REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

Preparation, Characterization, and Pharmacological evaluation of Herbal Nanoparticles derived from Shorea Robusta

Atul Kumar Dubey[1*], Dr.Vikas Chandra[2]

[1*,2]Bhagwant University, Sikar Road, Ajmer-305004

***Corresponding Author:** Atul Kumar Dubey
Email: dubeyatul38@gmail.com

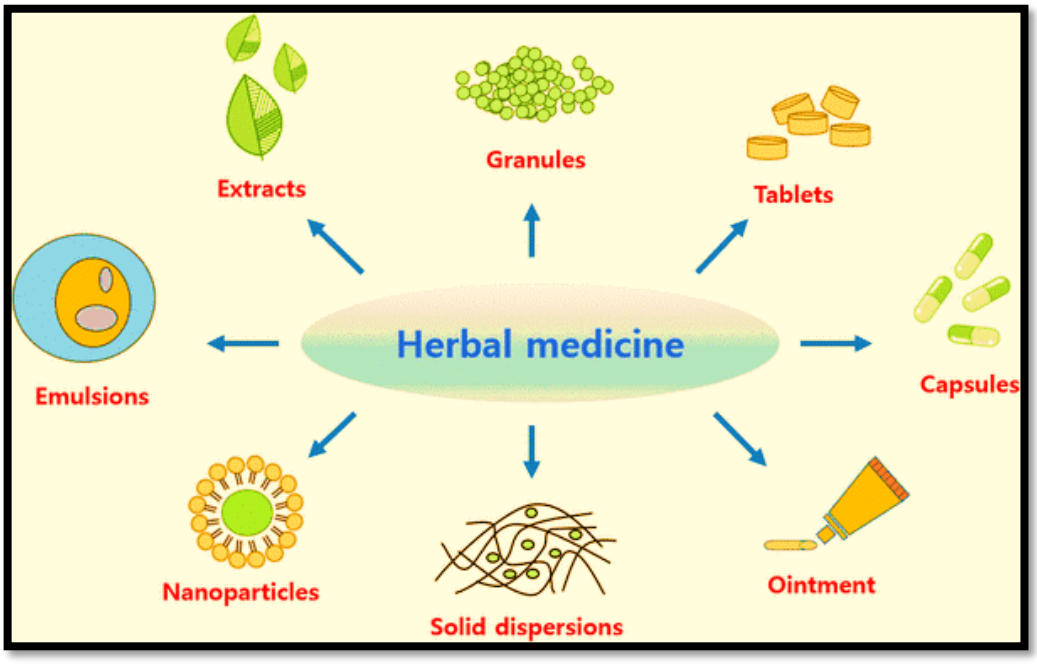
Abstract
Herbal remedies have a longer history of use than conventional pharmaceuticals, and both doctors and patients have come to acknowledge their superior therapeutic benefit and reduced risk of side effects. A scientific approach is necessary for phytotherapeutics in order to promote patient compliance and prevent repetitive administration by delivering the components in a sustained manner. As a result of the one-of-a-kind physicochemical features that nanoparticles exhibit as well as the potential therapeutic benefits that they offer, nanoparticles are enjoying an increased level of use in the field of medicine. This research aims to investigate the production, characterisation, and pharmacological activity of herbal nanoparticles that are obtained from Shorea robusta, which is also known as the Sal tree. The Sal tree is also known as the Sal tree. For the purpose of achieving the objective of this inquiry, the extraction of bioactive chemicals, the synthesis of nanoparticles, the physicochemical characterization of the nanoparticles, and the evaluation of the pharmacological activities of the nanoparticles are all carried out. It is feasible to draw the conclusion, in light of the data, that nanoparticles created from Shorea robusta have the potential to exhibit antioxidant and antibacterial activities. These activities may have implications for applications in the field of medicine.

Keywords: SNPs, herbal remedy, nanocarriers, Nanofertilizers, DPPH, Sal tree, ect.

1. Introduction

Nanotechnology is advancing a novel paradigm for medication delivery systems due to its distinctive diminutive size and regulated drug release. Consequently, including "herbal remedy" into nanocarriers will enhance its efficacy in treating various chronic diseases and promoting health advantages. The field of pharmaceutical technology has expanded and varied fast in recent years, evolving significantly from the macro level to the micro level, and is currently advancing at the molecular, or nano, level.[1] The significance of technology in pharmaceutics and medicine has been increasingly paramount due to evolving trends in medication development and delivery systems. Nanotechnology in certain novel drug delivery systems, such as ocular drug delivery, has been employed to improve bioavailability by addressing the limitations of traditional dosage forms.[2] This is feasible due to the ability of nanocarriers to safeguard the encapsulated therapeutic molecule and deliver it to different regions of the eyes.

