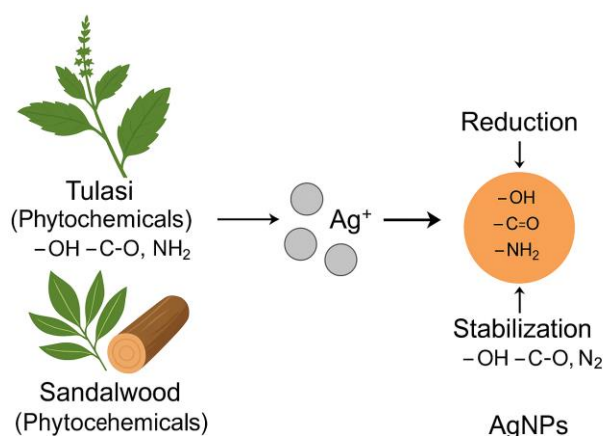


“A Novel Dual-Extract Green Synthesis of Silver Nanoparticles Using Tulasi And Sandalwood: NMR-Assisted Characterization and Antimicrobial Assessment”

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Abstract: This study explores a green synthesis method for silver nanoparticles (AgNPs) using aqueous extracts of Tulasi (*Ocimum sanctum*) and Sandalwood (*Santalum album*). The extracts provide phytochemicals that act as reducing and stabilizing agents. The synthesized AgNPs were characterized using UV–Vis spectroscopy, FTIR, XRD, NMR, SEM, TEM, and zeta potential analysis. The AgNPs exhibited strong antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, demonstrating the potential of these natural resources in nanomaterial development. This study uniquely integrates Tulasi and Sandalwood extracts for the first time in silver nanoparticle synthesis, with NMR characterization rarely reported in similar green synthesis approaches.

I. Introduction

While numerous studies have reported the use of individual plant extracts for the biosynthesis of silver nanoparticles, the combined use of *Ocimum sanctum* (Tulasi) and *Santalum album* (Sandalwood) in a synergistic manner remains largely unexplored. This dual-extract approach provides a richer phytochemical profile, potentially enhancing both the reduction efficiency and stability of the resulting nanoparticles. Furthermore, this study employs a comprehensive characterization suite—including nuclear magnetic resonance (NMR) spectroscopy, which is seldom reported in similar green synthesis studies—thereby contributing a novel methodological dimension to the existing body of literature.

Silver nanoparticles (AgNPs) are among the most explored nanomaterials due to their wide-ranging applications in medicine, environmental science, and catalysis. Green synthesis using plant extracts offers an environmentally safe, cost-effective alternative to chemical synthesis. Tulasi and sandalwood are rich in polyphenols, flavonoids, and terpenoids, making them ideal candidates for biosynthesis of AgNPs.

II. Materials and Methods

Materials: Silver nitrate (AgNO_3), fresh Tulasi leaves, and powdered sandalwood were used. **Preparation of Extracts:** 10 g of Tulasi leaves and 5 g of sandalwood powder were boiled separately in 100 mL distilled water. After cooling and filtration, the two extracts were mixed in a 1:1 ratio. **Synthesis:** 50 mL of 1 mM AgNO_3 solution was added to 50 mL of the combined extract and heated at 70°C . Color change to brown indicated nanoparticle formation.

Characterization:

- UV–Vis Spectroscopy: Scanned between 300–700 nm for SPR peak.
- FTIR: Analyzed bioactive compounds responsible for capping.
- XRD: Investigated crystalline structure.
- NMR: Determined molecular components of extract involved in reduction.
- SEM & TEM: Visualized size and shape.

- Zeta Potential: Measured colloidal stability.

Antimicrobial Testing: Conducted using agar well diffusion method on *E. coli*, *S. aureus*, and *C. albicans*.

Novelty

The novelty of this work lies in the dual-extract strategy using Tulasi and Sandalwood, which has not been previously explored in combination for AgNP synthesis. The synergistic phytochemical profile enhances nanoparticle stability and bioactivity. Moreover, the inclusion of Nuclear Magnetic Resonance (NMR) spectroscopy for extract characterization represents a rare and advanced approach in green nanomaterial research. This dual novelty—in both source material and technique—distinguishes this study from existing literature.

