

ORIGINAL ARTICLE

Antimicrobial Activity of a Cinnamon-Mediated ZnO Product Compared with the Crude Extract: A Preliminary Study

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ABSTRACT

Antimicrobial resistance continues to drive interest in agents that act through multiple mechanisms rather than depending on synthetic chemistry alone, and plant-mediated routes to zinc-containing nanomaterials are one such avenue. This preliminary study used aqueous *Cinnamomum verum* bark extract as a reducing and capping agent for zinc acetate, generating a cinnamon-ZnO product that was analyzed spectroscopically by FTIR and UV-Vis and tested for antibacterial and antifungal activity against *Bacillus subtilis*, *Escherichia coli*, and *Aspergillus niger* by agar well diffusion, alongside the crude extract itself. The product matched or exceeded the extract's inhibition zones at substantially lower applied volumes across all three organisms, reaching 28 mm against *B. subtilis* at 20 μ L, 32 mm against *E. coli* at 20 μ L, and 24 mm against *A. niger* at 40 μ L. Activity at these reduced volumes proved measurable and consistent across organisms, though whether it reflects a true mass-normalized dose advantage remains open, since the product's concentration was not independently quantified. Spectroscopic signals from FTIR and UV-Vis pointed to a phytochemical-associated surface but stopped short of establishing the product's crystalline structure or morphology, which would need XRD or electron microscopy to confirm.

Keywords: *Cinnamomum verum*, zinc oxide, green synthesis, antimicrobial activity, agar well diffusion.

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INTRODUCTION

Antimicrobial resistance ranks among the most pressing global health threats, driven in large part by antibiotic overuse across medical, agricultural, and environmental settings [4]. This has pushed interest toward nanotechnology-based antimicrobials, particularly metal oxide nanoparticles, which damage microbial membranes, generate reactive oxygen species, and release metal ions simultaneously, a multi-mechanism attack that microbes find harder to develop resistance against [4].

Zinc oxide (ZnO) stands out within this category. It acts against both Gram-positive and Gram-negative bacteria, combining this broad-spectrum activity with notable chemical stability and a favourable safety profile [4, 10]. Environmental and biocompatibility concerns increasingly surround the conventional routes to ZnO, which typically demand elevated temperatures alongside reducing agents and solvents that are far from benign [8]. Plant-extract-based green synthesis sidesteps much of this: compounds such as phenolics, flavonoids, and terpenoids double as reducing and capping agents in one step, a route reported to leave the resulting product better dispersed, more stable, and more bioavailable than material made the conventional way [11, 8].

Cinnamon (*Cinnamomum verum*) fits this role well. Cinnamaldehyde and eugenol, both present in the bark and essential oil, account for much of its antimicrobial character, acting mainly through damage to microbial cell membranes [10, 11, 5].

Combining cinnamon phytochemicals with ZnO appears to do more than simply add their individual effects. Cinnamon essential oil loaded onto ZnO nanoparticles has shown synergistic antimicrobial activity across Gram-positive, Gram-negative, and fungal pathogens [10], consistent with the broader pattern that essential-oil-loaded ZnO systems can outperform either component alone [1]. These hybrid systems have been proposed for antimicrobial wound dressings, surface coatings, and food packaging, applications where a sustained, low-level antimicrobial effect matters [10, 11].

Despite this promise, direct comparison between a cinnamon extract and its corresponding ZnO-mediated product, tested under the same assay conditions, is not something every green-synthesis report addresses. Studies combining synthesis with antimicrobial testing do not always examine closely how phytochemicals on the product surface change bioactivity relative to the crude extract they derive from [2].

This preliminary study prepared a cinnamon-mediated ZnO product using aqueous *Cinnamomum verum* bark extract as a green reducing and capping agent, analysed it spectroscopically by FTIR and UV-Vis, and directly compared its antibacterial and antifungal activity against *Bacillus subtilis*, *Escherichia coli*, and *Aspergillus niger* with that recorded for the crude cinnamon extract.

