

Nanoherbal-Antimicrobials in Modern Therapeutics: A Paradigm Shift in Healthcare Innovation

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ABSTRACT

Antimicrobial potential of silver nanoparticles (AgNPs) synthesized from herbal extracts of *Phyllanthus emblica* (amla), *Murraya koenigii* (kadhipatta), *Trachyspermum ammi* (ajwain) in the first experiment, and *Curcuma longa* (turmeric), *Syzygium aromaticum* (clove), *Zingiber officinale* (ginger), and *Ocimum sanctum* (tulsi) in the second experiment were evaluated. Also, their efficacy against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria was assessed. Methods: Silver nanoformulations were prepared using the aforementioned herbal extracts. Antibacterial activity was tested using the liquid broth culture to measure bacterial growth via absorbance on UV Spectrophotometry and confirmed further by the agar disc diffusion method to observe zones of inhibition. Concentrations tested included fortified herbal formulations in the range of 10-100 µg/ml. Comparative analysis was performed between crude extracts and corresponding fortified nanoformulations. The sample size for each plant was kept at 7, which was further performed in triplicate. Results: Nanoherbal formulations showed a significant reduction in bacterial growth, evidenced by lower absorbance values in broth cultures and larger zones of inhibition on agar plates. Also, fortified nanoformulations exhibited greater antibacterial activity than crude extracts. Gram-positive bacteria (*S. aureus*) responded more effectively than Gram-negative bacteria (*E. coli*). For *E. coli*, antibacterial potential at 80 µg/mL in the first experiment followed the order *Phyllanthus emblica* > *Murraya koenigii* > *Trachyspermum ammi*, and for *S. aureus*, at the same concentration was *Murraya koenigii* > *Trachyspermum ammi* > *Phyllanthus emblica*. For the second experiment, *Zingiber* > *Curcuma* > *Ocimum* > *Syzygium* was the trend for antibacterial potential. Conclusion: These findings advocate for the therapeutic application of nanoherbs in suppressing bacterial growth and associated infections.

Keywords: Nanoherb, antimicrobial, nanoparticle, multidrug resistant (MDR).

INTRODUCTION

Green nanotechnology, an interdisciplinary fusion of green engineering and green chemistry, represents a sustainable approach to materials science at the nanoscale. Nanotechnology itself deals with structures ranging from 1 to 100 nanometers and encompasses the manipulation and control of matter at this scale to develop novel materials, devices, and systems. An exciting and rapidly emerging application within this domain is nanoherbal therapeutics, which integrates principles of nanotechnology with herbal medicine. This approach involves encapsulating bioactive phytochemicals from medicinal plants into nanocarriers such as liposomes, nanoparticles, or nanofibers—

dramatically improving their solubility, stability, and bioavailability. As a result, these nanoscale formulations exhibit enhanced therapeutic efficacy against microbial pathogens, offering a promising strategy to combat antimicrobial resistance in modern healthcare. The present study focuses on the sustainable fabrication of nano-products derived from medicinal plants. Our goal is to conserve natural resources, minimize energy input, and eliminate toxic reagents, ensuring that both the process and its end-products remain environmentally benign. Nanoparticle synthesis can be carried out via physical, chemical, and biological methodologies. Physical methods include microwave irradiation, high-energy gamma radiation, and plasma techniques¹. Chemical methods span polyol synthesis, colloidal techniques,

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and electrochemical approaches^{2,3}. Biological methods, which align best with green nanotechnology principles, involve the use of bacteria, algae, fungi, viruses, plants, yeast, and human cell lines to produce nanoparticles in a non-toxic manner⁴⁻¹⁰. Biogenically synthesized nanoparticles often outperform those produced by conventional physical or chemical

means, largely due to their enhanced surface area-to-volume ratio and biofunctionalization with plant-derived compounds. Broadly, nanoparticle fabrication follows two main strategies: the top-down approach, which reduces bulk materials to the nanoscale, and the bottom-up approach, which assembles nanoscale structures from atomic or molecular precursors¹¹.