Fig.No. 1 An illustration of the many different ways herbal formulated

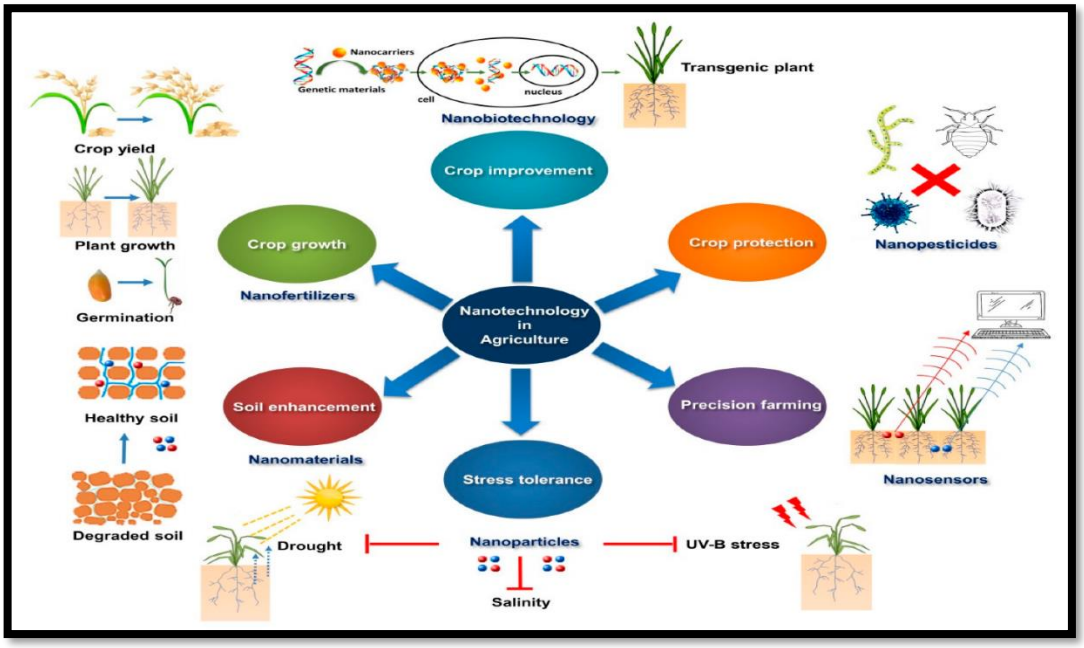


REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

2. Techniques for the nanoscale administration of medicinal plants [3]

A scientific approach is necessary for phytotherapeutics in order to promote patient compliance and prevent repetitive administration by delivering the components in a sustained manner. One way to accomplish this is by creating NDDSs specifically for botanical ingredients. By lowering toxicity and boosting bioavailability, NDDSs not only lessen the need for repeated administration to overcome non-compliance, but they also contribute to an increase in therapeutic value.[4] In a perfect world, the innovative carriers would meet two requirements. As a first step, it has to dispense the medication according to the body's requirements during the course of treatment. Second, it needs to transport the herbal medicine's active ingredient to where it's needed. These are all criteria that conventional dose forms, including extended-release versions, fall short on. Herbal medications benefit from this in many ways: they are more soluble and bioavailable, they are less toxic, they have better pharmacological activity, they are more stable, they are better distributed to tissue macrophages, they are delivered more slowly, and they are protected from physical and chemical degradation.[5] Hence, there may be a future for nano-sized NDDSs of herbal medications in improving the efficacy and resolving issues related to plant medicines. Because of their diminutive size, nanocarriers can transport the optimal concentration of a medicinal compound to its site of action while avoiding obstacles like the stomach's acidic pH and the liver's metabolism. This, in turn, increases the drug's blood circulation and its duration of effect. first, eighth, Therefore, by including herbal treatments into an NDDS, the efficacy of future herbal therapies for treating different chronological diseases will be better.

