



Green-synthesized ZnO nanoparticles modulating viscoelastic and antibacterial behavior in alginate-PVA hydrogels

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Abstract

This study establishes a structure-surface-property framework for sodium alginate (SA)/polyvinyl alcohol (PVA) hydrogels incorporating zinc oxide nanoparticles (ZnO-NPs) synthesized via an ethanolic extract of *Cnidioscolus aconitifolius* (chaya). ZnO formation proceeds through alkaline precipitation, while phytochemical species from the extract act as growth-modulating and surface-stabilizing agents rather than primary reductants. X-ray diffraction confirms preservation of the wurtzite crystalline phase with reduced crystallite size (~9.2 nm), and X-ray photoelectron spectroscopy together with thermogravimetric analysis demonstrate distinct surface-associated organic signatures in extract-assisted nanoparticles compared to extract-free ZnO. To isolate interfacial effects, nanoparticles synthesized with and without extract were incorporated into an identical SA/PVA dual-network matrix. Rheological analysis reveals an order-of-magnitude increase in storage modulus and expansion of the linear viscoelastic region, indicating nanoparticle-induced modification of network relaxation dynamics through interfacial polymer confinement rather than simple filler inclusion. The composite system also exhibits concentration-dependent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. These findings demonstrate that solvent-controlled phytochemical mediation during ZnO synthesis produces measurable surface modifications that directly influence hydrogel mechanics, providing a mechanistically grounded platform for environmentally benign nanocomposite development.

Keywords Phytochemical-assisted synthesis · ZnO-NPs · Surface chemistry modification · Dual-network hydrogel · Viscoelastic reinforcement · Nanoparticle-polymer interactions · Antibacterial nanocomposite

1 Introduction

Hydrogels constitute a class of soft functional materials for wound-related and topical interfaces because their three-dimensional polymeric networks retain substantial water content, providing a moist and protective environment. This hydration, together with the ability of hydrogel matrices to host and release embedded agents, has led to extensive research on hydrogel-based systems for accelerating repair processes and mitigating infection-related complications [1–5]. In addition, hydrogels can swell rapidly upon contact with water, forming a semi-solid material that conforms to irregular surfaces and facilitates uniform coverage [6]. Within this broader landscape, incorporation of zinc oxide nanoparticles (ZnO-NPs) is frequently explored to introduce antibacterial functionality (typically associated with zinc ion release and reactive oxygen species generation) while also modifying the microstructure and mechanical

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response of the polymer network [7–9]. Furthermore, the green synthesis of ZnO-NPs using plant extracts improves their morphology and stability, providing a synergistic effect that maximizes their efficacy in both biomedical and environmental applications [10, 11]. Accordingly, hydrogel-ZnO composite systems provide a framework to examine how green-synthesized inorganic nanofillers influence coupled mechanical and antibacterial performance in polymeric matrices.

Cnidoscopus aconitifolius (chaya) has been widely reported in the literature as a source of phenolic compounds, flavonoids, and other secondary metabolites, which are often associated with antioxidant and anti-inflammatory activity. In green synthesis routes, such phytochemicals are commonly reported to function as reducing and stabilizing agents, while phenolic- and flavonoid-rich plant extracts are widely reported in green ZnO synthesis, most studies rely on aqueous extraction protocols that favor highly polar metabolites. In contrast, the present work employs an ethanolic extract of *Cnidoscopus aconitifolius*, which selectively solubilizes the less polar phytochemicals, including triterpenes and carbonyl-containing compounds that are typically underrepresented in aqueous systems. This solvent-controlled extraction modifies the Zn^{2+} coordination environment during nucleation and results in ZnO nanoparticles with distinct surface chemistry, as later confirmed by XPS and TGA analyses, without altering the wurtzite crystalline phase. Therefore, the contribution of this study is not the botanical identity of the extract per se, but the demonstration that solvent-driven phytochemical selectivity translates into measurable differences in nanoparticle surface composition and subsequent composite viscoelastic behavior, facilitating nanoparticle nucleation while simultaneously limiting uncontrolled growth and aggregation [12–15]. Beyond their role in nanoparticle formation, plant-derived compounds may remain partially associated with the nanoparticle surface, influencing interfacial interactions with surrounding polymer matrices. In this sense, the incorporation of chaya-derived ZnO-NPs into hydrogel systems provides an opportunity to examine how phytochemical-assisted synthesis affects nanoparticle-polymer interactions, mechanical reinforcement, and antibacterial behavior at the composite level, without invoking additional functionalization steps. This dual role of the extract (as a synthesis-enabling component and as a modifier of nanoparticle surface chemistry) aligns with the broader objective of developing environmentally benign routes toward multifunctional hydrogel-based materials [16–18].

Numerous studies have explored composite hydrogels based on sodium alginate and polyvinyl alcohol, as this polymer combination provides a balance between ionic crosslinking, hydrogen bonding, and mechanical flexibility.

In particular, the incorporation of inorganic nanoparticles such as TiO_2 , CuO, and ZnO has been extensively investigated as a strategy to tailor the structural, mechanical, and functional properties of alginate-PVA matrices [19–23]. Despite the extensive literature on sodium alginate-polyvinyl alcohol (SA/PVA)-based nanocomposites, many reported approaches rely on synthesis routes that involve surfactants, stabilizers, or post-synthetic chemical treatments that increase process complexity and environmental impact. Although the SA/PVA polymer pair is widely reported in biomedical hydrogel applications, it is deliberately employed here as a structurally well-defined dual-network matrix rather than as a novel formulation. The combination of ionic crosslinking (alginate- Ca^{2+} interactions) and hydrogen-bonded PVA networks provides a reproducible and compositionally stable framework. This controlled architecture allows direct comparison between nanoparticle-free (P0) and nanoparticle-containing (P1) systems under identical polymer conditions. Within this defined matrix, any observed change in viscoelastic behavior can therefore be attributed to nanoparticle-polymer interfacial interactions rather than to formulation variability. While ZnO-reinforced SA/PVA hydrogels have been previously described for antibacterial and wound-healing applications, most studies focus predominantly on biological performance metrics, with comparatively limited mechanistic analysis of how nanoparticle surface chemistry influences hydrogel network mechanics. In the present work, the polymer matrix and crosslinking protocol are deliberately maintained constant, while the nanoparticle surface chemistry is modulated through plant-assisted synthesis. This controlled strategy enables isolation of the mechanical contribution of ZnO incorporation and establishes a quantitative link between nanoparticle surface modification and viscoelastic reinforcement within the dual-network hydrogel system. In this context, the present study develops and characterizes an SA/PVA hydrogel incorporating ZnO nanoparticles synthesized via a plant-assisted route using *Cnidoscopus aconitifolius* extract. The objective is not to introduce a new botanical source or polymer formulation, but to establish a controlled material platform in which nanoparticle surface chemistry can be systematically correlated with hydrogel network mechanics. Two nanoparticle systems were deliberately prepared: conventionally synthesized ZnO (M0) and extract-assisted ZnO (M1), followed by incorporation into an identical SA/PVA dual-network matrix. This strategy isolates the effect of solvent-controlled phytochemical mediation on nanoparticle surface composition (XPS, FTIR, TGA) while preserving the wurtzite crystalline phase confirmed by XRD. The resulting surface modifications are directly correlated with measurable changes in viscoelastic behavior, including expansion of the linear viscoelastic region and

significant enhancement of storage modulus. Thus, the distinguishing feature of this work lies in demonstrating that modulation of the phytochemical environment during ZnO nucleation produces quantifiable differences in nanoparticle surface chemistry that translate into altered interfacial interactions and mechanical reinforcement within a controlled hydrogel network.

2 Materials and methods

2.1 Ethanolic extract

Cnidioscolus aconitifolius (Mill.) I. M. Johnst. leaves (commonly known as chaya) were collected at an altitude of 10 m above sea level (21°03'25.2"N, 89°39'56.8"W) on March 6, 2025. The plant material was taxonomically identified at the Herbarium “U najil tiki xiw” and deposited under voucher number 001. The extract was prepared following a procedure from literature [24].

2.2 Synthesis of ZnO-NPs

ZnO-NPs were synthesized using zinc acetate (CIVEQ, 98%) and sodium hydroxide (CTR-Scientific, 98%) as precursors in an ethanol-water solvent system (ethanol 99.5%,

deionized water 18 M Ω). Two nanoparticle systems were prepared: M0, corresponding to ZnO synthesized in the absence of plant extract, and M1, synthesized in the presence of the extract. A 40 mM solution of zinc acetate was prepared in ethanol and labeled as S1. Separately, NaOH (8 g) was dissolved in 200 mL of a 1:1 (v/v) ethanol-water mixture to obtain solution S2. The optimized synthesis pathway for nanoparticle formation is shown in Fig. 1.

2.3 Patch fabrication

2.3.1 Preparation of the gelling solution and sol-gel

Gelling solutions of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, Sigma-Aldrich) were prepared in deionized water (18 M Ω) at concentrations of 1.5 M and 3.0 M. Hydrogel matrices were fabricated using a sol-gel approach. Sodium alginate (1 g) and polyvinyl alcohol (1 g) were each dissolved separately in 25 mL of deionized water. Each polymer solution was subjected to alternating cycles of mechanical stirring at 500 rpm for 5 min and heating in a recirculating water bath at 80 °C for 5 min until complete dissolution and homogenization were achieved. The resulting alginate and PVA sols were then combined in a 1:1 volume ratio at room temperature to obtain the final sol-gel precursor used for hydrogel formation.

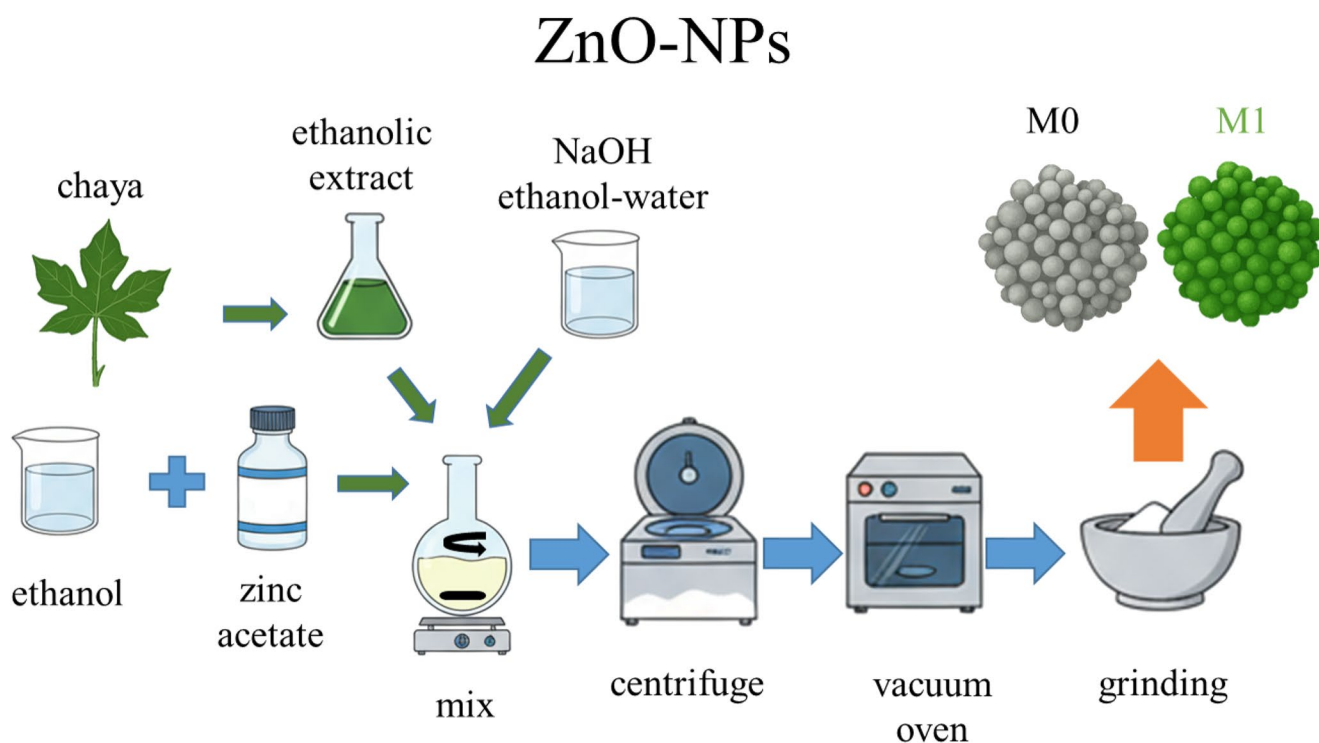


Fig. 1 Schematic representation of the optimized synthesis route for ZnO-NPs prepared without chaya extract (M0) and with ethanolic chaya extract (M1) through alkaline precipitation

2.3.2 Incorporation of the nanoparticles and patch formation

ZnO-NPs were incorporated into the sol-gel precursor through a dispersion step. An amount of ZnO-NPs corresponding to 0.5% (w/w) relative to 10 mL of sol-gel was dispersed in 2 mL of deionized water under mechanical stirring at 3000 rpm for 20 min. This dispersion was subsequently added to 10 mL of the sol-gel precursor and stirred under the same conditions for an additional 20 min to ensure homogeneous distribution of the nanoparticles.

