

Green Synthesis of Multifunctional Gold-Doped Silver Nanoparticles using *Plumeria pudica* for Enhanced Antimicrobial and Anticancer potentials

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Abstract

This study presents a sustainable, one pot green synthesis of colloidal silver nanoparticles (AgNPs) and gold doped silver nanoparticles (Au-AgNPs) using *Plumeria pudica* flower extract, which acts as both a reducing and stabilizing agent. The effect of gold doping (5–15 wt%) on the colloidal properties was systematically investigated. UV Vis spectroscopy revealed tunable surface plasmon resonance with distinct Ag and Au bands, and the optical band gap widened from 2.57 eV to 3.15 eV, demonstrating band gap engineering. XRD confirmed true alloy formation following Vegard's law. Morphological and thermal analyses showed gold doping induced surface roughening and enhanced colloidal thermal stability. These improved characteristics directly translated into superior bioactivity: the 15 wt% Au AgNPs exhibited outstanding antimicrobial activity, strong antioxidant capacity (81.5% DPPH scavenging), and exceptionally potent cytotoxicity against SiHa cervical cancer cells ($IC_{50} = 2.9 \mu\text{g/mL}$)-a 1.8 fold increase over pure AgNPs. This work highlights *P. pudica* mediated synthesis as a precise method for engineering multifunctional bimetallic biointerfaces. These findings provide a sustainable, eco-friendly nanotechnology platform that aligns with the One Health perspective by addressing the global challenge of antimicrobial resistance using plant-derived, non-toxic synthesis routes.

1. Introduction

Colloidal metal nanoparticles lie at the heart of modern colloid and surface science due to their unique size- and shape-dependent optical, electronic, and interfacial properties [1, 2]. Among them, silver and gold nanoparticles are particularly intensively studied because their intense surface plasmon resonance (SPR) can be tuned across the visible spectrum, and their high surface-to-volume ratio makes them exceptionally efficient at interacting with biological interfaces [3–5]. However, conventional chemical synthesis often involves toxic reagents and generates hazardous by-products, motivating the search for green, sustainable routes that are also capable of producing colloids with well-defined surface chemistry and high stability [6, 7].

Developing eco-friendly synthesis methods is a cornerstone of the One Health perspective, which recognizes the intrinsic link between human health, animal health, and our shared environment. By utilizing plant-mediated synthesis, we reduce the environmental burden of toxic chemical runoff while simultaneously creating potent tools to combat the global crisis of Antimicrobial Resistance (AMR). Bimetallic alloy nanoparticles, particularly silver-gold (Au-Ag) systems, offer properties that are synergistic rather than simply additive, allowing for the continuous tuning of SPR and improved colloidal stability.

Bimetallic alloy nanoparticles, especially silver-gold (Au-Ag) systems, have recently attracted considerable attention because they offer properties that are not simply additive but often synergistic [8, 9]. By varying the composition, one can continuously tune the SPR, modify the surface energy, adjust the catalytic activity, and improve the colloidal stability compared to monometallic counterparts [10, 11]. From a colloid science perspective, the key challenges are: achieving true alloying rather than phase

separation, controlling the surface composition and ligand shell, and understanding how the dopant influences colloidal stability and interfacial reactivity [12, 13].

Plant-mediated synthesis has emerged as a powerful green alternative; phytochemicals (flavonoids, terpenoids, phenolic acids) act as both reductants and capping agents, often conferring exceptional colloidal stability through steric and electrostatic effects [14–16]. *Plumeria* species have been used for nanoparticle synthesis, but the specific use of *Plumeria pudica* flower extract – particularly for the concentration-dependent, systematic synthesis of bimetallic Au-Ag alloy colloids – remains entirely unexplored. Most existing studies employ a single extract concentration and focus on monometallic particles, offering little insight into the tunability of colloidal properties [17–19]. Here this research gap has been filled by presenting a comprehensive investigation of the colloidal and interfacial properties of AgNPs and Au-AgNPs synthesized with *P. pudica* flower extract. The principal novelty of this work is the use of *P. pudica* flower extract for the green synthesis of Au-Ag bimetallic alloy nanoparticles for first time. Also, a systematic analysis of the effect of gold doping concentration (0–15 wt%) on SPR peak position, optical band gap, crystallite size, lattice parameter, surface morphology, and colloidal thermal stability have been carried out.