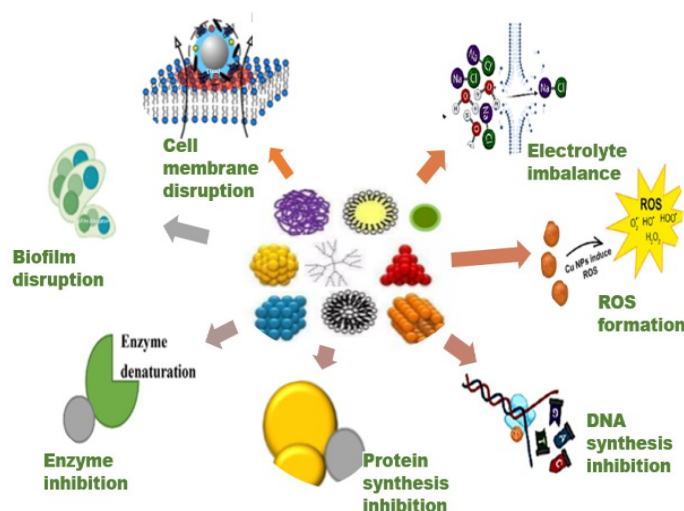


Figure 1. Proposed mechanism of action of phyto-nanoparticles illustrating their multifaceted antibacterial effects. The nanoparticles disrupt bacterial cell membranes and biofilms, induce electrolyte imbalance, generate reactive oxygen species (ROS), and inhibit the synthesis of DNA, proteins, and essential enzymes—collectively impairing microbial viability and proliferation

Regardless of the synthesis method, a broad spectrum of nanoparticle types exists, including polymeric, carbon-based, semiconductor, ceramic, metallic, and lipid-based nanoparticles. Additionally, a range of specialized nanocarriers has been developed for targeted delivery, such as solid-lipid particles, liposomes, dendrimers, polymeric micelles, carbon nanotubes (CNTs), as well as inorganic and magnetic nanoparticles^{12, 13}. Their size-dependent properties have enabled diverse applications across multiple sectors. These nanoparticles frequently exhibit potent biological activities—including antimicrobial, anticarcinogenic, antineoplastic, and anti-inflammatory effects, which make them highly valuable in advancing sustainable and precision-driven healthcare and environmental solutions^{14, 15}. Figure 1 illustrates how the phyto-assisted silver nanoparticles (AgNPs) mediate their antibacterial action¹⁶. In the present study, silver was employed to fabricate nanoherbals or phyto-nanoparticles, harnessing plant-derived compounds (Table 1) for enhanced antimicrobial activity. Silver ions (Ag^+)

exhibit bactericidal action by initiating cell wall lysis through disruption of membrane integrity, leading to intracellular leakage. Bioactive molecules such as polyphenols, organic acids, flavonoids, and terpenoids extracted from plants directly target bacterial cell walls and organellar membranes. This action results in cytoplasmic leakage and coagulation, protein denaturation, enzymatic dysfunction, including inhibition of DNA and RNA synthesis, disruption of the electron transport chain (ETC), impairing ATP generation, altered nutrient uptake, and lipid metabolism (Figure 1).

MATERIAL AND METHODS:

The first experimental setup involved synthesizing silver nanoparticles (AgNPs) using leaf extracts of *Murraya koenigii* (kadhi patta), *Phyllanthus emblica* (amla), and *Trachyspermum ammi* (ajwain). For the second experimental setup, rhizomes from *Curcuma longa* (turmeric), *Zingiber officinale* (ginger), *Ocimum sanctum* (tulsi) leaves, and dried floral buds

of *Syzygium aromaticum* (clove) were utilized. The plant leaves were collected from the Botanical Garden of Gargi College, and rhizomes and floral buds were procured from the nearby local market of Delhi. Taxonomic identification and authentication of plant species were done by the Botanists from the Botany department of Gargi College. These nanoparticles were evaluated for their antibacterial efficacy against pathogenic strains of *Escherichia coli* (5HD α) and *Staphylococcus aureus*. Figure 2 shows different parts of plants for the synthesis of fortified silver nanoparticles with their phyto constituents and applications.