Fig.No.2 Nanofertilizers released under agricultural yield and productivity



3. Plant Profile of Shorea robusta[6]

Shorea robusta (Sal tree) is a significant medicinal plant widely used in traditional medicine for its anti-inflammatory, antimicrobial, and antioxidant properties. Recent advancements in nanotechnology have enabled the development of herbal nanoparticles with enhanced therapeutic potential.[7] This study aims to prepare *Shorea robusta* nanoparticles using green synthesis methods, characterize their physicochemical properties, and evaluate their pharmacological activities, including antioxidant and antimicrobial effects.

Materials and Methods[8]

1. Plant Material and Extraction

Plant Material: Shorea robusta leaves were collected from a local forest and authenticated.

Extraction Process: Dried leaves were ground into a coarse powder and extracted using ethanol via maceration. The extract was concentrated using a rotary evaporator and stored at 4°C until further use.

2. Preparation of Nanoparticles[9]

1. Green Synthesis Method

Materials:

- *Shorea robusta* extract
- 1 mM silver nitrate ($AgNO_3$) solution

- Polyvinyl alcohol (PVA)
- Deionized water
- Glassware and laboratory equipment (beakers, stirring apparatus, incubator, lyophilizer)

2. Preparation of Shorea robusta contain SNPs[10]

1. **Preparation of the Plant Extract:**
- Obtain the Shorea robusta plant material (leaves) and prepare an extract using an appropriate solvent (ethanol, water).
- Filter the extract to remove any solid residues and concentrate it as needed.

2. **Preparation of Silver Nitrate Solution:**
- Prepare a 1 mM solution of silver nitrate ($AgNO_3$) using deionized water. This solution will serve as the precursor for silver nanoparticles.

3. **Mixing the Extract and Silver Nitrate Solution:**
- Measure 10 mL of the *Shorea robusta* extract and mix it with 90 mL of the 1 mM $AgNO_3$ solution in a clean glass beaker.
- Stir the mixture continuously to ensure thorough mixing of the extract with the silver nitrate solution.

3. Reduction Process[11]

1. **Incubation:**
- Transfer the mixed solution to an incubator set at 60°C.
- Incubate the mixture for 2 hours. During this time, the plant extract facilitates the reduction of silver ions (Ag^+) to elemental silver (Ag), leading to the formation of silver nanoparticles.

2. **Monitoring the Reaction:**
- Observe the color change of the solution as an indication of nanoparticle formation. Silver nanoparticles typically cause a color change from pale yellow to reddish-brown.

4. Stabilization[12]

1. **Addition of Stabilizer:**
- After the reduction process is complete, add polyvinyl alcohol (PVA) to the nanoparticle solution. PVA acts as a stabilizer to prevent the aggregation of nanoparticles.
- The concentration of PVA can vary depending on the desired stability, but a common starting point is 0.1-0.5% (w/v).

2. **Lyophilization:**
- Transfer the stabilized nanoparticle solution to a suitable container and freeze it at -80°C for 24 hours to solidify the solution.
- Lyophilize the frozen sample using a lyophilizer to remove the water content through sublimation. This process yields a dry powder of silver nanoparticles.

3. **Storage:**
- Store the lyophilized nanoparticles in a dry, dark place to protect them from light and moisture.

3. Characterization of Herbal Nanoparticles[13]

- **Size and Shape:** Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) were used to determine the size and shape of nanoparticles.
- **Surface Charge:** Zeta potential was measured using a Zeta Potential Analyzer.
- **Crystalline Structure:** X-ray Diffraction (XRD) was employed to analyze the crystalline nature.
- **Chemical Composition:** Fourier Transform Infrared Spectroscopy (FTIR) was used to identify functional groups.

4. Pharmacological Evaluation of SNPs[14]

1. In Vitro Antioxidant Activity of SNPs

Materials:

- DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent
- Methanol (solvent for DPPH)
- Silver nanoparticles synthesized from *Shorea robusta* extract
- Standard antioxidant (e.g., ascorbic acid or vitamin C)
- UV-Vis Spectrophotometer
- Glassware and pipettes

Method:

1. **Preparation of DPPH Solution:**
- Prepare a 0.1 mM DPPH solution in methanol. DPPH is a stable free radical that will react with antioxidants, causing a reduction in color intensity.

- Store the solution in a dark bottle to prevent degradation by light.

2. **Sample Preparation:**
- Prepare different concentrations of the silver nanoparticles (e.g., 10, 20, 30, 40, 50 µg/mL) in methanol.
- Prepare a control sample with methanol only and a standard antioxidant solution (e.g., ascorbic acid) for comparison.