MATERIAL AND METHODS

Plant material

Bark of *Cinnamomum verum* was sourced commercially from a local grocery store in Kishangarh, Ajmer, Rajasthan, India. Botanical identity was based on vendor labeling rather than formal taxonomic authentication. No herbarium voucher specimen was deposited for the bark.

Chemicals and reagents

Zinc acetate, ethanol, methanol, distilled water, potassium bromide, crystal violet dye, safranin dye, Gram's iodine, lactophenol cotton blue dye, and immersion oil were all of analytical grade, procured from CDH, India.

Preparation of cinnamon extracts

Two extracts were prepared from the bark, each for a different purpose.

The first was a crude extract used for direct antimicrobial testing. Fresh bark (10 g) was washed, crushed in a blender, and mixed with 10 mL of ethanol to form a paste. The paste then sat in a water bath held at 75°C for a period of 30 minutes. This step followed the general principles of heat-assisted solvent extraction described for medicinal plants [3]. The temperature, solvent ratio, and duration used here were specific to this study. Straining the heated paste recovered 4 mL of extract, stored at 4°C as a 50% solution until use.

The second was an aqueous extract used as the reducing and capping agent for synthesis. A separate batch of bark was washed with distilled water, air-dried for several days, and ground to a fine powder. Boiling 10 g of this powder in 100 mL of distilled water for 30 minutes produced the extract. Whatman filter paper was used to filter it, and the filtrate was kept at 4°C until required.

Synthesis of the cinnamon-ZnO product

Zinc acetate (4 g) was dissolved in 50 mL of distilled water with stirring. Ten milliliters of the aqueous cinnamon extract was then added to this solution. Using a magnetic stirrer, the mixture was maintained at 50°C under continuous stirring for 2 hours. Centrifugation followed, carried out three times at 5000 rpm with each cycle lasting 10 minutes. The resulting precipitate was collected and left to dry overnight inside an oven [7]. This is referred to throughout as the cinnamon-ZnO product.

Before antimicrobial testing, the dried product was dispersed in 1 mL of ethanol and sonicated in a digital ultrasonic bath for 30 minutes to form a uniform suspension. Its concentration was not independently determined as a mass value. Instead, it tracked the yield of the corresponding cinnamon extract. This constraint is addressed directly in the Discussion.

Microbial cultures and purity assessment

The bacterial panel comprised *Escherichia coli* and *Bacillus subtilis*. *Aspergillus niger* served as the fungal test organism. All three were lab-prepared stock cultures without assigned strain or accession numbers. Purity was assessed phenotypically, not through definitive species authentication.

For bacteria, heat-fixed smears were first stained with crystal violet for 60 seconds and then treated with Gram's iodine. A 60-second decolorization step with 95% alcohol followed, after which safranin

counterstain was applied for 30 to 40 seconds. Smears were examined at 100x [15]. *B. subtilis* stained purple. *E. coli*-stained pink. This matched their expected cell wall structures.

For fungi, a small portion of culture was mounted in lactophenol cotton blue and examined at 10x to 40x. Hyphae, spores, and conidial structures were visible [9].

Nutrient Broth and Luria-Bertani broth supported bacterial growth under agitation at 120 rpm, held at 37°C, while Nutrient Agar plates were incubated statically at the same temperature. Both formats were maintained for 16 to 24 hours. Fungal cultures took longer: Potato Dextrose Broth cultures were agitated at 120 rpm at 25 to 27°C, while Potato Dextrose Agar and Mueller-Hinton Agar plates were held statically over the same range, for 48 to 72 hours.

Antimicrobial activity assays

Antibacterial activity was tested by the agar well diffusion method [13] on Mueller-Hinton agar. Test organisms were swab-inoculated onto plates, and 6 mm wells were bored with a sterile puncture tool. Cinnamon extract was applied at 20, 50, and 100 µL per well. The cinnamon-ZnO product suspension was applied at 5, 10, and 20 µL per well. Plates were incubated at 37°C for 24 hours.