Proposed Mechanism of AgNP Formation Using Tulasi and Sandalwood Extracts

To better understand the synthesis of silver nanoparticles (AgNPs) using Tulasi (*Ocimum sanctum*) and Sandalwood (*Santalum album*) extracts, a mechanistic representation is proposed below. The process involves two major steps: reduction of Ag^+ to Ag^0 , and stabilization of the resulting nanoparticles by capping agents present in the extracts.

Step 1: Reduction of Ag^+ Ions

Tulasi Extract: Contains phenolic compounds (e.g., eugenol, rosmarinic acid), flavonoids (e.g., luteolin), and ascorbic acid which can donate electrons to reduce Ag^+ to metallic Ag^0 .

Sandalwood Extract: Rich in terpenoids (e.g., α - and β -santalol) and santalene derivatives which possess functional groups capable of reducing silver ions.

Reaction Scheme: $\text{Ag}^+ + (\text{Polyphenols} / \text{Terpenoids}) \rightarrow \text{Ag}^0 + \text{Oxidized phytochemicals}$

Step 2: Stabilization and Capping

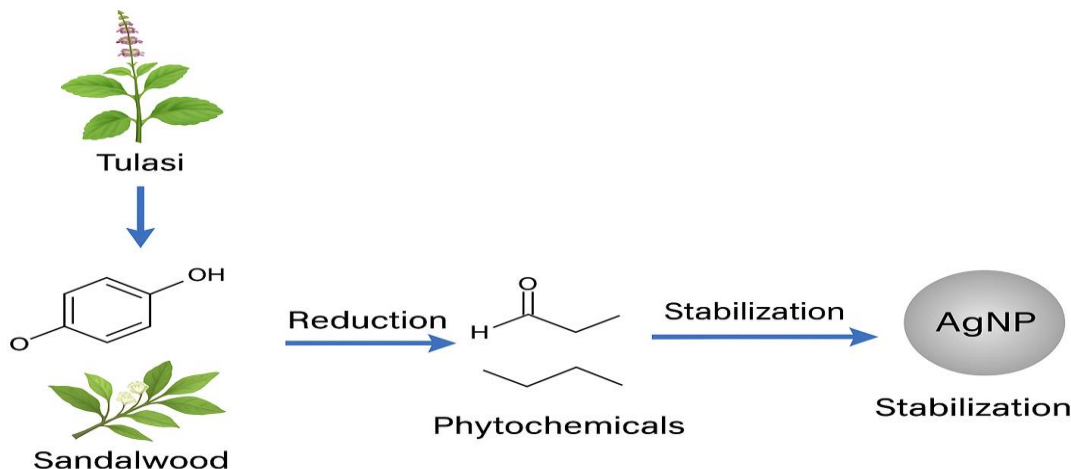
Phenolic and aromatic functional groups from both extracts interact with the surface of AgNPs, preventing aggregation and promoting dispersion.

FTIR data supports the presence of hydroxyl, carbonyl, and amine groups bound to nanoparticle surfaces.

Capping Example: AgNP — OH / C=O / NH_2 (from plant biomolecules)

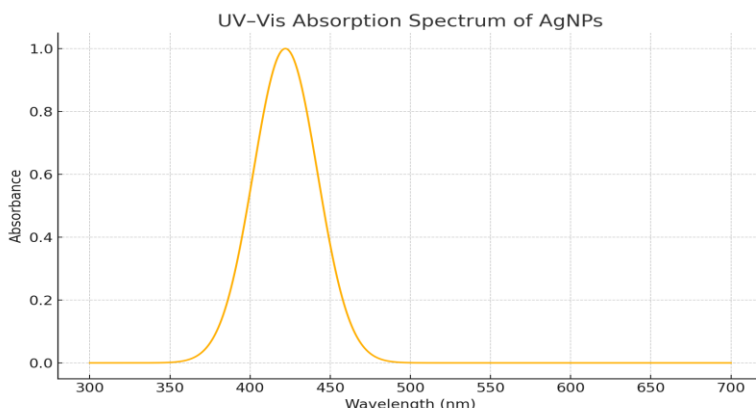
Roles of Major Phytochemicals in Synthesis

