoretic Light Scattering (ELS). The nanoparticle dispersion was diluted with double-distilled water in a 1:10 ratio to ensure appropriate conductivity and reduce signal interference. The diluted sample was loaded into a disposable zeta cell and analyzed using the same instrument (Horiba SZ-100) at 25°C after proper calibration. Zeta potential values were recorded in millivolts (mV).

Scanning Electron Microscopy (SEM) Analysis

The morphology and surface characteristics of the green-synthesized ZnO nanoparticles were examined using Scanning Electron Microscopy (SEM). For sample preparation, the synthesized nanoparticles were dispersed in methanol, an evaporating solvent, and a thin layer of the dispersion was placed onto a clean glass slide. The solvent was allowed to evaporate at room temperature, and excess liquid was removed using blotting paper prior to SEM analysis.

Formulation of Skincare Cream

Two topical cream formulations with different base compositions were prepared using the prepared ZnO nanoparticles exhibiting high withanolide entrapment efficiency. The formulations were designed to evaluate the influence of different cream bases on physicochemical properties, stability, and performance (Table 1).

Formulation 1: Stearic Acid-Based (Simple emulsion Type)

The cream was prepared by separately melting the oil phase, consisting of stearic acid and cetyl alcohol, and heating the aqueous phase containing glycerin, triethanolamine, and methyl paraben to 70°C. Zinc oxide nanoparticles were dispersed into the aqueous phase, after which the oil and aqueous phases were combined at 40°C under continuous stirring to form a uniform emulsion. Linseed oil and rose oil were subsequently incorporated, and the resulting cream was allowed to cool and stored at room temperature until further evaluation.

Formulation 2: Creamy Base with Emulsifying wax

The cream was prepared by separately heating the oil phase, consisting of beeswax and liquid paraffin, and the aqueous phase containing borax and methyl paraben to 70°C. Zinc oxide nanoparticles were dispersed into the aqueous phase, after which the oil and aqueous phases were combined at 40°C under continuous stirring to form a uniform emulsion. Linseed oil and rose oil were subsequently incorporated, and the prepared cream was allowed to cool and stored at room temperature until further evaluation.

Evaluation of Skincare Cream

Organoleptic Evaluation

The prepared cream formulations were evaluated for organoleptic properties, including color, odor, and physical state. The appearance of each formulation was assessed visually for color uniformity and surface smoothness and graded accordingly. Additional parameters such as homogeneity, texture, after-feel, type of smear, and ease of removal were also examined.

Spreadability Studies

The spreadability of the cream formulations was determined using two standard glass slides. A fixed amount of cream was placed on one slide and covered with a second slide. A weight of 140 g was applied uniformly over the slides to facilitate even spreading of the cream over a distance of 5 cm. Excess cream was removed, and the lower slide was fixed in position. A 30 g weight was then attached to the upper slide using a pulley system, and the time required for the slide to move a distance of 5 cm was recorded. Spreadability was calculated based on the recorded time, with shorter times indicating better spreadability.

$$\text{Spreadability} = \frac{M^{*}L}{T}$$

Where,

M = weight tied to the upper slide (30 g),

L = length of glass slide (5 cm),

T = time taken in seconds.

Homogeneity

The prepared cream formulations were evaluated for homogeneity by visual inspection and by touch to detect the presence of lumps, flocculates, or aggregates. Uniform appearance and smooth consistency indicated good homogeneity.

pH of the Cream

The pH of the cream formulations was measured using a calibrated pH meter. Prior to analysis, the pH meter was standardized using a suitable buffer solution. Approximately 0.5 g of the cream was dispersed in 50 mL of distilled water, and the pH of the resulting dispersion was recorded.

Viscosity

The viscosity of the cream formulations was determined using a Brookfield viscometer (DV-II+ Pro Model) equipped with an LV-64 spindle. The formulation was transferred into the viscometer adapter, and measurements were carried out by progressively increasing the rotational speed from 0.5 to 20 rpm. Viscosity values were recorded to assess the flow behavior and consistency of the formulations.

After-Feel

The after-feel of the cream was evaluated by applying a fixed quantity to the skin and assessing parameters such as emolliency, slipperiness, and the amount of residue remaining after application.

Ease of Removal

Ease of removal was assessed by washing the area of application with tap water after applying a fixed amount of cream and observing the extent to which the formulation could be removed without leaving residue.

Irritancy Test

An irritancy test was performed on the dorsal surface of the left hand. A 1 cm^2 area was marked, and the cream was applied to the site. The treated area was observed at regular intervals over a 24-h period for any signs of skin irritation, erythema, or edema.

Stability Testing

Stability studies were conducted in accordance with ICH guidelines. The cream formulations were stored in a stability chamber maintained at 40±2°C and 75±5% relative humidity for a period of three months. At the end of the study period, samples were evaluated for changes in physical appearance, homogeneity, and viscosity.

In vitro Diffusion Study

In vitro drug diffusion studies were carried out using a dialysis membrane method. 1 g of cream was placed in a dialysis

membrane and immersed in 100 mL of phosphate buffer (pH 7.4) contained in a 250 mL beaker. The diffusion medium was maintained at 37°C and stirred at 300 rpm using a magnetic stirrer. At predetermined time intervals, 5 mL aliquots were withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The samples were analyzed using High-Performance Liquid Chromatography (HPLC) to determine the percentage of drug released.

