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BIOGENIC SYNTHESIS AND EVALUATION OF SILVER NANOPARTCLES CONTAINING EUPHORBIA TITHYMALOIDES EXTRACT

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ABSTRACT
This study aimed to develop a green and sustainable method for synthesizing silver nanoparticles (AgNPs) using the leaf extract of *Euphorbia tithymaloides* and to evaluate their structural and antimicrobial properties. The plant's rich phytochemical profile, including flavonoids, terpenoids, alkaloids, and phenolic compounds, acted as natural reducing and stabilizing agents, facilitating an eco-friendly synthesis process. Leveraging the plant's traditional medicinal applications in wound healing, inflammation, and skin infections, this approach integrates ethnobotanical knowledge with modern nanotechnology to produce bioactive nanoparticles. Characterization of the synthesized AgNPs was performed using UV-Visible spectroscopy for confirmation of formation, Fourier-transform infrared spectroscopy (FTIR) to identify functional groups involved in reduction and stabilization, and Scanning Electron Microscopy (SEM) to assess particle size, shape, and distribution. The antimicrobial efficacy of the biosynthesized AgNPs was tested against various bacterial and fungal strains using agar well diffusion assays, demonstrating concentration-dependent inhibition zones. The results highlight the potential of *Euphorbia tithymaloides*-mediated AgNPs as effective, eco-friendly antimicrobial agents, providing a promising strategy to address microbial resistance while promoting sustainable nanotechnology.

KEYWORDS: Silver nanoparticles (AgNPs), Euphorbia tithymaloides, Green synthesis, Phytochemicals, Antimicrobial activity, Nanotechnology, Ethnobotany.

1.1 Novel Drug Delivery System (NDDS)
NDDS are advanced drug delivery methods designed to overcome limitations of conventional systems. They improve the efficacy of herbal and conventional medicines, ensure targeted delivery, reduce side effects, and enhance bioavailability. Integration of modern delivery technologies with traditional Ayurvedic medicines can optimize therapeutic outcomes. NDDS include systems like nanoparticles, liposomes, solid lipid nanoparticles, microemulsions, and matrix systems, allowing site-specific, controlled, and timely drug release (Saraf, 2010; Musthaba et al., 2009).

1.2 Novel Drug Delivery Approaches
Modern drug delivery strategies focus on.

- **Reducing drug degradation and loss**
- **Minimizing side effects**
- **Increasing bioavailability**

Drug carriers include polymers, microparticles, liposomes, micelles, and cells. Carriers can be designed for controlled degradation, stimuli-responsiveness (pH, temperature), and targeted delivery using ligand-receptor interactions. Drug release mechanisms include adsorption/desorption, diffusion, erosion, or a combination. Targeting can be **passive** (e.g., tumor accumulation via enhanced permeability) or **active** (ligand-mediated receptor targeting) (Sharma et al., 2019; Lai et al., 2022).

1.3 Nanoparticles
Nanoparticles (1–100 nm) are used to enhance drug delivery. They can be **nanocapsules** (drug enclosed in a polymer shell) or **nanospheres** (drug dispersed in a matrix). Biodegradable polymers, often PEG-coated, allow prolonged circulation, targeted delivery, and use in gene/protein therapy. Nanomedicine also includes nanofibers, carbon nanotubes, DNA-based nanostructures, and nanomachines (Anselmo & Mitragotri, 2016; Nikam et al., 2014).

Classification
1. **Organic nanoparticles:** Biodegradable and non-toxic (e.g., liposomes, micelles, dendrimers, ferritin), suitable for targeted drug delivery.

2. **Inorganic nanoparticles:** Metal-based (Ag, Au, Fe, Cu) and metal oxides (TiO_2, ZnO, SiO_2) with unique electrical, optical, and catalytic properties.
3. **Carbon-based nanoparticles:** Graphene, CNTs, fullerenes, and activated carbon, used in electronics, energy storage, and biomedical applications.

Preparation Methods

- **Polymer dispersion** (solvent evaporation, solvent diffusion)
- **Polymerization** (monomer polymerization in aqueous media)
- **Coacervation/Ionic gelation** (using chitosan, gelatin, alginate)
- **Other methods:** Supercritical fluids, particle replication, microemulsion, microwave-assisted, and green synthesis (Reverchon & Adami, 2013; Famili & Kompella, 2012).

