SUSTAINABLE BIMETALLIC NANOPARTICLES: MAKEOVER AND BIOLOGICAL ASSET

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ABSTRACT:
The green synthesis of nanoparticles is one step toward the sustainable development. Green tea extract was used as a source for synthesizing bimetallic Au-Ag Nanoparticles. These bimetallic nanoparticles were characterized by UV spectroscopy and TEM examination, which revealed the particles have core and shell structures. Bimetallic NPs have shown promising antibacterial, antifungal and antioxidant activity.

Keywords: green synthesis, silver, and gold bimetallic nanoparticles.

INTRODUCTION:
A vast variety of metal nanoparticles (NPs) have been formed in the multidisciplinary branch of study known as nanotechnology. The synthesized NPs are distinct in size and have a higher surface area to volume ratio, which encourages their reactivity with the nearby molecules.[1] Metal nanoparticles can be prepared by two routes. The physical approach utilizes several methods such as evaporation/condensation and laser ablation while a chemical reduction of the metal ions in solution, which encourages the eventual development of tiny metal aggregates or clusters.[2] Conventionally, both physical and chemical procedures are employed to create them; however, these processes have a number of drawbacks, such as high costs, poisonous byproducts, temperature and pressure requirements and lengthy reactions, etc. Instead environmentally acceptable, nontoxic, and widely accessible plant sources are used in the green synthesis of NPs from metal salt solution. The phytochemicals from plants are responsible for formation and stabilization of metal nanoparticles from the metal salts.[3]
Gold (Au) and silver (Ag) are noble metals that have been extensively used in the biosynthesis of NPs and examined therapeutically. In this study, green tea (*Camellia Sinensis*) leaves were used to synthesize bimetallic NPs. Green Tea is famous for its phenolic contents and good antioxidant activity.[4] Consequently, these properties are enhanced in the form of Ag-Au

bimetallic NPs; where these bioactive components are locked while reducing and capping of NPs. During the present work, Ag-Au bimetallic NPs were synthesized by using a leaf extract of *Camellia sinensis*, and its antibacterial and antifungal activity was evaluated. Further produced Ag-Au Bimetallic NPs were also characterized by UV–visible, FTIR, TEM, and SEM.[5]

Materials and method:

Materials:
Green Tea leaves (*Camellia sinensis*) was obtained as a gift sample from Nisarg Herbaceutical, Mumbai. $AgNO_3$ and $HAuCl_4$ was purchased from Alpha Chemika, Mumbai.

Methodology

Preparation of Green Tea Extract:
Clean and dried leaves of *Camellia sinensis*, about 20 gm, were soaked in 300 ml distilled water and boiled. This was boiled till the extract was reduced tol 100 ml and eventually filtered. This filtered extract was used as a starting material for the metal nanoparticle synthesis.

Synthesis of Nanoparticles
A 1: 50 dilution was used for the green tea extract. 0.5 to 3 mM aqueous solution of noble metal salt solutions (silver nitrate or auric chloride) were prepared and used for the synthesis of nanoparticles. The reaction medium contained extract: metal salt solutions in 1.2:1 with further dilution with equal volume of deionised water. The reaction was stirred and heated at 70 $^{\circ}$C for 2.5 hours. The color changes from colorless to characteristic reddish brown/ reddish purple indicated the formation of silver nanoparticles.
A combination of Gold and Silver nanoparticles were synthesized as bimetallic nanoparticles. Gold and Silver nanoparticle solution was prepared individually and mixed in various ratios. These were subjected to characterization of bimetallic nanoparticles.

Biological Activity studies:

Antibacterial Assay of Au-Ag Bimetallic Nanoparticles
The synthesized Ag-Au Bimetallic NPs were evaluated for antibacterial activity; a well diffusion method was applied to screen pathogenic microbes. For the antibacterial assay, the gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and gram-negative bacteria *Escherichia. coli* (*E. coli*) were spread uniformly on nutrient agar plates. Ag-Au Bimetallic NPs solution was introduced in the wells under sterilized conditions and incubated at 37°C for 24 h. The zone of inhibition (mm) around well was measured.[6]

Antifungal Assay of Bimetallic Nanoparticles

The synthesized Ag-Au Bimetallic NPs were evaluated for antifungal activity; against *Candida albicans* using similar procedure as that Żarowska etal.[7]

