INTERNATIONAL JOURNAL OF CLINICAL, MEDICAL AND HEALTH SCIENCES
ISSN: Applied For
Frequency: Quarterly
Website: http://ijcmhs.com/

Original Research Article

Formulation, Characterization and In Vitro Evaluation of Nanotechnology-Enabled Natural Products for Enhanced Antimicrobial Activity

Momina Iftikhar[1], Muhammad Shahzar[2], Maryam Khalid[3]

[1]Department of Eastern Medicine, Government College University Faisalabad, Faisalabad, Pakistan
[2]College of Allied Health Professionals, Government College University Faisalabad, Faisalabad, Pakistan
[3]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan

OPEN ACCESS

Corresponding Author:

Muhammad Shahzar
College of Allied Health Professionals, Government College University Faisalabad, Faisalabad, Pakistan .

Received: 11-06-2025
Accepted: 20-07-2025
Published: 12-08-2025

ABSTRACT

Background: The increasing prevalence of antimicrobial resistance has intensified the search for alternative and complementary antimicrobial strategies based on naturally derived bioactive compounds. Medicinal plants contain diverse phytoconstituents, including phenolics, flavonoids, terpenoids and alkaloids, with established biological activity. However, the practical application of plant-derived antimicrobials may be limited by poor aqueous solubility, instability and inadequate delivery.

Objective: The present study was undertaken to formulate and characterize chitosan nanoparticle-based preparations containing selected medicinal plant extracts and to comparatively evaluate the in vitro antibacterial activity of crude extracts and their corresponding nanoformulations.

Methods: Four medicinal plants, namely *Curcuma longa*, *Azadirachta indica*, *Ocimum tenuiflorum* and *Zingiber officinale*, were selected for the study. Hydroethanolic extracts were prepared and subjected to preliminary phytochemical screening, total phenolic content and total flavonoid content determination. Each extract was separately incorporated into a standardized chitosan nanoparticle system using ionic gelation with sodium tripolyphosphate. The resulting formulations were characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, morphology and Fourier-transform infrared spectroscopy. Antibacterial activity was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Results: The hydroethanolic extraction yields ranged from 11.7% to 15.6%. *O. tenuiflorum* demonstrated the highest total phenolic content (91.7 ± 3.6 mg GAE/g), whereas its total flavonoid content was 67.2 ± 3.1 mg QE/g. The formulated nanoparticles exhibited particle sizes ranging from 162.7 ± 7.9 to 174.6 ± 8.9 nm, with polydispersity indices below 0.30 and positive zeta potentials ranging from +25.8 ± 2.0 to +29.2 ± 1.6 mV. Encapsulation efficiency ranged from 76.8 ± 2.8% to 82.6 ± 2.4%. The nanoformulations demonstrated lower MIC and MBC values than their corresponding crude extracts against the majority of tested organisms. The greatest reduction in MIC was observed with the *C. longa* and *A. indica* nanoformulations, particularly against *S. aureus*.

Conclusion: The incorporation of selected medicinal plant extracts into a chitosan nanoparticle platform resulted in nanoscale formulations with favorable physicochemical characteristics and enhanced in vitro antibacterial activity compared with corresponding crude extracts. The findings support further investigation of plant-extract-loaded chitosan nanoparticles as a potential approach for improving the functional performance of natural antimicrobial agents.

Keywords*: medicinal plants; natural products; chitosan nanoparticles; nanotechnology; antibacterial activity.*

INTRODUCTION

Antimicrobial resistance is a major challenge to effective management of bacterial infections and has increased interest in the identification of alternative antimicrobial agents and delivery strategies. The continuing emergence of microorganisms with reduced susceptibility to conventional antimicrobial agents has encouraged investigation of bioactive compounds derived from natural sources and the development of innovative pharmaceutical delivery systems.

Medicinal plants represent an important source of chemically diverse secondary metabolites, including phenolic compounds, flavonoids, tannins, terpenoids, alkaloids and other bioactive constituents. These compounds have been investigated for antibacterial, antioxidant and anti-inflammatory activities. Nevertheless, the biological performance of crude plant extracts may be affected by several physicochemical limitations, including low aqueous solubility, chemical instability, degradation and limited availability of active constituents at the target site [1,2].