Antifungal activity against *A. niger* used the same method on Potato Dextrose Agar. Cinnamon extract was applied at 50, 100, and 200 µL per well, and the product suspension at 10, 20, and 40 µL per well. Plates were incubated at 25 to 27°C for 48 to 72 hours.

For both assays, zones of inhibition were measured in millimeters, including the well diameter. Poorly defined zones were recorded as "+."

Spectroscopic analysis

FTIR spectra were recorded by grinding the dried product with potassium bromide and compressing it into a pellet. Spectra were scanned over 4000 to 400 cm⁻¹ on a PerkinElmer Spectrum 65 spectrometer.

For UV-Vis analysis, the product was dispersed in ethanol. Dissolution was incomplete, but the resulting suspension was stable enough for optical measurement. Spectra were recorded on a Shimadzu UV-1800 double-beam spectrophotometer over 190 to 1100 nm.

Statistical analysis

All zone-of-inhibition measurements were performed in triplicate. Replicate consistency was assessed qualitatively, not through a formal statistical test. Inter-replicate variation stayed below 2% across conditions. Reported values represent the mean of the three readings for each condition.

RESULT AND DISCUSSION

Microbial identity and purity

The expected phenotypic features appeared for all three organisms (Fig. 1): Gram staining gave the expected results for the two bacteria, and lactophenol cotton blue examination did the same for the fungal culture. *B. subtilis* retained the crystal violet stain and appeared purple, in keeping with a thicker peptidoglycan cell wall, while *E. coli* stained pink, matching its thinner wall and the absence of that retention. *A. niger* showed dark, globose conidial heads on long conidiophores under lactophenol cotton blue. Together, these three organisms span Gram-positive, Gram-negative, and filamentous fungal cell envelope structures, a reasonable spread given that cell envelope interaction is reported as one of the main factors governing ZnO nanoparticle activity [10].

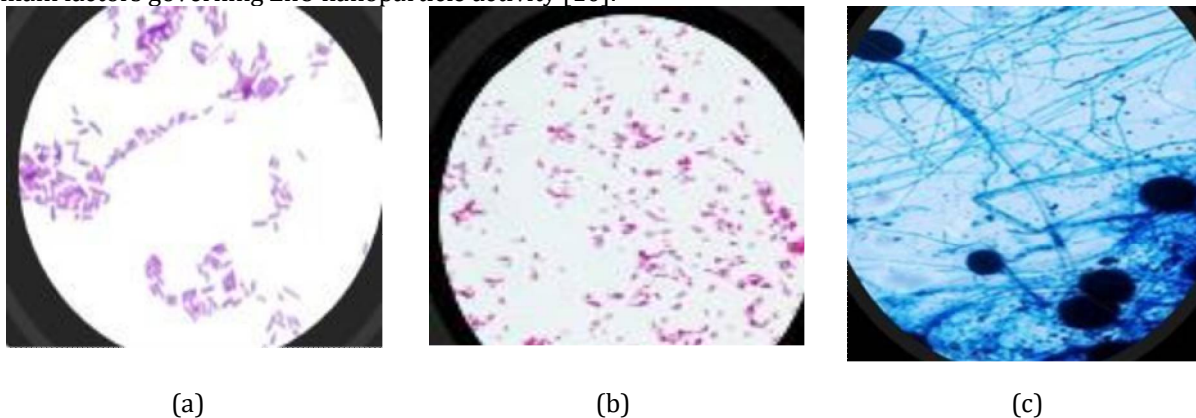


Fig. 1. Confirmation of microbial identity. (a) Gram stain of *Bacillus subtilis*, Gram-positive, 100x. (b) Gram stain of *Escherichia coli*, Gram-negative, 100x. (c) Lactophenol cotton blue stain of *Aspergillus niger* showing conidial heads, 40x.

Synthesis and spectroscopic analysis

Reacting zinc acetate with the aqueous cinnamon extract yielded a visible precipitate through the sequence of bark, bark powder, aqueous extract, and finally the dried cinnamon-ZnO product (Fig. 2).