Antimicrobial Susceptibility Test

The antibacterial and antifungal activities of ZnO nanoparticles and cream formulations were evaluated using the agar well diffusion method. ZnO nanoparticles synthesized using *Withania somnifera* and *Centella asiatica* were dispersed in distilled water to prepare solutions of 1 mg/mL concentration. *Escherichia coli, Staphylococcus aureus*, and *Candida albicans* were cultured in Luria-Bertani (LB), Brain Heart Infusion (BHI), and Sabouraud Dextrose Broth (SDB) media, respectively, and incubated for 24-48 hr prior to testing. Sterile agar plates were prepared, and wells were punched aseptically and exposed to UV light for 5-10 min. Microbial cultures were spread evenly on the agar surface, and 50 µL of each test sample was introduced into the respective wells. The plates were incubated at 35°C for bacterial strains and 25°C for the fungal strain for 24-48 hr. Zones of inhibition were measured to assess antimicrobial activity. The following samples were evaluated: (1) ZnO nanoparticles of *Withania somnifera*, (2) ZnO nanoparticles of *Centella asiatica*, (3) Cream containing ZnO nanoparticles, and (4) Cream containing herbal extracts only.

RESULTS AND DISCUSSION

Pre-formulation Studies of Herbal Extracts

Pre-formulation evaluation of *Withania somnifera* and *Centella asiatica* extracts confirmed their suitability for topical application and nanoparticle synthesis. The extracts exhibited skin-compatible pH values of 5.72 and 6.34, respectively, indicating minimal risk of irritation upon application. Both extracts showed good solubility in water and other polar solvents but were insoluble in chloroform, supporting their incorporation into aqueous-based topical systems. However, their hydrophilic nature necessitates a suitable carrier system to improve stability and controlled delivery, which was achieved through zinc oxide nanoparticle encapsulation.

Green Synthesis and Identification of Zinc Oxide Nanoparticles

Green synthesis of ZnO nanoparticles was successfully carried out using aqueous extracts of *W. somnifera* and *C. asiatica* as reducing and stabilizing agents. Dropwise addition of sodium hydroxide to achieve pH 8 resulted in the formation of a characteristic whitish-brown precipitate, confirming nanoparticle formation. The precipitate was separated by centrifugation at 3000 rpm and dried at 60°C to obtain fine ZnO nanoparticle powder.

Table 1: Composition for Cream Formulation 1 and 2.

Sl. No.	Ingredients	Quantity for Formulation 1	Quantity for Formulation 2
1	ZnO NP's	1 g	1 g
2	Stearic acid	6 g	--
3	Cetyl alcohol	1.5 g	--
4	Glycerin	3 mL	--
5	Methyl paraben	0.1 g	--
6	Triethanolamine	0.75 g	--
7	Bees wax	--	6 g
8	Liquid paraffin	--	18 mL
9	Borax	--	0.3 g
10	water	35.1 mL	6 mL
11	Linseed oil	1 mL	1 mL
12	Rose water	Q.S	Q.S

Table 2: Drug loading efficiency of ZnO NPs.

Trial No	Theoretical %	Practical %	% Drug loaded
01	2.81	0.938	33.38
02	2.96	1.356	45.81
03	2.80	1.761	62.89

Formula for determination of withanolides in gm/mg $$ \text{Quantity of Withanolide present in ZnO nanoparticle} = \frac{\text{Practical} %}{100} \times \text{quantity of ZnO np}