PREPARATION OF PLANT EXTRACT:

Plant extract preparation followed the established protocol^{17, 18}. 100 g of each of the plant parts as samples were washed with tap water, rinsed thoroughly in running water, and dried using filter

paper. Each sample was dried to a fine powder, and 10 g of each was placed in a beaker with 100 mL of deionized water. The mixture was heated at 60 °C in a water bath for 20 minutes, then allowed to cool at room temperature¹⁹. Filtration was performed using Whatman filter paper, followed by centrifugation at 81 $\times$ g for 20 minutes. The supernatant was stored under refrigeration at 4 °C for subsequent nanoparticle synthesis.

PREPARATION OF PRECURSOR:

A stock solution of 10 mM silver nitrate (AgNO₃) was prepared in distilled water. Serial dilutions ranging from 1.0 mM to 5.0 mM were created. AgNO₃ was used as a precursor for the synthesis of silver nanoparticles (AgNPs) from leaf extracts. For this, a 1mM solution of silver nitrate was prepared using double-distilled water and stored at 4 °C in a refrigerator²⁰.

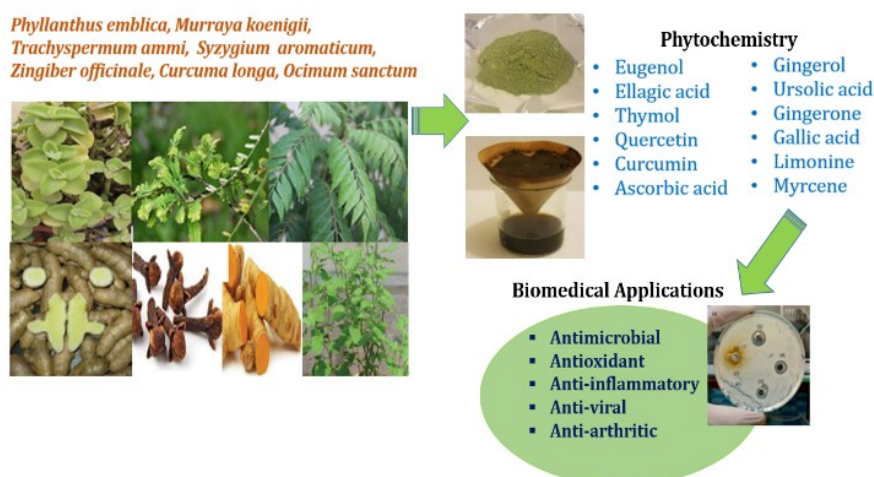


Figure 2. Overview of plant parts utilized in the synthesis of fortified silver nanoparticles (NPs), highlighting their key phytoconstituents and associated therapeutic applications. This schematic provides the anatomical and phytochemical rationale for plant selection, forming the basis for nanoformulation design in antimicrobial interventions.

BIOSYNTHESIS OF SILVER NANOPARTICLES (AGNPS)

10 ml of the extract was added dropwise into 90 ml of 1mM aqueous solution of AgNO₃. These flasks were wrapped in aluminum foil and incubated in a water bath for 5 hours. It is necessary to maintain conditions of constant stirring and a water bath at a temperature of 60 °C. Post-incubation, solutions were cooled to room temperature and observed for color change. Free

silver atoms from silver nitrate formed colloidal silver nanoparticles. Spectrophotometric analysis (UV-Vis spectrophotometer, LABTRONICS, Model LT 29) was performed after 24 hours of incubation, scanning from 200 nm to 700 nm. Antibacterial assays of both the plant extracts and phytochemical-enhanced silver nanoparticles were conducted using serial dilutions prepared in Luria Bertani (LB) broth. An equal volume of secondary *E. coli* culture was added to all



the triplicates of each of the samples, and microbial growth was monitored by measuring absorbance at 580 nm following incubation for 18–24 hours at 37 °C. Nanoparticle formation was evident across

various optical densities (O.D.) in the 200–700 nm range, with 380 nm identified as optimal for graph plotting.

Table 1*. Botanical information of plants used for phyto-herbal nanoparticles.