Hydrogel patches with and without nanoparticles were fabricated using a sequential ionic gelation process. The sol-gel precursor, the 1.5 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution, and the 3.0 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solutions were combined in a 2:1:1 volume ratio. Initially, the 1.5 M gelling solution was poured into a circular mold, followed by dropwise addition of the sol-gel precursor at the center, allowing radial spreading. After 60 min of gelation, the partially formed patch was transferred to a second mold containing the 3.0 M gelling solution, with the previously exposed surface oriented upward. Gelation was allowed to proceed for an additional 60 min. Patches prepared without nanoparticles and with ZnO-NPs were designated P0 and P1, respectively.

2.4 Microstructural characterizations

The morphology and elemental composition of the zinc oxide nanoparticles were examined by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS). SEM micrographs were acquired using a JEOL JSM-7600 F microscope equipped with a low-angle backscattered electron (LBE) detector and operated at an accelerating voltage of 15 kV. Prior to imaging, samples were coated with a thin Au-Pd layer by sputtering for 30 s to minimize charging effects. X-ray photoelectron spectroscopy (XPS) measurements were performed using a K-Alpha Surface Analysis system (Thermo Fisher Scientific). Survey spectra were collected over a binding energy range of 0–1350 eV with a step size of 1 eV and a pass energy of 100 eV, while high-resolution spectra were acquired with a step size of 0.1 eV and a pass energy of 50 eV. Measurements were conducted on both sputtered and non-sputtered surfaces to assess surface composition and potential atmospheric contributions. Binding energies were calibrated using the C 1s peak at 284.5 eV (C=C). Peak deconvolution was performed using OriginPro 2021 software employing the GaussAmp fitting function.

Crystalline phases were identified by X-ray diffraction (XRD) using a Bruker D-8 Advance diffractometer operated at 40 kV and 30 mA with Cu $K\alpha$ radiation ($\lambda=0.154$ nm). Diffractograms were recorded with a step size of 0.02° and a

step time of 0.1 s. Fourier-transform infrared (FTIR) spectra were acquired using an Agilent Cary 630 FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. Spectra were recorded in the $4000\text{--}650\text{ cm}^{-1}$ range and used to analyze functional groups present in the extract, nanoparticles, and hydrogel samples.

2.5 Bacterial tests

The antibacterial activity of zinc oxide nanoparticles was evaluated using standard in vitro assays. Tests were conducted against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). Bacterial suspensions were prepared and adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Preliminary antibacterial screening was performed using the disk diffusion method on Mueller-Hinton agar plates. Sterile disks were placed on the inoculated agar surface, and 10 μL of ZnO-NP suspensions in sterile water were applied to each disk. Amikacin was used as a positive control, and sterile water was used as a negative control. Plates were incubated at 37°C for 24 h, after which inhibition zones were measured. The minimum inhibitory concentration (MIC) was determined using the broth microdilution method in Mueller-Hinton broth at double concentration. A 45 mg/mL ZnO-NP stock suspension was prepared, and aliquots of 2, 4, and 6 μL were added to a final assay volume of 600 μL , corresponding to final ZnO-NP concentrations of 143.3, 286.7, and 430.0 $\mu\text{g/mL}$, respectively. All assays were conducted under sterile conditions. To clarify the possible antibacterial contribution of the ZnO core, extract-derived surface species, and the polymeric matrix, additional disk-diffusion assays were performed using extract-free ZnO-NPs (M0), extract-assisted ZnO-NPs (M1), washed extract-assisted ZnO-NPs (M1-C), ethanolic chaya extract (EE), and the nanoparticle-free SA/PVA hydrogel matrix. M1-C corresponds to M1 nanoparticles washed with isopropanol/ethanol to partially remove weakly associated extract-derived species. For the comparative inhibition-zone assays, 450 μg per disk of each nanoparticle sample was used. Amikacin (10 $\mu\text{g/mL}$) and DMSO were used as positive and negative controls, respectively. The nanoparticle-free SA/PVA matrix was evaluated under the same disk-diffusion conditions to determine whether the polymeric matrix itself contributed to antibacterial activity.

2.6 Rheology

The rheological behavior of the hydrogel samples was characterized to evaluate their viscoelastic response under oscillatory and steady shear conditions. Circular specimens of hydrogel patches with and without ZnO-NP were analyzed

using a Kinexus Pro+ rotational rheometer (Malvern Instruments, UK) equipped with a Peltier temperature control system. Measurements were performed using a cone-plate geometry with a diameter of 40 mm, a cone angle of 4.0107° , and a gap of 0.1473 mm.

Dynamic mechanical analysis was conducted to obtain the complex shear modulus (G^*), defined as the ratio of applied shear stress to shear strain:

$$G^* = G' \sin(\omega t) + G'' \cos(\omega t)$$

The storage modulus (G') and loss modulus (G'') were determined as measures of the elastic and viscous components of the viscoelastic response, respectively, while the loss tangent ($\tan \delta = G''/G'$) was used to quantify the relative contribution of viscous dissipation [25].

Amplitude sweep tests were first performed at a fixed frequency of 1 Hz over an oscillatory strain range from 0.01% to 100% in order to identify the linear viscoelastic region (LVR). Subsequently, frequency sweep tests were carried out within the LVR at a constant strain of 0.1%, covering a frequency range from 0.1 to 10 Hz. In addition, flow ramp tests were conducted by applying shear stresses from 0 to 50 Pa to assess the response of the hydrogel samples under increasing load. All measurements were performed at controlled temperature conditions.

2.7 Thermogravimetric analysis

The thermal stability of the ZnO-NP was evaluated by thermogravimetric analysis (TGA). Measurements were carried out under an air atmosphere using a heating rate of $10^\circ\text{C min}^{-1}$ over a temperature range from 30 to 800°C .

3 Results and discussion

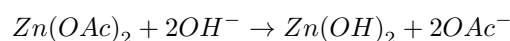
3.1 ZnO-NPs

To determine whether phytochemical mediation represents a simple botanical substitution or a material-level modification, two nanoparticle systems were synthesized under identical inorganic precursor conditions: M0 (without extract) and M1 (with ethanolic *chaya* extract). This controlled design isolates the effect of the phytochemical environment on ZnO nucleation and surface development. XPS and TGA analyses demonstrate that the extract-assisted route increases surface-associated organic content and modifies oxygen coordination states, while XRD confirms preservation of the wurtzite crystalline phase. These surface modifications are directly correlated with measurable differences in hydrogel viscoelastic behavior, including expansion of

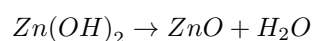
the linear viscoelastic region and a significant increase in storage modulus. Therefore, the conceptual contribution of this work lies in establishing a link between phytochemical-assisted surface modification and nanoparticle–polymer interfacial reinforcement. Whereas most reported green ZnO routes employ aqueous extracts enriched in highly polar metabolites, the present approach utilizes an ethanolic extract that alters the Zn^{2+} coordination environment during nucleation. The focus is thus on controlled surface chemistry modulation rather than botanical substitution.

3.1.1 Proposed formation mechanism

In the present system, ZnO formation proceeds through alkaline hydrolysis and precipitation of Zn^{2+} from zinc acetate upon NaOH addition, leading to $\text{Zn}(\text{OH})_2$ formation:



Subsequent dehydration under heating yields crystalline ZnO:



Thus, the process follows a hydroxide-induced precipitation pathway rather than a classical redox reduction mechanism. In the extract-assisted system (M1), phytochemical species present in the ethanolic *Cnidoscolus aconitifolius* extract interact with Zn^{2+} ions and/or adsorb onto early $\text{Zn}(\text{OH})_2/\text{ZnO}$ nuclei during nucleation and growth. These compounds are therefore interpreted as complexing, growth-modulating, and surface-stabilizing agents rather than primary reducing species. Phytochemical studies reported in the literature have identified oxygenated secondary metabolites, including the triterpenoid taraxerone, in ethanolic extracts of *Cnidoscolus aconitifolius*, as well as phenolic and flavonoid families, all containing functional groups capable of coordinating Zn^{2+} or binding to developing oxide surfaces [26, 27]. Consistent with this interpretation, XPS reveals changes in the oxygen-containing surface components and an additional Na contribution in M1, while TGA indicates increased organic-associated mass loss relative to M0. The smaller crystallite size observed for extract-assisted nanoparticles (9.2 nm vs. 10.4 nm) further supports partial surface adsorption that limits crystallite growth. Importantly, XRD confirms that these interfacial modifications occur without altering the final wurtzite crystalline phase. Overall, phytochemical mediation is therefore interpreted as regulating nucleation and early particle development through surface interaction mechanisms that confer a distinct interfacial chemistry to the resulting ZnO nanoparticles.

3.1.2 SEM-EDS analyses

To elucidate the effect of the plant-assisted synthesis route on nanoparticle morphology, aggregation behavior, and surface composition, two ZnO systems were compared: M0, synthesized without extract, and M1, synthesized in the presence of ethanolic chaya extract. This controlled comparison allows the influence of phytochemical mediation on ZnO particle development to be assessed independently of the hydrogel matrix. Representative SEM micrographs and particle/agglomerate size distribution histograms are shown in Fig. 2. Because both systems exhibit irregular morphology and evident aggregation, the SEM-derived values should be interpreted as the apparent size of visible particles/agglomerates rather than the size of primary crystallites. This distinction is important because XRD provides crystallite sizes

at the nanometric scale, whereas SEM and DLS reflect larger secondary structures generated by particle aggregation. For M0, the SEM images reveal predominantly submicrometric, irregularly shaped particles with a broad size distribution and a strong tendency to agglomerate. ImageJ analysis of 206 visible particles/agglomerates yielded an apparent mean size of 189.2 ± 127.0 nm, with a median value of 152.1 nm and a size range of 37.7–692.2 nm. In contrast, M1 exhibits a more heterogeneous morphology, including lamellar or flake-like structures with defined edges together with larger granular aggregates. ImageJ analysis of 131 visible particles/agglomerates yielded an apparent mean size of 460.7 ± 250.4 nm, with a median value of 392.6 nm and a size range of 125.5–1312.3 nm.