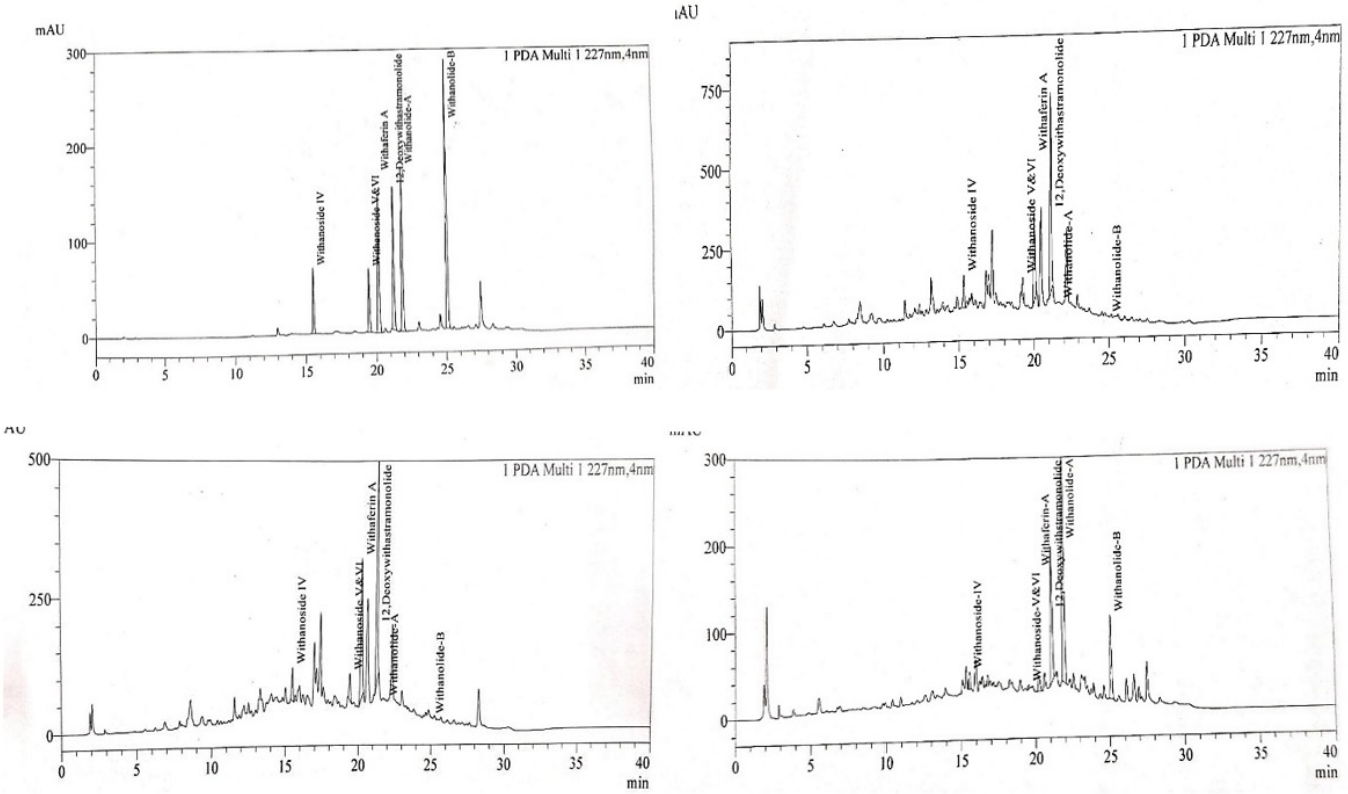

Figure 1: HPLC Chromatograms of i) Reference Standard (Withanolides) ii) Trial-1 iii) Trial 2 iv) Trial 3 [Chromatogram image showing peaks for Withanoside IV, Withanoside VandVI, Withaferin A, 12-Deoxywithastramonolide, Withanolide-A, Withanolide-B].

Table 3: ***In vitro*** **drug release profile in dissolution testing of nanoparticles and drug diffusion from the nanoparticles incorporated in the cream.**

Sl. No.	Time (mins)	% Withanolide content present in Formulation	Drug Dissolution from Pure Nanoparticles		Drug Diffusion from Nanoparticles in Cream	
			% Withanolide released	Withanolide released in mg	% Withanolide released	Withanolide released in mg
1	15	1.76	1.30±0.11	0.23	1.23±0.11	0.13
2	30	1.76	3.01±0.13	0.53	2.61±0.15	0.46
3	60	1.76	4.03±0.21	0.71	4.37±0.21	0.77
4	120	1.76	6.64±0.23	1.17	6.42±0.23	1.13
5	180	1.76	10.3±0.32	1.81	8.23±0.18	1.45
6	240	1.76	12.5±0.27	2.20	12.32±0.32	2.17
7	300	1.76	14.3±0.23	2.52	15.51±0.26	2.73
8	360	1.76	14.7±0.24	2.59	15.90±0.67	2.80
9	420	1.76	15.9±0.32	2.81	16.42±0.78	2.64
10	480	1.76	18.6±0.31	3.29	117.6±0.37	3.02

UV-visible spectroscopic analysis confirmed nanoparticle formation, with absorption peaks ranging between 364-374 nm for the preliminary trials. These absorption maxima correspond to band gap energies between 3.31-3.41 eV, consistent with nanosized ZnO particles. Among the trials, Trial 2 showed an optimal absorption peak at 364 nm, indicating effective nanoparticle formation. This confirms the successful synthesis and optical characteristics of ZnO nanoparticles.

Preparation of Zinc Oxide Nanoparticles

The amount of plant extract (1, 1.5, and 2 g) significantly influenced nanoparticle yield and intensity of precipitation. Higher extract concentrations produced more intense precipitates, indicating enhanced participation of phytochemicals in reduction and stabilization processes. This observation supports previous reports on plant-mediated green synthesis mechanisms (Iqbal *et al.*, 2019; Singh *et al.*, 2018; Singh *et al.*, 2016).

Drug Loading Efficiency of ZnO Nanoparticles

Drug loading efficiency of ZnO nanoparticles was quantified using HPLC analysis (Table 2). Three formulation trials demonstrated drug loading efficiencies of 33.38%, 45.81%, and 62.89%, respectively (Table 4). The increasing trend indicates improved encapsulation of withanolides with increasing extract concentration. The formulation (Trial 3) showed the highest loading efficiency (62.89%), confirming effective entrapment of bioactive compounds within the ZnO nanoparticle matrix. HPLC chromatograms of all trials are shown in Figure 1, validating consistent peak resolution and quantification. confirms successful phytoconstituent loading.

Optical Characterization by UV-visible Spectroscopy

The ZnO nanoparticles exhibited a prominent absorption peak at 359 nm in the UV-Visible spectrum. This corresponds to a calculated band gap energy of approximately 3.4 eV using Planck's equation, confirming nanoscale ZnO formation. The band gap value directly influences antimicrobial and UV-protective properties of ZnO nanoparticles (Sirelkhatim *et al.*, 2015).