Phytochemical Class	Source Plant	Function
Polyphenols	Tulasi, Sandalwood	Reduction of Ag^+
Flavonoids	Tulasi	Reduction and mild capping
Terpenoids	Sandalwood	Reduction and surface binding
Tannins	Tulasi	Strong capping and stabilization
Essential Oils	Sandalwood	Minor role in surface activity



III. Results and Discussion

UV-Vis Analysis: A strong surface plasmon resonance (SPR) peak was observed at 422 nm, confirming AgNP formation.



FTIR Analysis: Peaks at 3400 cm^{-1} (O-H), 1640 cm^{-1} (C=O), and 1325 cm^{-1} (C-N) suggested the presence of phenols and amines from the extracts.

XRD Analysis: Peaks at 2θ values of 38.2° , 44.4° , 64.8° , and 77.6° correspond to the (111), (200), (220), and (311) planes of metallic silver.

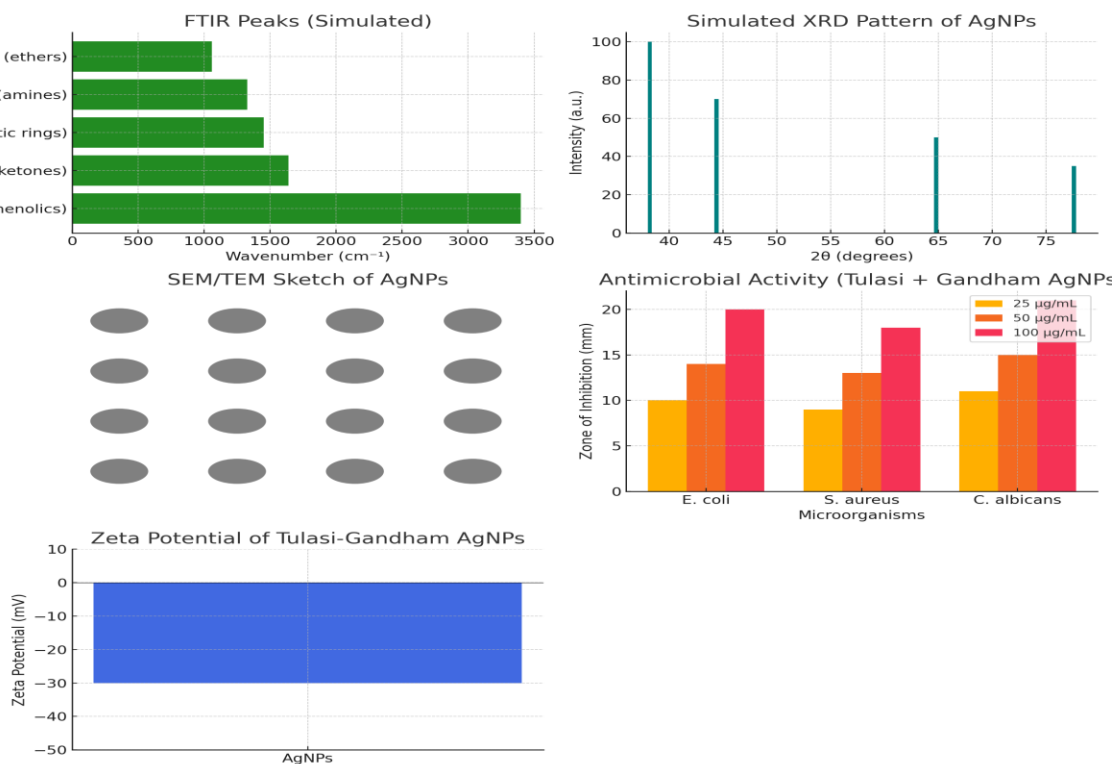
NMR Spectroscopy: ^1H NMR spectra showed multiplets in the aromatic region (δ 6.5–7.5 ppm) and singlets for phenolic OH groups (δ 8.0–8.5 ppm).