These results indicate that the ethanolic extract modifies ZnO particle growth and aggregation behavior. Although

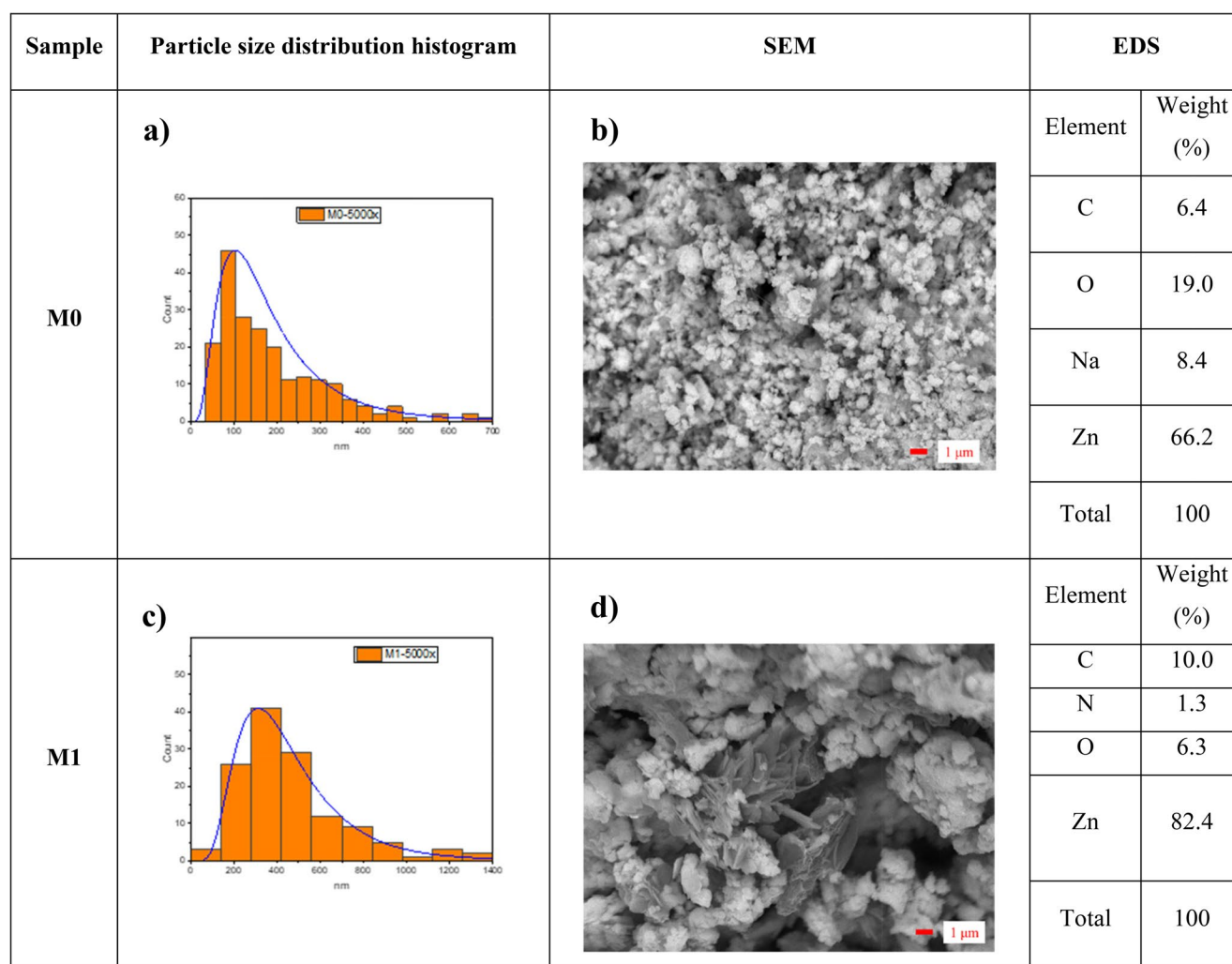


Fig. 2 SEM-based particle/agglomerate size distribution, representative SEM micrographs, and EDS elemental composition of ZnO-NPs synthesized without and with ethanolic chaya extract. **(a, c)** Particle/agglomerate size distribution histograms obtained from 5000x SEM micrographs using ImageJ software for M0 and M1, respectively. The apparent mean sizes were 189.2 ± 127.0 nm for M0 ($n=206$) and 460.7 ± 250.4 nm for M1 ($n=131$). **(b, d)** Representative SEM images and EDS elemental composition of extract-free ZnO-NPs (M0) and extract-assisted ZnO-NPs (M1), respectively. The values correspond to visible particles/agglomerates rather than primary crystallite size

XRD shows a slightly smaller crystallite size for M1 compared with M0, the SEM analysis reveals larger apparent secondary structures for M1. This suggests that extract-derived species influence nucleation and crystallite growth while also promoting the formation of larger aggregates or flake-like assemblies. Therefore, the extract does not simply reduce the apparent SEM particle size; rather, it modifies the morphology and aggregation state of the ZnO system. Additional SEM-based size distributions, DLS measurements, and high-magnification SEM images are provided in the Supporting Information file (Figs. S7–S10), further supporting the presence of secondary aggregates in both nanoparticle systems. Elemental composition was further evaluated by EDS. In M0, zinc and oxygen are the predominant elements, with minor carbon signals commonly attributed to adventitious carbon and residual species from the synthesis environment. In M1, zinc and oxygen also dominate the spectrum, but additional carbon and trace nitrogen contributions are detected, consistent with residual extract-derived species associated with the nanoparticle surface. Quantitative EDS analysis indicates zinc contents of 66.2 wt% for M0 and 82.4 wt% for M1, with corresponding oxygen contents of 19.0 wt% and 6.3 wt%, respectively. These compositional differences support the interpretation that the extract-assisted synthesis modifies the nanoparticle surface composition while preserving ZnO formation. Overall, the SEM-EDS results provide morphological and compositional evidence of extract-induced surface and aggregation changes, consistent with the XPS and TGA analyses discussed below.

3.1.3 XRD analysis

The crystalline structure of the ZnO-NPs synthesized with and without chaya extract was analyzed by X-ray diffraction. The diffraction patterns of both M0 and M1 samples, shown in Fig. 3, display a set of well-defined reflections characteristic of the hexagonal wurtzite structure of ZnO. All observed peaks can be indexed to the standard ZnO reference pattern (PDF 00–036–1451), indicating that the incorporation of the plant extract during synthesis does not alter the crystal phase of the nanoparticles.

The main diffraction peaks were located at $2\theta = 31.77^\circ$, 34.42° , 36.25° , 47.53° , 56.60° , 62.86° , 66.38° , 67.96° , and 69.10° , corresponding to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) crystallographic planes, respectively. Crystallite sizes were estimated using the Debye–Scherrer equation [28] applied to the (100), (002), (101), (102), and (110) reflections. The average crystallite size was determined to be 10.4 nm for the M0 sample and 9.2 nm for the M1 sample. These values fall within the range commonly reported for ZnO-NPs synthesized via

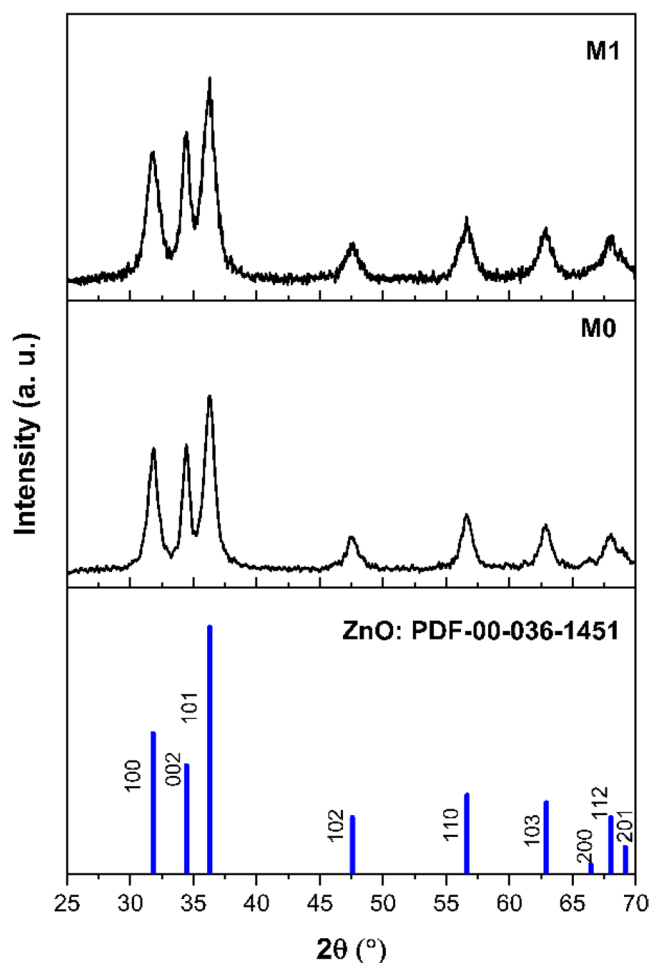


Fig. 3 X-ray diffraction patterns of ZnO-NPs synthesized without chaya extract (M0) and with ethanolic chaya extract (M1). The indexed reflections correspond to the hexagonal wurtzite ZnO phase according to PDF 00–036–1451

green routes and indicate that the presence of chaya extract during synthesis leads to a modest reduction in crystallite size [29]. This is because the ethanolic extract modifies the phytochemical composition involved in Zn^{2+} coordination during nucleation. By preferentially solubilizing less polar phenolic and triterpenoid compounds, the solvent indirectly alters the Zn^{2+} complexation environment prior to hydrolysis, which can influence nucleation density, crystallite growth, and surface chemistry. The relatively small crystallite dimensions observed for both samples are consistent with the pronounced peak broadening evident in the diffraction patterns and correlate with the nanoscale features observed in the SEM analysis. In particular, the smaller average crystallite size of the M1 sample suggests that plant-derived components present during synthesis may partially limit crystal growth, yielding ZnO domains with higher surface-to-volume ratios. While such nanoscale dimensions are commonly associated with increased surface-to-volume

ratios, they also contribute to the tendency toward particle agglomeration observed in electron micrographs. Additional insight into the electronic structure of the nanoparticles is provided by the UV-Vis absorption spectra (Figure S3). Both M0 and M1 samples exhibit optical band gap energies of approximately 3.6 eV, which are higher than the band gap of bulk ZnO ($E_{g,bulk} = 3.2$ eV [30]). The observed blue shift is commonly attributed to size-related effects, including quantum confinement and increased surface disorder in nanocrystalline ZnO. Differences in absorption maxima between the two samples further reflect variations in particle size distribution and aggregation state induced by the plant-assisted synthesis route [31–34].

3.1.4 XPS analysis

The surface chemical composition and zinc oxidation state of ZnO-NPs synthesized with and without chaya extract were examined by X-ray photoelectron spectroscopy. Survey spectra for M0 and M1 (Fig. 4a) are dominated by Zn, O, and C signals, as expected for ZnO nanoparticles exposed to ambient conditions. Compared with M0, the M1 sample shows additional Na contributions and a different relative surface composition, indicating that the extract-assisted synthesis modifies the nanoparticle surface chemistry. These differences are consistent with the partial retention of extract-derived species on the ZnO surface, generating a distinct interfacial environment relative to extract-free nanoparticles. The surface chemical composition obtained from the XPS survey spectra is summarized in Table 1.

High-resolution Zn 2p spectra (Fig. 4b) display two well-defined components at ~ 1021 eV and ~ 1044 eV, assigned to Zn 2p_{3/2} and Zn 2p_{1/2}, respectively. The energy separation of ~ 23 eV is characteristic of Zn²⁺ in ZnO [35]. A slight shift in the Zn 2p_{3/2} binding energy for M1 suggests modification of the local chemical environment at the nanoparticle surface [36]. To further confirm the zinc oxidation state, the Zn L₃M₄₅M₄₅ Auger region was analyzed and the modified Auger parameter (α') was calculated using the Zn 2p_{3/2} binding energy and the Zn L₃M₄₅M₄₅ Auger kinetic energy [37]:

$$\alpha' = BE(Zn\ 2p_{3/2}) + KE(Zn\ L_3M_{45}M_{45})$$

Since the Auger peak was recorded on the binding-energy scale, the kinetic energy was obtained as:

$$KE = h\nu - BE(Auger) \text{ using Al K}\alpha \text{ radiation } (h\nu = 1486.6 \text{ eV}).$$

Thus: $\alpha' = BE(Zn\ 2p_{3/2}) + (1486.6 - BE(Auger))$ For M0, using $BE(Zn\ 2p_{3/2}) = 1021.7$ eV and $BE(Auger) = 499.0$ eV, $\alpha' = 1021.7 + (1486.6 - 499.0) = 2009.3$ eV. For M1, using $BE(Zn\ 2p_{3/2}) = 1021.1$ eV and $BE(Auger) = 497.5$ eV, $\alpha' = 1021.1 + (1486.6 - 497.5) = 2010.2$ eV. These

values are consistent with Zn²⁺ in ZnO for both samples, confirming that the extract-assisted synthesis modifies the surface chemical environment without changing the zinc oxidation state. The O 1s spectra provide further information on the oxygen-containing surface species. For M0, the O 1s envelope was fitted with one main component assigned to the dominant oxygen contribution of the ZnO surface. For M1, two components at ~ 529.8 eV and ~ 531.3 eV were observed, assigned to lattice oxygen in Zn–O and surface hydroxyl/adsorbed oxygen species, respectively [38]. Importantly, the narrow feature observed near 535–536 eV was not assigned to O 1s, instead, it was attributed to a Na Auger contribution and excluded from the O 1s fitting. This correction avoids overestimating high-binding-energy oxygen species and improves the reliability of the O 1s deconvolution. The C 1s spectra for both samples were deconvoluted into components at ~ 284.7 eV, ~ 286.2 eV, and ~ 288.7 eV, corresponding to C–C/C–H, C–O, and O–C=O environments, respectively [39–41]. Because carbon-containing species may arise from adventitious carbon and residual acetate species from the zinc precursor, the C 1s region alone is not sufficient to distinguish the two nanoparticle systems. However, when considered together with the O 1s changes, the Na contribution detected in M1, and the TGA results showing higher organic-associated mass loss for M1, the XPS data support the presence of extract-derived surface species on the extract-assisted nanoparticles. Overall, XPS confirms that M1 possesses a differentiated surface chemistry relative to M0 while preserving the Zn²⁺ oxidation state characteristic of ZnO.