Botanical Name	Common Name	Family	Useful Plant Part	Major Metabolites
<i>Trachyspermum ammi</i>	Ajwain	Apiaceae	Seeds, Leaves, Stems	Thymol, γ -terpinene, α and β -pinene, p-cymene, Limonene, and Myrcene ²¹
<i>Phyllanthus emblica</i>	Indian Gooseberry (Amla)	Phyllanthaceae	Fruits, leaves, seeds, bark, roots, flowers	Ellagic acid, gallic acid, emblicanin A and B, phyllembein, quercetin, and ascorbic acid [22]. Alkaloids and hydrolysable tannins [23, 24]
<i>Murraya koenigii</i>	Curry leaves (Kadhipatta)	Rutaceae	Leaves	Calcium, phosphorus, Iron, Carotene as Vitamin A, Nicotinic Acid, and Vitamin C [25]. Murrayastne, Murrayaline, Pyrayafoline, Carbazole, Koenigin, Girinimbin, Iso-mahanimbin, Koenine, Koenidine, and Koenimbine, triterpenoids (Cyclomahanimbine, tetrahydromahanimbine) [26]
<i>Curcuma longa</i>	Turmeric	Zingiberaceae	Rhizome	Curcumin ²⁷
<i>Ocimum sanctum</i>	Tulsi	Lamiaceae	Leaves	Eugenol, ursolic acid [28]
<i>Zingiber officinale</i>	Ginger	Zingiberaceae	Rhizome	Gingerol, gingerone, shogaols, parasols [29]
<i>Syzygium aromaticum</i>	Clove	Myrtaceae	Flower buds	Eugenol [30]

Footnote*: The table provides a taxonomic and phytochemical overview of the selected medicinal plants, detailing their botanical families, predominant bioactive constituents, and the specific plant parts utilized in the present study. This foundational information supports the rationale for plant selection and informs the design of nano formulations by linking traditional therapeutic use with targeted antimicrobial potential.

Bacterial growth parameters were further analyzed by recording absorbance at 600 nm. Nanoparticles derived from each plant were diluted to concentrations of 20, 40, 60, 80, and 100 μ g/ml (10–100 μ g/ml range). These were tested against gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* strains using both liquid broth dilution and agar disc diffusion methods. For the growth of the bacteria in nutrient broth medium, fortified plant extract AgNPs (1mM) were added in varying amounts from 0.5 ml, 1ml, 1.5 ml, 2.0 ml, 2.5

ml, and 3 ml with corresponding volumes of liquid broth medium, making the final volumes a fixed value of 10 ml. The broth culture media were incubated at 60 °C, a constant pH of 8 for 72 hours, followed by centrifugation of the broth at 15000 rpm for 10 minutes. Absorbance (O.D.) was measured after a period of 24, 48, and 72 hours. Growth curves were obtained for both bacteria in triplicate for each of the sample concentrations. Liquid broth without any nanoparticles was used as a positive control for comparison. The results were shown in terms of mean



± S.D. and analyzed by Student's t-test; $P < 0.05$ was considered significant. For the Agar-disc-diffusion method, 6 mm diameter holes were made in each of the petri plates, which were previously smeared with bacterial inoculum with an L-shaped spreader. 20 μL of each sample of water, plant crude extract, and three different concentrations of fortified nanoparticles (40, 60, 80 $\mu\text{g/ml}$) were added in triplicate to the wells using a micropipette. The plates were incubated at 37 $^{\circ}\text{C}$ for 24 hours to see the bacterial growth. Crude leaf extract was taken as a positive control, and pure water as a negative control. The zone of inhibition in terms of diameters in mm was taken as a measure of reduced growth of bacteria.