Antioxidant Study using DPPH

The antioxidant activity was characterized utilizing DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) assay. A stock solution of DPPH in methanol was prepared. 1.25 mL of this stock solution was added to 3 mL of methanol. The mixture was shaken vigorously and allowed to stand at room temperature for 4 min. Then the absorbance was measured at 516 nm by using a UV-visible spectrophotometer.[8]

Results and discussion:

1) Characterization using Surface Plasmon Resonance (SPR):

The surface Plasmon Resonance Phenomenon was used to detect the formation of nanoparticles.[9] Surface plasmon resonance (SPR) is a phenomenon where the electrons in the metal surface layer are excited by photons of incident light with a certain angle of incidence, and then propagate parallel to the metal surface. Every nanoparticle has its unique resonance absorption wavelength.

The colour changes in aqueous solutions are due to the surface Plasmon Resonance phenomenon. SPR is the resonant oscillation of conduction electrons at the interface between negative and positive permittivity material stimulated by incident light. It is the basis of many standard tools for measuring the adsorption of material onto planar metal (typically gold or silver) surfaces or onto the surface of metal nanoparticles. It is the fundamental principle behind many colour-based biosensor applications, different lab-on-a-chip sensors and diatom photosynthesis.

SPR characterization of Au and Ag was done using UV spectrophotometer. UV absorbance of Au and Ag Nanoparticle was found to be 527 nm and 432 nm, respectively. The ideal range is 500-540 nm for Au and for 400-450 nm for Ag.

Fig no. 1: SPR of Au NP

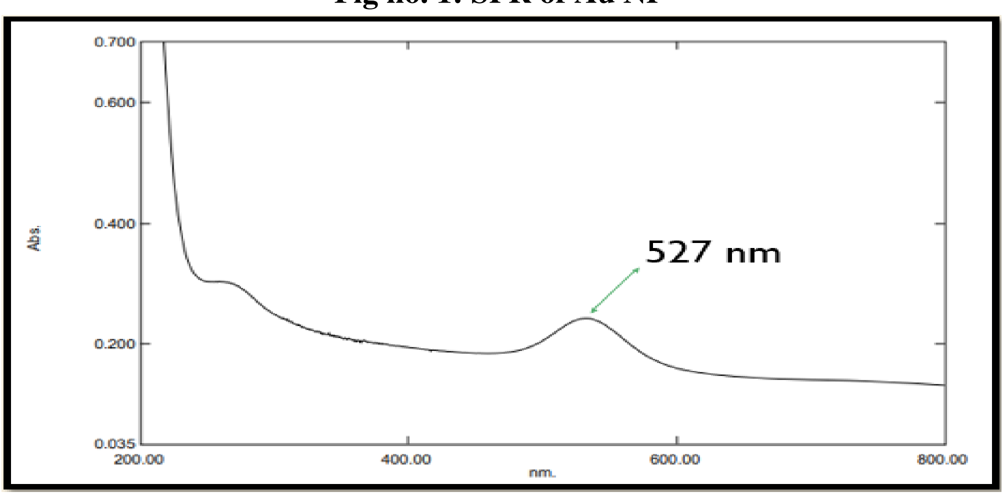
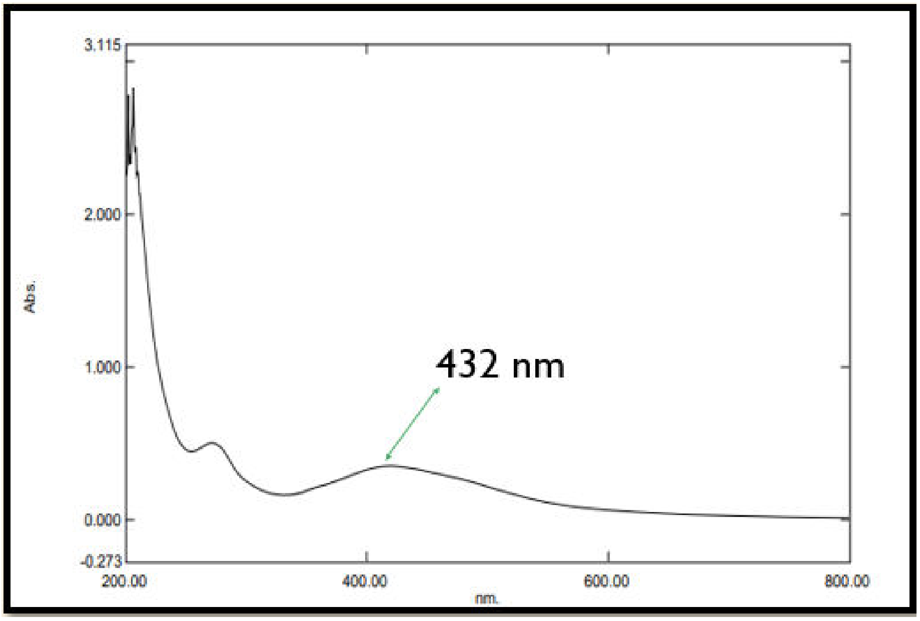


