



Research Article

Green Synthesis of Iron and Zinc Nanoparticles from *Centella Asiatica*, *Lawsonia Inermis*, *Ocimum Tenuiflorum* and Assessing their Biological Activity for Wound Healing Properties

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Green synthesis of nanoparticles has emerged as a sustainable and biocompatible approach for developing advanced therapeutic systems. In the present study, iron and zinc nanoparticles were synthesized using aqueous extracts of *Centella asiatica*, *Lawsonia inermis*, and *Ocimum tenuiflorum*, exploiting their rich phytochemical composition for reduction and stabilization. The synthesized nanoparticles were further incorporated into an Aloe vera-based nano-herbal formulation to enhance stability and therapeutic efficacy. The nanoparticles were characterized using UV–Visible spectroscopy, FTIR, and SEM analyses, confirming successful synthesis, functional group involvement, and nanoscale morphology. The biological potential of the nanoparticles and their formulations was evaluated through a series of in vitro assays. Antioxidant activity assessed by DPPH assay revealed strong radical scavenging potential, particularly in Vallarai-mediated nanoparticles. Anti-inflammatory activity, evaluated through HRBC membrane stabilization and protein denaturation assays, demonstrated significant inhibition, with enhanced performance observed in Aloe vera-incorporated formulations. Antimicrobial studies using MIC and agar well diffusion methods showed concentration-dependent bacterial inhibition, with improved efficacy in nano-herbal formulations. Toxicity and biocompatibility assessments, including hemolysis and blood clotting index assays, indicated acceptable safety profiles at optimized concentrations. The overall findings suggest that the synergistic interaction between plant-derived phytochemicals, metal nanoparticles, and Aloe vera significantly enhances biological activity. This study highlights the potential of green synthesized nano-herbal systems as eco-friendly, multifunctional candidates for wound healing and biomedical applications, warranting further in vivo validation.

Keywords: Hemolysis; Nano-herbal Formation; Carrier matrix; Biocompatibility; Flavanoids; Stabilizing agents; Synergistic; Wound healing; HRBC membrane stabilization; Nanobiotechnology.

INTRODUCTION

Wound healing is a complex and dynamic biological process involving a coordinated sequence of events, including hemostasis, inflammation, proliferation, and tissue remodeling. Any disruption in these phases, particularly prolonged inflammation or microbial infection, can delay healing and lead to chronic wounds (Guo & DiPietro, 2010; Eming et al., 2014).

Conventional therapeutic strategies, including synthetic anti-inflammatory drugs and antibiotics, are widely used to manage wound-related complications; however, their prolonged use is often associated with adverse effects such as cytotoxicity, antimicrobial resistance, and delayed tissue regeneration (Boateng & Catanzano, 2015; Frykberg & Banks, 2015). This has prompted increasing interest in the development of alternative, biocompatible, and multifunctional

therapeutic systems. In recent years, nanotechnology has emerged as a promising approach in biomedical applications, particularly in wound management. Nanoparticles exhibit unique physicochemical properties, such as high surface area-to-volume ratio, enhanced reactivity, and improved penetration ability, which enable them to interact effectively with biological systems (Rai et al., 2009; Zhang et al., 2016). Among various nanomaterials, metal nanoparticles such as iron and zinc have gained considerable attention due to their intrinsic antimicrobial, antioxidant, and anti-inflammatory properties (Wang et al., 2017; Raghupathi et al., 2011). Zinc, an essential trace element, plays a critical role in cell proliferation, collagen synthesis, and immune regulation, making it particularly important in wound healing processes (Lin et al., 2018). Similarly, iron-based nanoparticles are known to facilitate oxygen transport and cellular metabolism, contributing to tissue repair and regeneration (Mahmoudi et al., 2011). Green synthesis of nanoparticles using plant extracts has emerged as an eco-friendly and sustainable alternative to conventional physical and chemical methods. This approach utilizes bioactive phytochemicals such as flavonoids, phenolics, alkaloids, and terpenoids as natural reducing and stabilizing agents, eliminating the need for toxic reagents (Iravani, 2011; Ahmed et al., 2016). Plant-mediated synthesis not only enhances the biocompatibility of nanoparticles but also imparts additional therapeutic properties due to the synergistic effects of phytoconstituents (Shankar et al., 2004; Singh et al., 2018). Medicinal plants such as *Centella asiatica*, *Lawsonia inermis*, and *Ocimum tenuiflorum* have been extensively studied for their pharmacological activities. *Centella asiatica* is well known for its wound healing potential, attributed to compounds such as asiaticoside and madecassoside, which promote collagen synthesis and angiogenesis (Gohil et al., 2010; James & Dubery, 2009). *Lawsonia inermis* possesses strong antimicrobial and anti-inflammatory properties due to the presence of lawsone and other phenolic compounds (Habbal et al., 2005). *Ocimum tenuiflorum* (Tulsi) is recognized for its antioxidant, antimicrobial, and immunomodulatory activities, primarily due to its rich content of eugenol, flavonoids, and phenolic acids (Prakash & Gupta, 2005; Pattanayak et al., 2010). The incorporation of these plant extracts in nanoparticle

synthesis enhances their therapeutic efficacy through a synergistic mechanism. In addition to nanoparticles, natural biomaterials such as *Aloe vera* have been widely used in wound healing applications due to their moisturizing, anti-inflammatory, and antimicrobial properties. *Aloe vera* contains bioactive compounds such as polysaccharides, anthraquinones, and glycoproteins that promote fibroblast proliferation, collagen deposition, and epithelialization (Hamman, 2008; Chithra et al., 1998). Furthermore, *Aloe vera* can act as an effective carrier matrix for nanoparticles, improving their stability, controlled release, and bioavailability (Surjushe et al., 2008). The integration of plant-mediated nanoparticles with *Aloe vera*-based formulations represents a novel nano-herbal approach for wound healing. Such systems combine the advantages of nanotechnology with the therapeutic potential of medicinal plants and natural biomaterials, resulting in enhanced biological activity and reduced toxicity (Mittal et al., 2013; Thakkar et al., 2010). Despite significant progress in this field, there remains a need for comprehensive evaluation of the biological properties of such formulations, including their antioxidant, anti-inflammatory, antimicrobial, and hemocompatibility profiles. Therefore, the present study focuses on the green synthesis of iron and zinc nanoparticles using aqueous extracts of *Centella asiatica*, *Lawsonia inermis*, and *Ocimum tenuiflorum*, followed by their incorporation into an *Aloe vera*-based nano-herbal formulation. The synthesized nanoparticles were characterized and evaluated for their biological activities relevant to wound healing, including antioxidant, anti-inflammatory, antimicrobial, and toxicity assessments. The study aims to provide insights into the synergistic effects of plant-derived phytochemicals and metal nanoparticles, highlighting their potential as safe and effective alternatives for wound healing applications.