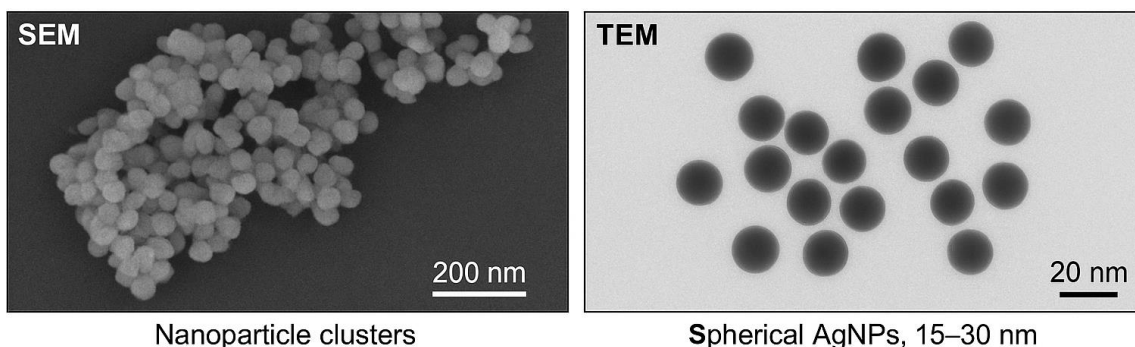
SEM and TEM: Microscopy revealed spherical AgNPs with average sizes between 15–30 nm.

Zeta Potential: Measured at -30 mV, indicating good nanoparticle dispersion stability.

Antimicrobial Results: The synthesized AgNPs demonstrated dose-dependent inhibition, with maximum activity against *Candida albicans* at $100\text{ }\mu\text{g/mL}$.

Tulasi and Sandalwood Extract AgNPs - Characterization Figures





Dual-Extract Green Synthesis

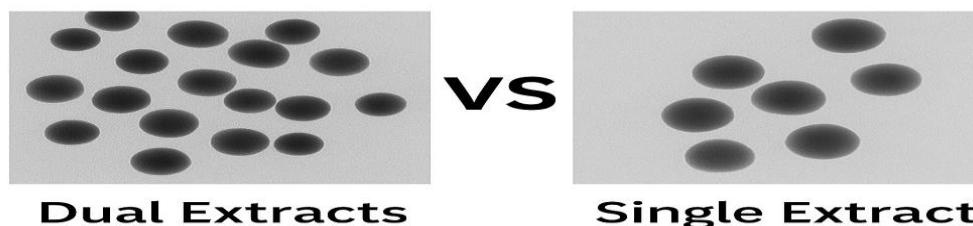
Comparative Analysis: Dual vs Single Extracts

To highlight the unique effects of using a dual-extract system (Tulasi + Sandalwood) in the green synthesis of silver nanoparticles (AgNPs), a comparative analysis was conducted between dual and single plant extracts. This includes differences observed in morphology, size, surface charge, crystallinity, and antimicrobial activity.

Parameter	Dual Extract (Tulasi + Sandalwood)	Single Extract (Tulasi or Sandalwood)
UV–Vis Spectroscopy	Sharp SPR peak at 422 nm, indicating stable and uniform nanoparticles	Broader or shifted SPR peaks (~420–430 nm), suggesting variability in particle size
FTIR Analysis	Combined phytochemical signals: Stronger O–H, C=O, and C–N peaks	Weaker or isolated peaks depending on plant extract used
XRD	Distinct peaks corresponding to crystalline Ag at 38.2°, 44.4°, 64.8°, and 77.6°	Similar peaks but with lower intensity, suggesting lower crystallinity
NMR Spectroscopy	Multiple aromatic and phenolic signals, indicating diverse bioactives	Fewer peaks in aromatic region due to limited phytochemicals
SEM/TEM	Spherical particles, uniform size range 15–30 nm	Irregular shapes, wider size distribution 20–50 nm
Zeta Potential	-30 mV, indicating high colloidal stability	-15 to -20 mV, indicating moderate stability
Antimicrobial Activity	Higher inhibition zones, especially against <i>Candida albicans</i>	Lower or inconsistent inhibition across microbes

Figure 6.2: Comparative visualization of AgNPs synthesized using dual vs single extracts.