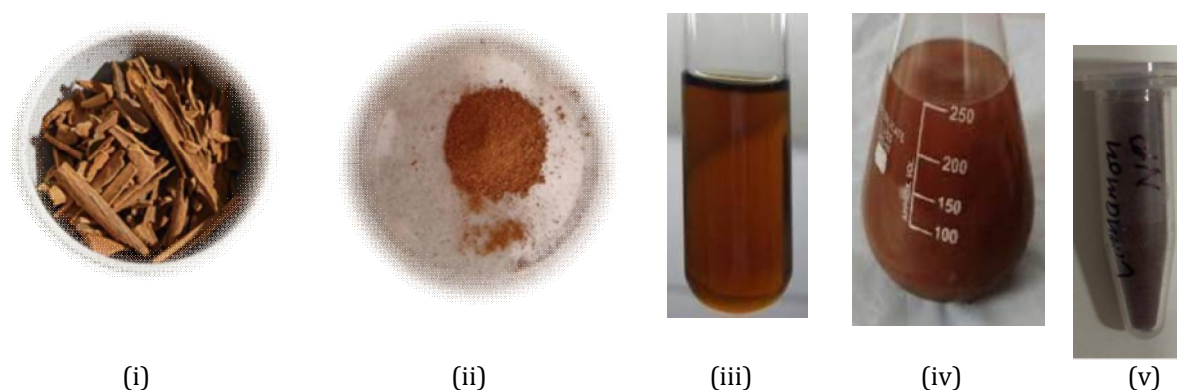


Fig. 2: Synthesis of the cinnamon-ZnO product: (i) cinnamon bark, (ii) cinnamon bark powder, (iii) extract obtained by boiling the cinnamon bark powder, (iv) addition of zinc acetate and cinnamon bark extract, (v) cinnamon bark-ZnO product.

FTIR analysis of this product showed bands at 3422, 1562, 1418, 1025, 691, and 618 cm^{-1} (Fig. 3a). A broad band at 3422 cm^{-1} is attributable to O-H stretching, most likely from phenolic compounds carried over from the extract. Bands at 1562 and 1418 cm^{-1} fall in the region expected for aromatic C=C stretching and O-H or C-H bending, pointing to organically bound biomolecules on the product surface. Two lower bands, at 691 and 618 cm^{-1} , align with Zn-O stretching, a pattern broadly in line with FTIR assignments reported for related plant-mediated ZnO systems (Motelica et al., 2023). Together, these bands indicate a product surface carrying both Zn-O-associated vibrations and organic functional groups attributable to cinnamon-derived constituents. This is more indicative of phytochemicals associated with the surface than proof of a defined core-shell nanostructure, since FTIR alone cannot resolve particle morphology or crystalline phase.

UV-Vis analysis gave a different picture than expected (Fig. 3b). Literature typically places the ZnO nanoparticle absorption maximum between 360 and 380 nm (Sirelkhatim et al., 2015), but no peak appeared in that window here. Instead, peaks were observed at 254.5 and 270.5 nm, in line with $\pi \rightarrow \pi^*$ transitions of aromatic phytochemicals, along with a slight peak at 594.5 nm. This absence is itself a notable result rather than a failed measurement. Three explanations could account for it: a small particle size producing a strong blue shift, strong absorption from organic constituents overshadowing it, or simply a low concentration of the product within the dispersion. None of these can be confirmed or ruled out with the data collected here. What FTIR and UV-Vis together support is that the product surface carries cinnamon-derived organic character. What they cannot establish is the crystalline identity or morphology of the underlying core, which would require XRD or electron microscopy.