MATERIALS AND METHODS

2.1 Materials and Preparation of Nano-Herbal Formulation

Fresh, mature, and disease-free leaves of *Lawsonia inermis*, *Ocimum tenuiflorum*, and *Centella asiatica* were collected, washed thoroughly with distilled water, shade-dried, and finely powdered. Aqueous

extracts were prepared by boiling 10 g of plant powder in 250 mL distilled water for 15–20 min, followed by filtration using Whatman No. 1 filter paper. The filtrates were stored at 4°C until further use. These extracts are known to contain bioactive phytochemicals such as flavonoids, phenolics, and tannins, which act as natural reducing and stabilizing agents in green nanoparticle synthesis (Iravani, 2011; Ahmed et al., 2016). Iron and zinc precursor solutions were prepared using ferrous chloride (FeCl_2) and zinc sulfate (ZnSO_4), respectively. Green synthesis of nanoparticles was performed by the dropwise addition of plant extract into heated metal salt solutions under constant magnetic stirring. The formation of nanoparticles was confirmed by a visible color change due to surface plasmon resonance. The synthesized nanoparticles were collected by centrifugation at 6000 rpm for 15 min, washed repeatedly with distilled water, and dried. Fresh *Aloe vera* gel was extracted, homogenized, and blended with the synthesized nanoparticles to obtain a uniform nano-herbal formulation. The formulation was dried and later rehydrated prior to experimental applications to improve stability and usability.

2.2 Phytochemical Quantification

The phytochemical composition of the plant extracts and synthesized formulations was evaluated by quantifying total flavonoid and alkaloid contents using established spectrophotometric methods. Total flavonoid content was determined using an aluminum chloride-based colorimetric assay, which relies on the formation of a stable flavonoid–aluminum complex (Chang et al., 2002). Briefly, different concentrations of the sample extracts (50–200 μL) were mixed with the reagent system and incubated under controlled conditions to allow color development. The absorbance of the resulting solution was measured at 415 nm using a UV–Visible spectrophotometer. The flavonoid content was calculated from a standard calibration curve and expressed as equivalent units, where increased absorbance corresponds to higher flavonoid concentration. Total alkaloid content was estimated using a colorimetric method involving the formation of a chromogenic complex with specific alkaloid-detecting reagents (Harborne, 1998). Sample aliquots (50–200 μL) were treated with the reagent system and incubated to allow complete reaction. The

absorbance was recorded at 570 nm using a UV–Visible spectrophotometer. Alkaloid concentration was calculated using a standard curve, providing an estimate of the total alkaloid content in the samples.

2.3 Nanoparticle Synthesis and Nano-Herbal Formulation

Fresh, mature, and disease-free leaves of *Lawsonia inermis*, *Ocimum tenuiflorum*, and *Centella asiatica* were collected and thoroughly washed with distilled water to remove surface impurities. The plant materials were shade-dried at ambient temperature and finely powdered. Aqueous extracts were prepared by boiling 10 g of each powdered sample in 250 mL of distilled water for approximately 15–20 min, followed by filtration through Whatman No. 1 filter paper. The filtrates were stored at 4°C for subsequent use. These plant extracts are known to contain diverse phytoconstituents such as flavonoids, phenolics, and tannins, which function as reducing and stabilizing agents during green nanoparticle synthesis (Iravani, 2011; Ahmed et al., 2016). Iron and zinc precursor solutions were prepared using ferrous chloride (FeCl_2) and zinc sulfate (ZnSO_4), respectively. The green synthesis of nanoparticles was carried out by the gradual addition of plant extract into the heated metal salt solution under continuous magnetic stirring. The reaction was monitored visually, and the formation of nanoparticles was indicated by a distinct color change attributed to surface plasmon resonance. The synthesized nanoparticles were separated by centrifugation at 6000 rpm for 15 min, followed by repeated washing with distilled water to remove unreacted constituents. The purified nanoparticles were then dried and stored for further applications. Fresh *Aloe vera* gel was extracted, homogenized, and incorporated with the synthesized nanoparticles to prepare a uniform nano-herbal formulation. The formulation was dried under controlled conditions and later reconstituted prior to experimental use to ensure consistency and stability.

2.4 Characterization of Nanoparticles

The synthesized nanoparticles were characterized using a combination of analytical techniques to determine their morphology, functional groups, and optical properties. Surface morphology and particle size distribution were examined using Scanning

Electron Microscopy (SEM) (Goldstein et al., 2003). Dried nanoparticle samples were mounted on carbon-coated stubs and sputter-coated with a thin layer of gold to improve conductivity. The samples were then observed under SEM at suitable magnifications, and images were analyzed to assess particle shape, size, and distribution. The presence of well-defined and uniformly distributed particles confirmed successful nanoparticle synthesis. Fourier Transform Infrared (FTIR) spectroscopy was employed to identify functional groups involved in the reduction and stabilization of nanoparticles (Stuart, 2004). The dried nanoparticle samples were mixed with potassium bromide (KBr) and compressed into pellets. Spectral analysis was performed in the range of 4000–400 cm^{-1} . Characteristic absorption peaks corresponding to hydroxyl, carbonyl, and amine groups were identified, indicating the involvement of plant-derived biomolecules in nanoparticle formation. UV–Visible spectroscopy was used to confirm nanoparticle formation and evaluate their optical properties (Kelly et al., 2003). The nanoparticle suspension was diluted appropriately and scanned over a wavelength range of 200–800 nm. The appearance of characteristic absorption peaks due to surface plasmon resonance provided confirmation of nanoparticle formation and insights into particle size and distribution.

2.5 DPPH Radical Scavenging Assay

Antioxidant activity was evaluated using the DPPH radical scavenging assay (Brand-Williams et al., 1995). A 1 mL DPPH solution in methanol was added to varying concentrations of samples (50–250 μL). The mixtures were incubated in the dark for 30 min at room temperature. Absorbance was measured at 520 nm, and the percentage inhibition of DPPH radicals was calculated.

2.6 Anti-inflammatory assay

2.6.1 Protein Denaturation Assay

Anti-inflammatory activity was assessed using the protein denaturation assay (Mizushima and Kobayashi, 1968). Reaction mixtures containing phosphate buffer (pH 7.4), bovine serum albumin (BSA), and sample extracts were incubated and heated at 70°C. After cooling, absorbance was

measured at 680 nm. The percentage inhibition of protein denaturation was calculated.

2.6.2 Human Red Blood Cell (HRBC) Membrane Stabilization Assay

The HRBC membrane stabilization assay was performed to evaluate anti-inflammatory activity (Shinde et al., 1999). Fresh human blood was processed to obtain RBC suspension. Samples at varying concentrations were incubated with RBCs under hypotonic conditions. After incubation and centrifugation, absorbance of the supernatant was measured at 540 nm. The percentage inhibition of hemolysis was calculated.