3.1.5 TGA analysis

The thermal behavior of the ZnO-NPs synthesized with and without chaya extract was evaluated by thermogravimetric analysis, and the corresponding thermograms are presented in Fig. 5. Both M0 and M1 samples exhibit three distinct regions of mass loss, which can be associated with different thermal events occurring upon heating under an air atmosphere.

Zone 1 is attributed to the removal of physically adsorbed moisture from the nanoparticle surfaces. In this region, the M1 sample shows a larger mass loss compared with M0, indicating a higher affinity for water, which is consistent with the presence of residual organic species introduced during the plant-assisted synthesis. Zone 2, extending from approximately 80 to 415 °C for M0 and from 95 to 327 °C for M1, corresponds to the decomposition of residual precursor species and organic components associated with the nanoparticles. For the M0 sample, this region is mainly related to the decomposition of zinc acetate residues and other synthesis by-products [42]. In contrast, the more

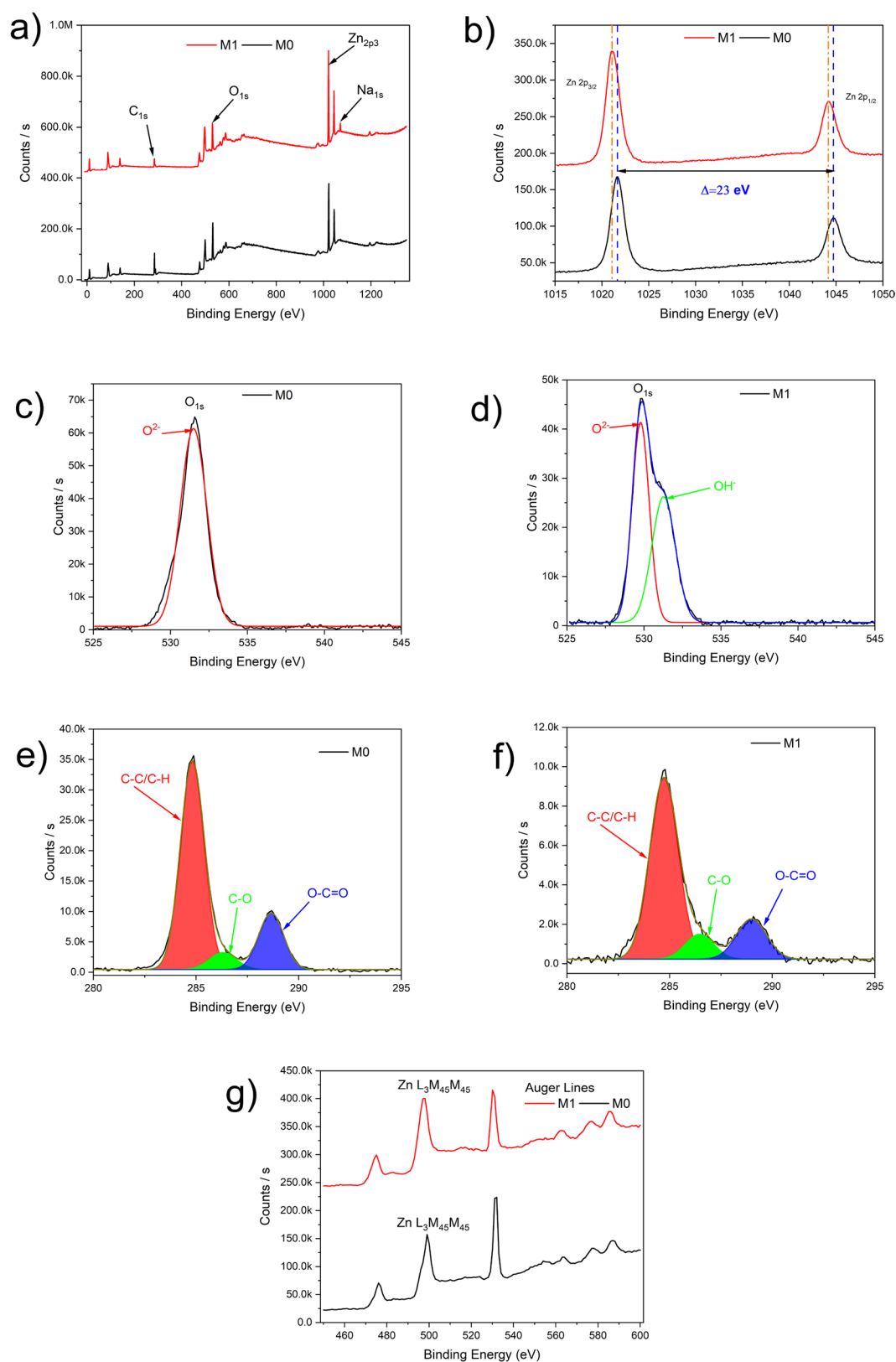


Fig. 4 XPS analysis of ZnO-NPs synthesized without extract (M0) and with ethanolic chaya extract (M1): **(a)** survey spectra, **(b)** high-resolution Zn 2p spectra showing the Zn 2p_{3/2} and Zn 2p_{1/2} components and their spin-orbit separation, **(c, d)** O 1s spectra for M0 and M1, respectively, **(e, f)** C 1s spectra for M0 and M1, respectively, and **(g)** Zn L₃M₄₅M₄₅ Auger spectra used to calculate the modified Auger parameter. Peak assignments are indicated in the fitted spectra

Table 1 Surface chemical composition of ZnO-NPs synthesized without extract (M0) and with ethanolic chaya extract (M1), as determined from XPS survey spectra. Values are reported as relative atomic percentages of the detected surface elements

Sample	C _{1s} (at%)	O _{1s} (at%)	Zn _{2p_{3/2}} (at%)	Na _{1s} (at%)
M0	49.41	35.98	14.6	--
M1	20.84	40.75	31.0	7.41

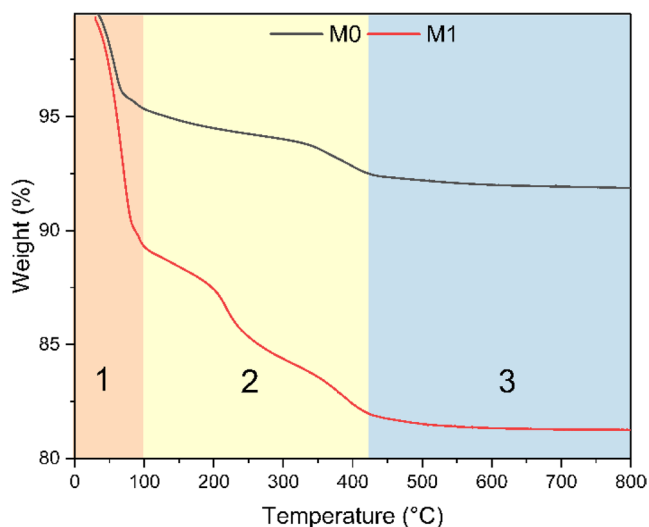


Fig. 5 Thermogravimetric analysis of ZnO-NPs synthesized without chaya extract (M0) and with ethanolic chaya extract (M1). The thermograms show three main mass-loss regions associated with adsorbed moisture, residual precursor/organic decomposition, and the thermally stable ZnO phase

pronounced mass loss observed for the M1 sample reflects the thermal degradation of organic compounds derived from the chaya extract that remain associated with the nanoparticle surface. Zone 3, occurring at temperatures above approximately 420 °C, is characterized by a relatively stable mass profile and corresponds to the thermally stable ZnO phase. The final residual masses were determined to be 92.1% for the M0 sample and 82.4% for the M1 sample. The lower residual mass observed for M1 is consistent with its higher organic content, as inferred from the preceding mass-loss steps. Quantitative values for the mass losses and associated temperature ranges are summarized in Table 2. The TGA results provide clear evidence that the use of chaya extract during synthesis leads to ZnO-NPs with increased organic surface content, which influences their thermal behavior

while preserving the thermal stability of the inorganic ZnO phase at elevated temperatures.

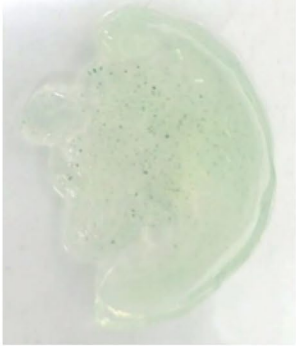
3.2 Visual assessment of the integration of the ZnO-NPs into the patches

The visual evaluation of the hydrogel patches revealed clear differences in shape, opacity, and apparent nanoparticle distribution, providing a qualitative assessment of ZnO-NPs integration within the polymer matrix. Representative images of the different patch configurations are shown in Fig. 6. For clarity, the nomenclature used to describe the nanoparticle incorporation sequences is as follows: M1 (AN-E), M2 (AEN), M3 (ANE), and M4 (ENA), where AN denotes the zinc acetate and sodium hydroxide mixture, A represents zinc acetate, N corresponds to sodium hydroxide, and E refers to the ethanolic chaya extract. Among the different configurations evaluated, one nanoparticle-loaded patch (P1) was selected for detailed rheological analysis based on its uniform macroscopic appearance and apparent homogeneity. Although visual inspection does not provide quantitative information on nanoparticle dispersion at the microscopic level, it offers an initial indication of successful incorporation of ZnO-NPs into the SA/PVA hydrogel network. Importantly, the observed macroscopic integrity of the patches suggests that the adopted fabrication method enables nanoparticle integration without compromising the structural continuity of the hydrogel matrix. Unlike most green ZnO synthesis routes that rely on aqueous plant extracts, the present work employs an ethanolic extract of chaya, which provides a distinct phytochemical profile. Ethanol selectively extracts less polar phenolics and triterpenoid compounds while minimizing the presence of polysaccharides and other high-molecular-weight hydrophilic components. This compositional selectivity alters the Zn²⁺ coordination environment during nucleation and growth, leading to ZnO nanoparticles with a distinct surface chemistry and modified aggregation behavior. Consequently, the extraction solvent plays an active role in directing the synthesis rather than serving merely as a green reducing medium. The novelty of this green synthesis lies in the use of an ethanolic extract as a phytochemical-selective medium that actively modulates ZnO nucleation and surface chemistry, rather than relying on conventional aqueous extracts.