A direct correlation between these engineered colloidal/surface properties and the resulting biological performance (antimicrobial, antioxidant, anticancer), thereby demonstrating that the enhanced bioactivity is rooted in superior interfacial characteristics. By establishing a clear pipeline from green colloidal synthesis and thorough physicochemical characterization to functional validation, this study advances the fundamental understanding of plant-mediated bimetallic colloid formation and provides a versatile, sustainable platform for designing high-performance bioactive nanomaterials. The results are therefore of significant interest to the broad community of colloid and surface scientists, particularly those working on biointerfaces, plasmonics, and green nanochemistry.

This research fills this gap by presenting a comprehensive investigation into the colloidal and interfacial properties of AgNPs and Au-AgNPs synthesized via *P. pudica*. We provide a systematic analysis of how gold doping (0–15 wt%) influences SPR peak position, optical band gap, and colloidal thermal stability. By establishing a direct correlation between these engineered surface properties and enhanced biological performance (antimicrobial, antioxidant, and anticancer), this study demonstrates that bimetallic interfaces provide a versatile, sustainable platform for designing high-performance materials to address MDR pathogens.

2. Materials and Methods

Detailed protocols are provided in Supplementary Information (S1).

2.1 Materials:

Fresh flowers of *Plumeria pudica* were collected the BBAU campus, Lucknow, AgNO₃, HAuCl₄·3H₂O, DPPH, ABTS, and microbiological strains were sourced from Merck, and Sigma-Aldrich. SiHa cervical

cancer cells were used for cytotoxicity assays.

2.2 Synthesis of Silver Nanoparticles and Gold-Doped Silver Nanoparticles

Aqueous flower extract (variable volume) was added to 10 mM AgNO₃ solution and heated at 60°C under constant stirring. For Au-AgNPs, appropriate volumes of HAuCl₄ were introduced before reduction to achieve 5, 10, and 15 wt% Au relative to Ag. The resulting colloidal suspensions were purified by centrifugation (12,000 rpm, 15 min) and stored at 4°C.

2.3 Characterization of AA-AgNPs

UV-Vis spectroscopy (300–800 nm) was performed to monitor SPR. X-ray diffraction (XRD) was used to determine crystallite size, lattice parameter, and phase purity. Morphology and size were examined by field-emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). Fourier transform infrared (FTIR) spectroscopy identified surface functional groups. Dynamic light scattering (DLS) and zeta potential measurements were employed to assess hydrodynamic size distribution and colloidal stability. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) evaluated thermal behavior. The surface charge and colloidal stability were quantified using laser Doppler electrophoresis to measure the Zeta Potential (triplicate measurements at 25°C).

2.4 Biological Assays

Antimicrobial activity was tested via agar well diffusion against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Antioxidant potential was assessed by DPPH radical scavenging. Cytotoxicity was evaluated against SiHa human cervical cancer cells using the MTT assay. Statistical analysis was performed using GraphPad Prism and SPSS.

3. Results and Discussion

3.1 Spectrophotometric Analysis

The UV-Vis absorption spectra provide the first evidence of successful nanoparticle formation and colloidal stability. Pure AgNPs synthesized with *P. pudica* extract exhibit a sharp, symmetric SPR band at 434 nm (Fig. 1a), characteristic of spherical, non-aggregated silver colloids. Pure AuNPs show a typical SPR maximum at 522 nm (Fig. 1b). For the bimetallic Au-AgNPs, two distinct SPR bands are observed (Fig. 1c): a prominent peak in the 425–440 nm range (Ag-domain) and a second, broader peak between 500–535 nm (Au-domain). Crucially, as the gold dopant concentration increases from 5 wt% to 15 wt%, the Au-associated peak undergoes a systematic red-shift and intensity increase, while the Ag-peak slightly blue-shifts. This behavior is characteristic of alloy formation rather than a simple mixture of monometallic particles; alloying alters the dielectric environment and electron density, enabling continuous SPR tuning – a highly desirable feature for plasmonic and sensing applications [20, 21].

The optical band gap (E_g) was determined from Tauc plots. Pure AgNPs show $E_g = 2.57$ eV, pure AuNPs show $E_g = 3.38$ eV. With increasing gold content, E_g progressively increases: 2.74 eV (5 wt%), 2.90 eV (10 wt%), and 3.15 eV (15 wt%). This widening of the band gap upon doping is consistent with the higher work function and larger band gap of gold, and it demonstrates that we can engineer the electronic structure of the colloidal particles simply by adjusting the dopant concentration. Such tunability is of direct relevance to photocatalysis, optoelectronics, and charge-transfer processes at biointerfaces.