Nanoparticle Types

- **Silver:** Antimicrobial, water treatment, cosmetics
- **Gold:** Diagnostic tracers, cancer detection
- **Alloy:** Enhanced properties compared to single metals
- **Magnetic:** Targeted therapy, MRI, hyperthermia (Rai et al., 2009; Giljohann et al., 2020; Huynh et al., 2020)

1.4 Silver Nanoparticles (AgNPs)

AgNPs have high electrical/optical conductivity, chemical stability, and catalytic activity. They are widely used in biomedical applications: antibacterial, antiviral, antifungal, anti-angiogenic, antitumor, biosensing, and bioimaging. AgNPs can also exhibit cytotoxicity depending on size, distribution, and cell type (Yu et al., 2013; Gurunathan et al., 2015).

Synthesis

- **Top-down:** Bulk material is broken into nanoparticles via grinding, milling, laser ablation.
- **Bottom-up:** Atoms self-assemble chemically or biologically (green synthesis).
- **Physical methods:** Evaporation-condensation, laser ablation
- **Microemulsion and microwave-assisted methods** provide control over size, shape, and homogeneity (Ahmed et al., 2016; Zhao et al., 2014).

Applications

- **Antimicrobial**: Effective against bacteria, viruses, fungi
- **Antioxidant**: Scavenges free radicals
- **Antidiabetic**: Reduces carbohydrate-hydrolyzing enzyme activity
- **Ag-based inks**: Flexible electronics, conductive printing (Abou et al., 2010; Chiolerio et al., 2012).

PLANT PROFILE

Figure 1: *Euphorbiatithymaloides.*

Scientific Classification

- **Kingdom:** Plantae
- **Order:** Malpighiales
- **Family:** Euphorbiaceae
- **Genus:** *Euphorbia*
- **Species:** *Euphorbiatithymaloides*

MATERIALS AND METHODS

This study involved the biosynthesis of silver nanoparticles (AgNPs) using *Euphorbia tithymaloides* leaf extract and the evaluation of their physicochemical and antimicrobial properties.

Reagents, Chemicals, and Instruments

Essential chemicals such as silver nitrate, solvents (petroleum ether, methanol, ethanol), acids, bases, and other reagents were used. Analytical and laboratory instruments included UV-Vis spectrophotometer, SEM, zeta sizer, magnetic stirrer, hot air oven, and lyophilizer, ensuring precise measurements, characterization, and visualization of nanoparticles.

Plant Material Collection and Extraction

Leaves of *Euphorbia tithymaloides* were shade-dried, powdered, and subjected to sequential Soxhlet extraction using petroleum ether and methanol to obtain non-polar and polar compounds. The extract was concentrated, dried, and stored for further analysis. Organoleptic studies and solubility testing were conducted to assess quality and solvent compatibility.

Phytochemical Screening

Qualitative tests confirmed the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins, phenolics, saponins, triterpenoids, and steroids, highlighting the plant's rich bioactive profile.

Biosynthesis of Silver Nanoparticles

A 1 mM $AgNO_3$ solution was prepared and mixed with different concentrations of plant extract (12.5–100 mg/mL). Formation of AgNPs was indicated by a color change from pale yellow to brown.

Characterization of AgNPs

- **Visual Observation:** Monitored color changes over time to confirm Ag^+ reduction.
- **UV-Vis Spectroscopy:** Detected surface Plasmon resonance (SPR) peaks to assess nanoparticle formation, size, and stability.
- **SEM:** Examined surface morphology, shape, and uniformity.
- **Zeta Potential:** Evaluated particle surface charge and colloidal stability.
- **Particle Size Analysis:** Determined average size and distribution via dynamic light scattering.

Stability Studies

Formulations were stored under accelerated conditions (25°C ± 2°C, 60% RH; 40°C ± 2°C, 70% RH) for three months, with periodic assessment of color, appearance, and particle size following ICH guidelines.

Antimicrobial Activity: The agar well diffusion method was employed against *Escherichia coli* and *Staphylococcus aureus*. Zones of inhibition were measured post-incubation (37°C, 18–24 h) to evaluate the antibacterial potential of AgNPs, silver nitrate, and plant extracts.