Table 2 Temperature ranges and mass losses obtained from TGA analysis of ZnO-NPs synthesized without chaya extract (M0) and with ethanolic chaya extract (M1)

NPs	Zone 1			Zone 2			Zone 3	Weight losses (%)
	T ₁ (°C)	T ₂ (°C)	Weight (%)	T ₁ (°C)	T ₂ (°C)	Weight (%)	Thermal stability temperature	
M0	30	79	4.6	80	415	3.3	416	7.9
M1	30	95	10.3	95	327	3.7	420	17.6
				328	419	3.6		

Fig. 6 Visual assessment of ZnO-NPs integration into SA/PVA hydrogel patches. P0 corresponds to the nanoparticle-free hydrogel, PA to the hydrogel containing extract-free ZnO-NPs (M0), and P1-P4 to hydrogels containing extract-assisted ZnO-NPs prepared under different reagent-addition sequences: P1 (M1), P2 (M2), P3 (M3), and P4 (M4)

Sample	P0	PA
		
Sample	P1	P2
		
Sample	P3	P4
		

3.3 FTIR characterization of chaya leaf extract, ZnO-NPs, and hydrogel

The normalized FTIR spectra of the chaya leaf extract, ZnO-NPs, and hydrogel patches with and without nanoparticles are shown in Fig. 7. All samples exhibit a broad O–H stretching band between 3550 and 3200 cm^{-1} , indicating the presence of hydroxyl groups [42, 43]. In the chaya extract, this broad band is mainly associated with ethanol, which is further supported by bands at 2922 and 1375 cm^{-1} assigned to C–H bending modes of CH_3 groups [42, 44]. For the nanoparticle samples, the O–H band reflects contributions from residual ethanol and/or adsorbed water. In particular, for M1, the O–H band is consistent with a combination of residual ethanol and adsorbed water, whereas for M0 (and for the hydrogel samples P0 and P1), the same band is mainly attributed to water retained within nanoparticle surfaces and hydrogel cavities. In agreement with this interpretation, a band at 1625 cm^{-1} is observed in P0 and P1, further supporting water retention in the hydrogel matrix. The spectra of the hydrogel samples also display characteristic vibrational features of the polymer components. Bands associated with alginate are observed at 1625 and 1300 cm^{-1} , while a band at 1080 cm^{-1} is assigned to C–O bonds in PVA. Notably, in M1 and P1, the absence of the C–H stretching doublet at 2922 and 2851 cm^{-1} , as well as the absence of the C=O band at 1737 cm^{-1} (both present in the ethanolic extract), is consistent with interactions between extract-derived species and the nanoparticle surface and becomes incorporated into the composite system. Finally, the C–O stretching region (1260–1000 cm^{-1}) may

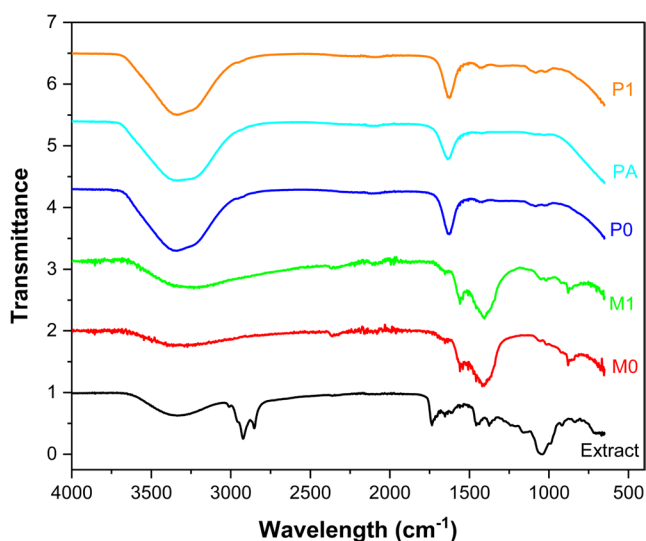


Fig. 7 Normalized FTIR spectra: P0, PA and P1. P0 corresponds to the hydrogel patches without ZnO-NPs. PA corresponds to the hydrogel patches with M0 (ZnO-NPs), and P1 corresponds to hydrogel patches with M1 (ZnO-NPs-extract). M0 and M1 correspond to ZnO-NPs synthesized without and with extract, respectively

include contributions from residual zinc acetate in M0 and M1, as well as ethanol-related features in M1 [44]. The FTIR results, together with the visual assessment, support the embedding of ZnO-NPs within the SA/PVA hydrogel patches.

3.4 Rheological analysis. Viscoelastic properties of the hydrogel patch

The rheological behavior of the hydrogel patches is presented in Fig. 8. Frequency-dependent shear moduli provide insight into network integrity and relaxation dynamics [45, 46]. In soft polymer systems, low-frequency responses are associated with chain mobility and network rearrangement [47], whereas high-frequency behavior reflects elastic, structure-dominated contributions [48]. The incorporation of ZnO nanoparticles produces a clear modification of the viscoelastic response, with the strongest reinforcement observed for the hydrogel containing extract-assisted ZnO-NPs (P1). Because P0, PA, and P1 were prepared using the same SA/PVA composition and crosslinking protocol, the differences in rheological behavior can be attributed primarily to nanoparticle incorporation and to differences in nanoparticle surface chemistry rather than to changes in the bulk hydrogel formulation. Amplitude sweep tests at 1 Hz (Fig. 9a) show that the linear viscoelastic region (LVR) expands from 25 Pa in P0 to 113 Pa in P1, indicating enhanced resistance to structural disruption under applied stress. Since polymer composition and crosslinking conditions are identical in both systems, this reinforcement is attributed to nanoparticle-polymer interfacial interactions. The magnitude of the storage modulus G' also increases

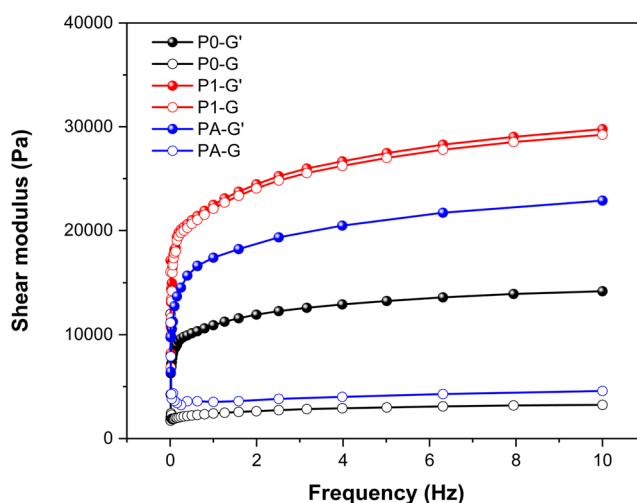


Fig. 8 Frequency-dependent shear modulus of SA/PVA hydrogel patches: nanoparticle-free hydrogel (P0), hydrogel containing extract-free ZnO-NPs (PA, M0), and hydrogel containing extract-assisted ZnO-NPs (P1, M1)

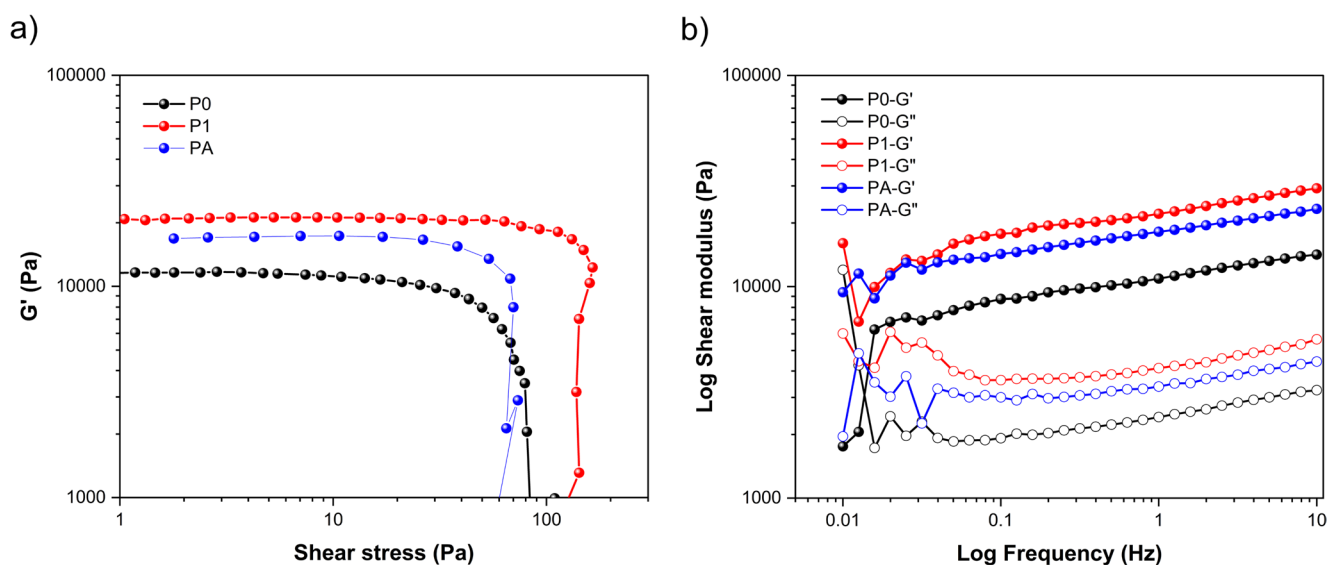


Fig. 9 Rheological behavior of SA/PVA hydrogel patches: **(a)** linear viscoelastic region determined by amplitude sweep at 1 Hz and **(b)** frequency-dependent storage and loss moduli plotted as log shear modulus versus log frequency. P0 corresponds to the nanoparticle-free hydrogel, PA to the hydrogel containing extract-free ZnO-NPs (M0), and P1 to the hydrogel containing extract-assisted ZnO-NPs (M1)

substantially upon nanoparticle incorporation, with PA and P1 exhibiting higher values than P0. At 0.01 Hz, G' rises from 1759 Pa (P0) to 9401 Pa (PA) and 16,040 Pa (P1), representing an approximately nine-fold enhancement for M1 NPs, and remains higher across the entire frequency range (Fig. 9b). This sustained increase indicates reduced polymer chain mobility and a more elastic-dominated network [49, 50]. The stronger response of P1 compared with PA indicates that reinforcement is not only a consequence of adding an inorganic filler but also depends on the surface chemistry of the nanoparticles. XPS analysis shows that M1 possesses a differentiated oxygen-containing surface environment, including surface hydroxyl/adsorbed oxygen contributions, while FTIR and TGA support the presence of extract-derived organic species associated with the nanoparticle surface. These surface features provide additional interaction sites for hydrogen bonding with PVA hydroxyl groups and alginate chains. In parallel, localized Zn^{2+} surface sites may interact with alginate carboxylates [51], although Ca^{2+} remains the primary ionic crosslinker of the hydrogel network. Together, these interactions favor polymer adsorption at the nanoparticle interface, restrict local chain mobility, and increase the effective stiffness of the surrounding polymer layer. This provides a mechanistic basis for the interfacial polymer confinement proposed for P1. Frequency-dependent behavior further distinguishes the two systems. P1 exhibits a low-frequency inflection between 0.01 and 0.1 Hz, suggesting relaxation processes associated with nanoparticle-polymer interactions [52], this behavior is not observed with the same intensity in PA. Across the full

frequency range, G' exceeds G'' in P1 and PA, with higher G'/G'' ratios relative to P0, confirming a more elastically dominated response [53–55]. Overall, the rheological data indicate that ZnO-NPs function as multifunctional physical reinforcement nodes within the SA/PVA dual-network architecture. The observed viscoelastic enhancement is therefore interpreted as arising from interfacial polymer confinement and modified relaxation dynamics rather than simple volumetric inclusion of rigid particles.

3.5 Proposed nanoparticle-polymer interaction mechanism

The significant increase in storage modulus and expansion of the linear viscoelastic region observed for P1 indicate that the reinforcement produced by extract-assisted ZnO-NPs extends beyond passive filler effects. This interpretation is supported by the comparison with PA, which contains extract-free ZnO-NPs (M0) in the same SA/PVA matrix but shows a lower reinforcement effect than P1. Since the polymer composition and crosslinking protocol are kept constant, the enhanced response of P1 is attributed to the distinct surface chemistry of M1. XPS shows that M1 contains surface hydroxyl/adsorbed oxygen contributions and a modified surface composition, while FTIR and TGA support the presence of extract-derived organic species associated with the nanoparticle surface. These features provide additional interaction points for hydrogen bonding with PVA hydroxyl groups and alginate chains. Localized Zn^{2+} surface sites may also contribute to interactions with alginate carboxylates, although Ca^{2+} remains the primary

ionic crosslinker in the bulk hydrogel network. Therefore, M1 is proposed to generate a more constrained interfacial polymer layer, reducing local chain mobility and altering relaxation dynamics. In this context, interfacial polymer confinement is presented as a mechanistic interpretation supported by the combined surface chemistry and rheological data, rather than as a direct structural measurement. To clarify the relationship between nanoparticle surface chemistry and hydrogel rheology, the proposed interfacial interaction mechanism is schematically illustrated in Fig. 10. In the PA system containing extract-free ZnO-NPs (M0), polymer-particle interactions are mainly associated with the inorganic ZnO surface and residual precursor-derived species, resulting in a lower density of effective interfacial interaction sites. In contrast, M1 contains surface hydroxyl/adsorbed oxygen contributions and extract-derived organic residues, as supported by XPS, FTIR, and TGA analyses. These surface features provide additional interaction points for hydrogen bonding with PVA hydroxyl groups and alginate chains, while localized Zn^{2+} surface sites may also interact with alginate carboxylates. As a result, the M1-containing hydrogel (P1) develops a more constrained interfacial polymer layer, reducing local chain mobility and enhancing elastic network response.