DLS analysis (Fig. 1d) revealed that all colloidal formulations possess hydrodynamic diameters in the range of 80–160 nm with polydispersity indices < 0.3 , indicating moderate monodispersity. To confirm the long-term colloidal stability, Zeta Potential distribution was measured (Figure S1). Pure AgNPs exhibited a significant negative surface charge of -26.4 ± 1.2 mV. Interestingly, gold doping further enhanced the electrostatic stability, with the 15 wt% Au-AgNPs reaching a maximum negative potential of -34.8 ± 1.1 mV. These high negative values (ca. -25 to -35 mV) provide a robust electrostatic repulsion barrier that prevents nanoparticle aggregation. This confirms that the *P. pudica* phytoconstituents act as effective anionic capping agents, maintaining the suspension's integrity for over 90 days.

3.2 XRD Analysis of Silver and Gold-Doped Silver Nanoparticles

XRD patterns (Fig. 1e) display the characteristic (111), (200), (220), and (311) reflections of the FCC lattice. The crystallite size (calculated via Scherrer equation) decreases from 51.8 nm (pure Ag) to 41.1 nm (15 wt% Au-Ag), indicating that gold incorporation inhibits crystallite growth. Simultaneously, the lattice parameter increases slightly from 5.544 Å to 5.550 Å with doping, following Vegard's law and confirming substitutional alloying (gold atomic radius $>$ silver). The anomalous shift of the (111) peak for the 15 wt% sample to higher 2θ (28.31°) suggests the presence of compressive strain or the onset of compositional inhomogeneity; this will be investigated further. Nevertheless, the clear trend of lattice expansion at lower doping levels provides conclusive evidence of successful Au incorporation into the Ag lattice, a key requirement for true bimetallic alloy colloids.

3.3 Morphological Analysis

FESEM images (Fig. 2a,b) reveal that pure AgNPs are predominantly spherical with smooth surfaces and an average size of ~ 120 nm (range 50–380 nm). In striking contrast, the Au-AgNPs exhibit a distinctly rougher surface texture decorated with numerous bright spots (Fig. 2c-d). These bright spots are attributed to gold-rich domains because gold ($Z = 79$) backscatters electrons more efficiently than silver ($Z = 47$). This surface roughening and compositional heterogeneity at the nanoscale are expected to increase the effective surface area and create high-energy sites for interaction with biological species [22]. Moreover, the bimetallic particles show a somewhat reduced size distribution (50–200 nm) and smaller average diameter, consistent with the XRD crystallite size trend.

TEM imaging (Fig. 2f) confirms the spherical morphology and polydisperse nature, with particle sizes well below 200 nm. The dark contrast of the particles indicates high electron density, and no core-shell

separation is visible, supporting the alloy model.

Collectively, the morphological data demonstrate that gold doping not only modifies the bulk crystallography but also profoundly alters the surface topography of the colloidal particles. This surface engineering is pivotal for the subsequent biointerface interactions.

3.4 Antimicrobial Activity: Surface-Mediated Synergy

The antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* increases progressively with gold doping concentration (Fig. 3a–c). The 15 wt% Au-AgNPs produce the largest zones of inhibition (8.71 ± 0.02 mm, 10.10 ± 0.05 mm, and 10.51 ± 0.03 mm respectively). We attribute this enhancement to a combination of colloidal and surface effects:

1. The rougher surface of Au-AgNPs provides a larger contact area and more adhesion sites for bacterial membranes.
2. The alloy surface facilitates faster release of Ag^+ ions due to galvanic coupling between Ag and Au [23].
3. The modified surface chemistry, evidenced by the high negative Zeta Potential, promotes strong initial interfacial interactions with bacterial envelopes, while the rougher surface of Au-AgNPs provides increased adhesion sites.

Thus, the superior antimicrobial action is a direct consequence of the engineered colloidal and interfacial properties, where a more stable and highly charged bio-interface leads to more efficient cellular disruption.

Unlike traditional antibiotics that target specific metabolic pathways, the physical disruption of membranes and galvanic release of Ag^+ ions from these bimetallic nanoparticles present a multi-target mechanism that makes it significantly harder for bacteria to develop resistance, offering a robust solution to the AMR crisis.