2.7 Toxicity analysis

2.7.1 Blood Clotting Index (BCI) Assay

Hemostatic activity was evaluated using the Blood Clotting Index (BCI) assay (Li et al., 2008). Fresh human blood (200 μL) was mixed with test samples and incubated to allow clot formation. Distilled water was added to lyse non-clotted RBCs, and absorbance was measured at 540 nm. Lower absorbance indicated better clotting ability.

2.7.2 Hemolysis Assay for Hemocompatibility

Hemocompatibility was assessed using the hemolysis assay (ISO 10993-4, 2017). RBC suspensions (2%) were treated with different sample concentrations. PBS and distilled water were used as negative and positive controls, respectively. After incubation and centrifugation, absorbance was measured at 540 nm. Percentage hemolysis was calculated to determine cytotoxicity.

2.8 Anti-Microbial activity

2.8.1 Minimum Inhibitory Concentration (MIC) Assay

Antimicrobial activity was evaluated using the broth dilution method (Andrews, 2001). LB broth was prepared, sterilized, and inoculated with bacterial cultures. Test samples were added at varying concentrations and incubated at 37°C for 24 h. Optical density was measured at 600 nm, and percentage inhibition was calculated.

2.8.2 Agar Well Diffusion Method

The antibacterial activity was further confirmed using the agar well diffusion method (Bauer et al., 1966). LB agar plates were prepared and inoculated with bacterial cultures. Test samples were introduced into wells, and plates were incubated at 37°C for 24 h. Zones of inhibition were measured to evaluate antimicrobial efficacy.

3. Results and Discussion

3.1 Extraction and Quantification of Phytochemical

3.1.2. Quantification of Flavonoids

The flavonoid quantification results (Table 1; Fig. 1) demonstrated a clear concentration-dependent increase across all plant extracts. Among the samples, *Ocimum tenuiflorum* (Tulsi) and *Centella asiatica* (Vallarai) exhibited comparatively higher flavonoid content at elevated concentrations, whereas *Lawsonia inermis* (Henna) showed a consistent increase throughout the tested range. The enhanced flavonoid levels at higher concentrations suggest improved extraction efficiency and availability of polyphenolic compounds. These findings are significant, as flavonoids are known to contribute to antioxidant and anti-inflammatory responses, which are critical in wound healing processes. The comparatively higher values observed in Tulsi and Vallarai indicate their stronger phytochemical potential, supporting their selection for nanoparticle synthesis.

Table 1. Total flavonoid content of plant extracts (mg RE/g extract)

Sample	50 μ L	100 μ L	150 μ L	200 μ L
Henna	18.4	32.6	45.8	52.3
Tulsi	14.2	28.9	41.6	58.7
Vallarai	10.6	30.4	38.2	60.1

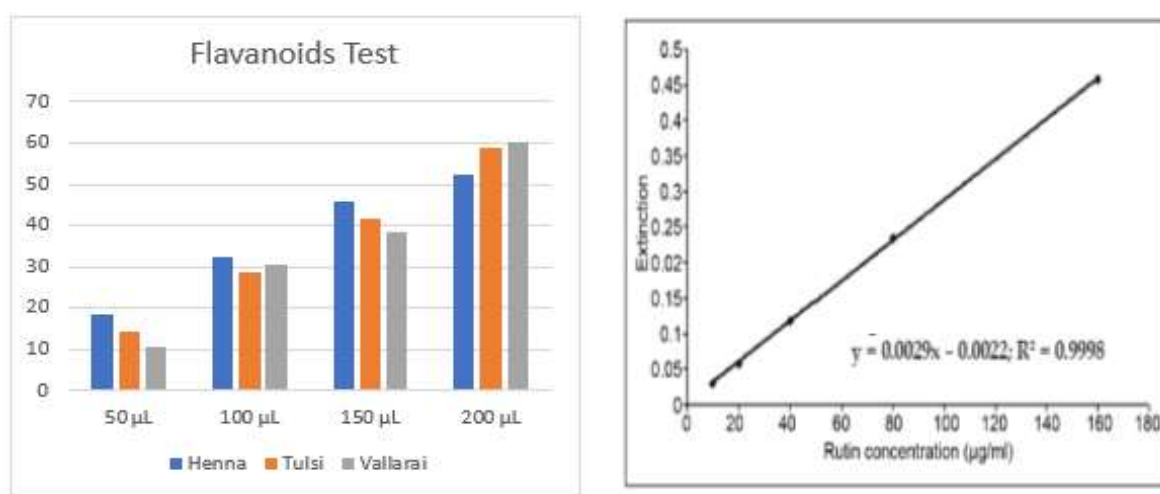


Figure 1. Concentration-dependent increase in flavonoid content of plant extracts.

3.1.2 Total Alkaloid Content

Table 2. Total alkaloid content of plant extracts (mg AE/g extract).

Sample	50 μ L	100 μ L	150 μ L	200 μ L
Henna	22.5	28.6	36.8	48.2
Tulsi	24.3	31.7	42.5	55.6
Vallarai	26.8	34.9	50.7	58.3

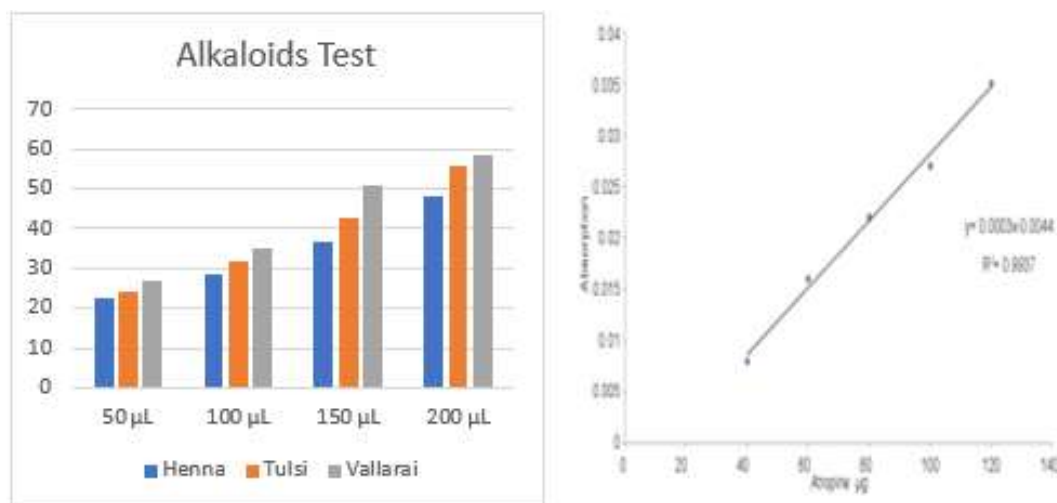


Figure 2. Variation in alkaloid content with concentration.