3.6 Antibacterial tests

The antibacterial activity of the ZnO-NPs synthesized with chaya extract (M1 and M4, see the Supporting Information File) was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using standard in vitro assays. These two bacterial strains were selected to represent microorganisms with distinct cell wall structures, which are known to influence their susceptibility to inorganic antibacterial agents.

Disk diffusion assays were first employed as a qualitative screening method to assess bacterial growth inhibition in the presence of ZnO-NPs. Representative inhibition zones observed for both bacterial strains at different nanoparticle concentrations are shown in Fig. 11, while additional images and measurements corresponding to replicate experiments are provided in the Supporting Information File. The inhibition halos are more pronounced for *Staphylococcus aureus* than for *Escherichia coli*, reflecting differences in the interaction between ZnO-NPs and Gram-positive vs. Gram-negative bacteria [42]. The nanoparticle-free SA/PVA matrix did not show detectable inhibition halos under the tested conditions, as shown in the Supporting Information File (Figure S4) (Table 3).

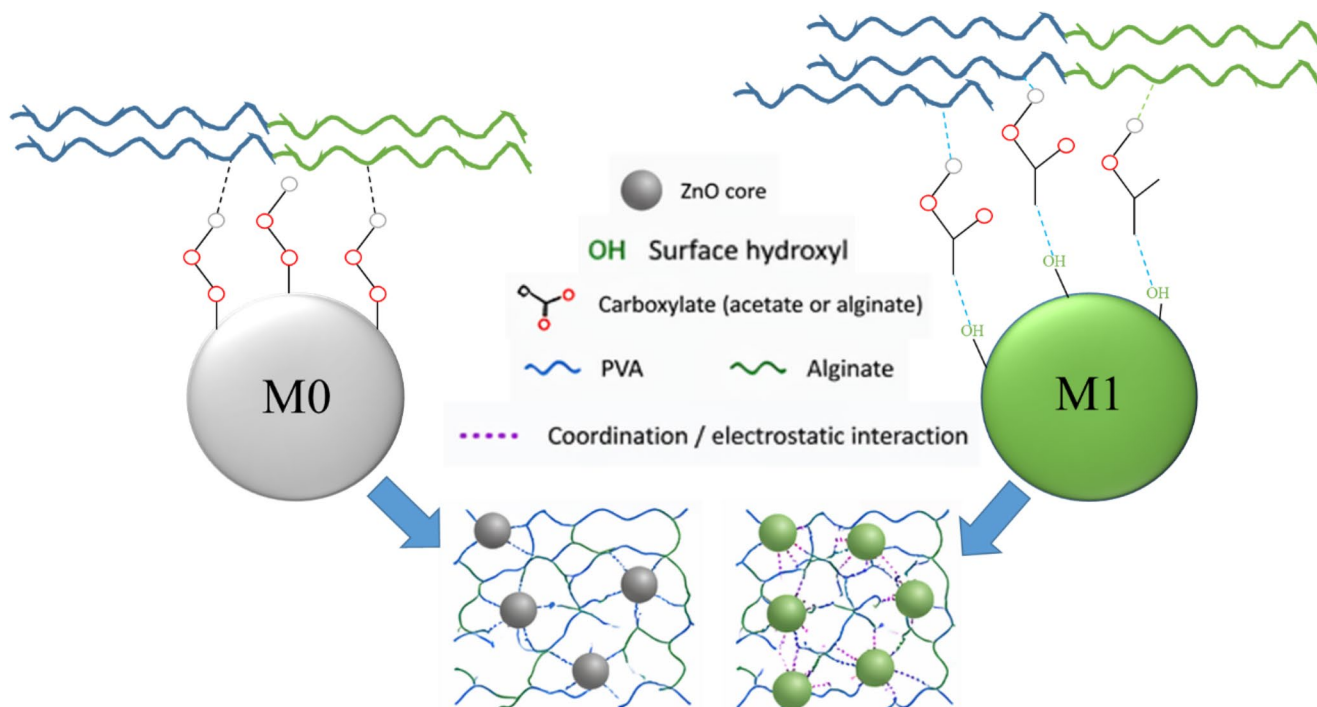


Fig. 10 Proposed interfacial interaction mechanism between ZnO-NPs and the SA/PVA hydrogel network. In the PA system, extract-free ZnO-NPs (M0) interact with PVA and alginate chains mainly through the inorganic ZnO surface and residual precursor-derived species. In contrast, extract-assisted ZnO-NPs (M1) present a modified surface chemistry, including surface hydroxyl/adsorbed oxygen contributions and extract-derived organic residues, promoting hydrogen bonding with PVA hydroxyl groups and alginate chains, as well as possible localized interactions between Zn^{2+} surface sites and alginate carboxylates. These interactions increase polymer adsorption and interfacial chain confinement, contributing to the enhanced elastic response observed for P1

Fig. 11 Inhibition zone assay of extract-assisted ZnO-NPs against *Staphylococcus aureus* and *Escherichia coli*. Representative inhibition halos obtained using 450 μg of M1 and M4 ZnO-NPs are shown for (a) *S. aureus* and (b) *E. coli*. The positive control corresponds to amikacin

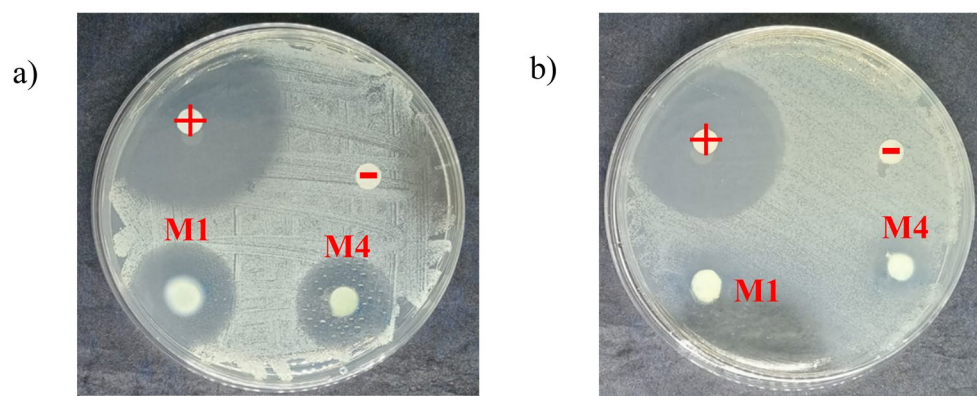


Table 3 Inhibition halo diameters obtained for extract-assisted ZnO-NPs against *Staphylococcus aureus* and *Escherichia coli*. Values are reported in millimeters for M1 and M4 nanoparticles, with amikacin used as the positive control

Halos (mm)			
Control +	M1	M4	
<i>Staphylococcus aureus</i>			
42.8	24.7	22.5	
<i>Escherichia coli</i>			
37.3	14.6	11.6	

The final ZnO-NP concentrations evaluated in the broth microdilution assays were 143.3, 286.7, and 430.0 $\mu\text{g/mL}$. Growth inhibition was assessed by absorbance at 600 nm and by plating on solid medium to distinguish turbidity reduction from bactericidal response. Based on the absorbance data shown in Table S1, both M1 and M4 produced concentration-dependent growth inhibition against *S. aureus*, with complete suppression of detectable turbidity at 430 $\mu\text{g/mL}$. At 286.7 $\mu\text{g/mL}$, M1 produced a marked reduction in bacterial growth compared with the negative control, but residual absorbance was still detected, therefore, this concentration was interpreted as strongly inhibitory under the tested conditions rather than as complete turbidity suppression. Plating results further supported a concentration-dependent decrease in viable colonies, with a stronger response for M1 than for M4 (Fig. 12). For *E. coli*, absorbance values remained relatively high across the tested concentration range for both nanoparticle systems, indicating that neither M1 nor M4 produced complete inhibition of bacterial growth under the evaluated conditions (Table S1, Supporting Information File). Although a concentration-dependent decrease was observed, particularly for M4 at the highest concentration tested, visible bacterial colonies were still detected upon plating on solid media (Fig. 12). This behavior indicates a bacteriostatic rather than bactericidal effect, and therefore a definitive MIC value could not be established for *E. coli* within the tested concentration range. The extract-free ZnO-NPs (M0) were also evaluated as a control to determine whether the ZnO core alone contributed

to antibacterial activity. As shown in Table S2 of the Supporting Information File, M0-treated cultures showed absorbance values comparable to the negative control for both *S. aureus* and *E. coli*, indicating that M0 did not inhibit bacterial growth under the tested conditions. Comparison with previously reported ZnO-based systems should therefore be interpreted cautiously. In the present work, extract-assisted ZnO-NPs showed a marked concentration-dependent inhibitory effect against *S. aureus*, with complete suppression of detectable turbidity at 430 $\mu\text{g/mL}$ for both M1 and M4 (Table S1, Supporting Information File). This behavior is within the concentration range reported for other plant-assisted ZnO systems, such as ZnO-NPs synthesized using *Artemisia absinthium* extract (312 $\mu\text{g/mL}$) [56], Aloe vera extract (ALE-ZnO-NPs, 2200 $\mu\text{g/mL}$) [57], and extract-free ZnO systems (0.5 mg/mL) [58]. However, because the present assays distinguish between turbidity reduction, colony formation, and strain-dependent bacteriostatic/bactericidal behavior, the comparison is used only as contextual reference rather than as a direct equivalence of MIC values.

To clarify the dominant antibacterial contribution in the present system, additional comparative inhibition-zone assays were performed using M0, M1, M1-C, EE, and the nanoparticle-free SA/PVA matrix. The corresponding results are provided in the Supporting Information (Figure S6 and Table S3). M0 did not produce detectable inhibition halos against either *S. aureus* or *E. coli*, whereas EE and M1 showed measurable inhibition against both bacterial strains. M1-C retained antibacterial activity against *S. aureus*, although with a reduced inhibition halo, and did not show detectable inhibition against *E. coli*. These results indicate that the antibacterial activity observed for M1 is not dominated by the ZnO core alone under the tested conditions. Instead, the response is mainly associated with extract-derived phytochemical species adsorbed or weakly bound to the ZnO surface. This interpretation is consistent with the surface characterization results. XPS analysis confirmed that M1 has a surface composition distinct from M0, including additional surface contributions associated with

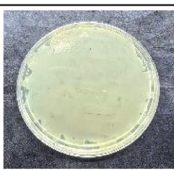
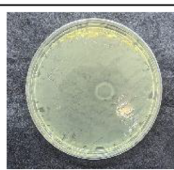
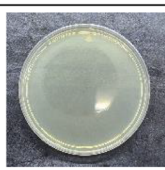
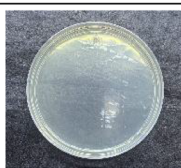
<i>Staphylococcus aureus</i>				
C-	M1 (µg/mL)			
	143.3 ± 7	286 ± 14	430 ± 10	
				
C+	M4 (µg/mL)			
	143.3 ± 7	286 ± 14	430 ± 10	
				
<i>Escherichia coli</i>				
C-	M1 (µg/mL)			
	143.3 ± 7	286 ± 14	430 ± 10	
				
C+	M4 (µg/mL)			
	143.3 ± 7	286 ± 14	430 ± 10	
				

Fig. 12 Concentration-dependent antibacterial evaluation of extract-assisted ZnO-NPs against *S. aureus* and *E. coli*. Representative cultures and plating results for M1 and M4 are shown to assess bacteriostatic and bactericidal responses under the tested conditions

extract-derived species and oxygenated functionalities. The corrected XPS analysis also confirmed Zn in the +2-oxidation state for both M0 and M1, indicating that the antibacterial differences are not due to a change in zinc oxidation state but rather to differences in surface chemistry. TGA further supports this interpretation, since M1 showed a lower final residual mass than M0 and larger mass losses associated with moisture and organic decomposition, consistent with a higher content of surface-associated organic material. Therefore, Zn^{2+} release and ROS-related pathways

may contribute secondarily, as commonly reported for ZnO-based antibacterial systems, but they do not appear to be the predominant mechanism under the present experimental conditions. Zeta potential measurements further support this cautious interpretation. M0 and M1 showed slightly negative zeta potential values of -4.66 and -6.93 mV, respectively (Figure S5). These values suggest limited electrostatic attraction between the ZnO-NPs and negatively charged bacterial surfaces. Previous work [59] has reported that negatively charged ZnO-NPs may be less favorable

for ROS generation at the bacteria-nanoparticle interface. Thus, the antibacterial behavior observed here is more consistently attributed to surface-bound phytochemical species associated with extract-assisted ZnO-NPs, rather than to dominant electrostatic interaction or ZnO-mediated ROS production alone. The nanoparticle-free SA/PVA matrix did not produce detectable inhibition halos against either bacterial strain under the investigated conditions (Figure S4). Therefore, the SA/PVA hydrogel matrix itself was not considered intrinsically antibacterial in this study. Its role is interpreted as a physical polymeric scaffold for nanoparticle incorporation, while the antibacterial response arises from the extract-assisted ZnO-NPs and, more specifically, from their phytochemical-modified surface. The antibacterial assays show that extract-assisted ZnO-NPs exhibit selective antibacterial activity, with a stronger effect against Gram-positive *S. aureus* than against Gram-negative *E. coli*. The combined inhibition-zone assays, broth microdilution results, zeta potential measurements, XPS, and TGA analyses support a mechanism in which surface-associated phytochemical species are the main contributors to the observed antibacterial response, while Zn^{2+} release and ROS-related pathways may contribute secondarily. The Supporting Information File provides complementary quantitative data, replicate measurements, and additional images supporting this interpretation.

