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Research Article

Exploration of Antioxidant and Anti-inflammatory Potential and Green Synthesis of Silver Nanoparticles Using *Holoptelea integrifolia*

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ABSTRACT

Holoptelea integrifolia (Indian elm) is a medicinal plant traditionally used in Ayurveda for managing inflammatory conditions, wounds, and pain. In this study, silver nanoparticles (AgNPs) were synthesized using the bark extract of *H. integrifolia* and their antioxidant and anti-inflammatory activities were compared with those of chemically synthesized AgNPs. The methanolic extract contained various phytochemicals including alkaloids, flavonoids, terpenoids, glycosides, phytosterols, tannins, and saponins, which acted as reducing and capping agents during nanoparticle synthesis. The formation of AgNPs was confirmed by a color transition to brown and a characteristic UV–visible absorption peak at 440 nm. TEM imaging showed predominantly spherical nanoparticles with sizes between 2 and 200 nm. HPLC analysis confirmed the presence of rutin as a major compound in the extract. In the DPPH assay, the bark extract and green-synthesized AgNPs showed IC_{50} values of 63.89 μg/mL and 70.45 μg/mL, respectively, whereas chemically synthesized AgNPs displayed 95.68 μg/mL, indicating better antioxidant potential in plant-mediated AgNPs. Anti-inflammatory evaluation revealed IC_{50} values of 188.74 μg/mL for chemically synthesized AgNPs, 994.69 μg/mL for biosynthesized AgNPs, and 2655.87 μg/mL for the crude extract. These findings suggest that *H. integrifolia* bark extract is an effective eco-friendly source for AgNP synthesis, and the incorporation of nanoparticles can enhance the biological potential of plant-derived compounds.

Keywords: *Holoptelea integrifolia*, Silver nanoparticles, Antioxidant activity, Anti- inflammatory activity, Phytochemicals, Green synthesis, TEM, HPLC.

INTRODUCTION

Holoptelea integrifolia (Roxb.), commonly known as Indian Elm or Jungle Cork Tree.[1] Widely distributed across Asia, it has been used in Ayurveda and Siddha medicine for centuries to treat inflammation, arthritis, skin diseases, fever, and gastrointestinal disorders.[2] Phytochemical investigations confirm the presence of alkaloids, flavonoids, tannins, saponins, and essential oils in its bark and leaves, compounds known for antioxidant and anti-inflammatory effects.[3,4] The green synthesis of AgNPs using *H. integrifolia* extracts thus presents a dual advantage: its phytochemicals act as both reducing/capping agents and therapeutic contributors, producing nanoparticles with improved stability and bioactivity.[5]

Nanomaterials have gained growing attention due to their unique physicochemical properties and wide-ranging applications in drug delivery, bio-imaging, antimicrobial therapy, biosensing, and environmental remediation.[6–8] Among the different types of nanomaterials, AgNPs are especially important because of their strong antimicrobial, antioxidant, and anti-inflammatory activities. They are increasingly explored as alternatives in the fight against multidrug-resistant pathogens, a global health challenge highlighted by the World Health Organization (WHO).[9] However, traditional physical and chemical synthesis methods for AgNPs are often energy-intensive, costly, and dependent on toxic reagents, raising environmental and safety concerns.

To address these challenges, medicinal plants have gained attention as natural resources for green nanotechnology. Their phytoconstituents such as flavonoids, tannins, phenolic acids, terpenoids, and sugars act as reducing and stabilizing agents in nanoparticle synthesis, resulting in eco-friendly, cost-effective, and biocompatible nanomaterials (Sh. Mohammed et al. 2023). Beyond their role in synthesis, plants themselves are rich in bioactive molecules that contribute inherent pharmacological properties, thereby enhancing the therapeutic properties of plant-mediated nanoparticles. The present study was therefore undertaken to

synthesize and characterize AgNPs from *H. integrifolia* bark extract using both green and chemical methods, and to evaluate and compare their antioxidant and anti-inflammatory activities.[10]

MATERIALS AND METHODS

This study follows an experimental design to analyse the phytochemical profile and assess the antioxidant and anti-inflammatory activities of *H. integrifolia* bark extract. The methodology includes extraction, qualitative and quantitative phytochemical analysis, and bioactivity evaluation using standard laboratory protocols. All chemicals and reagents used were of analytical grade. Major reagents such as silver nitrate, solvents, acids, and assay-specific chemicals were sourced from SRL (India), while BHT, DPPH, and diclofenac were obtained from Sigma Scientific. Fresh human red blood cell (HRBC) suspension was prepared and used for the anti-inflammatory assay.