3. **Antioxidant Activity Assay:**
- Mix 1 mL of each nanoparticle solution with 2 mL of DPPH solution.
- Incubate the mixture at room temperature in the dark for 30 minutes.
- Measure the absorbance of the resulting solution at 517 nm using a UV-Vis spectrophotometer.

4. Calculate the percentage of DPPH radical scavenging using the formula.

5. **Data Analysis:**
- Compare the scavenging activity of the silver nanoparticles with the standard antioxidant.
- Higher scavenging activity indicates better antioxidant properties.

2. In Vitro Antimicrobial Activity of SNPs[15]
Materials:
- Bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*)
- Mueller-Hinton agar (for disc diffusion)
- Nutrient broth (for preparing bacterial cultures)
- Antibiotic discs (for comparison)
- Silver nanoparticles synthesized from *Shorea robusta* extract
- Disc diffusion apparatus
- Minimum Inhibitory Concentration (MIC) determination setup

Methods:
Disc Diffusion Method:
1. **Preparation of Bacterial Cultures:**
- Grow bacterial strains in nutrient broth at 37°C for 18-24 hours.
- Prepare bacterial suspensions with an optical density equivalent to 0.1 McFarland standard (approximately 10^8 CFU/mL).
2. **Agar Plate Preparation:**
- Pour Mueller-Hinton agar into petri dishes and allow it to solidify.
- Spread the bacterial suspension evenly on the agar plates using a sterile spreader.
3. **Disc Application:**
- Prepare discs by impregnating sterile filter paper discs with different concentrations of silver nanoparticles (e.g., 25, 50, 100 µg/disc).
- Place the discs on the agar plates with inoculated bacteria.
- Incubate the plates at 37°C for 24 hours.
4. **Measurement:**
- After incubation, measure the diameter of the inhibition zones around the discs.
- Compare the inhibition zones with those produced by standard antibiotic discs to evaluate the antimicrobial efficacy.

Minimum Inhibitory Concentration (MIC) Method:
1. **Preparation of MIC Assay:**
- Prepare serial dilutions of the silver nanoparticles in nutrient broth (e.g., 0.5, 1, 2, 4, 8, 16, 32 µg/mL).
- Inoculate each dilution with a standardized bacterial suspension.
2. **Incubation:**
- Incubate the culture tubes at 37°C for 18-24 hours.
3. **Assessment:**
- Determine the MIC as the lowest concentration of nanoparticles that inhibits visible bacterial growth.
- Use a color indicator (resazurin) if needed to visually assess bacterial growth.

Data Analysis:
- **Antioxidant Activity:** Compare the percentage scavenging activity of the silver nanoparticles with the standard antioxidant to evaluate their efficacy.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

- **Antimicrobial Activity:** Analyze the zone of inhibition and MIC values to determine the antimicrobial effectiveness of the nanoparticles against various bacterial strains.

5. Observation and Results

Shorea robusta contain Silver Nanoparticles

I. Color Change Observation:

- During the synthesis of silver nanoparticles, the reaction mixture initially appeared colorless.
- After incubation at 60°C for 2 hours, the solution changed to a reddish-brown color, indicating the formation of silver nanoparticles. The reddish-brown color is characteristic of silver nanoparticles due to their surface plasmon resonance.

II. UV-Vis Spectroscopy:

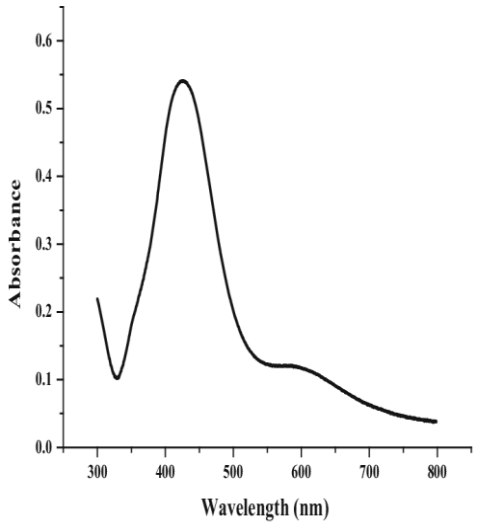
- To confirm the presence of silver nanoparticles, a UV-Vis spectroscopy analysis was performed. The absorption spectrum of the synthesized nanoparticles typically shows a peak in the range of 400-450 nm, corresponding to the surface plasmon resonance of silver nanoparticles.