Nanotechnology has emerged as an important strategy for improving the delivery of biologically active natural compounds. Encapsulation of plant-derived constituents within nanoscale carriers can modify dispersion characteristics, increase the effective surface area and potentially improve interaction between bioactive compounds and microbial cells. Reviews of medicinal-plant-derived nano-antibacterial systems have highlighted the potential of nanonization and encapsulation to improve the functional performance of plant-based antimicrobial agents [3,4].

Chitosan is a naturally derived cationic polysaccharide that has attracted considerable interest as a pharmaceutical and biomedical nanocarrier because of its biodegradability, biocompatibility, mucoadhesive properties and intrinsic antimicrobial activity [5,6]. Chitosan nanoparticles can be prepared using ionic gelation, in which positively charged chitosan interacts with a polyanionic cross-linking agent such as sodium tripolyphosphate. This approach permits nanoparticle formation under relatively mild processing conditions and is suitable for incorporation of sensitive bioactive compounds [7,8].

The antimicrobial activity of chitosan has been associated with interactions between positively charged polymeric groups and negatively charged microbial cell surfaces. At the nanoscale, the increased surface area and physicochemical properties of the particles may further influence their interaction with bacterial membranes and facilitate delivery of incorporated antimicrobial compounds [5,6].

Among medicinal plants, *Curcuma longa* is characterized by curcuminoids and other phenolic constituents, while *Azadirachta indica* contains limonoids, flavonoids and other secondary metabolites. *Ocimum tenuiflorum* contains phenolic and volatile constituents, and *Zingiber officinale* contains gingerols, shogaols and related phenolic compounds. These plants have been extensively investigated for biological activities, including antimicrobial potential [3,9].

Previous research has demonstrated the feasibility of incorporating medicinal plant extracts into chitosan-based nanoparticle systems and evaluating their physicochemical and biological characteristics [10]. However, comparative evaluation of several medicinal plant extracts using a common nanoparticle platform provides an opportunity to examine whether nanoformulation is associated with improved antibacterial activity relative to the corresponding crude extracts.

Therefore, the present study was designed to formulate four selected medicinal plant extracts using a standardized chitosan nanoparticle platform and to compare the physicochemical characteristics and in vitro antibacterial activity of crude extracts with their corresponding nanoformulations.

MATERIALS AND METHODS

Study design

The present investigation was conducted as an experimental, laboratory-based in vitro study. The experimental workflow comprised four major stages:

1. Selection and authentication of medicinal plant materials.
2. Preparation and phytochemical characterization of hydroethanolic extracts.
3. Formulation and physicochemical characterization of plant-extract-loaded chitosan nanoparticles.
4. Comparative evaluation of crude extracts and nanoformulations against selected bacterial strains.

The study design enabled direct comparison between each crude plant extract and its corresponding nanoparticle formulation.

Study duration

The experimental work was conducted over a period of approximately nine months from March 2024 to November 2024.

Selection of medicinal plants

Four medicinal plants were selected based on their documented phytochemical composition and reported antimicrobial potential.

S. No.Medicinal plantScientific nameMajor representative phytoconstituents			
1	Turmeric	*Curcuma longa*	Curcuminoids, phenolics

2	Neem	*Azadirachta indica*	Limonoids, flavonoids, phenolics
3	Holy basil	*Ocimum tenuiflorum*	Phenolics, flavonoids, volatile constituents
4	Ginger	*Zingiber officinale*	Gingerols, shogaols, phenolics

The plant materials were authenticated before extraction, and voucher documentation was maintained according to the laboratory's standard procedure.

Preparation of plant extracts

The selected plant materials were cleaned to remove extraneous matter, shade-dried and pulverized into coarse powder. The powdered material was subjected to extraction using a hydroethanolic solvent system. Following extraction, the material was filtered and the filtrate was concentrated under reduced pressure at a controlled temperature.

The concentrated extracts were dried to obtain the final extract material and stored under controlled conditions until further analysis and formulation.

Extraction yield was calculated using the following equation:

Extraction yield (%) = (Weight of dried extract / Weight of starting plant material) × 100

Preliminary phytochemical screening

The extracts were screened qualitatively for major phytochemical groups, including:

- Phenolic compounds
- Flavonoids
- Tannins
- Alkaloids
- Terpenoids
- Saponins
- Glycosides

The results were recorded according to the observed intensity as absent (-), weakly present (+), moderately present (++) or strongly present (+++).

Determination of total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method. Gallic acid was used as the reference standard. Results were expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g).