Fig no. 2: SPR of Ag NP



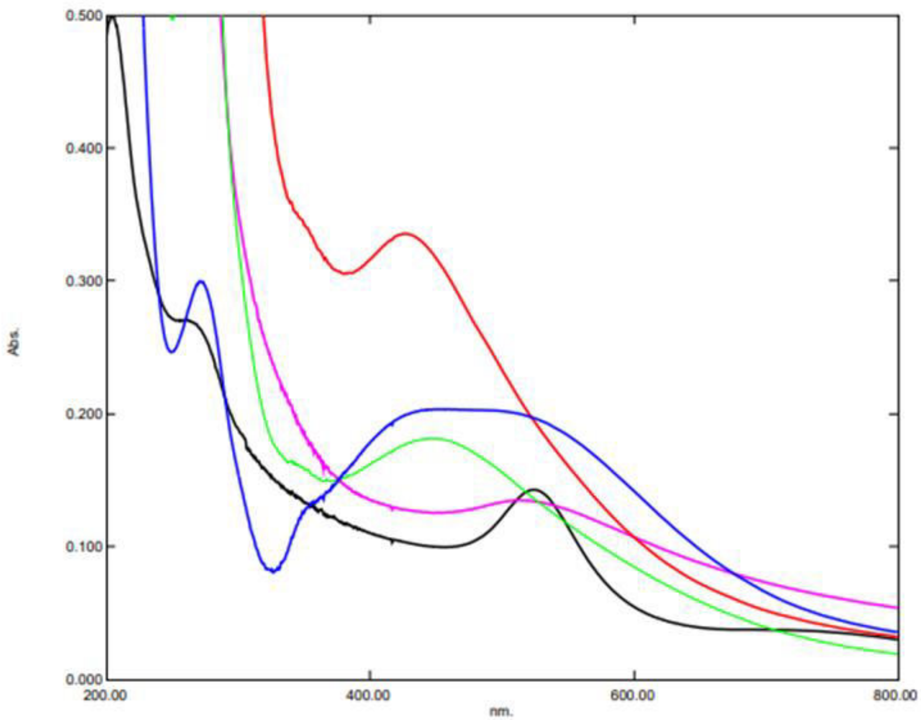
Synthesis of Bimetallic Nanoparticles

The bimetallic nanoparticle was formulated in different ratios of gold and silver. The gold and silver metallic salt solutions were mixed together in the above ratios. The concentration of the solutions was kept constant.

Table 1: Different Composition of Au and Ag

Sr. No	Color Code in graph	Au : Ag	λ max
A	Black	1:0	527.0 nm
B	Pink	3:1	515.0 nm
C	Blue	1:1	483.0 nm
D	Green	1:3	447.0 nm
E	Red	0:1	432.5 nm