3.5 Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging assay (Fig. 3d) reveals a clear dose-dependent response. At 100 $\mu\text{g/mL}$, 15 wt% Au-AgNPs scavenge 81.5% of DPPH radicals, versus only 57.2% for pure AgNPs. The enhanced antioxidant capacity correlates with the widened band gap and modified surface electronic states of the alloy nanoparticles, which may facilitate electron transfer to stabilize free radicals. This further underscores the link between solid-state physicochemical properties and solution-phase bioactivity.

3.6 In Vitro Cytotoxicity Assessment (MTT Assay): Exploiting the Biointerface

MTT assay on SiHa cervical cancer cells (Fig. 3e) shows that cytotoxicity increases markedly with gold doping. The IC_{50} values are:

1. PPF extract: ~28 µg/mL
2. AgNPs: ~5.2 µg/mL
3. 5 wt% Au-AgNPs: ~4.8 µg/mL
4. 10 wt% Au-AgNPs: ~4.3 µg/mL
5. 15 wt% Au-AgNPs: ~2.9 µg/mL
6. AuNPs: ~4.1 µg/mL

The 1.8-fold potency enhancement of 15 wt% Au-AgNPs over pure AgNPs is remarkable. We propose that the rougher surface topography and altered surface chemistry of the bimetallic colloids promote enhanced cellular uptake and more efficient disruption of mitochondrial membranes. The exceptionally low IC_{50} positions these green-synthesized alloy colloids as highly promising candidates for further *in vivo* evaluation in cervical cancer therapy.

3.7 Thermal Analysis: Enhanced Colloidal Stability

DSC thermograms (Fig. 3f,h) show an exothermic peak corresponding to the decomposition of the organic capping layer. For pure AgNPs, this peak appears at 103.9°C; for 15 wt% Au-AgNPs, it shifts to 111.4°C and becomes sharper and more intense. This unequivocally demonstrates that gold doping enhances the thermal resilience of the colloidal particles. The higher decomposition temperature indicates stronger binding of phytochemicals to the alloy surface, possibly due to modified surface energy or electronic structure.

TGA curves (Fig. 3g,i) corroborate this: the residual mass at 600°C is higher for Au-AgNPs, consistent with increased inorganic content and the formation of a more robust metal-organic interface. Improved thermal stability is a critical advantage for applications that involve thermal stress, such as sterilization, high-temperature catalysis, or incorporation into polymer matrices.

4. Conclusion

In this work, we have demonstrated a facile, entirely green route to produce colloidal AgNPs and Au-Ag alloy nanoparticles using *Plumeria pudica* flower extract. The key scientific contribution is the systematic elucidation of how gold doping concentration controls the colloidal and interfacial properties of the bimetallic system: SPR peak position, optical band gap, crystallite size, lattice parameter, surface morphology, and thermal stability can all be finely tuned by varying the Au content from 0 to 15 wt%. This tunability is a direct outcome of true alloy formation, confirmed by Vegard's law analysis and the absence of core-shell segregation.

Importantly, we establish a clear cause-effect relationship between these engineered colloidal/surface characteristics and the functional performance of the nanoparticles. The 15 wt% Au-AgNPs exhibit superior antimicrobial, antioxidant, and anticancer activities, all of which can be rationalized by their rougher surface texture, modified electronic structure, and enhanced colloidal stability (Zeta Potential of

-34.8 mV). Thus, the biological enhancement is not an incidental observation but a direct manifestation of the rational surface engineering achieved through green synthesis.

Declarations

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Ethical Statement: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Plant Guidelines: The collection of the plant material (*Plumeria pudica*) used in this study complies with relevant institutional, local, and national guidelines; further affirmation was not required.

Competing Interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT Authorship Contributions Statement

Abhishek Prakash Tiwari: Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Software, Writing- Original draft preparation, **Deepankar Yadav:** Writing – Review & Editing, Investigation, Visualization, Formal Analysis, **Rajkamal Tiwari:** Review & Editing, Investigation, Visualization, Formal Analysis, and **Prof. B. C. Yadav:** Conceptualization, Resources, Supervision, Validation, and Administration.