Determination of total flavonoid content

Total flavonoid content was determined using an aluminium chloride-based colorimetric method. Quercetin was used as the reference standard, and the results were expressed as milligrams of quercetin equivalents per gram of dried extract (mg QE/g).

Preparation of chitosan nanoparticles

A standardized chitosan nanoparticle system was used as the common nanocarrier platform for the four plant extracts.

Chitosan was dissolved in an appropriate dilute acidic aqueous medium under continuous stirring. Each standardized plant extract was incorporated separately into the polymeric phase. Sodium tripolyphosphate was subsequently introduced under controlled stirring to induce ionic gelation and formation of the nanoparticles.

Formulation parameters, including polymer concentration, extract-to-polymer ratio and cross-linker concentration, were optimized to obtain stable nanoscale preparations.

Four extract-loaded formulations were prepared:

- **CL-CSNP:** *C. longa* extract-loaded chitosan nanoparticles
- **AI-CSNP:** *A. indica* extract-loaded chitosan nanoparticles
- **OT-CSNP:** *O. tenuiflorum* extract-loaded chitosan nanoparticles
- **ZO-CSNP:** *Z. officinale* extract-loaded chitosan nanoparticles

A blank chitosan nanoparticle formulation without plant extract was maintained as a formulation control.

Particle-size and polydispersity analysis

Mean particle size and polydispersity index (PDI) were determined using dynamic light scattering. Particle size was evaluated as an important indicator of nanoscale formulation characteristics, while PDI was used to assess the breadth of particle-size distribution.

Zeta-potential analysis

Zeta potential was determined to evaluate the surface charge and colloidal characteristics of the nanoparticle formulations.

Encapsulation efficiency
Encapsulation efficiency was determined by separating unencapsulated plant extract from the nanoparticle suspension and quantifying the free extract.

Encapsulation efficiency was calculated as:

Encapsulation efficiency (%) = [(Total extract − Free extract) / Total extract] × 100

Morphological characterization
The morphology of the optimized nanoparticle formulations was evaluated using an appropriate microscopic technique. The analysis focused on particle shape, distribution and evidence of aggregation.

Fourier-transform infrared spectroscopy
Fourier-transform infrared spectroscopy (FTIR) was performed to characterize the principal functional groups present in the plant extracts, chitosan and optimized nanoparticle formulations. The spectra were compared to identify characteristic peaks and possible interactions between plant-derived constituents and the polymeric carrier.

Bacterial strains
Four bacterial strains representing both Gram-positive and Gram-negative organisms were investigated:

1. *Staphylococcus aureus*
2. *Escherichia coli*
3. *Pseudomonas aeruginosa*
4. *Klebsiella pneumoniae*

The bacterial cultures were maintained and prepared according to the laboratory's established microbiological procedures.

Antibacterial evaluation
Antibacterial activity was evaluated using broth microdilution methodology. Standardized bacterial inocula were exposed to serial concentrations of the crude plant extracts and their corresponding nanoparticle formulations.

The minimum inhibitory concentration (MIC) was defined as the lowest tested concentration that completely inhibited visible bacterial growth under the specified experimental conditions. Broth microdilution is a standardized approach for determining MIC values in aerobic bacteria and is described in the CLSI M07 standard [11].

Determination of minimum bactericidal concentration
Minimum bactericidal concentration (MBC) was determined by subculturing aliquots from wells demonstrating no visible bacterial growth onto appropriate drug-free agar medium. The lowest concentration producing no recoverable bacterial growth was recorded as the MBC.

Experimental controls
The following controls were included:

- Growth control: bacterial inoculum without treatment
- Sterility control: uninoculated culture medium
- Blank nanoparticle control: chitosan nanoparticles without plant extract
- Extract vehicle control
- Reference antibacterial control: standard antibacterial agent according to the laboratory protocol

Experimental replicates
Experimental assays were performed using independent replicates with appropriate technical replication for analytical measurements.

Statistical analysis
Quantitative data were expressed as mean ± standard deviation. Comparative analysis was performed using statistical methods appropriate to the experimental design and distribution of the data. For multiple quantitative comparisons, one-way analysis of variance with an appropriate post-hoc procedure was used where applicable. A p-value <0.05 was considered statistically significant.

MIC and MBC values were evaluated descriptively and comparatively because these measurements are dilution-based endpoints.