RESULTS

Among the three plant-derived silver nanoparticles (AgNPs), those synthesized from *Phyllanthus emblica* were found to exhibit the highest

antimicrobial activity against *Escherichia coli* at a concentration of 80 $\mu\text{g/ml}$, followed by nanoparticles from *Murraya koenigii* and *Trachyspermum ammi*. In contrast, antibacterial activity against *Staphylococcus aureus* was most prominent in AgNPs from *Murraya koenigii* and *Trachyspermum ammi* at 80 $\mu\text{g/ml}$, followed by *Phyllanthus* nanoparticles, as determined by the liquid culture broth method (Figure 3a & 3b). Stimuli-responsive nanoparticles for bacteria target via the recognition of bacterial microenvironment, such as chemical stimuli of variations in pH, enzymes, and redox state of the bacterial surroundings, and physical stimuli such as temperature, light, magnetic field, and acoustic waves around. Common bacteria for human infection used in testing of nanoparticles targeting are Gram-positive (*Staphylococcus aureus* and *Streptococcus*), Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Helicobacter pylori*, *Pseudomonas aeruginosa*), and others (*Mycobacterium tuberculosis*)³¹.

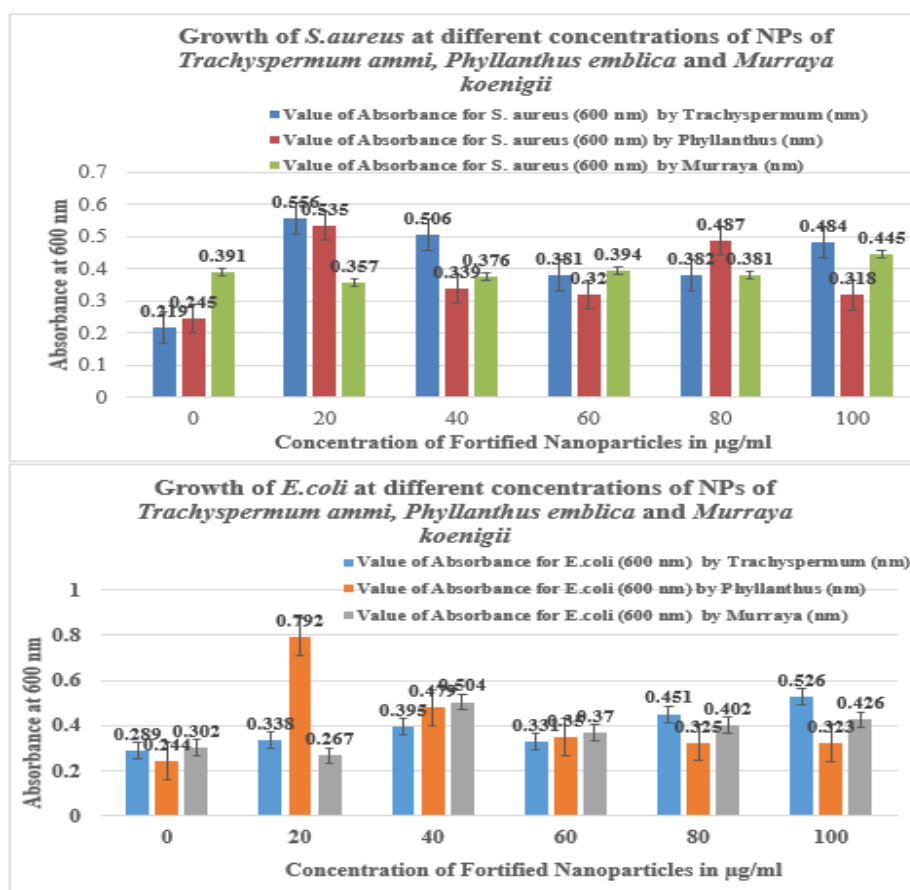


Figure 3. Growth response of *Staphylococcus aureus* (3a) and *Escherichia coli* (3b) following treatment with fortified silver nanoparticle (NP) formulations at varying concentrations (20, 40, 60, 80, and 100 $\mu\text{g/ml}$). The data illustrate dose-dependent antibacterial activity, reflecting the inhibitory effect of nanoformulations on bacterial proliferation.