As shown in Table 2 and Fig. 2, alkaloid content increased steadily with concentration for all samples. Vallarai exhibited the highest alkaloid levels, followed by Tulsi and Henna. The elevated alkaloid content suggests strong antimicrobial and pharmacological potential, as these compounds are known to interfere with microbial metabolism and inflammatory pathways. The higher initial and final values observed in Vallarai indicate its superior bioactive composition, making it particularly relevant for biomedical applications. Overall, the phytochemical results confirm that all three plant extracts are rich in therapeutic compounds, with concentration playing a key role in their availability.

3.2 Synthesis and Characterization of Nanoparticles

The green synthesis of iron and zinc nanoparticles using aqueous extracts of *Lawsonia inermis*, *Ocimum tenuiflorum*, and *Centella asiatica* was successfully achieved, as evidenced by visual, spectroscopic, and microscopic analyses. The formation of nanoparticles

was initially indicated by a distinct color change in the reaction mixture upon addition of plant extracts to the metal precursor solutions. This change is attributed to the reduction of metal ions into nanoscale particles mediated by plant-derived phytochemicals such as flavonoids, phenolics, and proteins, which also act as stabilizing agents.

3.2.2 UV–Visible Spectroscopy Analysis

The UV–Visible spectroscopic analysis (Fig. 3a–b) confirmed the successful formation of nanoparticles. Zinc nanoparticles exhibited a characteristic absorption peak in the range of 320–380 nm, corresponding to band-gap absorption of ZnO nanoparticles. In contrast, iron nanoparticles showed a broad absorption band between 250 and 350 nm. The increase in absorbance intensity indicates progressive nanoparticle formation and improved dispersion stability. The broad peak observed in FeNPs suggests polydispersity and possible formation of mixed iron oxide phases, which is commonly reported in green synthesis approaches.

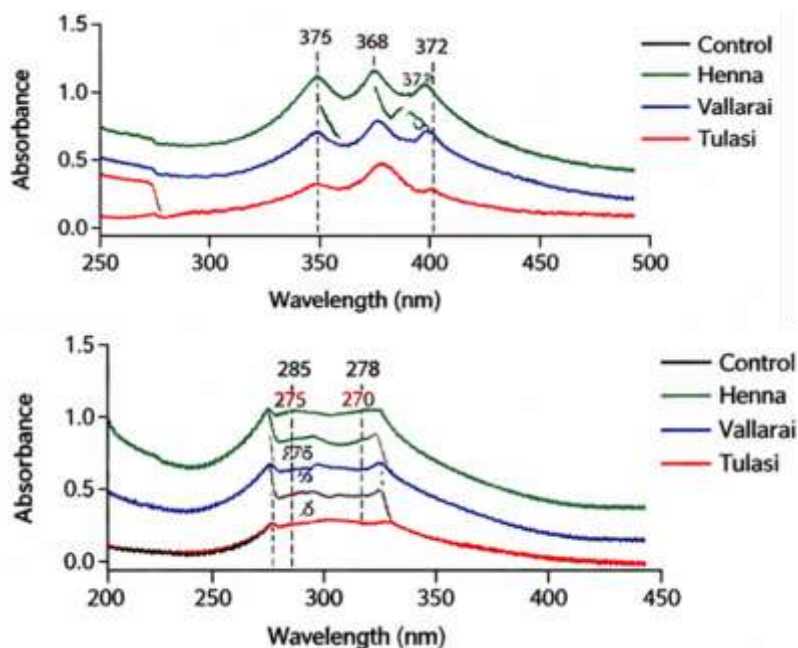


Figure 3. UV–Visible spectra of synthesized nanoparticles: (a) Zinc nanoparticles (ZnNPs); (b) Iron nanoparticles (FeNPs).

3.2.3 Fourier Transform Infrared (FTIR) Analysis

FTIR analysis (Fig. 4a–b) was performed to identify functional groups responsible for nanoparticle synthesis and stabilization. The spectra revealed a broad peak around 3400 cm^{-1} corresponding to O–H stretching vibrations of phenolic compounds. Peaks near 2920 cm^{-1} indicate C–H stretching, while bands observed at $1600\text{--}1650\text{ cm}^{-1}$ correspond to C=O

stretching of carbonyl groups. Additional peaks in the regions of 1400 cm^{-1} and $1000\text{--}1100\text{ cm}^{-1}$ were attributed to C–N and C–O stretching vibrations, respectively. The presence of distinct peaks below 600 cm^{-1} confirms metal–oxygen bonding (Zn–O and Fe–O), validating nanoparticle formation. These results clearly indicate the involvement of plant-derived biomolecules in reducing and capping the nanoparticles.

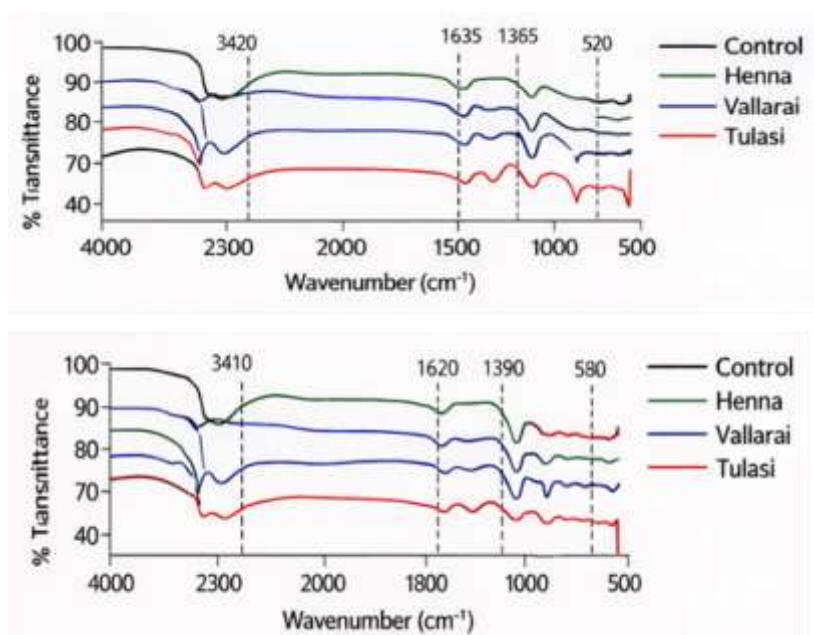


Figure 4. FTIR spectra of nanoparticles: (a) ZnNPs; (b) FeNPs.

3.2.4 Scanning Electron Microscopy (SEM) Analysis

SEM analysis (Fig. 5a–b) revealed that the synthesized nanoparticles predominantly exhibited spherical to irregular morphologies with noticeable agglomeration. The particle size ranged approximately between 20 and 100 nm. Zinc nanoparticles appeared relatively smaller and more

uniformly distributed compared to iron nanoparticles, which showed a higher tendency toward aggregation. This aggregation behavior may be attributed to high surface energy and the presence of biological capping agents derived from plant extracts. The observed morphology confirms the successful green synthesis of nanoparticles and reflects the influence of phytochemicals on particle formation and stabilization.