3.7 Comparison of our work with the literature

Table 4 summarizes a comparative analysis of the present study with recent reports on ZnO-based polymer composites and nanocomposite hydrogels. The comparison considers three main aspects: ZnO crystallite size determined by XRD, the polymer matrix used for composite preparation,

and the reported mechanical or antibacterial response. For antibacterial performance, the values are presented only as contextual references because experimental protocols, bacterial strains, inoculum conditions, exposure times, and endpoint criteria differ among studies. In the present work, the antibacterial response is described in terms of concentration-dependent inhibition, inhibition-zone diameter, and complete suppression of detectable turbidity against *S. aureus*, rather than as a direct MIC equivalence. This comparison highlights the distinctive contribution of the present study, the extract-assisted modulation of ZnO surface chemistry and its correlation with viscoelastic reinforcement within a controlled SA/PVA dual-network hydrogel system.

4 Conclusions

This study establishes a controlled structure-surface-property framework to evaluate how phytochemical-assisted ZnO synthesis modifies nanoparticle surface chemistry and influences the mechanical and antibacterial behavior of SA/PVA hydrogel nanocomposites. By preparing extract-free ZnO-NPs (M0) and extract-assisted ZnO-NPs (M1) under comparable inorganic precursor conditions, the effect of the ethanolic *Cnidioscolus aconitifolius* extract was isolated from changes in the polymer formulation. Structural and surface characterization confirmed that the extract-assisted route preserved the hexagonal wurtzite ZnO phase while producing measurable differences in crystallite size, morphology, aggregation state, surface composition, oxygen-containing surface species, and organic-associated mass loss. The inclusion of the Zn Auger region and modified Auger parameter calculation further confirmed that Zn remains in the Zn^{2+} oxidation state in both M0 and M1, indicating that

Table 4 Comparison of the present work with representative ZnO-based polymer composites and nanocomposite hydrogels reported in the literature. The comparison includes ZnO crystallite size, polymer matrix, rheological response, and antibacterial performance against *S. aureus*. Antibacterial values are provided as contextual references because experimental conditions and endpoint criteria differ among studies

System	Extract plant	ZnO size (nm) XRD	Polymer matrix	G' (Pa)	Concentration associated with complete turbidity suppression against <i>S. aureus</i> ($\mu\text{g/mL}$)	Inhibition (mm)	Reference
PVA-ZnO	--	--	PVA	10,000	--	--	[60]
PVA/PEG-AZO-NPs	--	18.96	PVA/PEG	--	8.35	--	[61]
CS/PVA/AV/ZnO	<i>Matricaria chamomilla</i>	24	CS/PVA	--	--	13	[62]
PVA-ZnO-NPs	<i>Moringa oleifera</i>	30	PVA	--	--	4.81	[63]
cellulose/PVA-ZnO and Ag/ZnO-NPs	<i>Eclipta prostrata</i>	24.55	Cellulose/PVA	--	--	18.83	[64]
SA/PVA/ZnO	<i>konjac extract</i>	25.1	SA/PVA	--	--	--	[65]
Pectin/alginate aerogel-ZnO	<i>beetroot</i>	--	Pectin/SA	--	--	--	[66]
ZnO-NPs aqueous leaf extracts of <i>Cnidioscolus aconitifolius</i>	<i>Cnidioscolus aconitifolius</i>	--	--	--	--	--	[12]
SA/PVA-ZnO-NPs synthesized with ethanolic extract*	<i>Cnidioscolus aconitifolius</i>	9.2	SA/PVA	16,040	430	24.7	This work

* For this work, the value corresponds to complete suppression of detectable turbidity, not direct MIC equivalence

the differences between both systems arise mainly from surface chemical modification rather than from a change in zinc oxidation state. When incorporated into the SA/PVA dual-network hydrogel, the surface-modified ZnO-NPs produced a clear viscoelastic reinforcement effect. The M1-containing hydrogel showed an expanded linear viscoelastic region, increased storage modulus, and enhanced elastic dominance compared with the nanoparticle-free matrix and the M0-containing system. This mechanical response is most consistently explained by nanoparticle-polymer interfacial interactions, including hydrogen bonding between surface hydroxyl/oxygenated species and PVA/alginate chains, together with possible localized interactions between Zn^{2+} surface sites and alginate carboxylates. Because interfacial confinement was not measured directly, it is presented as a mechanistic interpretation supported by the combined XPS, FTIR, TGA, and rheological evidence. Therefore, the observed reinforcement is interpreted as arising from interfacial polymer confinement and modified relaxation dynamics rather than from simple rigid filler incorporation or changes in bulk crosslinking chemistry. The antibacterial assays showed that extract-assisted ZnO-NPs exhibit concentration-dependent antibacterial activity, with a stronger response against *S. aureus* than against *E. coli*. Complete suppression of detectable turbidity was observed against *S. aureus* at 430 $\mu\text{g/mL}$, whereas a definitive MIC could not be established for *E. coli* within the tested concentration range. Additional comparative assays demonstrated that extract-free ZnO-NPs (M0) and the nanoparticle-free SA/PVA matrix did not produce detectable inhibition halos under the investigated conditions. In contrast, the ethanolic extract and M1 showed measurable antibacterial activity, and washed M1-C retained partial activity against *S. aureus*. These findings support a mechanism in which surface-associated phytochemical species are the main contributors to the observed antibacterial response, while Zn^{2+} release and ROS-related pathways may contribute secondarily but do not appear to dominate under the present experimental conditions. The main contribution of this work is the demonstration that solvent-controlled phytochemical mediation during ZnO synthesis generates measurable surface modifications that translate into altered nanoparticle-polymer interactions, enhanced viscoelastic reinforcement, and antibacterial functionality within a controlled SA/PVA hydrogel platform. This chemically and mechanically characterized system provides a foundation for future studies aimed at optimizing nanoparticle dispersion, loading, release behavior, cytocompatibility, and biological performance under more application-relevant conditions.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s42247-026-01441-4>.

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Author Contributions KO: Data curation, investigation, methodology, validation, writing-original draft. LFJ-C: Data curation, investigation, methodology, software, validation, writing-original draft. JR-S: Data curation, methodology, writing-review & editing. EO-V: Data curation, methodology, resources, validation, writing-review & editing. GR-G: Investigation, methodology, resources, writing-review & editing. LH: Conceptualization, data curation, formal analysis, investigation, software, supervision, validation, visualization, writing-original draft. MAF-H: Conceptualization, formal analysis, funding acquisition, project administration, resources, supervision, visualization, writing-review & editing.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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References

1. V. Gounden, M. Singh, Hydrogels and wound healing: current and future prospects. *Gels*. **10**(1), 43 (2024). <https://doi.org/10.3390/gels10010043>
2. E.I. Oprita, A. Iosageanu, O. Craciunescu, Natural polymeric hydrogels encapsulating small molecules for diabetic wound healing. *Gels*. **9**(11), 867 (2023). <https://doi.org/10.3390/gels9110867>
3. K. Sauer et al., The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nat. Rev. Microbiol.* **20**(10), 608–620 (2022). <https://doi.org/10.1038/s41579-022-00767-0>
4. P. Victor, D. Sarada, K.M. Ramkumar, Pharmacological activation of Nrf2 promotes wound healing. *Eur. J. Pharmacol.* **886**, 173395 (2020). <https://doi.org/10.1016/j.ejphar.2020.173395>