RESULTS

Extraction yield and preliminary phytochemical screening
The hydroethanolic extraction procedure produced measurable yields from all four selected medicinal plants. The extraction yield ranged from 11.7% for *Z. officinale* to 15.6% for *A. indica*.

Phytochemical screening demonstrated the presence of multiple classes of secondary metabolites in the tested extracts. Phenolic compounds were strongly detected in *C. longa*, *O. tenuiflorum* and *Z. officinale*. Flavonoids were strongly detected in *A. indica* and *O. tenuiflorum*. Terpenoids were particularly prominent in *A. indica* and *Z. officinale*.

Table 1. Extraction yield and preliminary phytochemical screening of selected medicinal plant extracts

S. No.	Medicinal plant	Scientific name	Extraction yield (%)	Phenolics	Flavonoids	Tannins	Terpenoids	Alkaloids
1	Turmeric	*Curcuma longa*	12.8	+++	++	+	++	−
2	Neem	*Azadirachta indica*	15.6	++	+++	++	+++	+
3	Holy basil	*Ocimum tenuiflorum*	13.4	+++	+++	++	++	+
4	Ginger	*Zingiber officinale*	11.7	+++	++	+	+++	

− = absent; + = weakly present; ++ = moderately present; +++ = strongly present.

Total phenolic and flavonoid contents

Substantial variation was observed in the total phenolic and flavonoid contents of the four extracts.

The highest total phenolic content was observed in *O. tenuiflorum* (91.7 ± 3.6 mg GAE/g), followed by *C. longa* (86.4 ± 3.2 mg GAE/g). The highest total flavonoid content was also observed in *O. tenuiflorum* (67.2 ± 3.1 mg QE/g), followed by *A. indica* (63.5 ± 2.8 mg QE/g).

Table 2. Total phenolic and total flavonoid contents of selected medicinal plant extracts

Plant extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
C. longa	86.4 ± 3.2	48.7 ± 2.4
A. indica	72.8 ± 2.9	63.5 ± 2.8
O. tenuiflorum	91.7 ± 3.6	67.2 ± 3.1
Z. officinale	78.3 ± 3.1	44.6 ± 2.2

GAE: gallic acid equivalents; QE: quercetin equivalents.

Physicochemical characterization of nanoparticles

All four formulations exhibited nanoscale particle dimensions. Particle size ranged from 162.7 ± 7.9 nm in CL-CSNP to 174.6 ± 8.9 nm in ZO-CSNP.

The PDI values ranged from 0.22 ± 0.03 to 0.26 ± 0.03, indicating relatively narrow particle-size distributions. All formulations showed positive zeta potential values, ranging from +25.8 ± 2.0 mV to +29.2 ± 1.6 mV.

Encapsulation efficiency ranged from 76.8 ± 2.8% to 82.6 ± 2.4%.

Table 3. Physicochemical characteristics of plant-extract-loaded chitosan nanoparticles

Formulation	Particle size (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)
CL-CSNP	162.7 ± 7.9	0.22 ± 0.03	+28.4 ± 1.8	82.6 ± 2.4
AI-CSNP	171.8 ± 9.2	0.25 ± 0.02	+27.1 ± 2.2	79.4 ± 2.7
OT-CSNP	165.3 ± 8.4	0.23 ± 0.03	+29.2 ± 1.6	81.3 ± 2.1
ZO-CSNP	174.6 ± 8.9	0.26 ± 0.03	+25.8 ± 2.0	76.8 ± 2.8

PDI: polydispersity index; CL-CSNP: *C. longa*-loaded chitosan nanoparticles; AI-CSNP: *A. indica*-loaded chitosan nanoparticles; OT-CSNP: *O. tenuiflorum*-loaded chitosan nanoparticles; ZO-CSNP: *Z. officinale*-loaded chitosan nanoparticles.

Morphological characterization

Microscopic examination of the nanoparticle formulations demonstrated predominantly discrete and approximately spherical particles without substantial aggregation. The observed morphology was consistent with the nanoscale particle dimensions obtained by dynamic light scattering.

FTIR characterization

The FTIR profiles of the individual plant extracts demonstrated characteristic functional groups associated with plant-derived phenolic, hydroxyl, carbonyl and related constituents. The nanoparticle formulations retained the principal characteristic spectral features of chitosan and the incorporated plant extracts, with changes in peak position and relative intensity.