Further examination revealed that *Murraya koengii* AgNPs were active against *S. aureus* within the 60–80 µg/ml range, with a maximum at 60 µg/ml, while *Phyllanthus* AgNPs showed efficacy between 40–60 µg/ml with a maximum at 40 µg/ml, and *Trachyspermum ammi* AgNPs demonstrated peak activity at 80 µg/ml. Overall, the nanoparticles from all three plant extracts exhibited stronger antibacterial potential against *S. aureus* (Gram-positive) than *E. coli* (Gram-negative), suggesting higher specificity or efficacy toward Gram-positive bacteria. This is attributed to the presence of thicker peptidoglycan layer interlinked with teichoic and lipoteichoic acid in the cell wall of gram positive bacteria which

correspond to a thinner peptidoglycan layer in case of gram negative bacteria outer and inner cell membranes along with a lipopolysaccharide (LPS) lining on the outer membrane of Gram negative bacteria that can resist the action of antibacterial drugs to some extent. These results validate the further exploration of *Phyllanthus embellica*, *Trachyspermum ammi*, and *Murraya koengii* nanoparticle-based solvent extracts as promising bioactive candidates for antimicrobial drug development. These findings were consistent with the trends observed in the agar disc diffusion assays (Figure 4: Results from Agar Disc Diffusion Method).

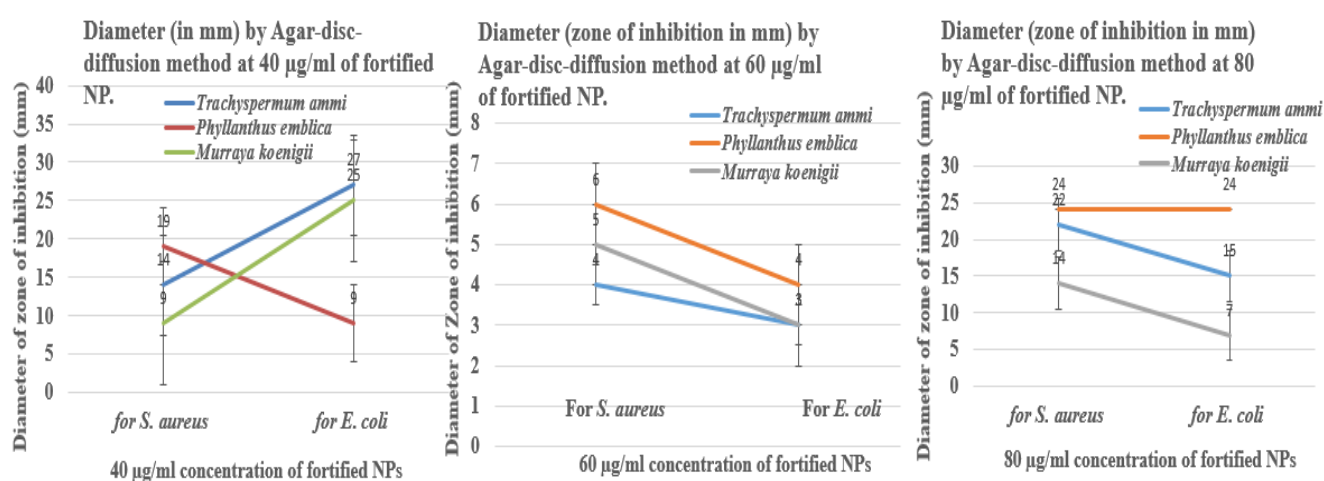


Figure 4. Zone of inhibition observed at 40, 60, and 80 µg/ml concentrations using the agar disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. The diameter of the inhibition zones reflects the antibacterial potential of the formulations, indicating their efficacy in suppressing bacterial growth.

In the second phase of the study, AgNPs were synthesized using phytochemical-rich extracts of *Curcuma longa* (Turmeric), *Ocimum sanctum* (Tulsi), *Syzygium aromaticum* (Clove), and *Zingiber officinale* (Ginger). The well-documented ability of plant-based compounds to reduce silver ions facilitated the efficient synthesis of AgNPs from AgNO₃. These nanosilver-fortified extracts were compared to their respective crude (unfortified) plant

extracts by assessing bacterial growth inhibition against *E. coli* (DH5α strain). Spectrophotometric readings at 540 nm revealed greater suppression of bacterial growth by the nanosilver-integrated extracts. Notably, *Zingiber* and *Curcumin* nanosilver formulations exhibited higher antibacterial activity as compared to their corresponding crude counterparts, followed by *Ocimum* and *Synzygium* (Figure 5a-5d).