In vitro drug release study of ZnO nanoparticle

In vitro release studies demonstrated sustained release of withanolides from ZnO nanoparticles over an 8-hr period. A total of 3.29 mg of withanolides was released from 17.6 mg of loaded drug (Table 3). The controlled release profile is attributed to strong binding of phytoconstituents within the ZnO matrix and their limited solubility in phosphate buffer. Sustained release is desirable for topical applications as it prolongs therapeutic action and reduces dosing frequency (Patil *et al.*, 2019; Rajendran *et al.*, 2010). Additionally, nanoparticle systems are known to improve skin retention and minimize systemic absorption (Kumar *et al.*, 2021).

FTIR studies: FTIR studies of ZnO NPs

The FTIR spectrum of the synthesized ZnO nanoparticles, recorded in the 4000-400 cm^{-1} range to observe the various functional groups involved in the preparation of the nanoparticles. As seen in the FTIR spectra, the research shows the characteristic absorption peak corresponding to Zn-O stretching vibrations at 539 cm^{-1} and is in agreement with known literature. The observation of functional groups, such as alkanes, alkenes, carbonyl, amide, carboxylic acid, and hydroxyl groups suggests the role of phytochemicals in reducing the zinc ions and further stabilizing the ZnO nanoparticles. Phytochemicals played a role in capping and preventing aggregation of the ZnO nanoparticles while observing different stretching bands in the various FTIR spectra (Gunalan *et al.*, 2012).

SEM, Particle Size, and Zeta Potential Analysis

SEM images revealed ZnO nanoparticles with flake- and rod-like morphology and slightly rough surfaces (Figure 2). Some degree of aggregation was observed, which is common in green-synthesized nanoparticles. Zeta potential analysis showed a surface charge of -25.6 mV (Figure 3), indicating moderate colloidal stability due to electrostatic repulsion. Particle size analysis confirmed nanoscale dimensions with uniform distribution (Figure 3). Similar stability profiles have been reported for green-synthesized ZnO nanoparticles (Sharma *et al.*, 2020).

Formulation and Evaluation of Skincare Cream

ZnO nanoparticle-loaded creams were successfully formulated using two bases: a stearic acid-based emulsion (Formulation 1) and a beeswax-paraffin base (Formulation 2). Both formulations exhibited good homogeneity, smooth texture, and no phase separation. Evaluation studies showed skin-compatible pH (5.18-5.70), moderate viscosity (25,000-40,000 cps), good spreadability, pleasant after-feel, ease of removal, and absence of skin irritation. Stability studies confirmed no significant changes after storage under accelerated conditions. Based on overall performance, Formulation 1 was selected for further *in vitro* diffusion and antimicrobial studies due to its lighter texture and superior spreadability.

In vitro Diffusion Study

The *in vitro* diffusion study was performed to evaluate the release behavior of bioactive constituents from the ZnO nanoparticle-loaded cream formulation. The diffusion profile demonstrated a gradual and sustained release of withanolides over a period of 8 hr, indicating effective encapsulation of the phytoconstituents within the ZnO nanoparticle matrix and their uniform distribution in the cream base. An initial moderate

Table 4: Data for the zone of inhibition.

Sl. No.	Sample	Zone of inhibition (mm)		
		E. coli	*S. aureus*	*C. albicans*
1	ZnO NP's (Withania somnifera)	36	26	12
2	ZnO NP's (Centella asiatica)	35	25	10
3	Cream with ZnO NP's	33	24	09
4	Cream with herbal extract	09	07	08