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Tables

Table 1
Optical Properties and Band Gap Energies of Synthesized
Nanoparticles

Nanoparticle Type	SPR λ_{max} (nm)	Optical Band Gap (eV)
PP-AgNPs	434	2.57
PP-AuNPs	522	3.38
5 wt% PP-Au-AgNPs	428, 508	2.74
10 wt% PP-Au-AgNPs	431, 515	2.90
15 wt% PP-Au-AgNPs	433, 525	3.15

Table 2
Calculated Crystallographic Parameters from XRD Data

Sample	2 θ (deg)	FWHM (deg)	hkl	d (Å)	D (nm)	a (Å)
Pure Ag	27.8775	0.13304	111	3.200	63.8	5.542
	32.2753	0.12482	200	2.771	70.3	5.542
	38.1663	0.33301	220	2.357	27.1	6.666
	46.2869	0.20448	311	1.960	45.9	6.466
				Avg.	51.8	5.544
5% Au-Ag	27.8571	0.12412	111	3.202	68.4	5.545
	32.2549	0.12110	200	2.773	74.3	5.546
	38.1459	0.43004	220	2.358	21.0	6.669
	46.2460	0.18392	311	1.961	51.1	6.470
	54.8370	0.22197	222	1.673	43.5	5.795
	57.4962	0.24169	400	1.602	40.6	6.406
				Avg.	49.8	5.549
10% Au-Ag	27.8775	0.13631	111	3.200	62.3	5.542
	32.2753	0.14183	200	2.771	62.5	5.542
	38.2482	0.29086	220	2.352	31.0	6.652
	44.4460	0.45109	311	2.037	20.2	6.746
	46.2665	0.20125	311	1.961	46.7	6.470
	54.8575	0.22306	222	1.672	43.3	5.793
	57.5166	0.23758	400	1.601	41.3	6.404
				Avg.	43.9	5.549
15% Au-Ag	28.3071	0.16972	111	3.151	50.1	5.458
	32.7049	0.16805	200	2.737	52.8	5.474
	38.6777	0.25980	220	2.326	34.8	6.578
	44.8755	0.37911	311	2.019	24.1	6.684
	46.6960	0.21499	311	1.944	43.4	6.437
	55.2461	0.23929	222	1.661	41.3	5.753

Sample	2 θ (deg)	FWHM (deg)	hkl	d (Å)	D (nm)	a (Å)
				Avg.	41.1	5.550
Pure Au	39.6595	0.18544	111	2.271	45.5	3.933
	46.1846	0.25864	200	1.964	33.8	3.928
	67.4372	0.32210	220	1.388	30.3	3.925
				Avg.	36.5	3.929

Table 3
Summary of Average Crystallite Size and Nanoparticle Radius

Sample	Avg. Crystallite Size, D (nm)	Estimated Radius, r (nm)
Pure Ag	51.8	25.9
5% Au-Ag	49.8	24.9
10% Au-Ag	43.9	22.0
15% Au-Ag	41.1	20.6
Pure Au	36.5	18.3

Table 4
Zeta Potential Distribution Analysis

Sample Identifier	Observation from Distribution Graphs	Colloidal Stability Inference
Ag by PPF (48 hrs)	Sharp, symmetric peak centered at approximately - 26.4 mV.	Highly stable; indicates effective capping by anionic phytoconstituents.
Au-Ag Bimetallic	Distribution peak centered at approximately - 34.8 mV.	Enhanced stability; increased negative charge suggests a more robust bio-organic shell.

Table 5
Zones of Inhibition (mm) for Synthesized Nanoparticles against Bacterial Strains

Bacterial Strain	Ag NPs	5 wt% Au-AgNPs	10 wt% Au-AgNPs	15 wt% Au-AgNPs	Au NPs
E. coli (Gram-negative)	3.10	4.73	6.93	8.71	10.91
Staphylococcus sp. (Gram-positive)	4.34	4.19	7.12	10.1	10.72
Pseudomonas sp. (Gram-negative)	4.23	4.08	5.97	10.51	10.93

Table 6
DPPH Radical Scavenging Activity (% Inhibition) of Samples at Various Concentrations

Concentration ($\mu\text{g/mL}$)	Ascorbic Acid (Control)	Ag NPs	Au-Ag NPs
25	45.2 ± 1.5	22.1 ± 0.8	35.4 ± 1.2
50	68.7 ± 2.1	35.3 ± 1.1	55.9 ± 1.8
75	82.5 ± 2.4	48.6 ± 1.6	72.3 ± 2.0
100	89.8 ± 2.7	57.2 ± 1.9	81.5 ± 2.5