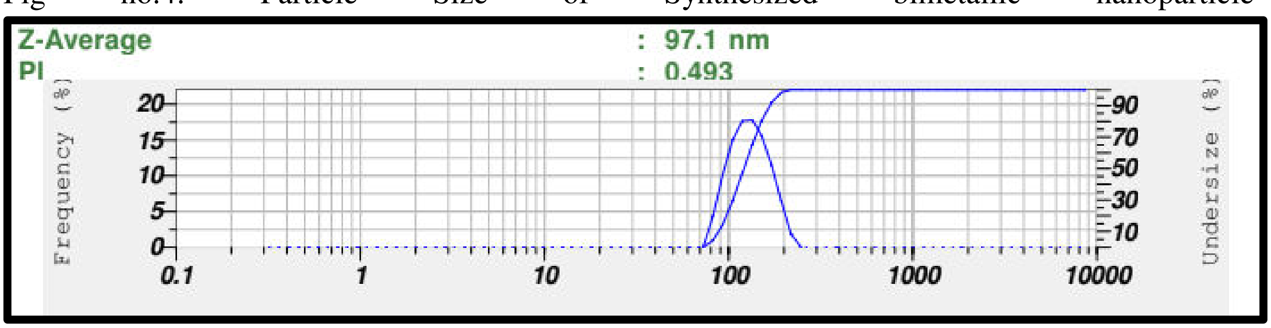
Fig no. 3: λ max of Synthesized Bimetallic Nanoparticles (A-E)



2) Particle Size Analysis and Polydispersity Index (PI) Data

The particle size and polydispersity index (PI) is a measure of the heterogeneity of a sample based on size. The average particle size was found to be 97.1 nm and PI 0.493 of bimetallic nanoparticle.

Fig no.4: Particle Size of Synthesized bimetallic nanoparticle

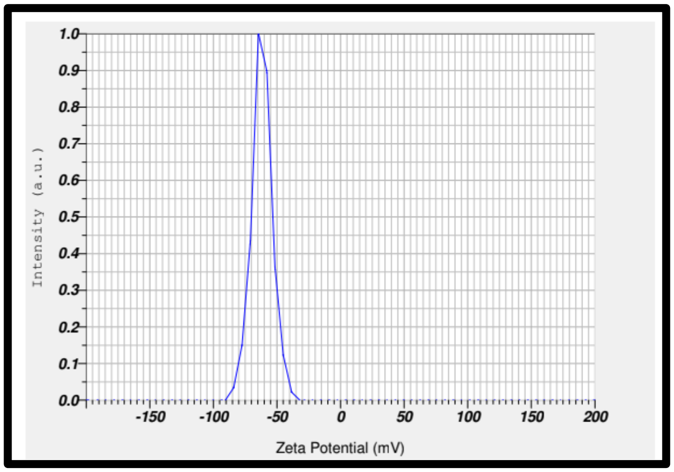


3) Zeta potential Data

The zeta potential of the Ag-Au bimetallic nanoparticles was -61.8 which signifies that the degree of electrostatic repulsion between adjacent similarly charged particles is high and there is no aggregation or flocculation. The high zeta potential indicates the stability of the Ag-Au bimetallic nanoparticles irrespective of the charge on it.

This suggests that the synthesized Ag-Au bimetallic nanoparticles are primarily made up of negative charge groups and are also accountable for the nanoparticle's modest stability. The negative zeta potential values of Au-Ag bimetallic nanoparticles formed using green tea leaves extract correspond to negatively charged plant phytochemicals capping the nanoparticles.

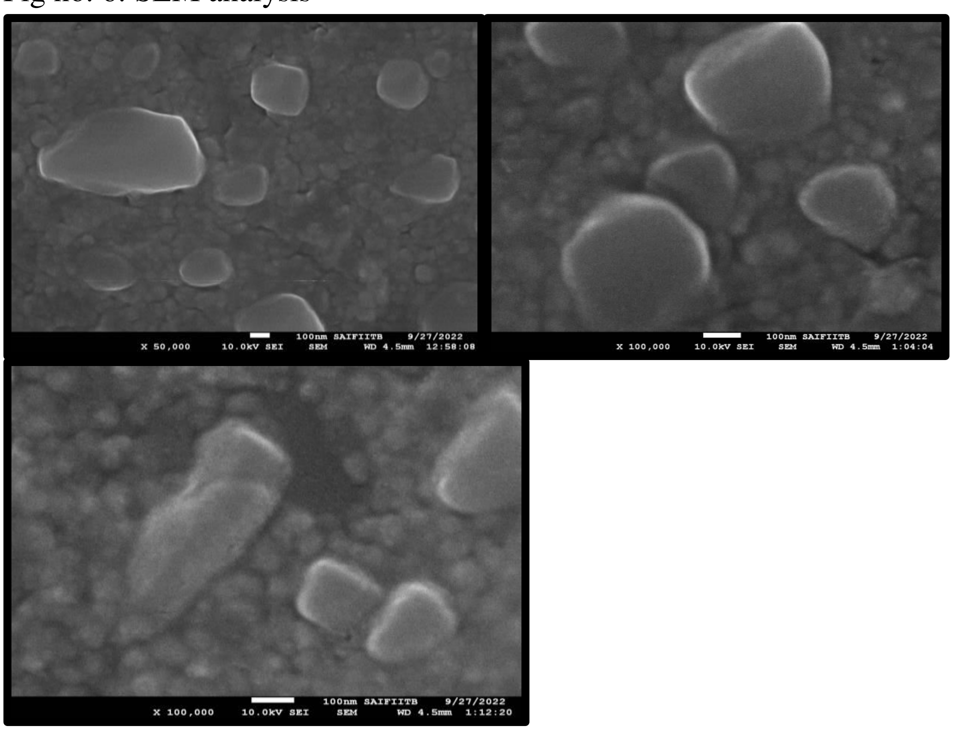
Fig no. 5: Zeta potential of Synthesized bimetallic nanoparticle