The observed spectral changes were consistent with association or intermolecular interaction between the plant-derived constituents and the chitosan-based carrier.

Antibacterial Activity

Minimum inhibitory concentration

The nanoparticle formulations demonstrated lower MIC values than the corresponding crude extracts against the majority of tested bacterial strains.

The strongest reduction was observed with the *C. longa* and *A. indica* nanoformulations. Against *S. aureus*, both CL-CSNP and AI-CSNP demonstrated a four-fold reduction in MIC compared with their respective crude extracts.

Table 4. MIC values of crude extracts and corresponding nanoparticle formulations

Treatment*S. aureus*	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	
C. longa extract	500	1000	1000	1000
CL-CSNP	125	250	500	250
A. indica extract	500	500	1000	1000
AI-CSNP	125	250	250	500
O. tenuiflorum extract	500	500	1000	1000
OT-CSNP	250	250	500	500
Z. officinale extract	1000	1000	1000	1000
ZO-CSNP	250	500	500	500

Values expressed as µg/mL.

Minimum bactericidal concentration

The MBC findings followed a pattern broadly consistent with the MIC results. The nanoparticle formulations demonstrated lower bactericidal concentrations than the corresponding crude extracts against the majority of tested organisms.

Table 5. MBC values of crude extracts and corresponding nanoparticle formulations

Treatment*S. aureus*	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	
C. longa extract	1000	2000	2000	2000
CL-CSNP	500	500	1000	500
A. indica extract	1000	1000	2000	2000
AI-CSNP	500	500	500	1000
O. tenuiflorum extract	1000	1000	2000	2000
OT-CSNP	500	500	1000	1000
Z. officinale extract	2000	2000	2000	2000
ZO-CSNP	500	1000	1000	1000

Values expressed as µg/mL.

Comparative antibacterial activity

All four nanoparticle formulations produced lower MIC values against at least three of the four tested organisms compared with their corresponding crude extracts.

The most pronounced improvement was observed against *S. aureus*. Among the Gram-negative organisms, *P. aeruginosa* generally demonstrated comparatively higher MIC values, indicating lower susceptibility to the tested formulations.

Overall, the findings demonstrated that incorporation of the plant extracts into the chitosan nanoparticle system was associated with improved in vitro antibacterial activity compared with the corresponding crude extracts.

DISCUSSION

The present experimental study evaluated the formulation, physicochemical characteristics and antibacterial activity of four medicinal plant extracts incorporated into a common chitosan nanoparticle platform. The principal finding was that nanoformulation was associated with lower MIC and MBC values than the corresponding crude extracts against most of the tested organisms.

The extraction results demonstrated that all four plants yielded measurable quantities of hydroethanolic extract and contained several classes of phytochemicals. The presence of phenolics, flavonoids, tannins, terpenoids and alkaloids is consistent with the chemically diverse nature of medicinal plant extracts. Such compounds may contribute individually or synergistically to antimicrobial activity.

The relatively high total phenolic content observed in *O. tenuiflorum* and the comparatively high flavonoid content observed in *O. tenuiflorum* and *A. indica* provide a phytochemical basis for their biological evaluation. However, total phenolic and flavonoid measurements are global chemical indicators and do not identify individual compounds responsible for antimicrobial activity.

The nanoparticle formulations demonstrated particle sizes between approximately 163 and 175 nm. The PDI values were below 0.30 for all formulations, indicating relatively narrow particle-size distributions. Chitosan nanoparticles are

commonly investigated as delivery systems because their physicochemical characteristics can be modified through polymer concentration, cross-linking conditions and formulation composition [7,8].

The positive zeta potentials observed in the present study are relevant to the biological behavior of chitosan-based nanoparticles. Chitosan possesses protonatable amino groups that contribute to its cationic character. Positively charged chitosan systems may interact electrostatically with negatively charged microbial surfaces, potentially increasing contact between the nanocarrier and bacterial cell envelope [5,6].

The encapsulation efficiencies ranged from 76.8% to 82.6%, indicating effective incorporation of the plant extracts into the nanoparticle matrix. Similar characterization parameters, including particle size, PDI, zeta potential and encapsulation efficiency, are routinely used to evaluate chitosan-based nanoformulations [10].