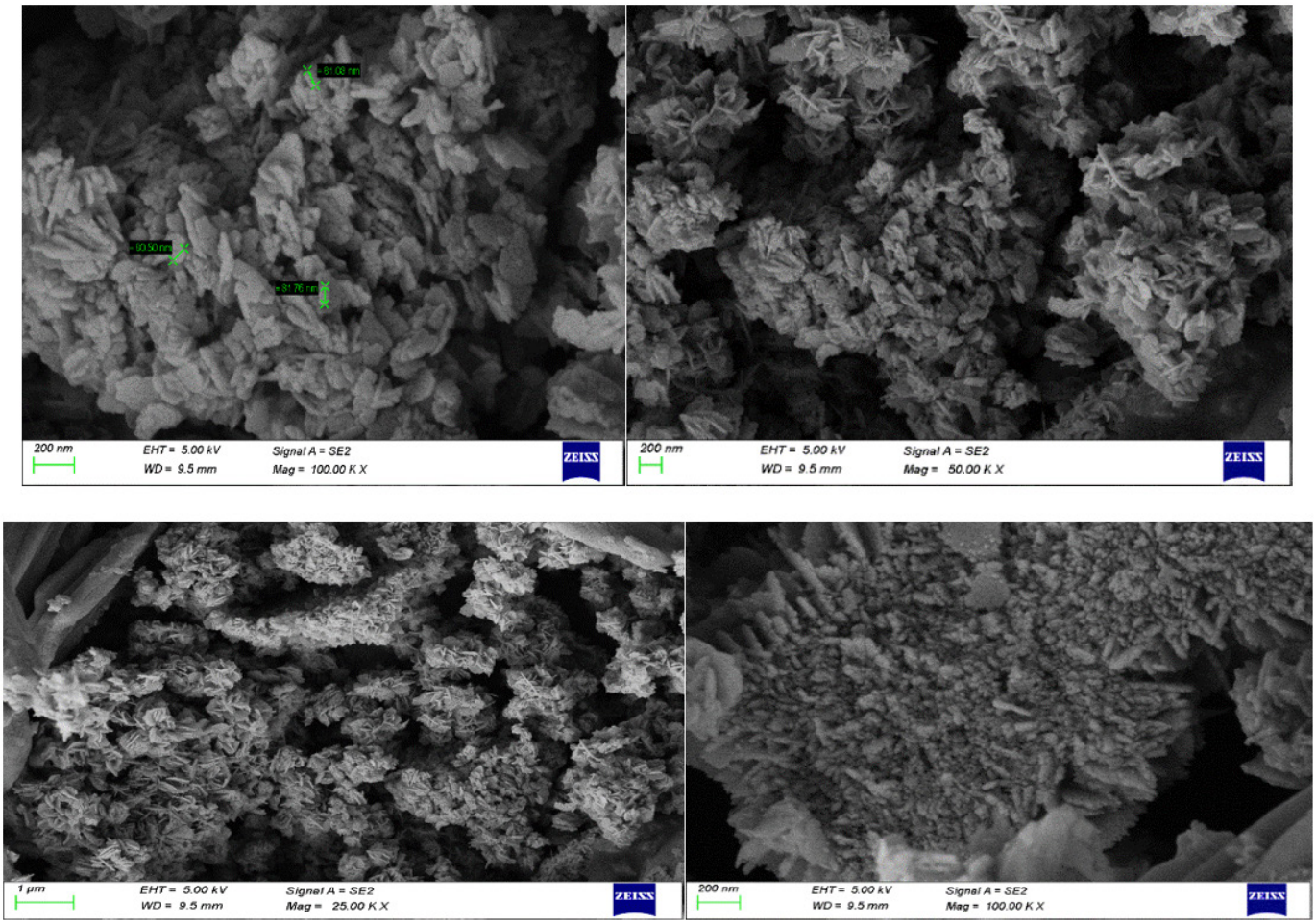

Figure 2: SEM images of ZnO nanoparticles.

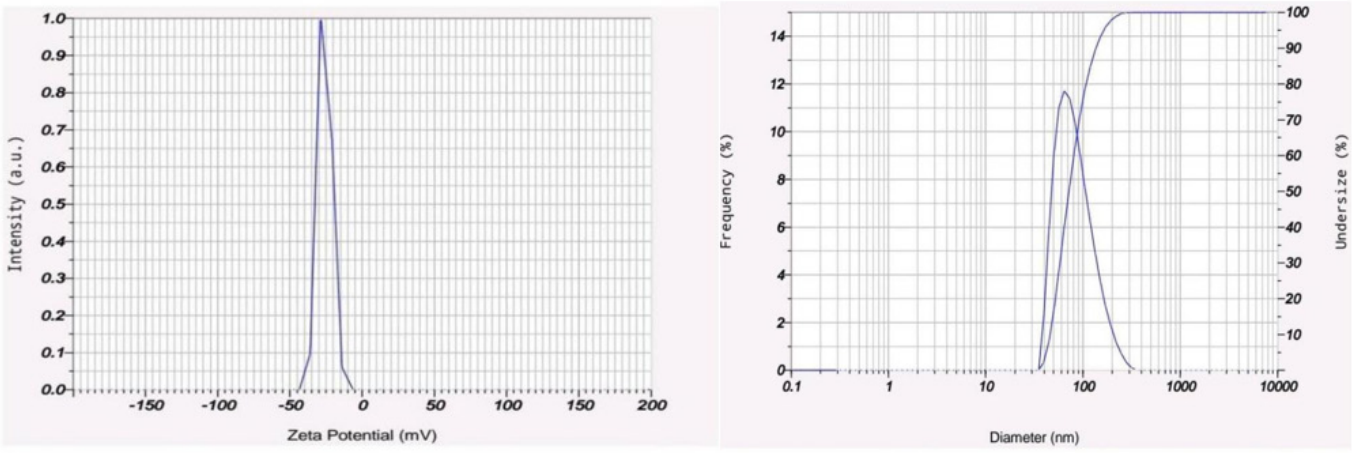

Figure 3: Zeta potential of ZnO NPs and Particle size of ZnO NPs. Sample 1 - ZnO NP's (*Withania somnifera*) Sample 2 - ZnO NP's (*Centella asiatica*)

release observed during the early time points may be attributed to the diffusion of surface-associated phytochemicals, followed by a controlled release phase governed primarily by diffusion from the nanoparticle core. The sustained release pattern suggests strong interaction between the phytochemicals and the ZnO nanoparticles, likely resulting from phytochemical capping during the green synthesis process. In addition, the viscosity and structural properties of the cream base contributed to slower diffusion by creating a barrier to rapid drug migration into the receptor medium. The absence of a pronounced burst release indicates formulation stability and uniform drug distribution, which are desirable characteristics for topical delivery systems. Overall, the *in vitro* diffusion results confirm that incorporation of green-synthesized ZnO nanoparticles effectively modulates

the release of herbal bioactives from the cream formulation. The controlled release behavior supports the suitability of ZnO nanoparticles as carrier systems for topical applications, offering prolonged availability of active constituents and improved formulation performance when compared to conventional herbal creams.