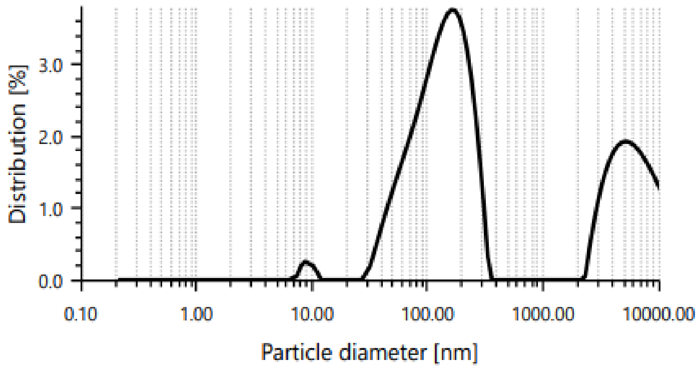
RESULTS

Table 1: Phytochemical testing of extract.

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff's test	Undetected	Detected
1.2	Mayer's reagent test	Undetected	Detected
1.3	Wagner's reagent test	Undetected	Detected
1.3	Hager's reagent test	Undetected	Detected
2.	Glycoside		
2.1	Borntrager test	Undetected	Detected
2.2	Killer-Killiani test	Undetected	Detected
3.	**Carbohydrates**		
3.1	Molish's test	Undetected	Detected
3.2	Fehling's test	Undetected	Detected
3.3	Benedict's test	Undetected	Detected
3.4	Barfoed's test	Undetected	Detected
3.5	Iodine Test	Undetected	Detected
4.	**Flavonoids**		
4.1	Shinoda Test	Detected	Detected
5.	**Tannin and Phenolic Compounds**		
5.1	Ferric Chloride test	Detected	Undetected
5.2	Lead Acetate Test	Detected	Undetected
5.3	Gelatin Test	Detected	Undetected
6.	**Saponin**		
6.1	Foam test	Undetected	Detected
6.2	Froth Test	Undetected	Detected
7.	**Test for Triterpenoids and Steroids**		
7.1	Salkowski's test	Detected	Detected
7.2	Libbermann-Burchard's test	Detected	Detected

The synthesized SNPs showed the following absorption spectrum at the wavelength range of 400-800 nm. The surface Plasmon resonance peak at range 300 to 800 nm was confirmed the formation of silver nanoparticle as shown in above Figure UV analysis of silver nanoparticle. Surface Plasmon resonance at 682.0 nm (F5) was represented best nanoparticle synthesis. Analysis was help to identify the time of nanoparticle synthesis initiation and progressive increase in intensity of peak was help to ascertain the extent of nanoparticles formed.

Particle size



Result

Hydrodynamic diameter	330.6 nm	**Mean intensity**	96.6 kcounts/s
Polydispersity index	23.4 %	**Absolute intensity**	96.6 kcounts/s
Diffusion coefficient	1.5 µm²/s	**Intercept g1²**	0.8954
Transmittance	88.1 %	**Baseline**	1.989

Figure 2: Particle size (F1).

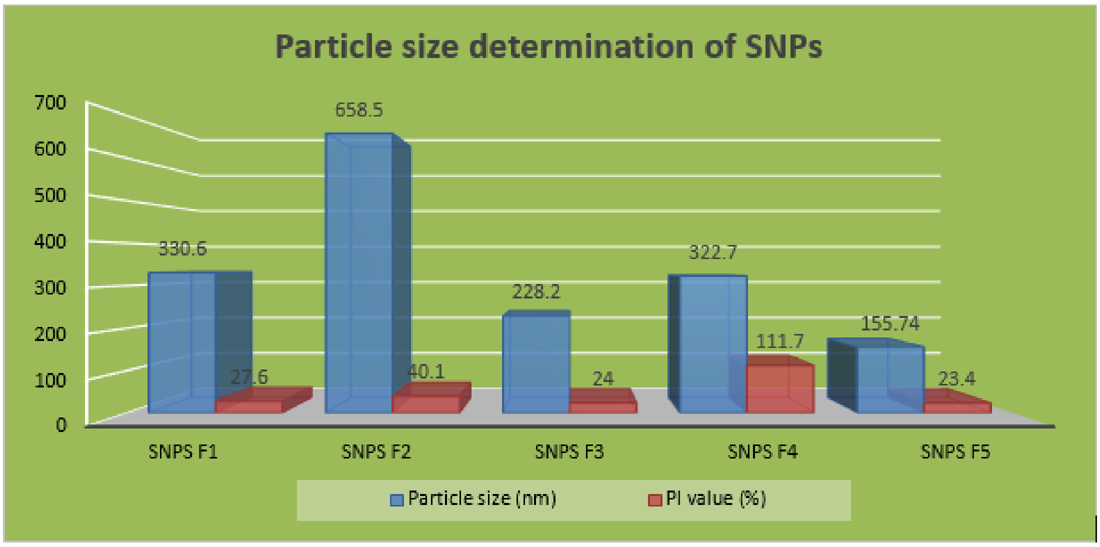


Figure 3: Graphical representation of Particle size determination of SNPs.