Zeta potential revealed -61.8 mV for formulated bimetallic NP.

4) Scanning Electron Microscopy

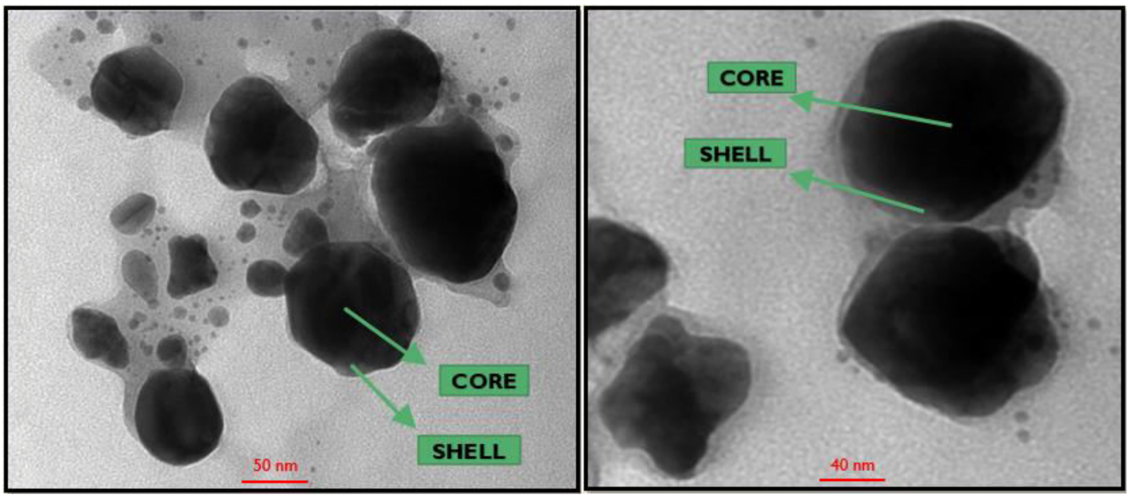
Fig no. 6: SEM analysis



The SEM images of the bimetallic nanoparticle showed a smooth surface morphology. The structure observed was irregular but with a prominent boundary indicating that nanoparticles have been formed and are within the ideal size range 10-100 nm.

5) Transmission Electron Microscopy

Fig no. 7: TEM analysis



The TEM analysis showed a circular shape of particles with a dark Core and a lighter Shell.
Hence we can conclude the formation of a bimetallic nanoparticle

6) Energy-dispersive X-ray analysis (EDAX)

Energy Dispersive X-Ray Spectroscopy (EDS or EDX) is a chemical microanalysis technique used in conjunction with scanning electron microscopy (SEM).

EDS functions with a series of three major parts: an emitter, a collector, and an analyzer. These parts are additionally typically equipped on an electron microscope such as SEM or TEM. The combination of these three pieces enables analysis of both how many X-rays are released, as well as what their energy is (in comparison to the energy of the initial X-rays that were emitted).

The EDS data is presented as a graph with KeV on the x-axis and peak intensity on the y-axis. The peak location on the x-axis are converted into the atoms that the energy changes represent by a computer program.