Antimicrobial Activity

The antimicrobial activity of green-synthesized ZnO nanoparticles and their corresponding cream formulations was evaluated against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. The ZnO nanoparticle-based samples exhibited noticeably larger zones of inhibition compared to formulations containing only herbal extracts, indicating enhanced antimicrobial effectiveness (Table 4, Figure 4). This improvement suggests a synergistic

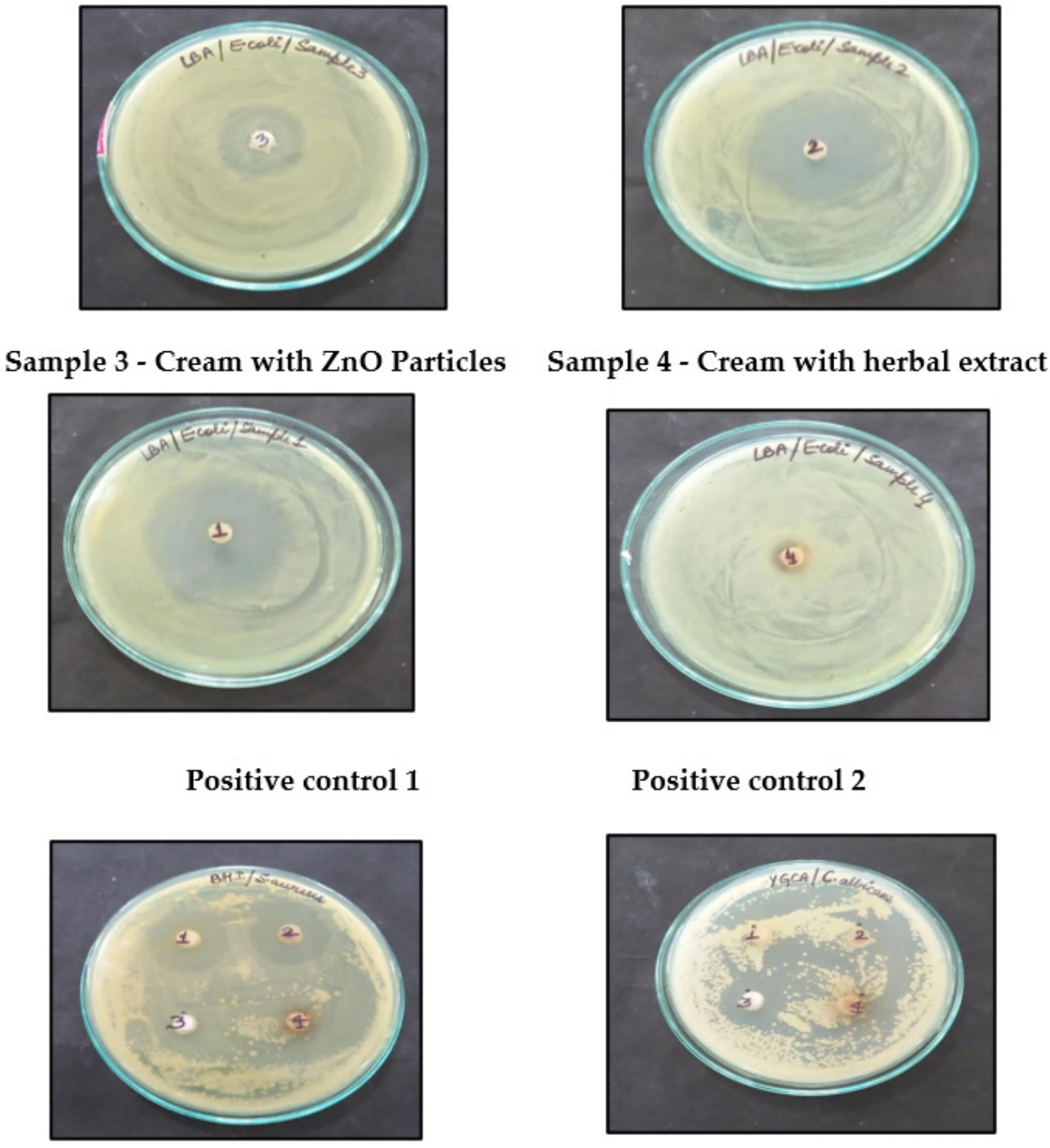

Figure 4: Antimicrobial Susceptibility Test of the given sample against the *E. coli, S. aureus,* and *C. albicans*.

interaction between ZnO nanoparticles and plant-derived phytoconstituents in suppressing microbial growth.

The superior antimicrobial performance of the ZnO nanoparticle-loaded formulations can be attributed to multiple complementary mechanisms. ZnO nanoparticles are known to generate reactive oxygen species, disrupt microbial cell membranes, and release Zn^{2+} ions, all of which interfere with essential cellular and metabolic functions. When combined with phytochemicals from *Withania somnifera* and *Centella asiatica*, which possess inherent antibacterial and antifungal properties, the nanoparticles exert a multi-target antimicrobial effect. Phytochemical capping during green synthesis is also likely to enhance nanoparticle stability and promote closer interaction with microbial cell surfaces, thereby improving antimicrobial activity.