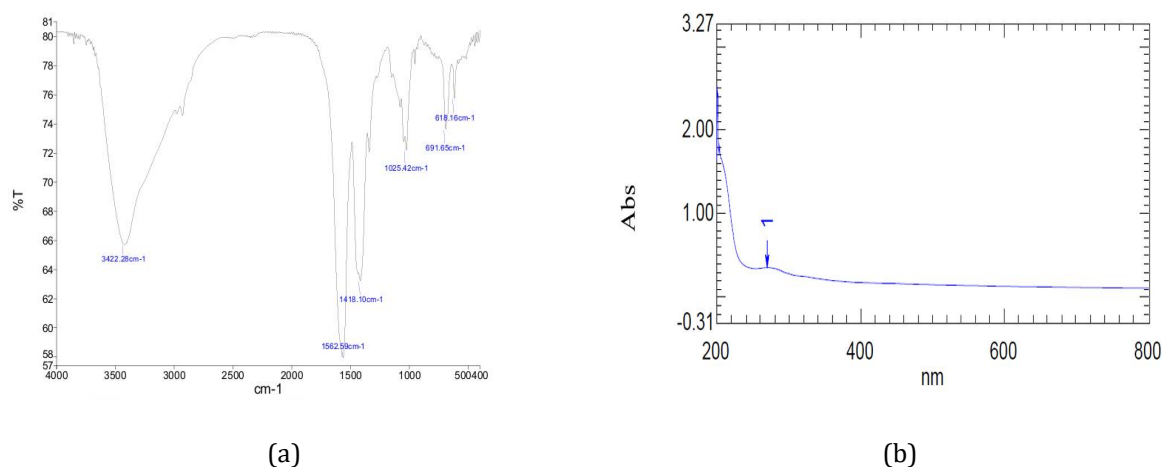


Fig. 3. Spectroscopic characterisation of cinnamon-ZnO product. (a) FTIR spectrum. (b) UV-Vis absorption spectrum.

Antibacterial Activity

The zone of inhibition data for the cinnamon extract and cinnamon-ZnO product against *B. subtilis* and *E. coli* (Fig. 4) are summarised in Table 1.

Table 1. Zone of inhibition (mm) against bacterial strains. Values represent the mean of three replicate measurements (Section 2.8).

Agent	Concentration (μL)	<i>B. subtilis</i>	<i>E. coli</i>
Cinnamon extract	100	26	28
Cinnamon extract	50	20	15
Cinnamon extract	20	14	12
Cinnamon-ZnO product	20	28	32
Cinnamon-ZnO product	10	20	21
Cinnamon-ZnO product	5	14	10

Inhibition zones for the crude extract increased steadily with applied volume across both organisms, reaching 26 mm against *B. subtilis* and 28 mm against *E. coli* only at the highest tested volume of 100 μL . At 20 μL , one-fifth the extract volume needed for a similar result, the cinnamon-ZnO product reached comparable or larger zones, 28 mm against *B. subtilis* and 32 mm against *E. coli*. Smaller zones of 10 to 21 mm were still evident for the product at 5 to 10 μL against both organisms. Against *E. coli* specifically, the effect was most pronounced: the product produced the largest zone recorded across these assays, worth noting because plant extracts alone are often weaker against Gram-negative bacteria, whose outer membrane limits penetration by many phytochemicals [12].

