SUPPLEMENTARY MATERIAL

In vitro* Antioxidant, Antimicrobial, Cytotoxic Potential of Gold and Silver Nanoparticles prepared using *Embelia ribes

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Abstract:

In recent years, the green synthesis of gold (GNPs) and silver (SNPs) nanoparticles has gained great interest among chemists and researchers. The present study reports an eco-friendly, cost-effective, rapid and easy method for the synthesis of gold and silver nanoparticles using the seed extract of *Embelia ribes* (SEEr) as capping and reducing agent. The synthesized GNPs and SNPs were characterized using the following techniques: UV-Vis spectroscopy, DLS, HR-TEM, FT-IR and XRD. The free radical scavenging potential of GNPs and SNPs was measured by DPPH assay and Phosphomolybdenum assay. Further, the antimicrobial activity against two microorganisms were tested using disc diffusion method and cytotoxicity of GNPs and SNPs was determined against MCF-7 cell lines at different concentrations by MTT assay. Both the GNPs and SNPs prepared from *Embelia ribes* comparatively showed promising results thereby proving their clinical importance.

Keywords: *Green synthesis, Gold nanoparticles, Silver nanoparticles, Seed extract, Embelia ribes*

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Experimental

Materials

All chemicals used in this experiment were of high level purity and obtained from Sigma chemicals (Bangalore, India). Silver nitrate salts, Chloroauric acid salts, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Synthesis of silver and gold nanoparticles from plant extract

The seeds of *Embelia ribes* (family: Myrsinaceae) plants were harvested from Vellore district of Tamil Nadu. The seed extract was first prepared by mixing 1.25 gm of dried powder with 50 mL of deionized water suspended in 250 mL conical flask and kept for boiling at 60°C for 1 hour. A constant volume of 1 mL of the prepared seed extract was taken in ten different boiling tubes, followed which 1×10^{-3} M AgNO_3 and 1×10^{-4} M HAuCl_4 was added in 0.1 to 1 mL range in two different sets respectively and the final volume of the reaction was made up to 5 mL with deionized water. The stability of gold and silver nanoparticles were checked by varying the pH ranging from 7 to 13 (data not shown). The optimum synthetic conditions were determined based on their characterization.

Characterization studies of synthesized gold and silver nanoparticles using seed extract.

The absorbance of SEEr-GNPs and -SNPs were characterized using UV-Visible spectrophotometer UV-1601 (Shimadzu Corporation, Japan). The nanoparticle solution in the reaction tube showing distinct characteristic peak was considered as optimum concentration, respectively. The hydrodynamic size of SEEr-GNPs and -SNPs was analyzed using particle size analyzer (Malvern NanoZS). Fourier transform infra-red spectroscopy analysis was carried out to identify the functional moiety responsible for the reduction of gold and silver ions to form nanoparticles. The FT-IR range was set between $500 - 4000 \text{ cm}^{-1}$. The X-ray diffraction was used to investigate the crystal structure of seed extract based synthesized gold nanoparticles. The

sample was ground and pressed in the sample holder to get a smooth plane surface and the diffraction pattern was observed over 2θ range of 30° - 120° . High resolution Transmission Electron Microscopy (HR-TEM) analysis was obtained with TEM-D950 TECHNAI G2-S-TWIN (FEI Company). The sample was added to the carbon-coated copper grid and dried at room temperature.

Antioxidant activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

The free radical scavenging activity of the extract, SEEr-GNPs and SEEr-SNPs was determined using DPPH assay (Wang et al. 2008). The dilution series of the test solutions (10-100 $\mu\text{g/mL}$) were incubated with freshly prepared DPPH solution in methanol (0.004% w/v). The reaction mixture was mixed vigorously and allowed to stand for 30 mins at room temperature. The change in the absorbance was measured photometrically at 517 nm using a Lambda 25 UV-Vis (Perkin Elmer, USA) spectrophotometer. The decrease in the absorbance denotes higher DPPH free radical scavenging activity. The procedure was repeated thrice to get triplicate values. Ascorbic acid dissolved in distilled water was used as reference standard. 95% of methanol was used as blank reagent. The ability of the sample to scavenge DPPH radical was calculated using the following formula. $[(Ac-At)/Ac] \times 100$, where Ac represents the absorbance of the control and At represents the absorbance of the test samples.

Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto et al (1999). An aliquot of 100 μL of sample solution was combined with 1 mL of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in a 4 mL vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as %PRP (Phosphomolybdenum Reducing Potential). Percent inhibition was

calculated by the following formula, $PRP (\%) = (1 - At/Ac) \times 100$, where At refers to the absorbance of the test sample, Ac refers to the absorbance of the control.