Incorporation of ZnO nanoparticles into the cream base further contributed to sustained antimicrobial performance. The controlled release of bioactive constituents from the nanoparticle-loaded formulation ensured prolonged exposure of microorganisms to inhibitory concentrations. In contrast, creams containing only herbal extracts exhibited comparatively lower zones of inhibition, possibly due to rapid diffusion and reduced stability of the active constituents. The nanoparticle-based system therefore offers improved retention and gradual release of antimicrobial agents at the site of application.

Overall, the antimicrobial findings confirm that ZnO nanoparticle-loaded formulations provide superior microbial inhibition compared to conventional herbal formulations. These results support the potential of green-synthesized ZnO nanoparticles as

effective antimicrobial carriers for topical applications, offering enhanced efficacy through synergistic action and controlled delivery of herbal bioactives (Jayaseelan *et al*., 2012; Nagarajan and Kuppusamy, 2013; Padalia *et al*., 2015; Rajiv *et al*., 2013).

CONCLUSION

The present study successfully demonstrated the green synthesis of zinc oxide nanoparticles using aqueous extracts of *Withania somnifera* and *Centella asiatica*, highlighting an eco-friendly, sustainable, and chemically safe approach to nanoparticle fabrication. The use of plant-derived phytochemicals as natural reducing and stabilizing agents eliminated the need for hazardous chemicals, resulting in a biocompatible ZnO nanocarrier system well suited for topical applications.

The green-synthesized ZnO nanoparticles showed efficient encapsulation of phytoconstituents and were successfully incorporated into cream formulations with acceptable physicochemical properties, homogeneity, and storage stability. Notably, *in vitro* drug release studies demonstrated a controlled and sustained release of withanolides from the nanoparticle-loaded cream over an extended period. This release behavior confirms effective entrapment within the nanoparticle matrix and underscores the ability of the green-synthesized ZnO nanoparticles and cream base to regulate diffusion and maintain prolonged availability of bioactive compounds at the site of application, without an initial burst release.

Overall, the findings emphasize the dual advantage of green synthesis and nanotechnology in enhancing formulation performance, drug release control, and antimicrobial efficacy. This environmentally sustainable nanotechnological strategy offers a promising platform for the development of advanced herbal skincare and dermatological formulations. Further investigations, including extended stability studies and comprehensive biological evaluations, are warranted to support translational and commercial applications.

ABBREVIATIONS

API: Active Pharmaceutical Ingredient; **BHI:** Brain Heart Infusion; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **DLS:** Dynamic Light Scattering; **ELS:** Electrophoretic Light Scattering; **FTIR:** Fourier-Transform Infrared Spectroscopy; **HPLC:** High-Performance Liquid Chromatography; **IAEC:** Institutional Animal Ethics Committee; **LB:** Luria-Bertani; **NP:** Nanoparticles; **PDI:** Polydispersity Index; **ROS:** Reactive Oxygen Species; **SDB:** Sabouraud Dextrose Broth; **SEM:** Scanning Electron Microscopy; **UV:** Ultraviolet; **ZnO:** Zinc Oxide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

Financial Support: I Would like to express my sincere gratitude to Kapiva Ayurveda company, Bangalore for providing herbal drug extracts and also for HPLC analysis support and research access for completing my work.

AUTHORS CONTRIBUTION

Conceptualization: Nimisha Jain Data curation: Akash Nayaka M, Nimisha Jain Formal analysis: Akash Nayaka M, Madhan M Funding acquisition: Nimisha Jain Investigation: Nimisha Jain Methodology: Akash Nayaka M, Madhan M Project administration: Akash Nayaka M, Madhan M Resources: Nimisha Jain Supervision: Nimisha Jain Validation: Nimisha Jain Visualization: Nimisha Jain Writing-original draft: Pryanka Sarah, Madhan M Writing-review and editing: Nimisha Jain

SUMMARY

What is the current knowledge?

- Zinc oxide nanoparticles are frequently utilized in topical creams due to their effective protective and microbial properties.
- Many traditional synthesis processes utilize toxic chemicals, which pose potential toxicity risks for use on the skin, as well as general environmental concerns.
- The traditional plant medicines *Withania somnifera* and *Centella asiatica*, both commonly used cosmeceuticals, are demonstrated to have anti-inflammatory, antioxidant, and collagen stimulating properties.

What is new here?

- We demonstrate a novel green synthesis of nanoparticles from a combined extract of *W. somnifera* and *C. asiatica*, which act as reducing and capping agents, and is sustainable and eco-friendly.
- The resulting skin cream was physically stabilized, compatible with skin, and possessed good spreadability.
- Studies showed significant contributions of antioxidant capacity and antimicrobial activity of the green synthesized formulation compared to the base formulation, demonstrating the strong synergism of the two extracts.

REFERENCES

Azizi, S., Ahmad, M. B., Namvar, F., and Mohamad, R. (2014). Green biosynthesis and characterization of zinc oxide nanoparticles using *Coriandrum sativum* leaf extract. *Materials Letters*, *116*, 275-277.

Babayevska, N., Przysiecka, Ł., Iatsunskyi, I., Nowaczyk, G., Jarek, M., Janiszewska, E., and Jurga, S. (2022). ZnO size and shape effect on antibacterial activity and cytotoxicity profile. *Scientific Reports*, *12*, 1-13.