Identification and Collection of Plant Material

Plants with therapeutic potential, particularly those possessing antioxidant and anti-inflammatory properties, were screened using the IMPPAT database. *H. integrifolia* was selected based on research objectives, supported by a literature review conducted using PubMed, Google Scholar, and ScienceDirect. Fresh bark samples of *H. integrifolia* were collected in December 2024 from Veermata Jijabai Udyaan, Byculla, Mumbai, India, and the plant was identified with assistance from the head of the nursery department.

Preparation of Extracts

Hot Extraction

Freshly collected bark samples of *H. integrifolia* were washed thoroughly with tap water to remove surface impurities and shade-dried at room temperature. The dried material was ground into fine powder using a mechanical grinder. For extraction, 2 g of powdered bark was accurately weighed, and 100 mL of methanol was measured in a pre-weighed beaker. The powdered bark was enclosed in a muslin cloth pocket and placed in a clean, dry Soxhlet apparatus containing 100 mL of methanol. Extraction was carried out at 100°C for 5 to 6 days[11].

Cold Extraction

The bark samples were shade-dried at room temperature, pulverized into a fine powder, and 2 g of the material was transferred into a pre-weighed conical flask containing 30 mL of methanol. The mixture was gently agitated and left for maceration at room temperature for seven days. Following maceration, the extract was filtered through Whatman No. 1 filter paper, and the filtrate was collected for further analysis.[11]

Preliminary Phytochemicals Screening[12]

Preliminary phytochemicals were screened for the detection of alkaloids, carbohydrates, reducing sugars, tannins, and phenolic compounds. The following qualitative tests were performed for phytochemicals screening (Figure 1).

Preparation of Silver Nanoparticles[13]

After dissolving 1.7 g of AgNO3 in 100 mL of distilled water, the mixture was left for reflux. Additionally, 0.7 g of citric acid was gradually added to the boiling solution after being dissolved in 1.5 mL of distilled water. Until all Ag^{+} ions were reduced and nanoparticles were formed, the reaction was kept under constant stirring and boiling. The pellet was repeatedly cleaned with ethanol and distilled water after the mixture was cooled to room temperature and centrifuged for ten minutes at 12,000 rpm. The cleaned nanoparticles were stored for additional analysis after being dried at 100°C.

Figure 1: Preliminary phytochemical screening

Synthesis of AgNPs from Plant Extracts[14]

A 200 mM silver nitrate ($AgNO_3$) solution was prepared in distilled water. For the synthesis of silver nanoparticles, 2 mL of concentrated plant extract was added to the $AgNO_3$ solution in a reaction vessel and heated to 200°C with continuous stirring. The $NaBH_4$ solution was then added dropwise while maintaining constant stirring until a noticeable colour change confirmed the formation of silver nanoparticles. The resulting mixture was centrifuged at 12,000 rpm for 10 minutes, and the pellet was washed with distilled water followed by ethanol to eliminate any unreacted residues. The purified nanoparticles were dried at 100°C and stored for further characterization.

Evaluation and Characterization of Synthesized Nanoparticles of H. integrifolia

UV–Vis spectrophotometry was performed (200–800 nm) for plant extract, plant-mediated AgNPs, and chemically synthesized AgNPs to confirm formation and detect characteristic peaks. HPLC analysis of *H. integrifolia* extract was carried out using a Thermo Scientific Dionex Ultimate 3000 system with a Hypersil Gold column (260 × 4.6 mm, 5 µm) under gradient elution (A – KH_2PO_4 pH 3.15, B – acetonitrile, C – methanol, D – water). FTIR spectroscopy was used to identify functional groups. TEM analysis was conducted at ICON Lab (Navi Mumbai) using an FEI TEM operated at 120 kV, providing high-resolution imaging (0.5 nm) and morphology analysis at magnifications from 20× to 3.5 lac.