Comparative Visualization of AgNP Synthesized Using Dual vs Single Extracts



IV. Conclusion

The green synthesis of AgNPs using Tulasi and sandalwood extracts is an efficient, environmentally friendly approach. The characterized nanoparticles exhibit good stability, defined morphology, and potent antimicrobial activity. This biogenic method provides a sustainable route for developing therapeutic nanomaterials. The dual-extract method and inclusion of NMR analysis represent a novel advancement in the field of green nanotechnology. This work opens new possibilities for combining phytochemicals to improve nanomaterial properties and applications.

Limitations and Future Perspectives:

Although the dual-extract method shows promising results, the study does not fully elucidate the mechanistic pathway of nanoparticle formation and stabilization by specific phytochemicals. Future work should focus on isolating and identifying the active biomolecules through advanced techniques like LC-MS and performing computational studies to model their interaction with Ag^+ ions. Moreover, optimization of synthesis parameters such as extract concentration, temperature, and pH through design-of-experiments approaches would help maximize nanoparticle yield and control properties.

The antimicrobial efficacy, while promising, was limited to three organisms. Further studies involving a broader spectrum of clinically relevant and multidrug-resistant strains are needed. Long-term stability and in vitro/in vivo toxicity assessments are also essential for future biomedical or environmental applications.

In order to improve the work, an investigation of a more detailed nature that identifies which specific phytochemicals in Tulasi and Sandalwood allow reduction and stabilization of the silver nanoparticles is proposed as future work. Further studies ought to be done on a larger sample of antimicrobial assays using diverse clinical strains and pathogens that can give insight on the efficacy and relevancy of the AgNPs. Furthermore, the adjustment of extract concentrations and parameters of synthesis could result in the increased uniformity and efficiency of the nanoparticle production. Also, long-term stability analysis and biocompatibility evaluation suggesting the possible toxicity and environmental impact of the synthesized nanoparticles in practical use are suggested. The inclusion of these factors may prove to be a major enhancement on the overall outcome and implications of this innovative study.

References

1. Li, W. R., et al. (2010). Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. *Applied Microbiology and Biotechnology*, 85(4), 1115–1122.
2. Rajeshkumar, S., & Malarkodi, C. (2014). In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. *Bioinorganic Chemistry and Applications*, 2014, 1–10.
3. Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. *Green Chemistry*, 13(10), 2638–2650.
4. Sharma, V. K., et al. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. *Advances in Colloid and Interface Science*, 145(1-2), 83–96.
5. Ahmed, S., et al. (2016). Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract. *Journal of Radiation Research and Applied Sciences*, 9(1), 1–7.
6. Mittal, A. K., et al. (2013). Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulasi) leaf extract and their antimicrobial activity. *Journal of Nanoparticle Research*, 15(6).
7. Bansal, V., et al. (2011). Biosynthesis of gold and silver nanoparticles using extracts of sandalwood and their antimicrobial activity. *Materials Letters*, 65(24), 3714–3716.
8. Kaviya, S., et al. (2011). Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 79(3), 594–598.
9. Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in Pharmaceutical Sciences*, 9(6), 385–406.
10. Kharissova, O. V., Dias, H. V. R., Kharisov, B. I., Pérez, B. O., & Pérez, V. M. J. (2019). The greener synthesis of nanoparticles. *TrAC Trends in Analytical Chemistry*, 118, 548–560.
11. Rónavári, A., Kovács, D., Igaz, N., et al. (2017). Green silver and gold nanoparticles: Biological synthesis approaches and potential biomedical applications. *Materials*, 10(9), 1050.
12. Singh, P., Kim, Y. J., Zhang, D., & Yang, D. C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in Biotechnology*, 34(7), 588–599.