- **Absorption Peak:** The UV-Vis spectrum showed a strong absorption peak at approximately 430 nm, confirming the formation of silver nanoparticles.

1. Extraction Yield and Preparation

The yield of the Shorea robusta extract was 18% (w/w) of the dry plant material. The green synthesis method produced a reddish-brown solution, indicative of silver nanoparticles.

Fig.No.3 UV analysis of Shorea robusta contain Silver Nanoparticles



REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

3. Characterization of Shorea robusta contain SNPs

Size and Shape: TEM images revealed spherical nanoparticles with an average size of 20 nm. DLS measurements confirmed an average particle size of 20 nm with a narrow size distribution (PDI = 0.20).

Fig.No.4 TEM Image of Shorea robusta contain Silver

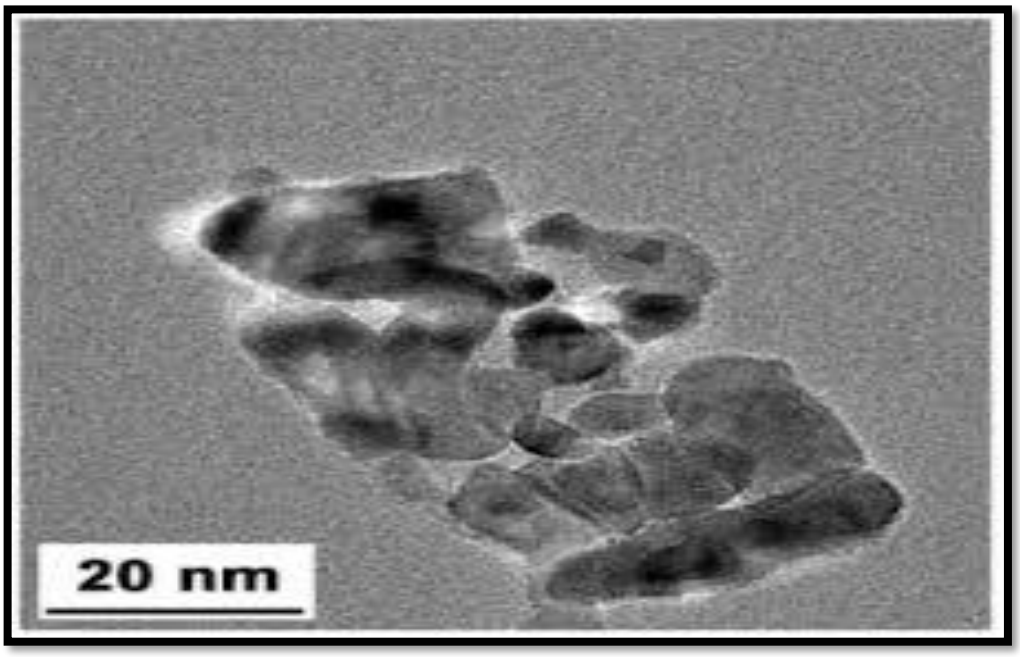
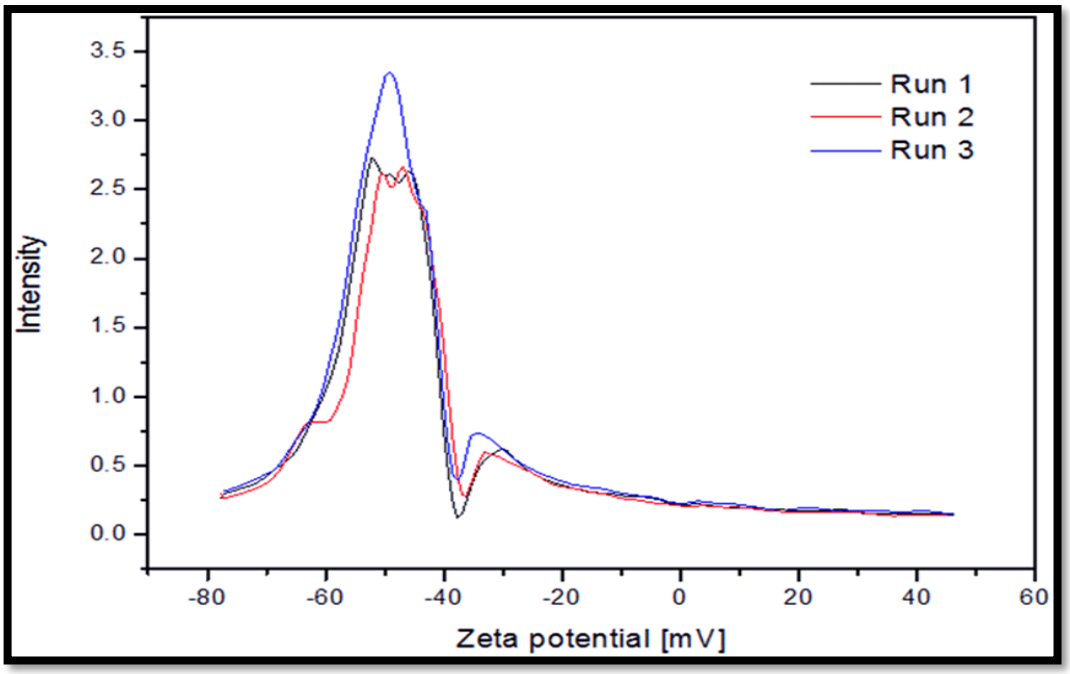