Antioxidant Assay

The antioxidant efficacy of the bark extract and synthesised AgNPs was assessed using the DPPH technique. A 1-mL aliquot of 100 ppm DPPH solution in methanol was combined with test samples at concentrations between 5 and 1000 ppm and incubated in darkness at ambient temperature for 30 minutes. Absorbance was measured at 517 nm using a UV–visible spectrophotometer. Butylated hydroxytoluene (BHT) functioned as the standard, while a DPPH–methanol solution acted as the control. Reduced absorbance readings indicated enhanced free radical scavenging capability.[15,16]

Anti-inflammatory Assay

The anti-inflammatory action was evaluated by the human red blood cell (HRBC) membrane stabilisation technique, which measures the capacity of test materials to inhibit erythrocyte haemolysis under hypotonic conditions. About 5 mL of human blood was combined with three millilitres of Alsever's solution, centrifuged at 3000 rpm for ten minutes, and the erythrocyte pellet was then washed three times with isotonic saline. A 10% HRBC suspension was created by diluting 1-mL of packed cells with 9 mL of isotonic saline.[17]

A phosphate buffer (pH 7.5) was made by combining Na_2HPO_4 and NaH_2PO_4 solutions, followed by increasing the final volume to 200 mL. For the assay, 1-mL of the test sample at varying concentrations was combined with 0.5 mL of 10% HRBC solution, 1-mL of phosphate buffer, and 1 mL of iso-saline. The mixtures were incubated at 37°C for 30 minutes and then centrifuged at 3000 rpm for 30 minutes. The absorbance of the supernatant was measured at 560 nm. Diclofenac served as the reference medication. A reduced absorbance signified enhanced membrane stabilisation and anti-inflammatory efficacy.[18-20]

RESULTS

Extraction of Plant Extract

Methanolic extraction of *H. integrifolia* bark using Soxhlet at 100°C yielded a dark yellow extract under hot conditions, whereas the cold extract gradually developed a light-yellow color. The crude extract, preserved at room temperature, indicated successful isolation of bioactive constituents. This suggests the presence of secondary metabolites with potential antioxidant and anti-inflammatory activities for further pharmacological evaluation.

Preliminary Phytochemical Investigation

The preliminary phytochemical screening of the methanolic bark extract of *H. integrifolia* confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phytosterols, and glycosides. Carbohydrates, reducing sugars, proteins and amino acids, phenolic compounds, and quinones were not detected. These findings (Table 1) indicate that the extract contains several secondary metabolites of phytochemical significance.

Synthesis of AgNPs from Plant Extracts

Silver nanoparticles were effectively synthesised using the methanolic extract of *H. integrifolia*. The introduction of silver nitrate solution to the plant extract resulted in a progressive colour shift from light yellow to brown, signifying the reduction of Ag^+ ions and the synthesis of AgNPs. The reaction mixture was then centrifuged and thoroughly washed to eliminate residual phytochemicals and unreacted metal ions. The emergence of brown colouration validated the reduction of silver ions by phytochemicals in the extract and the stabilisation of the nanoparticles. The synthesised AgNPs underwent characterisation by spectroscopic and microscopic methods.

Characterization of synthesized silver nanoparticles

UV–Vis spectral analysis

The plant-based AgNPs showed a color change from pale yellow to brown, while the chemically synthesized nanoparticles changed to dark grey. UV-vis spectral analysis confirmed the formation of silver nanoparticles, with characteristic absorption peaks observed

Table 1: Preliminary phytochemical screening

S. No.	*Preliminary test*	*Result*
1	Alkaloids	+
2	Carbohydrates	-
3	Reducing sugars	-
4	Proteins and amino acids	-
5	Flavonoids	+
6	Phenolic compounds	-
7	Tannins	+
8	Quinones	-
9	Saponins	+
10	Terpenoids	+
11	Phytosterols	+
12	Glycosides	+

at 440 nm for plant-based nanoparticles and 440 nm for chemically synthesized nanoparticles (Figure 2).