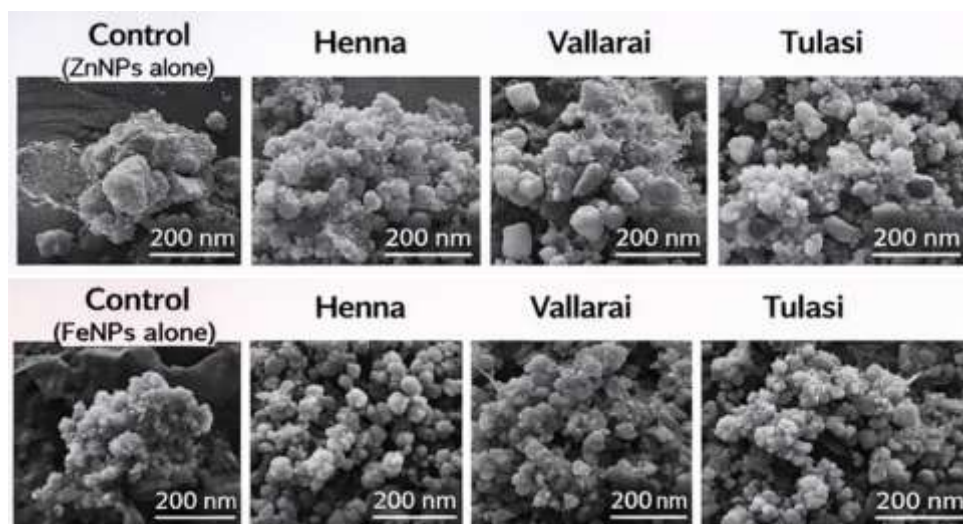


Figure 5. SEM micrographs of synthesized nanoparticles: (a) ZnNPs; (b) FeNPs.

3.3 Anti-Inflammatory Activity

3.3.1 HRBC Membrane Stabilization Assay

The HRBC assay results (Table 3) demonstrated that iron nanoparticles exhibited higher membrane stabilization compared to zinc nanoparticles. Henna-mediated FeNPs showed the highest stabilization

(64.3%), indicating strong anti-inflammatory potential. The incorporation of Aloe vera significantly enhanced the activity, with inhibition values reaching up to ~90% (Fig. 9). This enhancement may be attributed to the synergistic interaction between nanoparticles and Aloe vera, which improves membrane integrity and reduces hemolysis.

Table 3. HRBC membrane stabilization (%) of nanoparticles.

Sample	Nanoparticle Type	100 μ L (% Stabilization)	200 μ L (% Stabilization)
Henna	ZnNP	38.5%	46.2%
Tulsi	ZnNP	32.4%	41.8%
Vallarai	ZnNP	28.7%	36.5%
Henna	FeNP	52.6%	64.3%
Tulsi	FeNP	48.9%	59.7%
Vallarai	FeNP	45.2%	55.8%

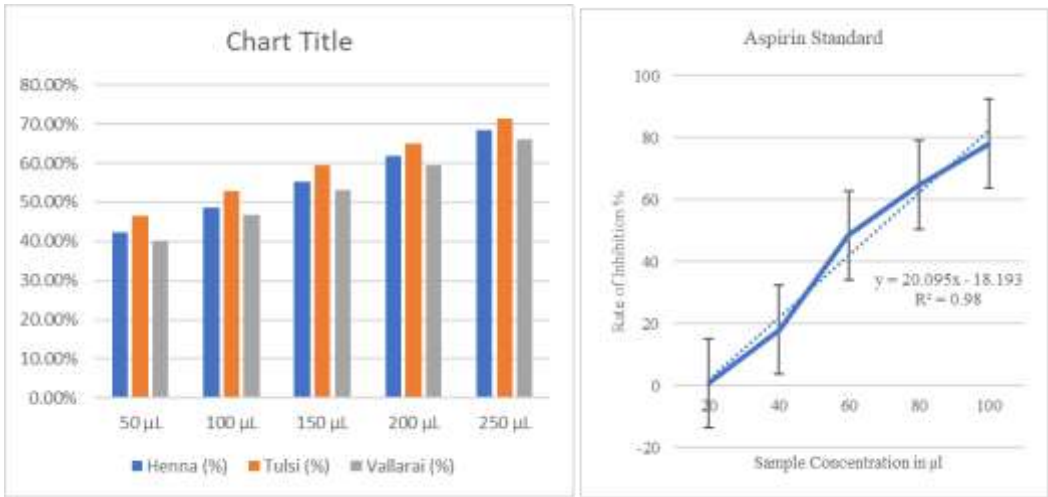


Figure 9. Percentage membrane stabilization of nanoparticle formulations.

3.3.2 Protein Denaturation Assay

The protein denaturation assay (Table 4; Fig. 10) showed that FeNPs exhibited higher inhibition compared to ZnNPs at elevated concentrations. Tulsi-

based FeNPs demonstrated the highest inhibition (74.8%). The results indicate that nanoparticles effectively stabilize proteins under stress conditions, thereby reducing inflammatory responses.

Table 4. Percentage inhibition of protein denaturation.

Sample	Nanoparticle Type	100 µL (% Inhibition)	200 µL (% Inhibition)
Henna	FeNP	48.6%	65.2%
Tulsi	FeNP	55.4%	74.8%
Vallarai	FeNP	50.2%	62.7%
Henna	ZnNP	32.5%	44.6%
Tulsi	ZnNP	35.1%	48.3%
Vallarai	ZnNP	30.4%	42.2%

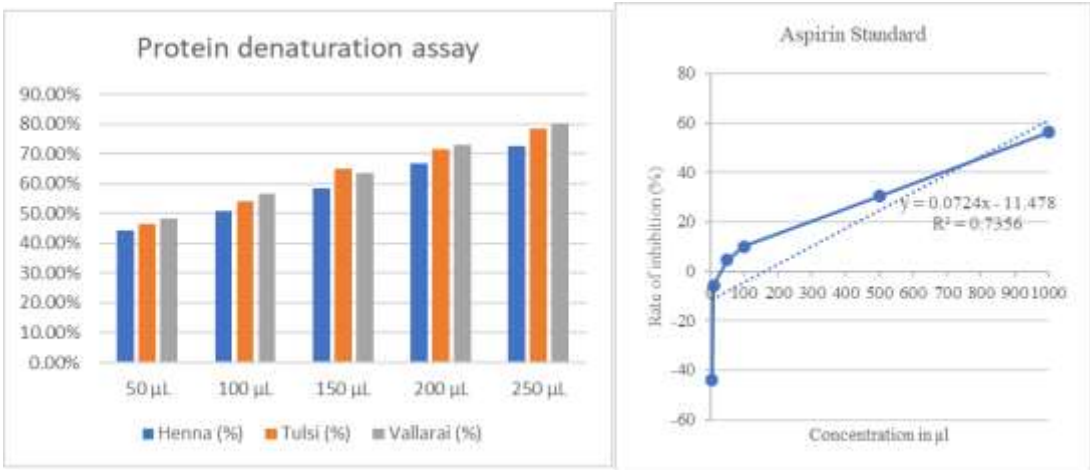


Figure 10. Protein denaturation inhibition profile.