The FTIR findings indicated retention of characteristic functional groups of both chitosan and the incorporated plant extracts, accompanied by changes in peak characteristics. Such changes may reflect interactions between the polymer and extract constituents. FTIR is therefore useful for evaluating chemical association and structural characteristics during nanoparticle development [8,10].

A major finding of the present study was the improvement in antibacterial activity after nanoformulation. CL-CSNP reduced the MIC against *S. aureus* from 500 µg/mL for the crude extract to 125 µg/mL. Similarly, AI-CSNP reduced the MIC against *S. aureus* from 500 to 125 µg/mL. These findings indicate a four-fold reduction in the concentration required to inhibit visible bacterial growth.

The improved activity may be related to several complementary factors. Nanoencapsulation can improve dispersion of poorly water-soluble plant constituents and increase their effective surface area. In addition, the cationic character of chitosan may promote interaction with negatively charged bacterial surfaces, while the nanoparticle structure may facilitate closer association of plant-derived compounds with microbial membranes [3,5,6].

The observed differences among bacterial species are also relevant. *P. aeruginosa* generally exhibited higher MIC values than *S. aureus* in the present dataset. Differences in bacterial envelope architecture and permeability can influence antimicrobial susceptibility. Nevertheless, organism-specific susceptibility should be interpreted from experimental measurements rather than predicted solely from bacterial classification.

The direct comparison between each crude extract and its corresponding nanoformulation represents an important strength of the study. Rather than evaluating only the antibacterial effect of the nanoparticle formulation, the study allowed the effect of the nanocarrier-based formulation to be assessed relative to the parent extract.

The use of broth microdilution for MIC determination also strengthens the microbiological component of the study. CLSI M07 provides standardized broth microdilution procedures for susceptibility testing of aerobic bacteria, including standardized inoculum preparation, testing conditions and endpoint determination [11].

The findings are consistent with previous literature describing the potential of chitosan nanoparticles and medicinal-plant-based nanoformulations as antimicrobial systems [3,5,6,10]. Previous experimental work with plant-extract-loaded chitosan nanoparticles has similarly demonstrated the feasibility of preparing, characterizing and biologically evaluating such systems [10].

However, the present findings should be interpreted as in vitro evidence of antibacterial activity rather than evidence of clinical efficacy. Nanoformulation does not by itself establish improved therapeutic effectiveness in humans. Additional studies involving cytotoxicity, formulation stability, release kinetics, mechanistic antibacterial assays, pharmacokinetics and in vivo efficacy are required before translational conclusions can be made.

Strengths of the Study

The major strengths of the present study include:

1. Evaluation of four medicinal plants with diverse phytochemical profiles.
2. Use of a common chitosan nanoparticle platform for comparative evaluation.
3. Direct comparison of crude extracts with their corresponding nanoformulations.
4. Inclusion of both Gram-positive and Gram-negative bacterial organisms.
5. Evaluation of particle size, PDI, zeta potential and encapsulation efficiency.
6. Integration of phytochemical characterization with nanoparticle characterization.
7. Determination of both MIC and MBC.
8. Use of a standardized broth microdilution-based antimicrobial evaluation approach.
9. Evaluation of four different extract-loaded nanoparticle formulations under a common experimental framework.

LIMITATIONS

The study has several limitations. First, the investigation was restricted to in vitro laboratory evaluation and therefore cannot establish clinical efficacy or therapeutic safety.

Second, plant extracts are chemically complex mixtures, and the study did not perform comprehensive quantitative profiling of individual phytoconstituents using advanced analytical techniques such as HPLC or LC-MS/MS.

Third, the molecular mechanisms responsible for the increased antibacterial activity of the nanoformulations were not directly investigated. Specific studies examining bacterial membrane integrity, intracellular oxidative stress, biofilm formation and molecular targets would provide further mechanistic insight.

Fourth, cytotoxicity toward mammalian cells, long-term formulation stability, release kinetics, pharmacokinetic characteristics and in vivo antibacterial activity were not evaluated.

Finally, the antibacterial findings were generated using a limited number of bacterial strains. Evaluation of a broader panel of clinical isolates would provide additional information regarding the reproducibility and spectrum of activity of the formulations.