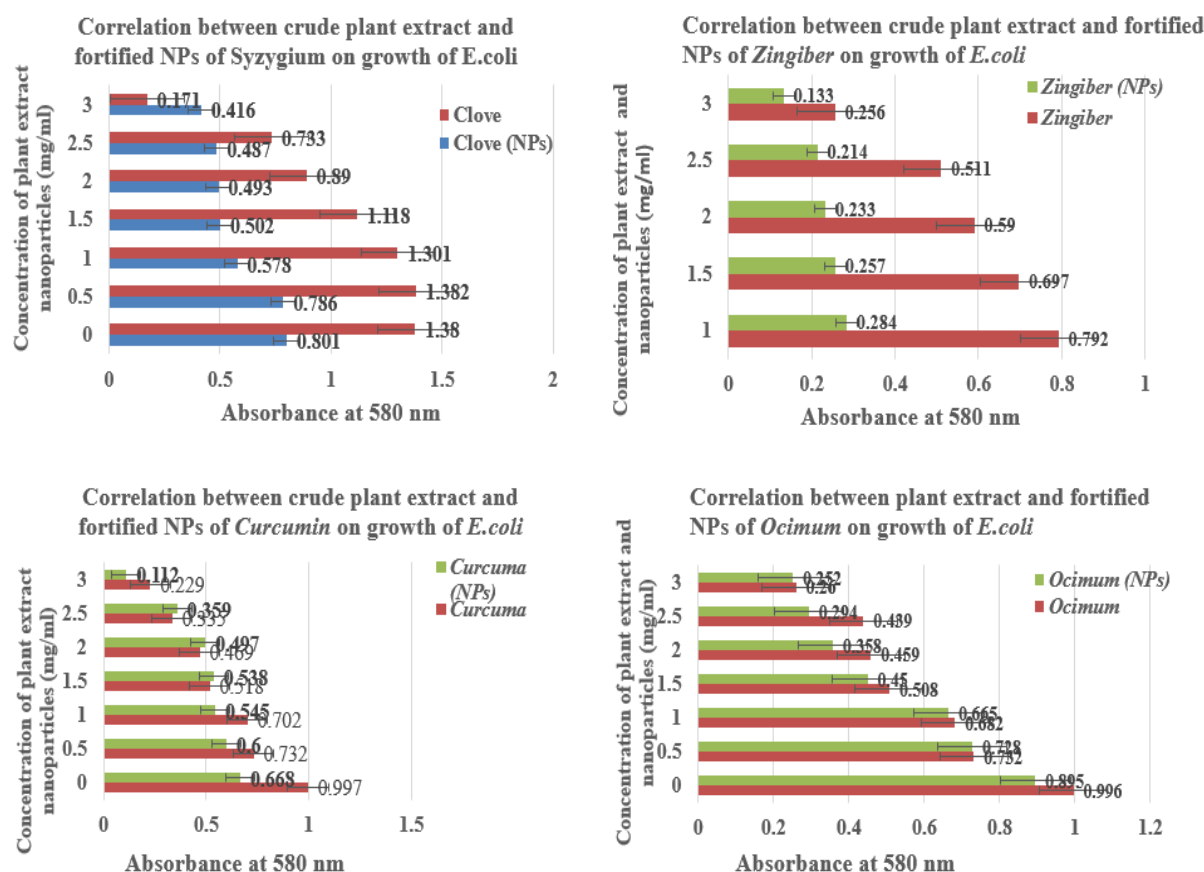


Figure 5a–5d. Comparative growth inhibition of *Escherichia coli* treated with fortified silver nanoparticles (NPs) synthesized from (a) *Syzygium aromaticum*, (b) *Zingiber officinale*, (c) *Curcuma longa*, and (d) *Ocimum sanctum*, versus their respective crude plant extracts. Data are presented as mean $\pm$ SD and were analyzed using Student's *t*-test; $P < 0.05$ was considered statistically significant. The comparative analysis highlights that fortified nanoparticle formulations derived from *Syzygium aromaticum*, *Zingiber officinale*, *Curcuma longa*, and *Ocimum sanctum* exhibited enhanced antibacterial activity against *E. coli* relative to their respective crude extracts. This correlation underscores the improved efficacy conferred by nanoencapsulation of phytoconstituents.