SEM analysis

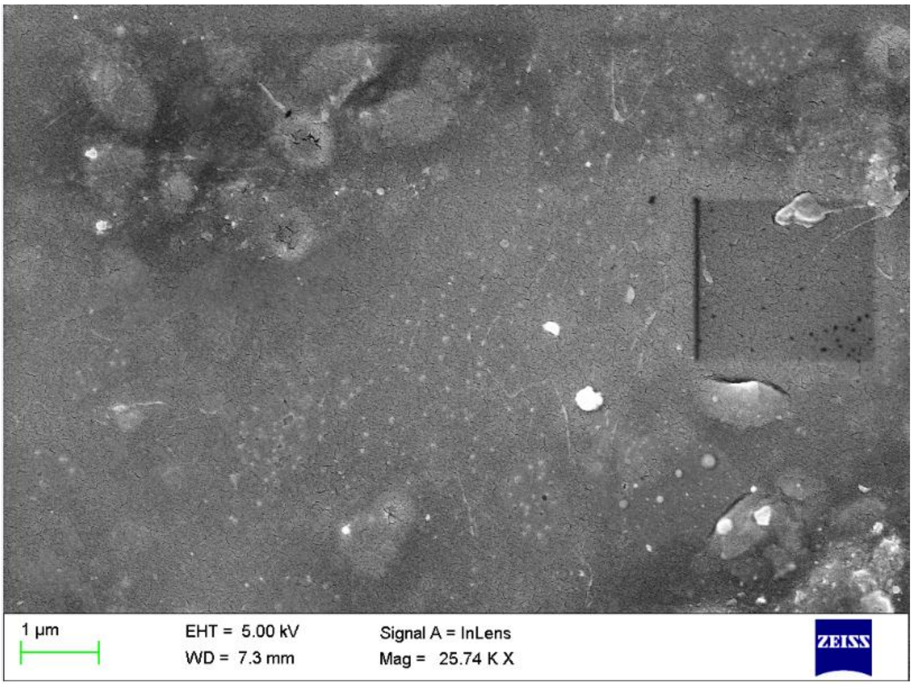


Figure 4: SEM.

This SEM image, taken at a magnification of 25.74KX, reveals the surface morphology of the sample at the microscale. The image shows a relatively smooth surface with some dispersed particles, pores, and agglomerates, which could indicate the presence of surface roughness, impurities, or phase separation. A few white spots seen may correspond to denser particles or possible contaminants, as they reflect more electrons. The scale bar of 1 µm indicates that the features observed are in the nanometer to micrometer range.

Antimicrobial activity of Ag Nanoparticle

Table 2: Antimicrobial activity of Ag Nanoparticle *against E.coli and S. aureus.*

S. No.	Sample Name (mg/ml)	Zone of Inhibition (mm) of *E. coli*	Zone of Inhibition (mm) of*S. aureus*
1.	(control)	0mm	0mm
2.	AgNO3 (1mg/ml)	7 mm	6 mm
3.	Extract (1mg/ml)	4 mm	5 mm
4.	Silver NPs (1ml)	2 mm	3 mm

Figure 5: Photograph showing zone of inhibition of planr extract mediated synthesis of AgNPs against A: *Staphylococcus aureus*, B: *Escherichia coli.*