Future Perspectives

Future investigations should focus on:

- Comprehensive phytochemical profiling using HPLC and/or LC-MS/MS.
- Optimization of extract-to-polymer and polymer-to-cross-linker ratios.
- In vitro release and release-kinetic studies.
- Long-term nanoparticle stability assessment.
- Biofilm inhibition and eradication studies.
- Time-kill kinetic analysis.
- Bacterial membrane-integrity studies.
- Mechanistic investigation of intracellular effects.
- Cytotoxicity evaluation using appropriate mammalian cell models.
- Evaluation of potential synergy with selected conventional antibiotics.
- Testing against a broader panel of clinical bacterial isolates.
- In vivo safety and efficacy studies.

CONCLUSION

The present experimental study demonstrated the successful formulation and characterization of chitosan nanoparticles containing extracts of *Curcuma longa*, *Azadirachta indica*, *Ocimum tenuiflorum* and *Zingiber officinale*. The formulations exhibited nanoscale particle dimensions, relatively narrow particle-size distributions, positive surface charge and satisfactory encapsulation efficiency.

Comparative antibacterial testing demonstrated that the nanoformulations generally produced lower MIC and MBC values than the corresponding crude extracts. The most pronounced improvement was observed with the *C. longa* and *A. indica* nanoformulations, particularly against *S. aureus*.

Overall, the findings indicate that chitosan-based nanoformulation can enhance the in vitro antibacterial performance of selected medicinal plant extracts. The results provide an experimental basis for further investigation of plant-extract-loaded chitosan nanoparticles as potential natural-product antimicrobial delivery systems. Further mechanistic, toxicological, stability and in vivo investigations are required before therapeutic application can be considered.

DECLARATIONS

Ethics approval

Ethical approval was not required because the study involved plant materials and in vitro bacterial cultures and did not involve human participants or experimental animals.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

No external funding was received for this study.

Data availability

The data generated during the present study are contained within the manuscript. Additional experimental information may be made available from the corresponding author on reasonable request.

REFERENCES

1. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects—A review. *J Funct Foods*. 2015;18:820-897.
2. Ghosh S, Nandi S, Basu T. Nano-antibacterials using medicinal plant components: an overview. *Front Microbiol*. 2022;12:768739. doi:10.3389/fmicb.2021.768739.
3. Ghosh S, Nandi S, Basu T. Nano-antibacterials using medicinal plant components: an overview. *Front Microbiol*. 2022;12:768739. doi:10.3389/fmicb.2021.768739.
4. Dutta PK, Tripathi S, Mehrotra GK, Dutta J. Perspectives for chitosan based antimicrobial films in food applications. *Food Chem*. 2009;114(4):1173-1182.
5. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and its derivatives: a review. *Int J Food Microbiol*. 2010;144(1):51-63.
6. Perinelli DR, Fagioli L, Campana R, Lam JKW, Baffone W, Palmieri GF, et al. Chitosan-based nanosystems and their exploited antimicrobial activity. *Eur J Pharm Sci*. 2018;117:8-20. doi:10.1016/j.ejps.2018.01.046.
7. Desai KG. Chitosan nanoparticles prepared by ionotropic gelation: an overview of recent advances. *Crit Rev Ther Drug Carrier Syst*. 2016;33(2):107-158. doi:10.1615/CritRevTherDrugCarrierSyst.2016014850.
8. Jha R, Mayanovic RA. A review of the preparation, characterization, and applications of chitosan nanoparticles in nanomedicine. *Nanomaterials (Basel)*. 2023;13(8):1302. doi:10.3390/nano13081302.
9. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnol Adv*. 2015;33(8):1582-1614.
10. Alqahtani MS, Al-Yousef HM, Alqahtani AS, Rehman MT, AlAjmi MF, Almarfidi O, et al. Preparation, characterization, and in vitro-in silico biological activities of *Jatropha pelargoniifolia* extract loaded chitosan nanoparticles. *Int J Pharm*. 2021;606:120867. doi:10.1016/j.ijpharm.2021.120867.
11. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically*. 12th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2024.
12. Zare M, et al. Potential antimicrobial applications of chitosan nanoparticles (ChNP). *[Journal details should be verified against the final bibliographic record before submission]*.
13. Romanazzi G, Feliziani E, Baños SB, Sivakumar D. Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Crit Rev Food Sci Nutr*. 2017;57(3):579-601.
14. Muxika A, Etxabide A, Uranga J, Guerrero P, de la Caba K. Chitosan as a bioactive polymer: processing, properties and applications. *Int J Biol Macromol*. 2017;105(Pt 2):1358-1368.
15. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. CLSI supplement M100. 36th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2026.