Antibacterial and anticancer activities of silver nanoparticles biosynthesized using *Embelia ribes* Burm.f. berries extract

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Silver nanoparticles (AgNPs) have gained considerable attention in the field of medicine and water treatment owing to their physiochemical properties. Biosynthesis of AgNPs is favourable over chemical synthesis for issues of environmental concern. Here, we synthesized AgNPs using aqueous seed extract of berries of *Embelia ribes* Burm.f. and analyzed their antibacterial and anticancer activities. The phytochemicals present in the seed extract of *E. ribes* berries were used as reducing agent for the formation of AgNPs. The biosynthesized AgNPs were characterized using UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and HR-TEM analysis. The Surface Plasmon Resonance (SPR) peak observed at 430 nm from UV-spectrum further confirms the formation of AgNPs. The presence of phytochemical adhering to AgNPs was confirmed from FTIR spectrum. XRD and SAED analysis showed that the AgNPs are of crystalline in nature. TEM images showed the AgNPs were roughly spherical shaped and approximately around 30 nm in size. The AgNPs thus synthesized, were evaluated for antibacterial and anticancer activities. The results revealed significant antibacterial activity against *Bacillus subtilis* and also dose dependent inhibition of cell proliferation in MCF-7 cell line. Higher concentration showed low cytotoxicity and maximum inhibition was at 10 ng/mL after 24 h.

**Keywords:** AgNP, Cancer, False black pepper, Vidanga

Application of green chemistry protocols is gaining increasing importance towards environment protection. Nanotechnology is one such area with its wide range of applications, particularly in the electronic and medical appliances. Nanoparticles, such as gold, silver, platinum and palladium are extensively studied due to their unique characteristics when reduced to nano-size from the bulk material.⁴⁻⁸ Among the various nanoparticles, silver nanoparticles attain more importance in the field of medicine and water treatment due to physiochemical properties.⁴⁻⁸ As eco-friendly and green chemistry methods gain more importance in the current scenario, synthesis of silver nanoparticles using biomolecules is considered better. The biomolecules obtained from microorganisms and plants act as reducing agent to convert Ag⁺ ions to AgNPs. The interaction between metal and microbes helps the researcher to explore the synthesis of metallic nanoparticles, such as platinum, gold and silver.

Synthesis of nanoparticles using microbes can be divided into intracellular and extracellular based on the place where the conversion of nanoparticles occurs.⁹⁻¹⁰ Though microbial synthesis of nanoparticles are considered as cost-effective and eco-friendly, they have drawbacks such as culturing of microbes, uneven size of nanoparticles and the duration of conversion process. Plant extract is also used for the synthesis of metal nanoparticles.¹¹ There are many reports on the biosynthesis of nanoparticles using extract of various plant parts.¹²⁻¹⁵ It suggests that the biomolecules present in the aqueous extract acts as reducing agent in conversion of bulk materials to nanoparticles. Sadia *et al.* has shown that the biomolecules adhere to nanoparticles as capping agent and stabilize the nanoparticles in the solution.¹⁰ Biomolecules from the plants proved to be a significant alternative source for the synthesis of stable metal nanoparticles. The biomolecules like protein, phenols, vitamins, organic acids, polysaccharides and flavonoids from the plant extract plays important role in conversion and stability of nanoparticles and the protocol is regarded as simple and eco-friendly method.¹⁷
Embelia ribes Burm.f. (Myrsinaceae), commonly known as False black pepper or vidanga, is widely used as ayurvedic medicine. The aqueous extract of dried seeds of berries from E. ribes is used for the synthesis of silver nanoparticles. In this study, we attempted a simple, cost-effective and eco-friendly method for synthesis of silver nanoparticles. Further, we analyzed the antimicrobial and anticancer potential of the biosynthesised nanoparticles as well.

Materials and Methods

All the chemicals were of reagent grade. Silver nitrate (AgNO₃) was purchased from Merck, Mumbai, India. The raw material Embelia ribes berries were purchased from M/s Abirami Botanical Corporation, Tuticorin, Tamil Nadu, India. Nutrient Agar medium was purchased from Hi-Media, Mumbai, India. Bacillus subtilis was isolated from (Clinical Samples, Department of Microbiology Taramani Campus, University of Madras, Chennai Tamil Nadu, India) for antibacterial assay. MTT (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) was purchased from Invitrogen, USA for cytotoxicity studies.

Preparation of seed extract of Embelia ribes berries

Twenty-five grams of seeds of berries of E. ribes were washed with distilled water, dried under shade (3 days) and ground into powder using a mixer grinder. About 10 g of the seed powder was transferred to 250 mL conical flask and 100 mL of distilled water was added. The mixture was heated on a water bath until the colour of solution changes to dark yellowish brown (45 min.). The change in colour of the mixture indicated the conversion of Ag⁺ ions to silver nanoparticles (AgNPs).

Synthesis of silver nanoparticles

About 10 mL of aqueous seed extract of E. berries was added to 90 mL of 1 mM silver nitrate solution in 250 mL taken in conical flask added via dropper over a period of 30 s. The mixture was heated at 70°C on a water bath until the colour of solution changes to dark yellowish brown (45 min.). The change in colour of the mixture indicated the conversion of Ag⁺ ions to silver nanoparticles (AgNPs).

UV–visible spectroscopic studies

The biosynthesised AgNPs was characterized by UV-visible spectroscopy. The reduction of Ag⁺ ions to AgNPs ions was confirmed by measuring the spectrum of the AgNPs after dilution using Jasco V-630 spectrophotometer operated within the range of 300-600 nm.