3.4 Antioxidant Activity (DPPH Assay)

The antioxidant results (Table 5 and Table 6; Fig. 11) showed a concentration-dependent increase in radical scavenging activity. Vallarai-mediated nanoparticles

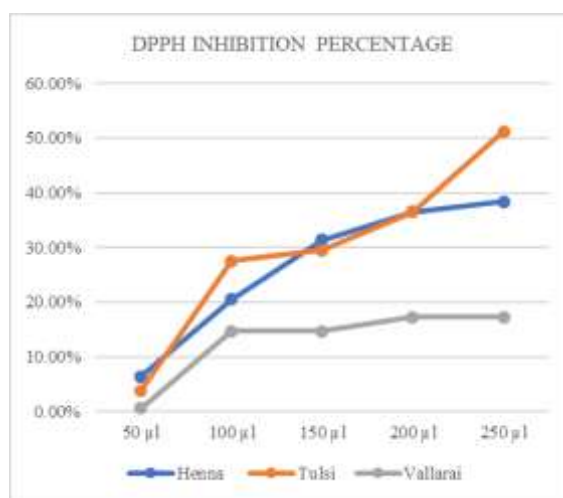
exhibited the highest activity (71.5%), indicating strong antioxidant potential. Interestingly, Aloe vera formulations showed slightly reduced activity, likely due to dilution or interaction effects within the gel matrix.

Table 5. DPPH radical scavenging activity of iron nanoparticles.

Sample	50µL	100µL	150µL	200µL	250µL
Henna	38.2%	41.4%	41.4%	54.4%	65%
Tulsi	8.9%	11.3%	29.2%	44.7%	57.7%
Vallarai	26.0%	51.2%	67.4%	67.4%	71.5%

Table 6. DPPH activity of Aloe vera-based formulations.

Sample	50µL	100µL	150µL	200µL	250µL
Henna	6.41%	20.51%	31.41%	36.54%	38.46%
Tulsi	3.85%	27.56%	29.49%	36.54%	51.28%
Vallarai	0.64%	14.74%	14.74%	17.31%	17.31%

**Figure 11. DPPH radical scavenging activity.**

3.5 Toxicity Analysis

3.5.1 Hemostatic Activity (BCI Assay)

The BCI results (Table 7 and Table 8; Fig. 12) indicated that lower concentrations exhibited better

clotting efficiency. Zinc nanoparticles showed comparatively better hemostatic performance than iron nanoparticles. Higher concentrations resulted in reduced clotting efficiency, emphasizing the importance of dose optimization.

Table 7. Blood clotting index (%) of nanoparticles.

Sample	Type	50 µL (%)	100 µL (%)	150 µL (%)	200 µL (%)	250 µL (%)
Henna	FeNP	72.5%	64.3%	55.8%	46.2%	38.4%
Tulsi	FeNP	75.1%	66.7%	58.9%	49.3%	41.2%
Vallarai	FeNP	78.3%	70.2%	61.5%	52.6%	44.8%
Henna	ZnNP	85.4%	80.6%	76.2%	71.5%	66.8%
Tulsi	ZnNP	87.2%	83.4%	79.6%	75.2%	70.5%
Vallarai	ZnNP	89.1%	85.3%	81.7%	77.6%	73.4%

Table 8. Blood clotting index (%) of Aloe vera formulations.

Sample	Type	50 µL (%)	100 µL (%)	150 µL (%)	200 µL (%)	250 µL (%)
Henna	FeNP + Aloe	68.2%	58.6%	49.7%	38.9%	30.4%
Tulsi	FeNP + Aloe	70.5%	60.8%	52.1%	41.6%	33.2%
Vallarai	FeNP + Aloe	72.4%	63.7%	54.6%	44.2%	35.8%
Henna	ZnNP + Aloe	82.6%	78.4%	73.6%	68.9%	63.5%

Tulsi	ZnNP + Aloe	84.3%	80.1%	75.8%	71.2%	66.7%
Vallarai	ZnNP + Aloe	86.1%	82.5%	78.6%	74.3%	69.8%

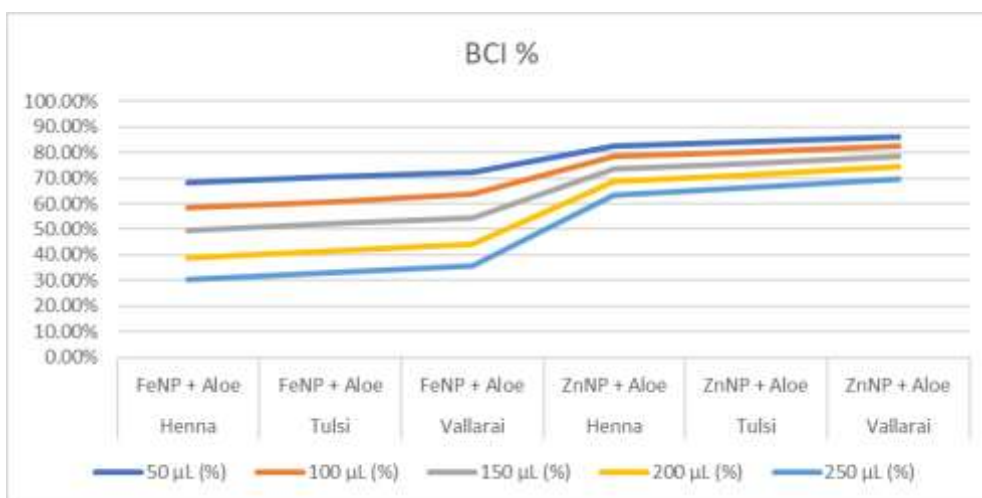


Figure 12. Blood clotting index comparison.

3.5.2 Hemocompatibility (Hemolysis Assay)

The hemolysis assay (Table 9 and Table 10; Fig. 13) demonstrated that Vallarai-mediated nanoparticles

exhibited the lowest hemolysis (2.6%), indicating superior biocompatibility. Aloe vera incorporation improved hemocompatibility across all samples, likely due to its membrane-protective properties.

Table 9. Percentage hemolysis of nanoparticles.

Sample	50µL	100µL	150µL	200µL	250µL
Henna	27.2%	31.9%	31.9%	31.9%	31.9%
Tulsi	42.1%	42.1%	47.7%	47.7%	53.4%
Vallarai	2.6%	24.4%	27.2%	31.9%	36.5%

Table 10. Percentage hemolysis of Aloe vera formulations.