TEM analysis

TEM analysis was carried out to determine the morphology and size distribution of the synthesized silver nanoparticles. The TEM micrographs (Figure 3) revealed that the nanoparticles were predominantly quasi-spherical or polyhedral in shape, with a tendency for slight aggregation. The average particle size was in the range of 2–200 nm. In addition, the images demonstrated the presence of a nanocrystalline structure, confirming that the AgNPs were successfully synthesized using the bark extract of *H. integrifolia*.

HPLC analysis

HPLC analysis of the methanolic bark extract of *H. integrifolia* (Figures 4 and 5) revealed a distinct chromatographic profile with three major peaks at retention times 2.338, 4.271, and 35.329 minutes, indicating the presence of multiple phytochemical constituents in the extract. A detailed plot of retention time versus peak intensity showed clear differentiation of compounds.

The peak at retention time 4.796 minutes corresponded to rutin, consistent with previously reported data for *H. integrifolia*. A peak detected at 2.646 min showed similarity to gallic acid based on literature; however, preliminary phytochemical screening did not indicate the presence of phenolic compounds, suggesting the need for further analytical confirmation[21]. The late-eluting peak at 35.346 minutes is presumed to represent a non-polar compound, which requires further structural elucidation. These findings align with earlier reports by Alli Kavitha et al. and indicate that additional characterization using techniques such as LC–MS is necessary for accurate compound identification.

Antioxidant Assay Analysis

With the IC_{50} values of 63.89 µg/mL, the plant extract and plant-based nanoparticles is 70.45 µg/mL samples show moderate antioxidant activity, compared with the IC_{50} of the standard, which was 23.01 µg/mL. The produced nanoparticles exhibited moderate antioxidant activity, with an IC_{50} value of 95.68 µg/mL. According to these results, Plant extract and plant-based nanoparticles and chemically synthesis nanoparticles have is less IC50 than the standard; thus, chemically manufactured nanoparticles and plant extract and chemically synthesized nanoparticles have lower antioxidant potential (Figures 6 and 7).