Gunalan, S., Sivaraj, R., and Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International*, *22*(6), 693-700.

Iqbal, J., Abbasi, B. A., Ahmad, R., Mahmood, T., Kanwal, S., Ali, B., Khalil, A. T., Shah, S. A., Alam, M. M., and Badshah, H. (2019). Green synthesis of ZnO nanoparticles using *Withania somnifera* extract: Characterization and evaluation of antimicrobial activity. *Applied Nanoscience*, *9*, 1367-1374.

James, J. T., and Dubery, I. A. (2009). Pentacyclic triterpenoids from *Centella asiatica*: Recent developments in extraction, analysis, and bioactivity. *Phytochemistry Reviews*, *8*, 61-77.

Jayaseelan, C., Ramkumar, R., Rahuman, A. A., and Perumal, P. (2012). Green synthesis of zinc oxide nanoparticles using *Ixora coccinea* leaf extract and its antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *96*, 1025-1030.

Kumar, R., Kumar, V., Singh, J., and Kumar, P. (2021). Green synthesis of zinc oxide nanoparticles using *Ocimum sanctum* leaf extract and their application in cosmetic formulations. *Materials Today: Proceedings*, *43*, 287-293.

Mishra, L. C., Singh, B. B., and Dagenais, S. (2000). Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. *Alternative Medicine Review*, *5*(4), 334-346.

Nagarajan, S., and Kuppusamy, K. A. (2013). Extracellular synthesis of zinc oxide nanoparticle using seaweeds of Gulf of Mannar, India. *Journal of Nanobiotechnology*, *11*, 39.

Padalia, H., Moteriya, P., and Chanda, S. (2015). Green synthesis of silver nanoparticles from marigold flower (*Tagetes erecta*) extract and their comparative antibacterial efficacy. *Journal of Bacteriology and Mycology: Open Access*, *2*(1), 1-7.

Patil, S. S., Deshmukh, P. K., and Patil, M. S. (2019). Solid lipid nanoparticles of *Withania somnifera* for topical delivery: Formulation and characterization. *Journal of Drug Delivery Science and Technology*, *52*, 642-649.

Raghupathi, K. R., Koodali, R. T., and Manna, A. C. (2011). Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of ZnO nanoparticles. *Langmuir*, *27*(7), 4020-4028.

Rajendran, R., Balakumar, C., Jayakumar, S., Vaideki, K., and Rajeshwari, S. (2010). Use of zinc oxide nanoparticles for antimicrobial finishing of cotton fabrics. *International Journal of Engineering Science and Technology*, *2*(3), 202-208.

Rajiv, P., Rajeshwari, S., and Venckatesh, R. (2013). Bio-fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant pathogens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *112*, 384-387.

Sharma, D., Rajput, J., Kaith, B. S., Kaur, M., and Sharma, S. (2020). Synthesis of ZnO nanoparticles using *Azadirachta indica* leaf extract and their characterization. *Journal of Alloys and Compounds*, *750*, 908-918.

Sharmila, G., Thirumarimurugan, M., and Muthukumaran, C. (2018). Green synthesis of ZnO nanoparticles using *Tecoma castanifolia* leaf extract: Characterization and evaluation of antimicrobial activity. *Materials Science in Semiconductor Processing*, *83*, 143-151.

Shukla, A., Rasik, A. M., and Dhawan, B. N. (1999). Asiaticoside-induced wound healing: Studies on the rat punch wound model. *Planta Medica*, *65*(3), 263-265.

Singh, B. N., Rawat, A. K. S., Bhagat, R. M., and Singh, B. R. (2018). Green synthesis of ZnO nanoparticles using *Azadirachta indica* leaf extract and their application in antimicrobial activity. *Applied Nanoscience*, *8*, 105-112.

Singh, P., Kim, Y. J., Zhang, D., and Yang, D. C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in Biotechnology*, *34*(7), 588-599.

Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., Hasan, H., and Mohamad, D. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters*, *7*(3), 219-242.

Vijayakumar, S., Krishnakumar, C., Vidhya, E., Arulmozhi, P., Mahadevan, S., and Praseetha, P. K. (2019). Green synthesis of zinc oxide nanoparticles using *Aloe barbadensis* miller leaf extract and evaluation of its antimicrobial activity. *Materials Science in Semiconductor Processing*, *103*, 104622.

Vijayakumar, S., Vaseeharan, B., Malaikozhundan, B., Shanthini, S., Thangaraj, M. P., Karthiyaini, T., Balasubramanian, S., and Gobi, N. (2018). Green synthesis of ZnO nanoparticles using *Atalantia monophylla* leaf extract: Antibacterial, antifungal and cytotoxic activity. *Microbial Pathogenesis*, *116*, 124-132.

Zhang, L., Jiang, Y., Ding, Y., Povey, M., and York, D. (2007). Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles. *Journal of Nanoparticle Research*, 9, 479-489.

Cite this article: Jain N, Nayaka A, Priyanka S, Madhan M. Green Synthesis of Zinc Oxide Nanoparticles Using *Withania somnifera* and *Centella asiatica* for Topical Herbal Skincare Applications. Pharmacog Res. 2026;18(2):404-14.