Sample	50µL	100µL	150µL	200µL	250µL
Henna	32.86%	35.5%	46.6%	46.6%	59.3%
Tulsi	31.3%	31.3%	34%	34%	34%
Vallarai	4%	11.3%	21.3%	30%	42%

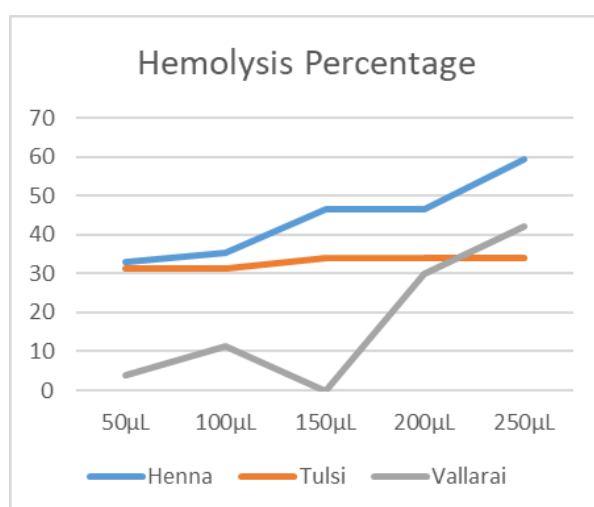


Figure 13. Hemolysis percentage of samples.

3.6 Antimicrobial activity

3.6.1 MIC Assay

The MIC results (Table 11 and Table 12; Fig. 14) revealed moderate antibacterial activity, with Henna showing relatively higher inhibition. Aloe vera formulations demonstrated enhanced activity, particularly at higher concentrations.

Table 11. MIC-based bacterial inhibition (%) of nanoparticles.

Sample	50µL	100µL	150µL	200µL	250µL
Henna	13.64%	15.91%	18.18%	20.4%	22.73%
Tulsi	6.82%	11.36%	11.36%	18.18%	90.91%
Vallarai	4.55%	6.82%	13.64%	15.91%	15.91%

Table 12. MIC-based inhibition of Aloe vera formulations.

Sample	50µL	100µL	150µL	200µL	250µL
Henna	6.82%	6.82%	9.09%	18.18%	59.09%
Tulsi	2.27%	18.18%	22.73%	31.82%	36.36%
Vallarai	9.09%	15.91%	15.91%	22.73%	27.27%

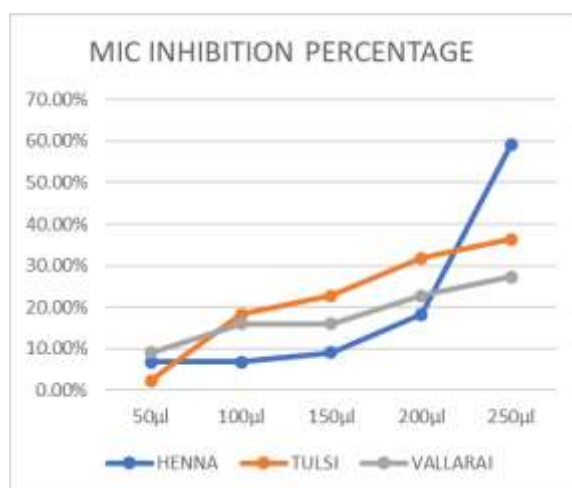


Figure 14. Bacterial growth inhibition (OD600).

3.6.2 Agar Well Diffusion Assay

Table 13. Zone of inhibition (mm) of nanoparticle formulations.

Concentration (mL)	NP (Without Gel)	NP + Aloe vera Gel	Unit
0.05	6.2 ± 0.3	7.8 ± 0.4	mm
0.10	8.9 ± 0.4	10.5 ± 0.5	mm
0.15	11.6 ± 0.5	13.2 ± 0.6	mm
0.20	14.8 ± 0.6	17.1 ± 0.7	mm
0.25	17.9 ± 0.7	20.4 ± 0.8	mm

The antimicrobial activity of green synthesized nanoparticles and nanoparticle-incorporated Aloe vera gel was evaluated using the agar plate method against bacterial culture. The effectiveness of the formulations was determined by measuring the zone

of inhibition (mm) surrounding the sample wells after incubation.

- Clear circular zones were observed around sample wells

- Zone diameter increased with concentration
- NP + Aloe gel showed larger zones than NP alone
- Control plate showed no inhibition zone

The present study demonstrates that green synthesized nanoparticles exhibit significant antimicrobial activity, which is further enhanced when incorporated with Aloe vera gel. The results clearly show a **concentration-dependent increase in zone of inhibition**, indicating that higher concentrations of nanoparticles lead to stronger antibacterial effects. At the highest concentration (0.25 mL), the nanoparticle formulation exhibited a maximum inhibition zone of 17.9 mm, whereas the nanoparticle–Aloe vera gel formulation showed an even higher inhibition zone of 20.4 mm. The enhanced antimicrobial activity of the nanoparticle-based formulations can be attributed to multiple mechanisms. Metal nanoparticles such as iron and zinc are known to generate reactive oxygen species (ROS), which damage bacterial cell membranes, proteins, and DNA. Additionally, nanoparticles can penetrate bacterial cell walls and disrupt intracellular processes, leading to cell death. The plant extracts used for nanoparticle synthesis—*Lawsonia inermis*, *Ocimum tenuiflorum*, and *Centella asiatica*—are rich in bioactive compounds such as flavonoids, phenolics, and terpenoids. These phytochemicals contribute to antimicrobial activity by interfering with microbial enzymes and membrane integrity. The incorporation of Aloe vera gel significantly improved antimicrobial effectiveness. Aloe vera contains bioactive components such as anthraquinones, saponins, and phenolic compounds, which possess inherent antimicrobial properties. Moreover, Aloe vera acts as a **natural carrier matrix**, enhancing nanoparticle dispersion and ensuring sustained release of active components at the site of action. The larger zones of inhibition observed in nanoparticle–Aloe vera gel formulations indicate a **synergistic effect** between plant-derived nanoparticles and Aloe vera. This combination not only improves antimicrobial potency but also makes the formulation more suitable for wound healing applications by preventing microbial infection.

DISCUSSION

The findings of the present study clearly demonstrate that the green synthesized nanoparticles derived from *Lawsonia inermis*, *Ocimum tenuiflorum*, and *Centella asiatica*, along with their incorporation into Aloe vera gel, significantly contribute to enhancing the therapeutic potential of the developed formulation. The experimental results collectively indicate that these nano-herbal systems possess promising biological activities, including anti-inflammatory, antioxidant, antimicrobial, hemostatic, and biocompatible properties. The observed activities are primarily attributed to the synergistic interaction between plant-derived phytochemicals and metal nanoparticles, which improved membrane stabilization, protein protection, free radical scavenging, and microbial inhibition. Furthermore, the incorporation of Aloe vera gel acted as an effective natural delivery matrix, enhancing stability, bioavailability, and overall biological performance of the nanoparticles. The concentration-dependent responses observed across experiments highlight the importance of dosage optimization, as lower to moderate concentrations exhibited better efficacy and safety compared to higher concentrations. Phytochemical analysis further supported these findings by confirming the presence of bioactive compounds such as flavonoids and alkaloids, which play a crucial role in mediating these biological effects. Overall, the study proves that the developed nano-herbal formulation is a promising, eco-friendly, and effective approach for wound healing applications, offering a potential alternative to conventional therapies with improved safety and multifunctional benefits.