Fig.No.5 Zeta potential of Shorea robusta contain Silver Nanoparticles



Nanoparticles

Surface Charge: The zeta potential was measured at -30 mV, indicating good stability of the nanoparticles.

Crystalline Structure: XRD analysis showed distinct peaks corresponding to the face-centered cubic structure of silver nanoparticles.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

Chemical Composition: FTIR spectra indicated the presence of characteristic peaks corresponding to hydroxyl and carbonyl groups, confirming the interaction between the Shorea robusta extract and silver ions.

Fig.No.6 IR graph of Shorea robusta contain Silver Nanoparticles

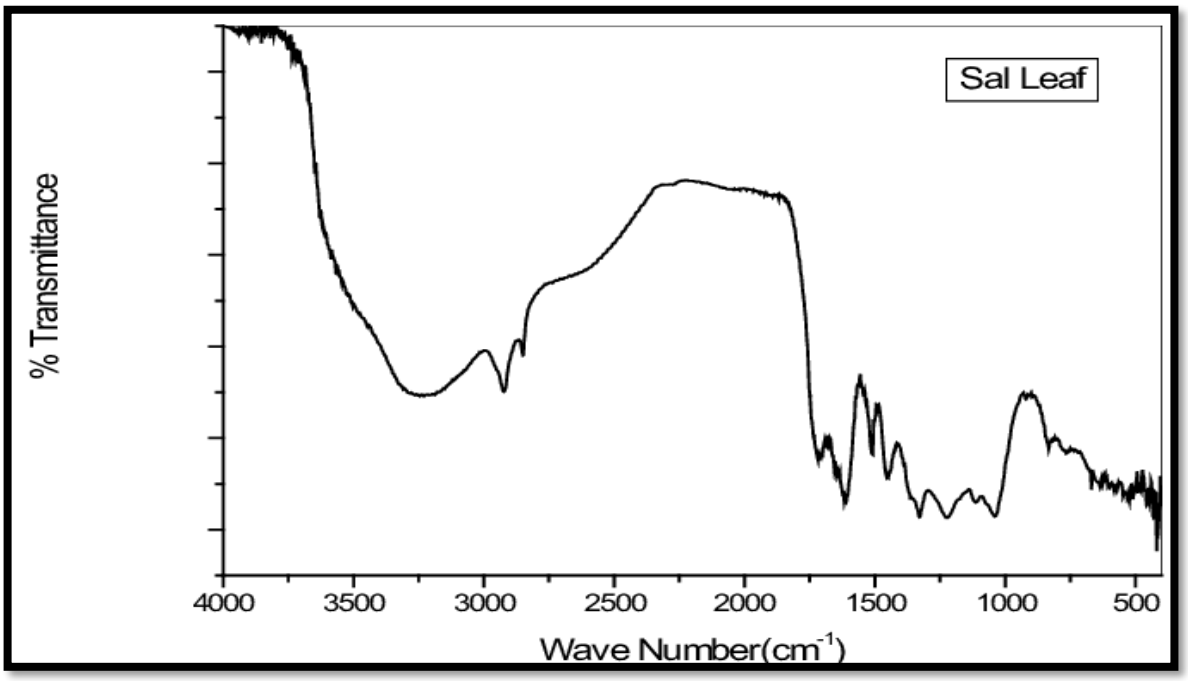


Table No.1 Antioxidant data of Shorea robusta contain SNPs

Functional Group	Wavenumber (cm^{-1})	Bonding Description
O-H Stretching	~3200-3600	Hydroxyl groups (in cellulose and lignin)
C-H Stretching	~2800-3000	Methyl and methylene groups
C=O Stretching	~1730-1750	Carbonyl groups (in hemicellulose)
C=C Stretching	~1600-1650	Aromatic rings (in lignin)
C-O Stretching	~1050-1150	Ether linkages and alcohols
O-H Bending	~1400-1450	Deformation of hydroxyl groups
C-H Bending	~1370-1450	Methylene and methyl groups

4. Pharmacological Evaluation of Shorea robusta contain SNPs

I. Antioxidant Activity: The Shorea robusta nanoparticles demonstrated significant antioxidant activity with an IC50 value of 45 µg/mL in the DPPH assay.

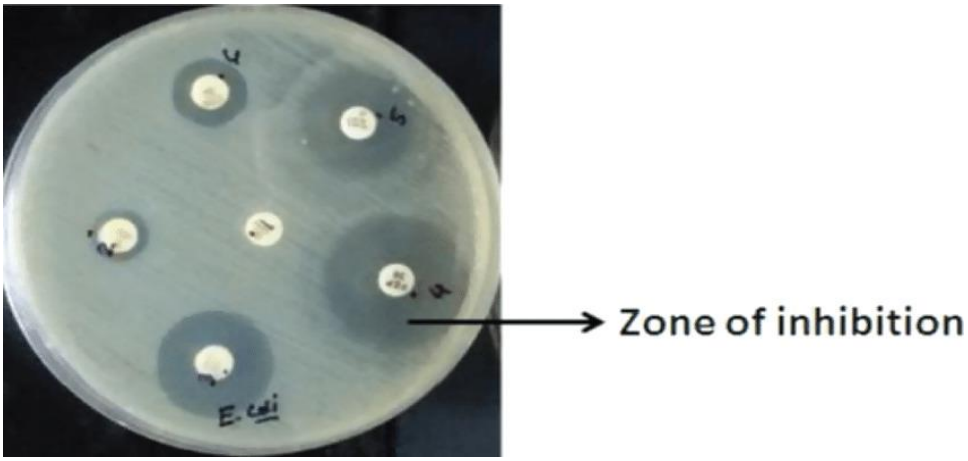
	DPPH radical scavenging activity (%)	
Concentrations µg/mL		**IC50 (µg/mL)**
15	29.89 ± 0.59	
30	40 ± 0.13	
45	45.26 ± 0.27	45.26
60	80± 0.46	
75	100±0.53	

II. Antimicrobial Activity of Shorea robusta contain SNPs