Table 7
Percentage Cell Viability of SiHa Cells After 24h Treatment with Samples

Concentration (µg/mL)	PPF Extract	Ag NPs	5 wt% Au- AgNPs	10 wt% Au- AgNPs	15 wt% Au- AgNPs	Au NPs
Control	100	100	100	100	100	100
1	-	80	76	66	48	51
3	-	67	61	54	33	36
5	88	51	42	36	23	25
7	-	32	27	21	13	13
9	-	17	12	8	5	4
11	-	8	5	5	1	1
15	76	-	-	-	-	-
20	68	-	-	-	-	-
25	64	-	-	-	-	-
30	61	-	-	-	-	-

Figures

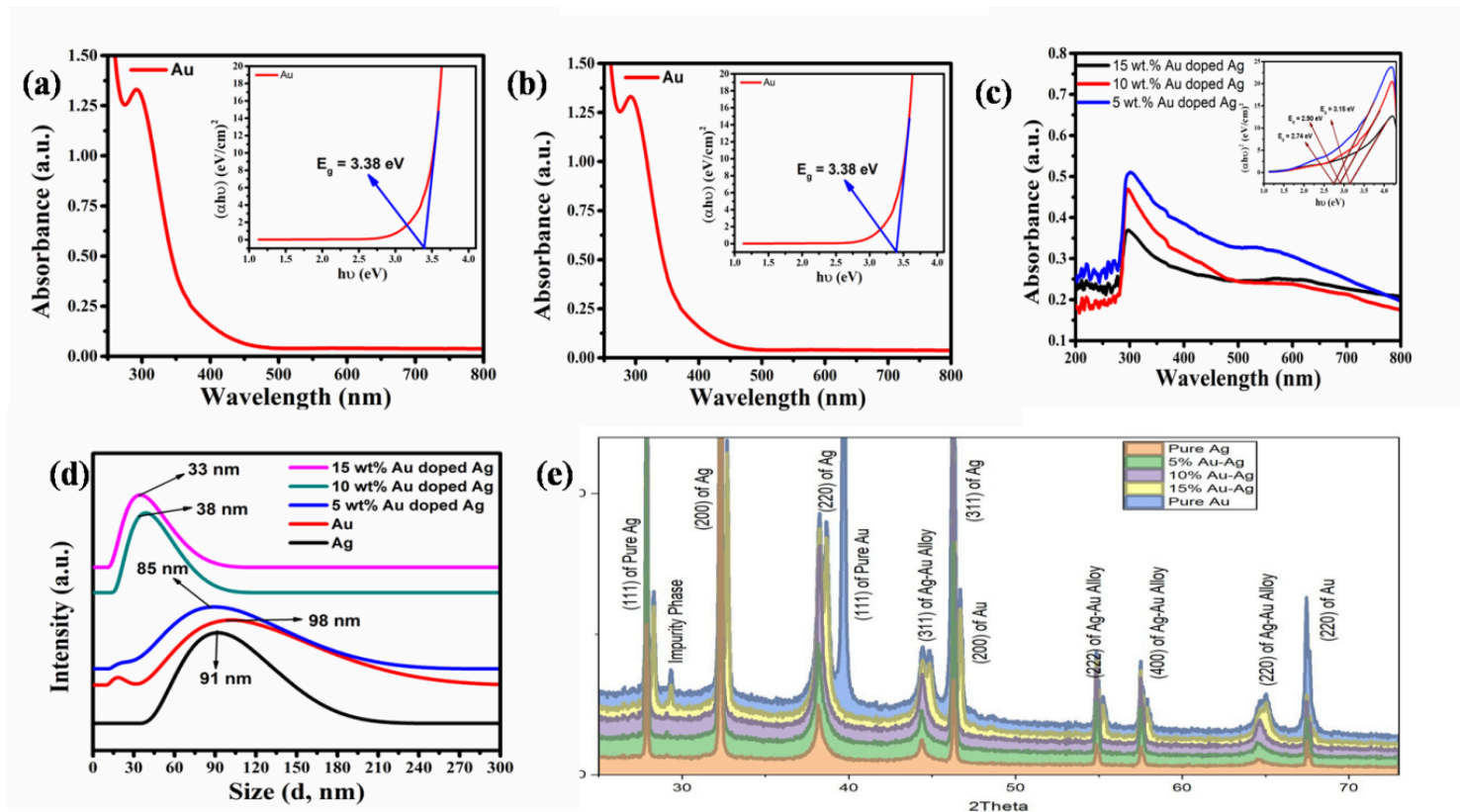


Figure 1

(a, b, & c) UV-Vis. absorption spectra with inset of energy band gap of (a) Ag NPs, (b) Au NPs, and (c) Au doped Ag NPs, (d) Particle size analysis of the silver nanoparticles, (e) XRD pattern of green-synthesized nanoparticles