DISCUSSION

Plant-derived fortified nanoparticles have emerged as promising agents in targeted antibacterial therapeutics. By inducing membrane perforation and oxidative stress, these nano systems effectively compromise bacterial integrity while minimizing harm to host cells. Silver–curcumin nanocomposites, for instance, demonstrate pore-forming capability and ROS-mediated damage against *Escherichia coli*. In contrast, silver ions released from AgNPs extend their cytotoxic action to *E. coli* and *S. aureus* through disruption of genetic and enzymatic machinery [32]. Curcumin is known as a reducing and capping agent, forms curcumin silver nanoparticles (curAgNPs or

cAgNPs) which bind to bacteria as nanocomposites and release Ag^+ easily, generates reactive oxygen species (ROS), causes damage to the bacterial cell wall, and hence cell death. The impact of curcumin is enhanced along with polyvinyl pyrrolidone (PVP) cAgNPs. This is the synergistic effect of nanoparticles of curcumin and PVP together towards enhancing antibacterial potential³¹. The efficacy of phytochemicals is further evident in synergistic pairings with antibiotics—*Embllica officinalis* significantly restricts bacterial growth, showing up to 91.5% inhibition of *S. aureus*, with inhibition zones aligning with prior reports³³. Likewise, *Murraya koenigii* (Kadhi Patta) leaf oil and its nanoparticle formulations yield robust zones of inhibition,



corroborating earlier findings within equivalent concentration ranges³⁴⁻³⁶. Collectively, these data strengthen the case for phyto-nanoparticle formulations as sustainable and potent alternatives in combating microbial resistance.

CONCLUSIONS

Green synthesis of nanosilver, when integrated with plant-based extracts, offers a dual advantage—significantly reducing production costs while promoting environmental sustainability. This fusion holds immense potential for antibacterial strategies across medicine, food safety, and pharmaceutical applications. Medicinal plants, long revered as reservoirs of natural bioactive compounds, are increasingly recognized for their safe, cost-effective, and efficient antimicrobial properties, especially in the face of growing multidrug resistance that complicates conventional antibiotic treatments. Nanoherbal formulations can be tailored to match individual microbiome profiles and infection dynamics, aligning seamlessly with the principles of personalized medicine. Their ability to exert therapeutic effects at lower doses translates to reduced adverse reactions and greater biocompatibility. However, for the long-term safety and cytotoxicity of silver nanoparticles in mammalian cells, their retention, excretion, and metabolism, deeper research in this field is required, with their use in clinical translations. To curb the cytotoxicity attributed to metal nanoparticles, infection-specific targeting strategies must be adopted. Interaction between NPs and host cells needs to be thoroughly explored with the help of sequencing technologies such as RNA-sequencing and proteomics for complicated interaction studies¹². Plant-based nanomedicines illuminate a sustainable path in pharmaceutical innovation, leveraging eco-friendly production methods and naturally derived compounds. This strategy not only reduces environmental impact but also enhances drug efficacy, marking a shift toward future-proofing healthcare through integrative natural science. In essence, the nanoherbal revolution represents a bold leap into an era where biotechnology meets botanical wisdom, ensuring precision, safety, and sustainability in therapeutic development. Therefore, the most tangible outcome of this synthesis-fortification synergy is the enhanced

antibacterial efficacy of phytochemical-rich formulations, positioning them as potent therapeutic candidates for the treatment of microbial infections within the healthcare sector and bringing about a shift in the entire paradigm, adopting more of herbal formulations in synergistic therapeutic interventions.

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CONTRIBUTIONS

NKD conceptualized the experiments, wrote the manuscript, edited, and reviewed.

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