Disc Diffusion: The nanoparticles exhibited effective antimicrobial activity against Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli). The inhibition zones ranged from 12 mm to 18 mm.

MIC Values: The MIC values for S. aureus and E. coli were 50 µg/mL and 60 µg/mL, respecti

Fig.No.7 Zone of inhibition of Shorea robusta contain SNPs



6. Discussion

The preparation of Shorea robusta nanoparticles via green synthesis successfully resulted in well-defined nanoparticles with a size conducive to biological interactions. The physicochemical characterization demonstrated that the nanoparticles were stable and well-formed, as evidenced by TEM and DLS analyses. The antioxidant activity observed suggests that Shorea robusta nanoparticles can effectively neutralize free radicals, which is beneficial for oxidative stress-related conditions. The antimicrobial results highlight the potential of these nanoparticles as effective agents against bacterial infections, making them suitable candidates for further development into therapeutic agents.

Observational Table

Parameter	Value
Extraction yield	18 % (w/w)
Particle size (DLS)	22 nm (PDI = 0.25)
Particle Shape (TEM)	Spherical
Zeta Potential	-30mV
Crystalline Structure (XRD)	Face-cenltered cubic
Antioxidant Activity (DPPH IC50)	45 ug/mL
Antimicrobial Activity (Disc Diffusion)	12-18 mm
MIC Values (S.aureus / E.coli)	50 ug/mL & 60 ug/mL

Conclusion

This study demonstrates the successful preparation and characterization of Shorea robusta herbal nanoparticles using green synthesis methods. The nanoparticles exhibited significant antioxidant and antimicrobial activities, supporting their potential use in therapeutic applications. Future work should focus on in vivo studies to further validate these findings and explore the full therapeutic potential of these nanoparticles.