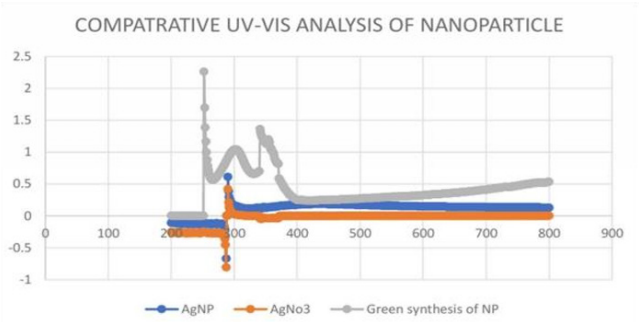

Figure 2: Comparative UV- vis analysis of nanoparticles

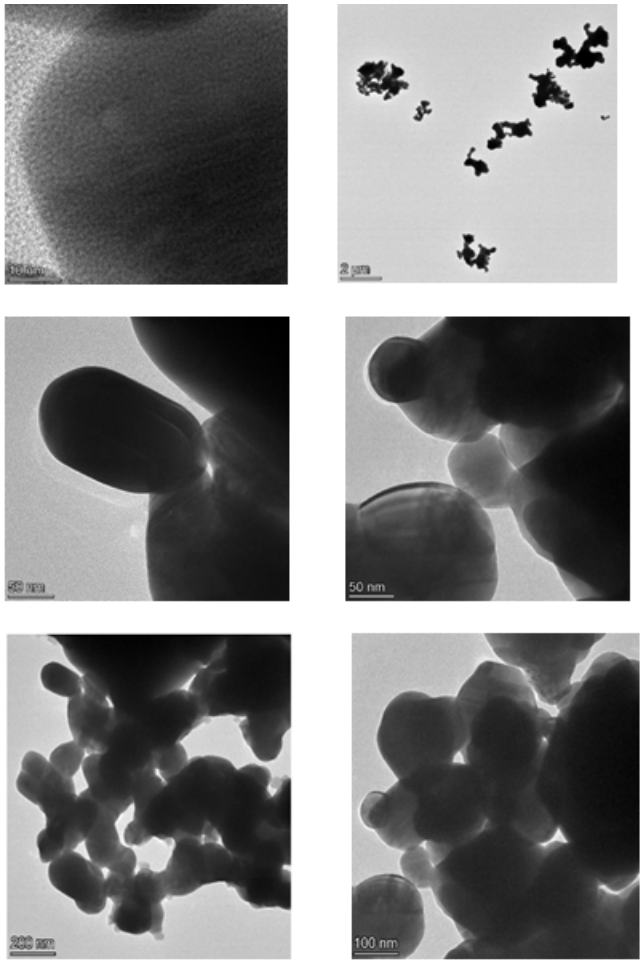

Figure 3: TEM analysis of silver nanoparticles

Anti-Inflammatory Assay Analysis

The IC_{50} values obtained from the study of the samples show that they are effective in their anti-inflammatory activity. As evidenced by the lowest value, the standard with the strongest anti-inflammatory activity had an IC_{50} value of 24.80 to 188.74 µg/mL, which was the chemically manufactured nanoparticles' IC_{50} value, which was much lower than the moderate activity shown by the nanoparticles. When compared to *H. integrifolia* bark extract, which had an IC_{50} value of 2655.87 µg/mL, the AgNPs extracted from *H. integrifolia* exhibited somewhat lesser antioxidant activity, with an IC_{50} value of 994.69 µg/mL. According to these findings, even though *H. integrifolia* plant-based AgNPs exceeded the plant extract in terms of anti-inflammatory potential, they still fall short of the standard and chemically synthesized AgNPs in terms of anti-inflammatory potential (Figures 8 and 9).[22,23]

DISCUSSION

The present study demonstrated the successful extraction of bioactive compounds from the bark of *H. integrifolia* using methanolic soxhlet extraction. Preliminary phytochemical screening confirmed the presence of Flavonoids, alkaloids, tannins, saponins, glycosides, phytosterols, and terpenoids, indicating the plant's richness in secondary metabolites with pharmacological importance.