CONCLUSION

The present study successfully developed and evaluated green synthesized iron and zinc nanoparticles using medicinal plant extracts (*Lawsonia inermis*, *Ocimum tenuiflorum*, and *Centella asiatica*) and demonstrated their enhanced therapeutic potential when incorporated into Aloe vera gel. The results confirmed that the nano-herbal formulations exhibit significant anti-inflammatory, antioxidant, antimicrobial, hemostatic, and biocompatible properties, which are essential for effective wound healing. The synergistic interaction between plant phytochemicals, metal nanoparticles,

and Aloe vera gel contributed to improved biological activity and stability of the formulation. Overall, this study proves that the developed nano-herbal system is a promising, eco-friendly, and cost-effective alternative for wound healing applications. Further studies, including in vivo and clinical evaluations, are recommended to validate its potential for practical biomedical use.

Ethics approval and consent to participate

Not applicable.

This study did not involve human participants, human data, or animal experiments.

Consent for publication

Not applicable.

This manuscript does not contain data from any individual person in any form (including images or videos).

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files. Additional raw data are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HJ, SM, and NJ performed the experimental analysis. HJ, SM, NJ wrote, interpreted the results, and organized the manuscript. AG and NP contributed to the review process, and KG reviewed the final version of the manuscript.

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REFERENCES

1. Guo, S., & DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of Dental Research*, 89(3), 219–229. <https://doi.org/10.1177/0022034509359125>
2. Eming, S. A., Martin, P., & Tomic-Canic, M. (2014). Wound repair and regeneration: Mechanisms, signaling, and translation. *Science Translational Medicine*, 6(265), 265sr6. <https://doi.org/10.1126/scitranslmed.3009337>
3. Boateng, J., & Catanzano, O. (2015). Advanced therapeutic dressings for effective wound healing—A review. *Journal of Pharmaceutical Sciences*, 104(11), 3653–3680. <https://doi.org/10.1002/jps.24610>
4. Frykberg, R. G., & Banks, J. (2015). Challenges in the treatment of chronic wounds. *Advances in Wound Care*, 4(9), 560–582. <https://doi.org/10.1089/wound.2015.0635>
5. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), 76–83. <https://doi.org/10.1016/j.biotechadv.2008.09.002>
6. Zhang, L., Gu, F. X., Chan, J. M., Wang, A. Z., Langer, R. S., & Farokhzad, O. C. (2016). Nanoparticles in medicine: Therapeutic applications and developments. *Clinical Pharmacology & Therapeutics*, 100(5), 543–552. <https://doi.org/10.1002/cpt.502>
7. Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: Present situation and prospects. *Journal of Biomedical Nanotechnology*, 13(1), 1–14. <https://doi.org/10.1166/jbnn.2017.2334>
8. Raghupathi, K. R., Koodali, R. T., & Manna, A. C. (2011). Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. *Langmuir*, 27(7), 4020–4028. <https://doi.org/10.1021/la104825u>

9. Lin, P. H., Sermersheim, M., Li, H., Lee, P. H. U., Steinberg, S. M., & Ma, J. (2018). Zinc in wound healing modulation. *Nutrients*, 10(1), 16. <https://doi.org/10.3390/nu10010016>
10. Mahmoudi, M., Sant, S., Wang, B., Laurent, S., & Sen, T. (2011). Superparamagnetic iron oxide nanoparticles (SPIONs): Development, surface modification and applications in chemotherapy. *Chemical Reviews*, 111(2), 253–280. <https://doi.org/10.1021/cr1002596>
11. Irvani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, 13(10), 2638–2650. <https://doi.org/10.1039/c1gc15386b>
12. Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using plant extracts: A review. *Journal of Advanced Research*, 7(1), 17–28. <https://doi.org/10.1016/j.jare.2015.02.007>
13. Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic nanoparticles using neem leaf broth. *Journal of Colloid and Interface Science*, 275(2), 496–502. <https://doi.org/10.1016/j.jcis.2004.03.003>
14. Singh, P., Kim, Y. J., Zhang, D., & Yang, D. C. (2018). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in Biotechnology*, 36(4), 412–424. <https://doi.org/10.1016/j.tibtech.2017.11.006>
15. Gohil, K. J., Patel, J. A., & Gajjar, A. K. (2010). Pharmacological review on *Centella asiatica*: A potential herbal cure-all. *Indian Journal of Pharmaceutical Sciences*, 72(5), 546–556. <https://doi.org/10.4103/0250-474X.78519>
16. James, J. T., & Dubery, I. A. (2009). Pentacyclic triterpenoids from the medicinal herb *Centella asiatica*. *Journal of Ethnopharmacology*, 123(1), 89–94. <https://doi.org/10.1016/j.jep.2009.02.016>
17. Habbal, O. A., Al-Jabri, A. A., El-Hag, A. H., Al-Mahrooqi, Z. H., & Al-Hashmi, N. A. (2005). In vitro antimicrobial activity of *Lawsonia inermis*. *Journal of Ethnopharmacology*, 100(3), 250–253. <https://doi.org/10.1016/j.jep.2005.02.034>
18. Prakash, P., & Gupta, N. (2005). Therapeutic uses of *Ocimum sanctum* Linn (Tulsi). *Indian Journal of Physiology and Pharmacology*, 49(2), 125–131.
19. Pattanayak, P., Behera, P., Das, D., & Panda, S. K. (2010). *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications. *Pharmacognosy Reviews*, 4(7), 95–105. <https://doi.org/10.4103/0973-7847.65323>
20. Hamman, J. H. (2008). Composition and applications of Aloe vera leaf gel. *Molecules*, 13(8), 1599–1616. <https://doi.org/10.3390/molecules13081599>
21. Chithra, P., Sajithlal, G. B., & Chandrakasan, G. (1998). Influence of Aloe vera on collagen characteristics. *Molecular and Cellular Biochemistry*, 181(1–2), 71–76. <https://doi.org/10.1023/A:1006819506949>
22. Surjushe, A., Vasani, R., & Saple, D. G. (2008). Aloe vera: A short review. *Indian Journal of Dermatology*, 53(4), 163–166. <https://doi.org/10.4103/0019-5154.44785>
23. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Journal of Nanoparticle Research*, 15, 1667. <https://doi.org/10.1007/s11051-013-1667-8>
24. Thakkar, K. N., Mhatre, S. S., & Parikh, R. Y. (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine*, 6(2), 257–262. <https://doi.org/10.1016/j.nano.2009.07.002>

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