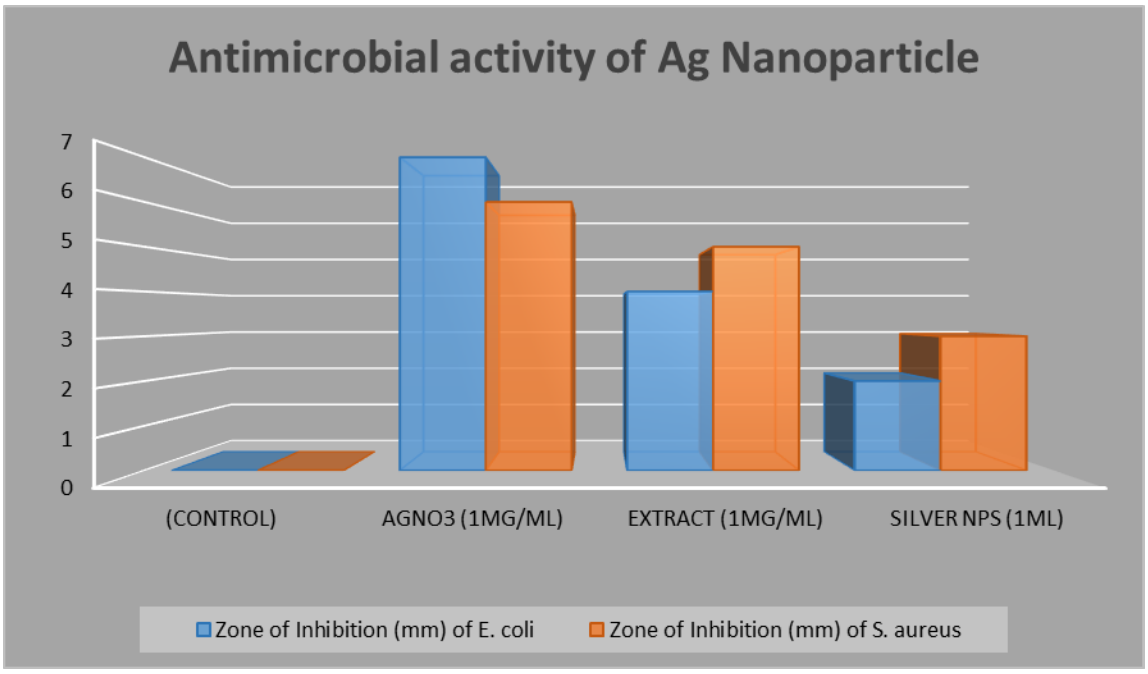


Figure 6: Antimicrobial activity of Ag Nanoparticle against *E. coli* and *S. aureus*.

SUMMARY AND CONCLUSION

This study focused on the green synthesis of silver nanoparticles (AgNPs) using *Euphorbia tithymaloides* leaf extract and the evaluation of their physicochemical characteristics and antimicrobial properties. A total of 300 grams of leaves were used for extraction, and the methanolic extract showed a higher yield (2.63%) compared to petroleum ether (0.79%), confirming methanol's efficiency in extracting polar bioactive compounds such as alkaloids, glycosides, flavonoids, and saponins.

Organoleptic analysis revealed that the extract possessed a dark green color, characteristic herbal odor, and a clear, slightly viscous appearance, supporting the presence of phytochemicals. Solubility tests further confirmed the extract's strong affinity for polar solvents like methanol, DMSO, and acetone. Phytochemical screening demonstrated a rich presence of medicinally important compounds in the methanolic extract, affirming its potential for nanoparticle synthesis.

Among the five formulations prepared, Formulation F5 was identified as the most stable and efficient. It exhibited the smallest particle size (155.74 nm) and a low polydispersity index (23.4%), indicating a uniform distribution. Furthermore, F5 showed the most negative zeta potential (-27.8 mV), suggesting excellent electrostatic stability and low risk of aggregation. UV–Vis spectrophotometric analysis confirmed nanoparticle formation with a surface plasmon resonance peak at 682.0 nm.

The stability study conducted over 90 days under both ambient and accelerated conditions showed no significant change in particle size, color, or appearance, confirming the long-term stability of F5. However, the antimicrobial assay revealed only mild antibacterial activity of the synthesized AgNPs against *E. coli* and *S. aureus* (2 mm and 3 mm zones of inhibition, respectively), which was less than that observed with silver nitrate or the crude extract alone.

In conclusion, the study successfully demonstrated that *Euphorbia tithymaloides* extract can be used for the biogenic synthesis of stable silver nanoparticles. While F5 emerged as the most promising formulation in terms of physical stability and nanoparticle characteristics, its antimicrobial activity was modest at the tested concentration. These findings suggest potential for further optimization possibly through concentration adjustments or synergistic formulation—for improved antimicrobial applications in nanomedicine.

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