The presence of silver and gold in the synthesized Ag-Au bimetallic nanoparticle sample was analyzed by the EDS technique. Figure 5 shows the EDS spectrum of synthesized Ag-Au bimetallic nanoparticles in the ratio 1:1. This pattern shows the presence of both Ag and Au atom signals at the respective position. These characteristic absorption peaks of Ag and Au confirm the formation of Ag-Au bimetallic nanoparticles.

Fig no. 8: EDS analysis

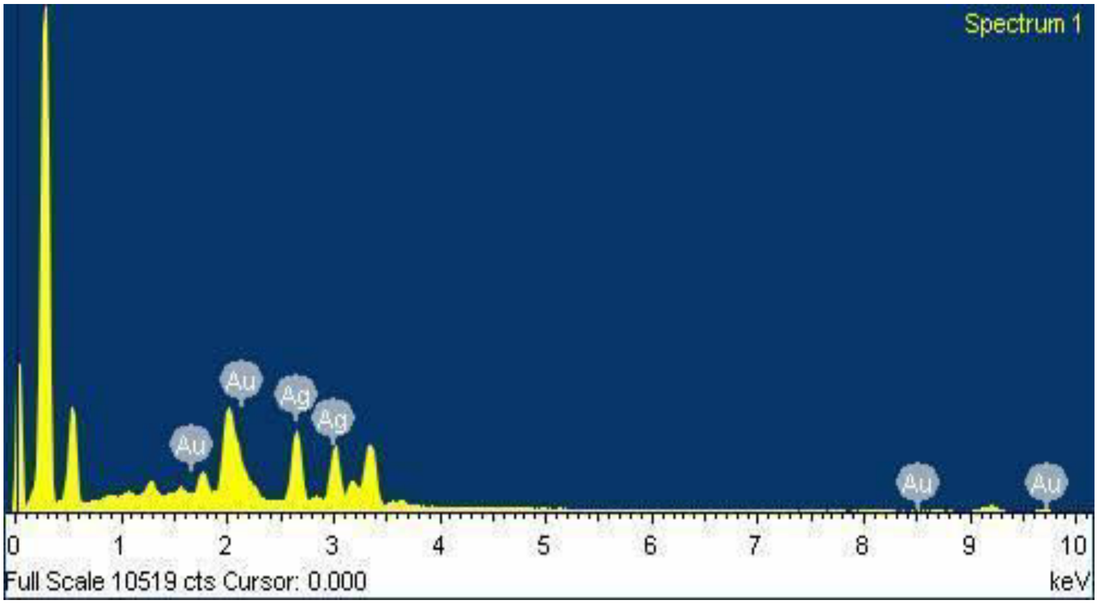


Table No. 2: EDS analysis

Element	**Weight%**	**Atomic%**
Ag	47.2	62.0
Au	52.8	38.0
Total	**100.00**	

It was observed that peaks of Ag and Au were present in the bimetallic nanoparticle sample.

Biological activity of bimetallic Nanoparticles:

1) Antibacterial and antifungal activity for Ag-Au Bimetallic nanoparticles[10,11]

Biological activity against	Zone of inhibition for Control (green tea extract)	Zone of inhibition for Bimetallic Np sample
S. aureus	Min zone of inhibition	16 ± 2 mm
E. coli	Min zone of inhibition	14 ± 2 mm
C. albicans	12± 2 mm	17 ± 2 mm

A very minute zone of inhibition was observed for tea extract. Whereas zone of Inhibition of 16 ± 2 mm for *S. aureus* and 14 ± 2 mm *E. coli* was found in antibacterial studies. A prominent zone of inhibition of 17 ± 2 mm was found in *C. albicans* for Antifungal studies.

3) Antioxidant Study using DPPH[12,13]

The IC_{50} value of standard ascorbic acid and bimetallic nanoparticle found to be 1.77 µl and 124 µl, respectively.

CONCLUSION:

Synthesis of novel nanomaterial from herbal plant source was successfully carried out, resulting in synthesis of gold, silver as well as bimetallic nanoparticles. SPR Phenomenon was made use of to detect the formation and the particle size and zeta potential results were in ideal expected range. The procedure applied for synthesis was sustainable, nontoxic and cost effective. Green Tea Extract was used as a source of herbal raw material that helped in bio-modification and formation of the nanoparticle. Various functional evaluations of the green synthesized nanoparticles were undertaken that gave positive results. The bimetallic particles showed good antibacterial and antioxidant activity.

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