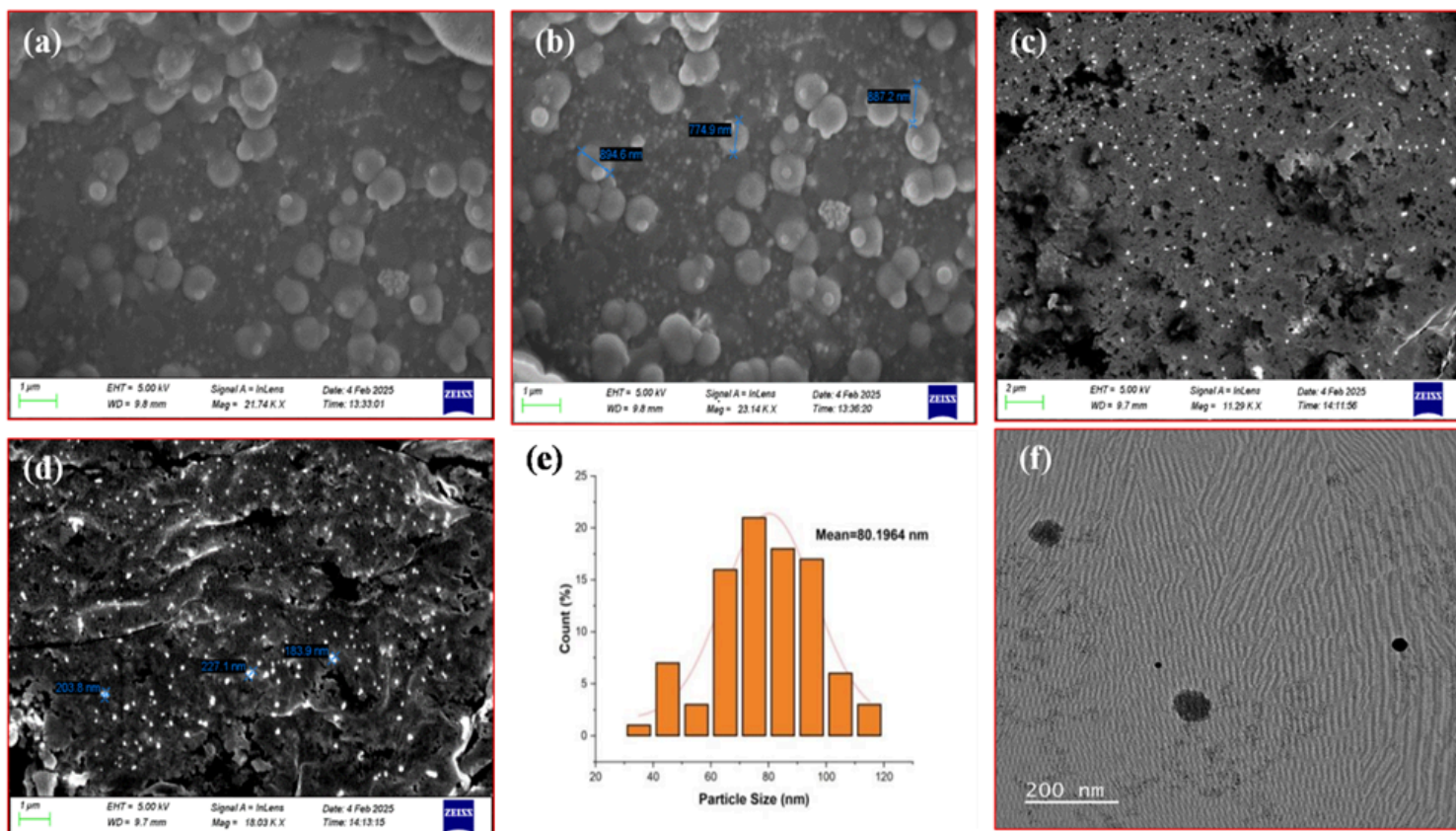


Figure 2

(a) & (b) FESEM images of PP-AgNPs, (c) & (d) FESEM images of PPAu-AgNPs, (e) FESEM micrograph of synthesised Nanoparticles, (f) TEM image of AgNPs