MTT Assay

Cell viability was measured with the conventional MTT reduction assay. Briefly, MCF-7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h in 200 μ L of RPMI with 10% FBS. Then culture supernatant was removed and various concentrations (0.11–100 μ g/mL) of test sample (SEEr-GNPs and -SNPs) was added and incubated for 48 h. After treatment cells were incubated with MTT (10 μ L, 5 mg/mL) at 37 °C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments (Evelyn et al. 2012).

$$\text{Cell viability (\%)} = \text{Mean OD/Control OD} \times 100$$

Antibacterial Activity

The antibacterial activity of SEEr-GNPs and –SNPs were determined using two bacterial cultures viz. Escherichia coli and Staphylococcus aureus by well diffusion method. The different concentrations of the test sample used were (250 μ g/mL, 500 μ g/mL, 750 μ g/mL and 1000 μ g/mL) added in the wells made on the nutrient agar plate. The antibiotic Tetracycline was used as standard. The plates were incubated at 37°C for 24-48 hrs. After incubation, the zone of inhibition was measured to determine the extent of antibacterial activity.

Statistical analysis

The data was expressed in percent inhibition with respect to control. All the tests were performed in triplicates and data expressed are as mean $\pm$ SD.

References

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Fig. S1. UV-Visible Spectroscopy, HR-TEM and PSA. UV-Visible absorbance graph for (a) gold nanoparticles (b) silver nanoparticles synthesized from different volumes of seed extract *Embelia ribes*, (c) TEM image of SEEr-GNPs, (d) TEM image of SEEr-SNPs, (e) Particles Size Analysis (PSA) of SEEr-GNPs and (f) PSA of SEEr-SNPs.

Figure S2: FTIR and XRD. (a), (b), (c) showing FTIR peaks observed from *Embelia ribes* seed extract (SEEr), SEEr-GNPs and SEEr-SNPs, respectively. (d), (e) showing XRD peaks for SEEr-GNPs and SEEr-SNPs, respectively.

Figure S3: Antioxidant Assay. (a) DPPH radical scavenging activity of SEEr-GNPs and SEEr-SNPs compared with control, (b) Phosphomolybdenum reducing potential of SEEr-GNPs and SEEr-SNPs compared with control.

Figure S4: Dose dependent cytotoxicity assay using SEEr-GNPs. Dose dependent cytotoxicity effect of SEEr-GNPs over cell viability (a) control, (b) 10 $\mu\text{g/mL}$, (c) 20 $\mu\text{g/mL}$, (d) 40 $\mu\text{g/mL}$, (e) 80 $\mu\text{g/mL}$, (f) 100 $\mu\text{g/mL}$.

Figure S5: Dose dependent cytotoxicity assay using SEEr-SNPs. Dose dependent cytotoxicity effect of SEEr-SNPs over cell viability (a) control, (b) 10 $\mu\text{g/mL}$, (c) 20 $\mu\text{g/mL}$, (d) 40 $\mu\text{g/mL}$, (e) 80 $\mu\text{g/mL}$, (f) 100 $\mu\text{g/mL}$.

Figure S6: MTT assay. Cytotoxicity of different concentration (10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL and 100 µg/mL) of SEEr-GNPs and SEEr-SNPs measured by MTT assay on MCF-7 cell lines.

Figure S7: Antibacterial Activity. Images of nutrient agar plate showing zone of inhibition when treated with SEEr-GNPs against (a) *E.coli*, (c) *S.aureus* and SEEr-SNPs against (b) *E.coli*, (d) *S.aureus* S-Standard (Tetracycline-30 $\mu\text{g/mL}$) (1-250 $\mu\text{g/mL}$, 2-500 $\mu\text{g/mL}$, 3-750 $\mu\text{g/mL}$, 4-1000 $\mu\text{g/mL}$).

Figure S8: Antibacterial Assay. Graph showing the antibacterial activity of SEEr-GNPs and SEEr-SNPs against *E.coli* and *S.aureus*.

Table S1: Zone of inhibition of gold and silver nanoparticles synthesized from seed extract against two bacterial species.

S.No	Samples	Zone of Inhibition (mm)									
		<i>Escherichia coli</i>					<i>Staphylococcus aureus</i>				
		250	500	750	1000	Standard	250	500	750	1000	Standard
1.	SEEr-GNPs	28	30	32	34	11	22	24	26	27	17
2.	SEEr-SNPs	20	24	26	28	11	22	25	26	27	17

Note : SEEr – Seed Extract Embelia ribes; GNPs – Gold nanoparticles; SNPs – Silver nanoparticles