5. Y. Zheng et al., A bacteria-responsive nanoplatform with biofilm dispersion and ROS scavenging for the healing of infected diabetic wounds. *Acta Biomater.* **193**, 545–558 (2025). <https://doi.org/10.1016/j.actbio.2024.12.042>
6. A.C. Afonso et al., Biofilms in diabetic foot ulcers: impact, risk factors and control strategies. *Int. J. Mol. Sci.* **22**(15), 8278 (2021). <https://doi.org/10.3390/ijms22158278>
7. R. Ishwarya et al., Microbial synthesis of zinc oxide nanoparticles and their potential biological application as an antimicrobial and anticancer agent. *Biocatal. Agric. Biotechnol.* **62**, 103417 (2024). <https://doi.org/10.1016/j.bcab.2024.103417>
8. V.J.S. Vázquez et al., S. hispanica mucilage/ZnO based films: Preparation, characterization, and potential application. *Food Hydrocoll.* **159**, 110637 (2025). <https://doi.org/10.1016/j.foodhyd.2024.110637>
9. D. Xiao et al., Zinc oxide nanoparticles for skin wound healing: a systematic review from the perspective of disease types. *Mater. Today Bio.*, 102221, 102221 (2025). <https://doi.org/10.1016/j.mtbio.2025.102221>
10. K. Greeshma et al., Sustainable ZnO NPs for a Greener Future: An Integrative Review of Emerging Frontiers in Environmental, Biomedical, and Nanoinformatics Applications. *Biomedical Mater. Devices.* 1–28 (2025). <https://doi.org/10.1007/s44174-025-00624-7>
11. Green synthesized ZnO and ZnO-based composites for wound healing applications. *Bioprocess Biosyst. Eng.* **48**(4), 521–542 (2025). <https://doi.org/10.1007/s00449-024-03123-z>
12. The biosynthesis of zinc oxide nanoparticles using aqueous leaf extracts of *Cnidioscolus aconitifolius* and their biological activities. *Green Chem. Lett. Rev.* (2023). <https://doi.org/10.1080/17518253.2023.2169591>
13. Green Biosynthesis of Nanoparticles Using Plant Extracts: Mechanisms, Advances, Challenges, and Applications. *Bionanoscience* (2025). <https://doi.org/10.1007/s12668-025-01883-w>
14. Green Essence of Plant Resources Capped Zinc Oxide Nanoparticles: Renewable Distinctions, Size-Shape-Modulated Physicochemical Diversity and Emerging Biomedical-Environmental Usefulness. *Part. Part. Syst. Charact.* (2025). <https://doi.org/10.1002/ppsc.202400145>
15. J. Hou et al., Plant-based metal nanomaterials: green synthesis, biosynthesis mechanisms, biomedical and environmental applications. *Planta.* **263**(2), 47 (2026)
16. Y. Iqbal et al., Chitosan-based functional materials combined with plant extract: A promising strategy in the stimulation of wound healing process. *J. Drug Delivery Sci. Technol.* **101**, 106314 (2024). <https://doi.org/10.1016/j.jddst.2024.106314>
17. O.T. Somade et al., *Cnidioscolus aconitifolius* leaf extract and ascorbate confer amelioration and protection against dimethyl nitrosamine-induced renal toxicity and testicular abnormalities in rats. *Toxicol. Rep.* **8**, 1098–1108 (2021). <https://doi.org/10.1016/j.toxrep.2021.05.011>
18. Synergistic effect of zinc oxide-cinnamic acid nanoparticles for wound healing management: in vitro and zebrafish model studies. *BMC Biotechnol.* (2024). <https://doi.org/10.1186/s12896-024-00906-w>
19. M.A. Ibrahim et al., Physical characterization, biocompatibility, and antimicrobial activity of polyvinyl alcohol/sodium alginate blend doped with TiO₂ nanoparticles for wound dressing applications. *Sci. Rep.* **14**(1), 5391 (2024). <https://doi.org/10.1038/s41598-024-55818-8>
20. E.-R. Kenawy et al., Biosynthesized ZnO NPs loaded-electropun PVA/sodium alginate/glycine nanofibers: synthesis, spinning optimization and antimicrobial activity evaluation. *Sci. Rep.* **15**(1), 1882 (2025). <https://doi.org/10.1038/s41598-024-81939-1>
21. S. Reisi et al., Biocompatible ZnO nanoflower-infused chitosan/alginate/PVA composite for accelerated skin regeneration. *J. Mater. Sci. - Mater. Med.* **36**(1), 87 (2025). <https://doi.org/10.1007/s10856-025-06950-y>
22. K. Shalumon et al., Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. *Int. J. Biol. Macromol.* **49**(3), 247–254 (2011). <https://doi.org/10.1016/j.ijbiomac.2011.04.005>
23. S. Veeramani et al., Development and characterization of ZnO-, CuO-, and CuO–ZnO-incorporated porous sodium alginate–polyvinyl alcohol–gelatin hybrid bio-nanocomposite scaffolds for wound healing applications. *Polym. Bull.* 1–23 (2025). <https://doi.org/10.1007/s00289-025-05875-4>
24. L. Hernandez et al., The anti-corrosion performance of chaya leaf extracts on pure Mg substrates in Ringer's solution. *Colloids Surf.* **688**, 133554 (2024). <https://doi.org/10.1016/j.colsurfa.2024.133554>
25. F. Cilurzo, C.G. Gennari, P. Minghetti, Adhesive properties: a critical issue in transdermal patch development. *Expert Opin. Drug Discovery.* **9**(1), 33–45 (2012). <https://doi.org/10.1517/17425247.2012.637107>
26. de R.G. Oliveira-Junior et al., Phytochemical and pharmacological aspects of *Cnidioscolus Pohl* species: A systematic review. *Phytomedicine.* **50**, 137–147 (2018). <https://doi.org/10.1016/j.phymed.2017.08.017>
27. M. Díaz-Fernández et al., Conformational Analysis and NMR Data Assignment of Taraxerone Isolated From *Cnidioscolus aconitifolius* (Chaya). *Magn. Reson. Chem.* **64**(1), 146–159 (2026). <https://doi.org/10.1002/mrc.70055>
28. S.T. Karam, A.F. Abdulrahman, Green synthesis and characterization of ZnO nanoparticles by using thyme plant leaf extract. *Photonics.* 2022. MDPI
29. M. Mahajan et al., Correction: Green synthesis of ZnO nanoparticles using *Justicia adhatoda* for photocatalytic degradation of malachite green and reduction of 4-nitrophenol. *RSC Adv.* **15**(10), 7843–7843 (2025). <https://doi.org/10.1039/D4RA08632E>
30. A. Vega-Poot et al., The nucleation kinetics of ZnO nanoparticles from ZnCl₂ in ethanol solutions. *Nanoscale.* **2**(12), 2710–2717 (2010). <https://doi.org/10.1039/c0nr00439a>
31. D. Singh et al., A study of ZnO nanoparticles and ZnO-EG nanofluid. *J. Exp. Nanosci.* **8**(5), 731–741 (2013). <https://doi.org/10.1080/17458080.2011.602369>
32. P.R. Verma, F. Khan, S. Banerjee, *Salvadora persica* root extract-mediated fabrication of ZnO nanoparticles and characterization. *Inorg. Nano-Metal Chem.* **51**(3), 427–433 (2020). <https://doi.org/10.1080/24701556.2020.1793355>
33. L. Wu, Y. Wu, Synthesis and optical characteristic of ZnO nanorod. *J. Mater. Sci.* **42**(1), 406–408 (2007). <https://doi.org/10.1007/s10853-006-0727-y>
34. A. Yadav et al., Functional finishing in cotton fabrics using zinc oxide nanoparticles. *Bull. Mater. Sci.* **29**(6), 641–645 (2006). <https://doi.org/10.1007/s12034-006-0017-y>
35. Y. Li et al., Phyto-mediated synthesis of ZnO/attapulgit nanocomposites using *C. limon* peel extract for enhancing the resistance against multidrug-resistant bacteria and fungi. *Inorg. Chem. Commun.* **170**, 113470 (2024). <https://doi.org/10.1016/j.inoche.2024.113470>
36. R. Al-Gaashani et al., XPS and optical studies of different morphologies of ZnO nanostructures prepared by microwave methods. *Ceram. Int.* **39**(3), 2283–2292 (2013). <https://doi.org/10.1016/j.ceramint.2012.08.075>
37. N. Nedyalkov et al., Laser Deposition of Metal Oxide Structures for Gas Sensor Applications. *Materials.* **19**(1), 176 (2026). <https://doi.org/10.3390/ma19010176>
38. A. Sahai, N. Goswami, Probing the dominance of interstitial oxygen defects in ZnO nanoparticles through structural and optical characterizations. *Ceram. Int.* **40**(9), 14569–14578 (2014). <https://doi.org/10.1016/j.ceramint.2014.06.041>

39. A review on C1s XPS-spectra for some kinds of carbon materials. Fullerenes Nanotubes Carbon Nanostruct. **28**(12), 1048–1058 (2020). <https://doi.org/10.1080/1536383x.2020.1794851>
40. T. Foophow et al., Preparation and characterization of dextran-modified ZnO and Cu-doped ZnO nanohybrid material for enhanced antimicrobial delivery and activity. Carbohydr. Polym. **349**, 122947 (2025). <https://doi.org/10.1016/j.carbpol.2024.122947>
41. L. Yang et al., Catal. Sci. Technol. (2026). <https://doi.org/10.1039/D6CY00211K>
42. E. Tilahun, Y. Adimasu, Y. Dessie, Biosynthesis and optimization of ZnO nanoparticles using Ocimum lamifolium leaf extract for electrochemical sensor and antibacterial activity. ACS omega. **8**(30), 27344–27354 (2023). <https://doi.org/10.1021/acsomega.3c02709>
43. S. Vaezifar, E. Etemadi, P. Heydari, Chitosan nanoparticles as a smart carrier for Capparis spinosa L. root extract: a targeted approach against hepatocellular carcinoma. Emergent Mater. **9**(5), 112 (2026). <https://doi.org/10.1007/s42247-026-01320-y>
44. J. Suresh et al., Green synthesis and characterization of zinc oxide nanoparticle using insulin plant (Costus pictus D. Don) and investigation of its antimicrobial as well as anticancer activities. Adv. Nat. Sci. Nanosci. Nanotechnol. **9**(1), 015008 (2018). <https://doi.org/10.1088/2043-6254/aa6f1>
45. P. Dadashi, K. Torbatinejad, A. Babaei, Hybridization as a promising approach to engineering the desired performance of bio-nanocomposites: GO-ZnO hybrid reinforced PCL. Sci. Rep. **15**(1), 17259 (2025). <https://doi.org/10.1038/s41598-025-02087-8>
46. J. Liu, H. Liang, Morphological and structural properties of isotactic polypropylene filled with nano-zinc oxide as investigated by dynamic rheology, creep and recovery in shear. Polym. Bull. **79**(9), 7923–7937 (2022). <https://doi.org/10.1007/s00289-021-03890-9>
47. H. Malektaj, A.D. Drozdov, J. deClaville, Christiansen, Mechanical properties of alginate hydrogels cross-linked with multivalent cations. Polymers. **15**(14), 3012 (2023). <https://doi.org/10.3390/polym15143012>
48. Y. Wang et al., Progress in research on metal ion crosslinking alginate-based gels. Gels. **11**(1), 16 (2024). <https://doi.org/10.3390/gels11010016>
49. X. Li et al., Strong, tough and mechanically self-recoverable poly (vinyl alcohol)/alginate dual-physical double-network hydrogels with large cross-link density contrast. RSC Adv. **8**(30), 16674–16689 (2018). <https://doi.org/10.1039/C8RA01302K>
50. W. You, W. Yu, Slow linear viscoelastic relaxation of polymer nanocomposites: contribution from confined diffusion of nanoparticles. Macromolecules. **52**(23), 9094–9104 (2019). <https://doi.org/10.1021/acs.macromol.9b01538>
51. G.D. Hattemer, G. Arya, Viscoelastic properties of polymer-grafted nanoparticle composites from molecular dynamics simulations. Macromolecules. **48**(4), 1240–1255 (2015). <https://doi.org/10.1021/ma502086c>
52. S. Asadpour, M. Kooravand, A. Asfaram, A review on zinc oxide/poly (vinyl alcohol) nanocomposites: Synthesis, characterization and applications. J. Clean. Prod. **362**, 132297 (2022). <https://doi.org/10.1016/j.jclepro.2022.132297>
53. M.C. Burroughs et al., Gelation dynamics during photo-cross-linking of polymer nanocomposite hydrogels. ACS Polym. Au. **3**(2), 217–227 (2022). <https://doi.org/10.1021/acspolymersau.2c00051>
54. Dynamics and viscoelastic behavior of waterborne acrylic polymer/silica nanocomposite coatings. Prog. Org. Coat. **129**, 125–132 (2019). <https://doi.org/10.1016/j.porgcoat.2019.01.017>
55. J. Song, N. Holten-Andersen, G.H. McKinley, Non-Maxwellian viscoelastic stress relaxations in soft matter. Soft Matter. **19**(41), 7885–7906 (2023). <https://doi.org/10.1039/D3SM00736G>
56. N. Akhras, A. Çelekli, H. Bozkurt, Enhanced Antimicrobial Activity of Green-Synthesized Artemisia-ZnO Nanoparticles: A Comparative Study with Pure ZnO Nanoparticles and Plant Extract. Foods. **14**(14), 2449 (2025). <https://doi.org/10.3390/foods14142449>
57. K. Ali et al., Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. J. Colloid Interface Sci. **472**, 145–156 (2016). <https://doi.org/10.1016/j.jcis.2016.03.021>
58. Z. Emami-Karvani, P. Chehrizi, Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria. Afr. J. Microbiol. Res. **5**(12), 1368–1373 (2011). <https://doi.org/10.5897/AJMR10.159>
59. M. Arakha et al., The effects of interfacial potential on antimicrobial propensity of ZnO nanoparticle. Sci. Rep. **5**(1), 9578 (2015). <https://doi.org/10.1038/srep09578>
60. M. Arab et al., Synthesis, rheological characterization, and antibacterial activity of polyvinyl alcohol (PVA)/zinc oxide nanoparticles wound dressing, achieved under electron beam irradiation. Iran. Polym. J. **30**(10), 1019–1028 (2021). <https://doi.org/10.1007/s13726-021-00952-7>
61. S. Al-Shehri et al., Synthesis of multifunctional flexible polymeric PVA/PEG blend nanocomposite films filled with AZO nanoparticles. Polym. Bull. **81**(16), 14561–14585 (2024). <https://doi.org/10.1007/s00289-024-05407-6>
62. H. Alvandi et al., Incorporation of Aloe vera and green synthesized ZnO nanoparticles into the chitosan/PVA nanocomposite hydrogel for wound dressing application. Polym. Bull. **81**(5), 4123–4148 (2024). <https://doi.org/10.1007/s00289-023-04874-7>
63. R. Murtafi'atin, A. Kusumaatmaja, Evaluation of Moringa oleifera Incorporated With Zinc Oxide Nanoparticles/Poly (Vinyl) Alcohol Nanofibers as Antibacterial Material. J. Appl. Polym. Sci. **142**(11), e56606 (2025). <https://doi.org/10.1002/app.56606>
64. Effect of biosynthesized ZnO and Ag/ZnO nanoparticles on bacterial cellulose/polyvinyl alcohol films for strawberry preservation. Cellulose **32**(2), 1049–1070 (2025). <https://doi.org/10.1007/s10570-024-06316-7>
65. Sodium alginate/polyvinyl alcohol/ZnO nanoparticles incorporated konjac extract for active food packaging. Polym. Bull. **82**(13), 8565–8588 (2025). <https://doi.org/10.1007/s00289-025-05870-9>
66. S. Mottola et al., Pectin/alginate aerogel containing ZnO produced from beetroot extract mediated green synthesis for potential applications in food packaging. J. CO₂ Utilization. **91**, 103003 (2025). <https://doi.org/10.1016/j.jcou.2024.103003>

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