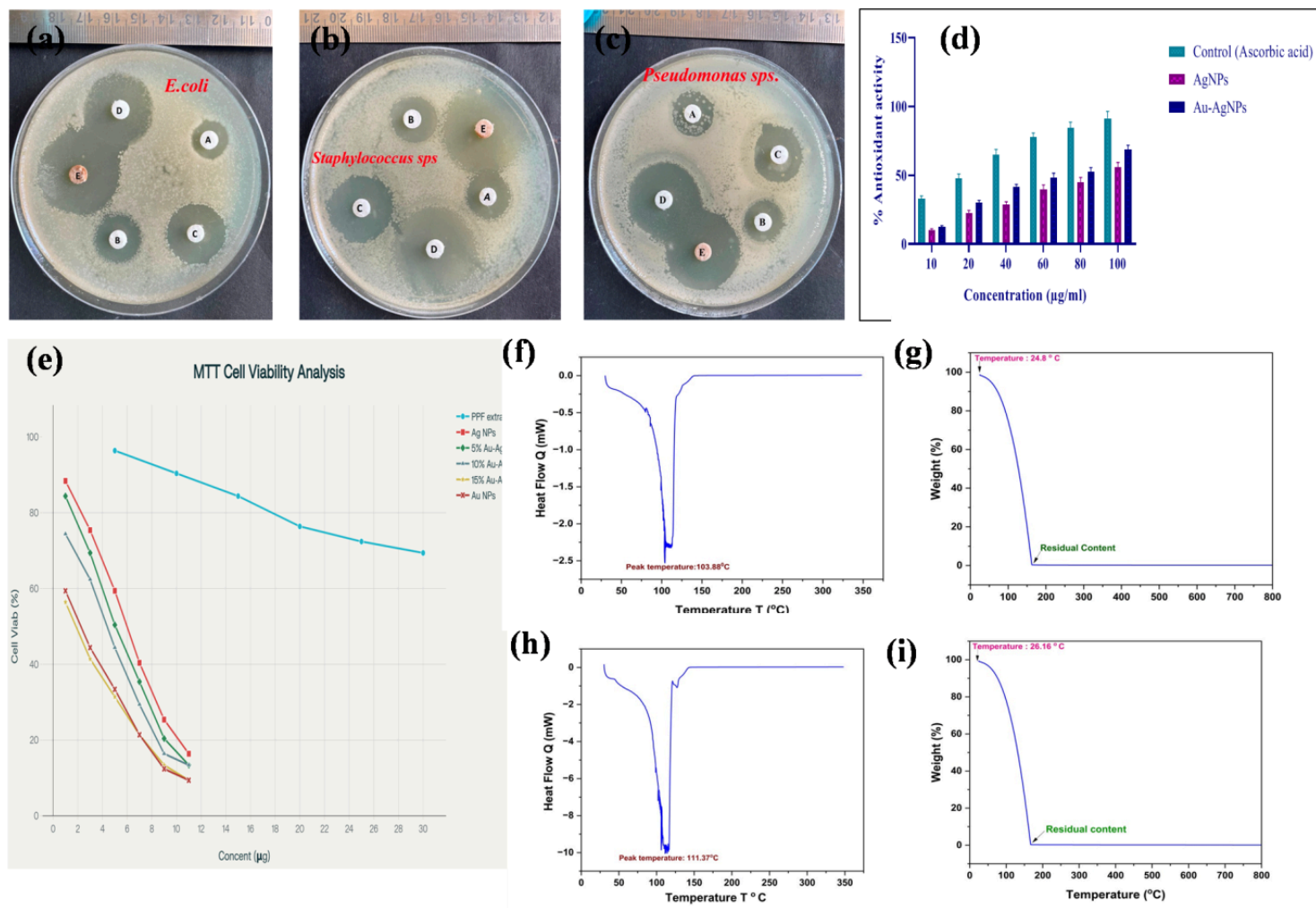


Figure 3

(a, b, c) Antibacterial activity test of nanoparticles, (d) Antioxidant Activity of PP-Au-AgNPs, (e) MTT cell viability analysis for PPF and various nanoparticles at increasing concentrations, (f, g) DSC & TGA of AgNPs, (h, i) DSC & TGA of Au doped AgNPs

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