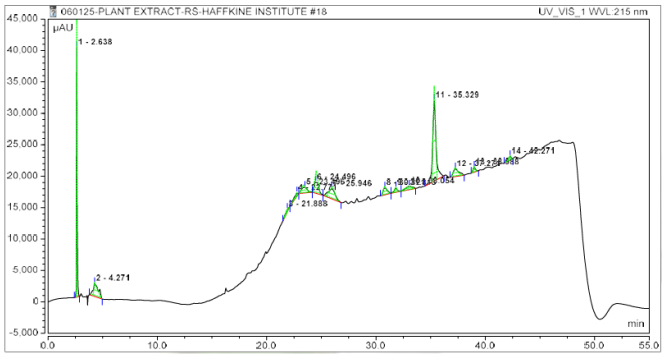

Figure 4: HPLC chromatogram of hot extract

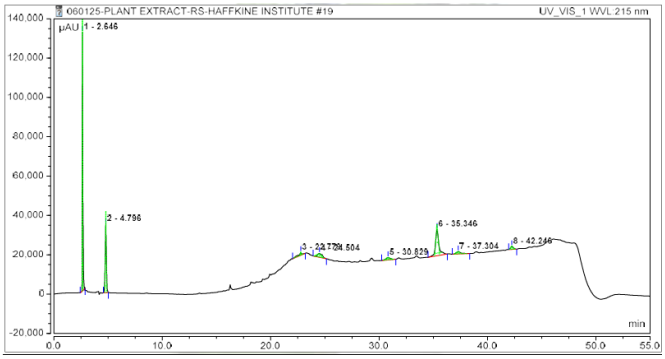

Figure 5: HPLC chromatogram of cold extract

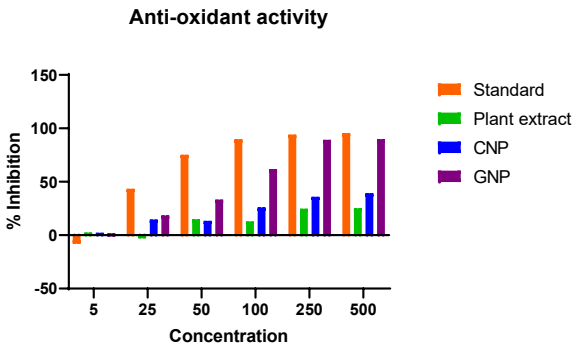

Figure 6: Antioxidant activity of *H. integrifolia*

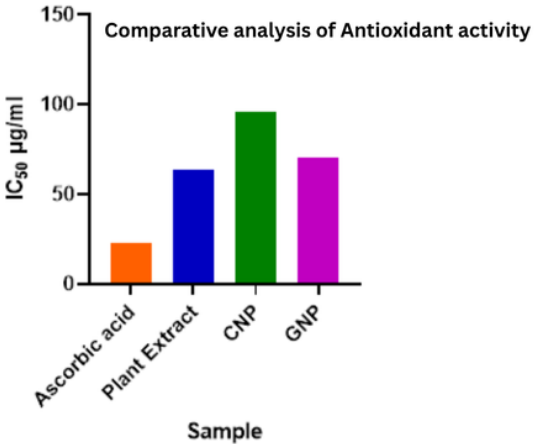

Figure 7: Comparative analysis of antioxidant activity

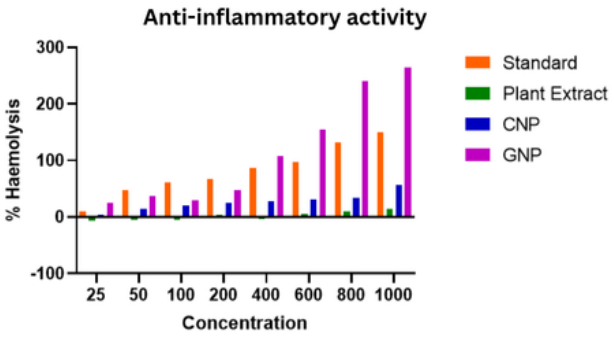

Figure 8: Anti-inflammatory activity of *H. integrifolia*

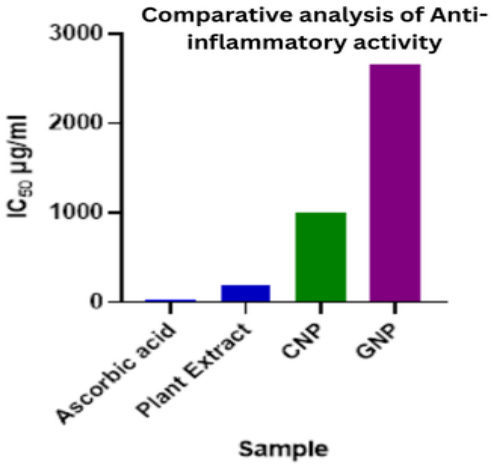

Figure 9: Comparative analysis of anti-inflammatory activity

The bark extract was further utilized for the green synthesis of silver nanoparticles, which was confirmed by a visible colour transition from yellow to brown and a distinct UV-vis absorption peak at 440 nm. TEM analysis revealed predominantly quasi-spherical and polyhedral nanoparticles with an average size of 2 to 200 nm, further supporting the successful formation of stable nanostructures.

Among the flavonoids, rutin was present in notable amounts in the methanolic extract of *H. integrifolia*. The peak of rutin in the chromatogram highlights its importance as a bioactive compound of this plant. Previous studies have reported rutin to exhibit multiple pharmacological effects, including anticancer, antioxidant, anti-inflammatory, and antimicrobial activities[24]. In addition to rutin, other flavonoids such as kaempferol, quercetin, and luteolin—commonly reported in stem and leaf plant extracts—are also of therapeutic relevance.

Overall, the HPLC profiling of *H. integrifolia* methanolic extract revealed the presence of diverse flavonoid constituents, with rutin being the predominant compound. Further studies using mass spectrometry and NMR spectroscopy are needed to confirm and characterize the compound detected at RT 35.346 minutes and to establish a complete phytochemical profile.