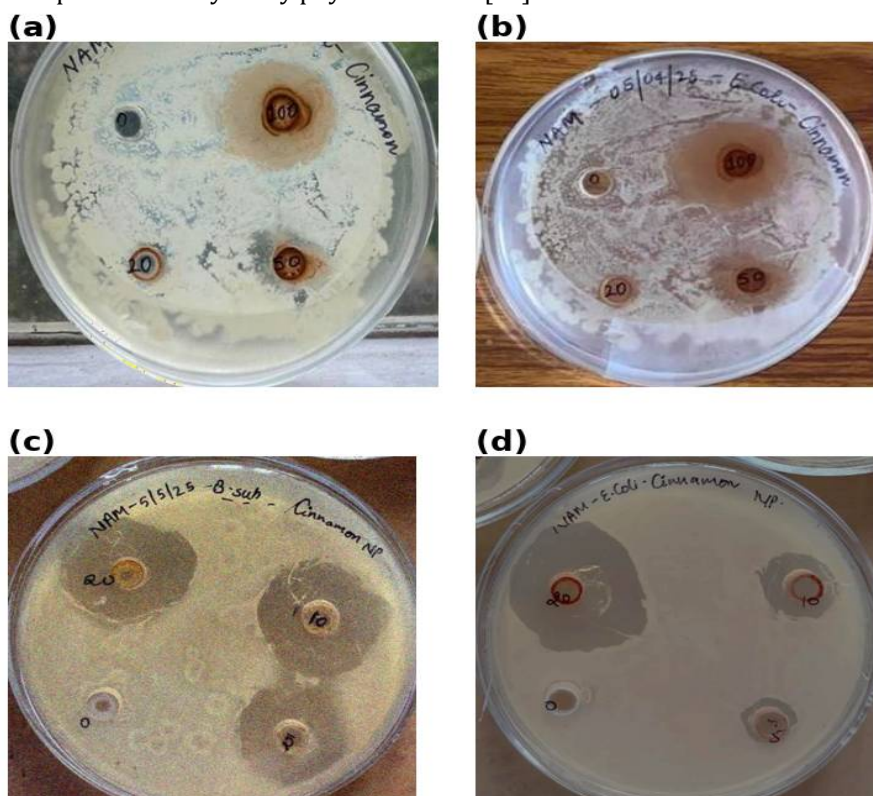


Fig. 4. Zone-of-inhibition assays against bacterial strains. (a) Cinnamon extract vs. *B. subtilis*. (b) Cinnamon extract vs. *E. coli*. (c) Cinnamon-ZnO product vs. *B. subtilis*. (d) Cinnamon-ZnO product vs. *E. coli*.

Antifungal Activity

Antifungal results against *A. niger* (Fig. 5) are summarised in Table 2. The same concentration-dependent pattern held here: extract activity rose from a minimal, poorly defined zone at 50 μL to 18 mm at 100 μL and 36 mm at 200 μL , while the product reached 24 mm at 40 μL , with measurable zones of 16 mm and 12 mm at 20 and 10 μL .

Table 2. Zone of inhibition (mm) against *Aspergillus niger*. Values represent the mean of three replicate measurements (Section 2.8).

Agent	Concentration (μL)	ZOI (mm)
Cinnamon extract	200	36
Cinnamon extract	100	18
Cinnamon extract	50	+ (minimal/incomplete zone)
Cinnamon-ZnO product	40	24
Cinnamon-ZnO product	20	16
Cinnamon-ZnO product	10	12

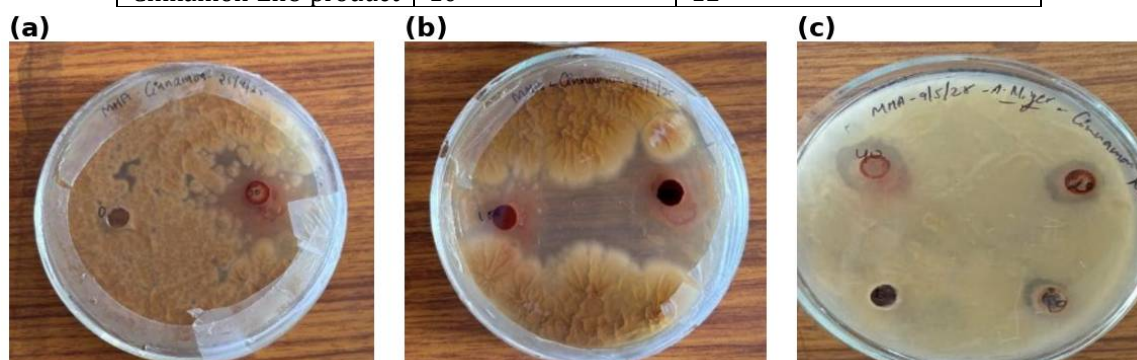


Fig. 5. Zone-of-inhibition assays against *Aspergillus niger*. (a) Cinnamon extract at 0 and 50 μL. (b) Cinnamon extract at 100 and 200 μL. (c) Cinnamon-ZnO product.