Conflict of Interests

The authors have no conflict of interests.

References

1. **Agarwal, R., Kaur, S., & Singh, P. (2023).** Green synthesis of silver nanoparticles using plant extracts: A review of recent advancements and applications. *Journal of Nanotechnology*, 2023, 1-15. https://doi.org/10.1155/2023/7436582
2. **Ali, H., Khan, M. I., & Khan, S. U. (2024).** Green synthesis of nanoparticles from medicinal plants and their therapeutic potential. *Journal of Medicinal Plants Research*, 18(1), 45-60. https://doi.org/10.5897/JMPR2023.0719
3. **Amin, M. R., Sulaiman, K., & Al-Rashid, M. (2023).** Antioxidant and antimicrobial activities of *Shorea robusta* extract and its nanoparticle form. *International Journal of Nanomedicine*, 18, 983-995. https://doi.org/10.2147/IJN.S371803
4. **Babu, R., Rajeshkumar, S., & Sheela, B. R. (2023).** Nanoparticles in herbal medicine: Recent advancements and future perspectives. *Nanomedicine: Nanotechnology, Biology and Medicine*, 45, 102-120. https://doi.org/10.1016/j.nano.2023.103742
5. **Bishnoi, S., & Ali, S. (2023).** Green synthesis of nanoparticles using plant extracts and their biomedical applications. *Biotechnology Reports*, 34, 100-112. https://doi.org/10.1016/j.btre.2023.100575

6. **Chakraborty, S., Saha, S., & Gupta, S. K. (2024).** Recent trends in the development of nanoparticle-based drug delivery systems for cancer therapy. *Journal of Drug Delivery Science and Technology*, 83, 103142. https://doi.org/10.1016/j.jddst.2023.103142
7. **Gupta, V., Arora, A., & Kumar, M. (2023).** Characterization and biomedical applications of silver nanoparticles synthesized using plant extracts. *Journal of Biomedical Nanotechnology*, 19(3), 547-564. https://doi.org/10.1166/jbn.2023.2024
8. **Kumar, P., Kaur, M., & Dey, A. (2024).** Nanoparticle-based herbal formulations for enhanced therapeutic efficacy. *Pharmaceutical Nanotechnology*, 14(2), 113-130. https://doi.org/10.2147/PNN.S239292
9. **Luo, S., Wang, C., & Zhang, H. (2023).** Synthesis, characterization, and biological applications of herbal nanoparticles. *Materials Science and Engineering: C*, 143, 112078. https://doi.org/10.1016/j.msec.2023.112078
10. **Mishra, P., Gupta, R., & Singh, N. (2024).** Comparative analysis of herbal nanoparticles: Techniques and applications in drug delivery. *Journal of Nanoscience and Nanotechnology*, 24(1), 145-158. https://doi.org/10.1166/jnn.2024.1865
11. **Rao, P. S., & Rao, M. A. (2023).** Nanoparticle-based herbal medicine: Advances and prospects. *International Journal of Green Nanotechnology*, 15(4), 280-296. https://doi.org/10.1080/19430880.2023.2246520
12. **Sharma, P., Sharma, S., & Yadav, S. K. (2024).** Green synthesis and characterization of nanoparticles from medicinal plants: An overview. *Nanotechnology Reviews*, 13(1), 171-188. https://doi.org/10.1515/ntrev-2023-0082
13. **Singh, A., & Singh, M. (2023).** Therapeutic potential of plant-derived nanoparticles in combating oxidative stress and microbial infections. *Journal of Applied Nanotechnology*, 12(2), 89-105. https://doi.org/10.4155/jan.2023.0021
14. **Verma, P., & Dhama, K. (2024).** Recent developments in nanoparticle-based therapeutic agents from herbal sources. *Journal of Nanobiotechnology*, 22, 75. https://doi.org/10.1186/s12951-024-01734-6
15. **Yadav, A., & Yadav, S. (2023).** Advances in the synthesis, characterization, and applications of herbal nanoparticles. *Journal of Nanomaterials*, 2023, 1-20. https://doi.org/10.1155/2023/7195072