The DPPH assay confirmed that *H. integrifolia* bark extract possesses moderate antioxidant activity, with an IC_{50} of 63.89 µg/mL, attributable to its phytoconstituents such as flavonoids, tannins, and

phenolic compounds. The bio-green synthesized AgNPs retained comparable activity (IC_{50} = 70.45 µg/mL), suggesting that the plant-derived biomolecules involved in nanoparticle capping and stabilization help preserve radical scavenging ability. In contrast, the chemically synthesized AgNPs exhibited weaker activity (IC_{50}= 95.68 µg/mL), indicating the advantage of green synthesis over chemical methods in maintaining antioxidant properties. As expected, the standard BHT showed the strongest activity (IC_{50} = 23.01 µg/mL).

The standard drug exhibited the strongest anti-inflammatory activity (IC_{50} = 24.80 µg/mL), thereby validating the reliability of the assay system. In contrast, the test samples displayed a gradient of activity, with chemically synthesized nanoparticles demonstrating higher potency (IC_{50} = 188.74 µg/mL) compared to plant-based nanoparticles (IC_{50} = 994.69 µg/mL) and the crude plant extract (IC_{50} = 2655.87 µg/mL). This trend highlights the significant role of nanotechnology in enhancing the pharmacological properties of phytoconstituents, primarily through improvements in solubility, stability, and cellular uptake.

The superior activity of chemically synthesized nanoparticles may be attributed to favourable physicochemical characteristics such as smaller particle size, higher uniformity, and greater purity. Conversely, the comparatively reduced efficacy of green-synthesized nanoparticles may result from residual organic components, incomplete encapsulation of bioactive compounds, or partial degradation during the synthesis process. These observations suggest that the biological performance of plant-based nanoparticles is influenced by both the phytochemical composition of the source material and the synthesis conditions employed.

The weak activity of the crude plant extract further indicates the limitations of phytochemicals in their natural state, likely due to their low concentration, poor solubility, and limited bioavailability. Taken together, the findings emphasize the potential of nanoparticle-based formulations, particularly chemically synthesized systems, in significantly enhancing the therapeutic efficacy of plant-derived substances for inflammatory disorders. Future studies should focus on optimizing green synthesis methods to balance eco-friendliness with pharmacological efficiency. Overall, the findings establish *H. integrifolia* bark as a valuable natural source of bioactive compounds and confirm green-synthesized AgNPs as sustainable candidates for antioxidant and anti-inflammatory applications. These results reinforce the relevance of eco-friendly nanoparticle synthesis in developing therapeutic agents from medicinal plants.

CONCLUSION

The study confirmed that the methanolic bark extract of *H. integrifolia* contains several phytochemicals and can successfully mediate the green synthesis of silver nanoparticles. The formation of AgNPs was verified by a color shift to brown, a UV-vis peak at 440 nm, and TEM results showing quasi-spherical nanoparticles of 2 to 200 nm. Both the extract and biosynthesized AgNPs exhibited antioxidant activity, with IC_{50} values of 63.89 and 70.45 µg/mL, respectively. In the anti-inflammatory assay, plant-mediated AgNPs showed greater activity (IC_{50} = 994.69 µg/mL) than the crude extract. These findings indicate that *H. integrifolia* bark and its green-synthesized AgNPs hold potential for antioxidant and anti-inflammatory applications and support the use of eco-friendly nanoparticle synthesis in medicinal research.

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CONFLICT OF INTEREST

There is no statement on conflict of interest.

FUNDING SOURCE

Nil

ETHICS OF HUMAN AND ANIMAL EXPERIMENTATION

Human blood samples were used for HRBC membrane stabilization assay after ethical approval and informed consent.

AUTHOR CONTRIBUTIONS

Ashish Asrondkar, Harshada Sawant: Conceptualization, Methodology, Writing – Original Draft, Editing, Data Collection, Analysis

Jayshree Kale, Chhaya Bhagat, Suvarna Kanade: Visualization, Methodology, Data Collection, Analysis.

Urmila Odhekar, Priya Patil, Ketkai Patil: Visualisation, Methodology, Analysis

Prashant More: Plant Identification, Plant Collection

Chetan Jain, Vrushali Patil: Supervision, Review & Editing, Project Administration

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