Comparative pattern, possible mechanisms, and interpretive limits

The cinnamon-mediated ZnO product demonstrated antimicrobial activity comparable to or greater than that of the corresponding crude extract against all three tested organisms, despite being evaluated at substantially lower applied volumes. The consistency of this response across the tested organisms suggests that the observed activity was not restricted to a single microbial species. However, the findings should be interpreted in the context of the experimental design, particularly the distinction between applied-volume efficiency and concentration-normalised antimicrobial potency.

Previous studies have proposed several mechanisms underlying the antimicrobial activity of ZnO nanoparticles, including disruption of microbial membrane integrity, generation of reactive oxygen species (ROS), and the release of Zn^{2+} ions [10, 12]. These mechanisms provide a plausible theoretical basis for interpreting the activity observed in the present study. Furthermore, the phytochemical groups indicated by FTIR analysis may contribute to the surface chemistry of the product and potentially influence its interaction with microbial cells. Nevertheless, these explanations remain hypothetical within the present experimental framework. Neither ROS generation, zinc-ion release, nor the direct involvement of surface-associated phytochemicals was experimentally quantified. Accordingly, the observed antimicrobial response cannot be attributed conclusively to any individual mechanism or to a synergistic interaction between ZnO and cinnamon-derived constituents.

Consequently, the comparisons presented in Tables 1 and 2 represent antimicrobial performance at different applied volumes under the specified assay conditions rather than a mass-normalised dose-response assessment. The ability of the cinnamon-ZnO product to produce comparable or greater inhibition at a lower applied volume is therefore a meaningful experimental observation. However, it does not, by itself, establish greater intrinsic potency, higher specific activity, or superior antimicrobial efficiency on a mass basis. Such conclusions require the independent determination of product concentration and the evaluation of antimicrobial responses across defined, comparable doses. This distinction is essential for avoiding overinterpretation of the findings and for establishing a reliable basis for comparison with other nanoparticle-based antimicrobial formulations.

Despite this limitation, consistent inhibition across the three tested organisms supports further investigation of cinnamon-mediated ZnO as a potential antimicrobial material. Future studies should employ XRD, TEM, and SEM to characterise its crystalline structure, morphology, and

nanoscale properties. The atypical UV–visible absorption profile also requires further investigation, including assessment of particle size, aggregation, and surface-associated phytochemicals. Mechanistic studies evaluating reactive oxygen species (ROS) generation, Zn²⁺ release, and phytochemical contributions, alongside appropriate controls, are needed to clarify antimicrobial activity. Furthermore, mass-normalised dose–response testing, additional microbial strains, and statistical validation are essential to establish antimicrobial potency, reproducibility, and potential practical applicability.

CONCLUSION

The product carries phytochemicals from the cinnamon extract on its surface, per the FTIR evidence discussed above. During synthesis the extract did double duty, reducing the zinc precursor while also capping the resulting surface, which places the product somewhere between organic and inorganic character rather than as a purely inorganic phase.

In every organism tested, the cinnamon-ZnO product matched or exceeded the crude extract's antimicrobial activity while using a substantially smaller applied volume, a pattern most pronounced against *E. coli*, where the product needed only a fraction of the extract's volume to achieve comparable inhibition. This study did not directly test the phytochemicals retained on the product surface, but membrane disruption, oxidative stress, and zinc ion release are mechanisms documented elsewhere for ZnO, and remain plausible contributors here rather than confirmed findings of this work.

These results support cinnamon-mediated green synthesis as a route toward antimicrobial materials that require less applied volume than their source extract, with potential relevance to applications such as wound dressings or food packaging, where minimizing material load matters. What remains open is the product's underlying structure, since the evidence gathered here is spectroscopic rather than structural. Confirming that structure, and extending these findings further, would require XRD or electron microscopy.

Conflicts of Interest: Nil

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