FTIR spectroscopic studies

A pellet for FTIR analysis was made by mixing, grinding and pelleting the AgNPs with potassium bromide (KBr). The FTIR spectrum was recorded using PerkinElmer FTIR spectrometer. The FTIR analysis of AgNPs reveals the presence of biomolecules adhered to the biosynthesised AgNPs.

X-Ray Diffraction (XRD)

X-ray diffraction (XRD) measurement of the biosynthesised AgNPs solution was drop-coated onto glass substrates for determination of the formation of AgNPs by an X’PertPro analytical X-ray diffractometer instrument with X’pert high score plus software operating at a voltage of 45 kV and a current of 40 mA with Cu Kα radiation.

HR–TEM analysis

A drop of biosynthesised AgNPs was placed on copper grid and vacuum evaporated to dry it. After drying the AgNPs, nanoparticles were photographed using Philips CM20 Transmission Electron Microscope equipped with selected area electron diffraction pattern (SAED).

Antibacterial activity of silver (Ag) nanoparticles

Antibacterial activity of silver nanoparticles was evaluated by the plate agar diffusion method. The test bacterial strain was inoculated into Mueller-Hinton broth and incubated at 37°C for 3 to 6 h after that a sterile cotton swab was immersed in the bacterial suspension and swabbed aseptically on the surface of sterile plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100 μL of extracts (100 mg/mL water). Wells loaded with 100 μL of Gentamicin (100 mg/mL water) served as positive control. The plates were incubated at 37°C for overnight in an incubator. Antibacterial activity was determined as diameter of the zone of inhibition (ZOI). ZOI measurements were made three times for each disc at different orientation and the average was recorded.

Cytotoxicity assay on MCF-7 cell lines

Cell culture

MCF-7 cells obtained from National Centre For Cell Science (NCCS) Pune, were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% Fetal bovine serum (FBS), streptomycin, gentamycin and amphotericin B were obtained from Hi-media, Mumbai. All cell cultures
were maintained at 37°C in a humidified atmosphere of 5% CO₂. Cells were allowed to grow to confluence (90%) over 24 h before use.

**Cell growth inhibition studies by MTT assay**

Cell viability was measured with the MTT test, as portrayed already with slight change. Quickly, MCF-7 cells were seeded at a thickness of 5×10³ cells/well in 96-well plates for 24 h, in 200 μL of RPMI with 10% FBS. At that point culture supernatant was expelled and RPMI containing different fixations (0.11-100 μg/mL) of test compound was included and hatched for 48 h. After treatment cells were incubated with MTT (10 μL, 5mg/mL) at 37°C for 4 h and further with DMSO at room temperature for 1 h. The plates were perused at 595nm on an examining multi-well spectrophotometer. Information spoke to the mean qualities for six autonomous tests.

\[
\text{Cell viability inhibition} = \left(1 - \frac{\text{OD}_{\text{Treated}}}{\text{OD}_{\text{Control}}}\right) \times 100
\]

**Results and Discussion**

**Synthesis of silver nanoparticles**

Berries of *Embelia ribes* contains various phytochemical constituents, such as embelic acid, tannin and phenolic acids which exhibits different biological activities such as antioxidant, antimicrobial and inhibition of cancer growth. The phytochemical present in the berries has tendency to reduce Ag⁺ ions in the silver nitrate solutions to silver nanoparticles (AgNPs). In the present study, the aqueous seed extract of berries of *E. ribes* is mixed with 1 mM aqueous AgNO₃ solution and the colour change to dark brown colour indicates the formation of AgNPs. Generally, free electrons in the nanoparticles exhibits Surface Plasmon Resonance (SPR) absorption band in the presence light which leads to colour change. Usually, the formation of AgNPs was confirmed by the colour change of the reaction mixture to dark brown colour and it is characterized by UV-visible spectrophotometer.

**UV-visible spectroscopic studies**

The UV-visible spectrum of the reaction mixture is depicted in the Fig. 1. It reveals sharp SPR band at 430 nm confirms the formation of AgNPs. The UV absorption observed matched well with the previous study conducted with the extracts of *Tridax procumbens* with the absorption spectra range at 410-430 nm²⁰.

**FTIR spectroscopic studies**

FTIR spectroscopy study was carried out for the biosynthesized AgNPs to investigate the interaction of biomolecules present in the extract and AgNPs. The FTIR spectrum of AgNPs synthesized using seed extract of berries of *E. ribes* showed absorption peaks at 3339, 2103, 1633, 145, 1394, 1232 and 1050 cm⁻¹ (Fig. 2). The peak at 3339 cm⁻¹ is characteristic of the presence of hydroxyl group (−OH) of various biomolecules present in the biosynthesized AgNPs. A sharp band at 1633 cm⁻¹ is assigned to C=C stretching vibrations of aromatic ring. The weak absorption bands at 1450 cm⁻¹ and 1394 cm⁻¹ are due to C−O stretching vibrations in carboxyl group. The absorption band at 1050 cm⁻¹ corresponds to C−N stretching of aliphatic amines. The various absorption bands in the FTIR spectrum manifest the presence of biomolecules attached to AgNPs. The biomolecules present in the seed extract of berries of *E. ribes* reduces the Ag⁺ ions to AgNPs and it is attached to AgNPs as capping agent that stabilizes the AgNPs.

**X-Ray diffraction (XRD)**

Fig. 3 shows X-Ray diffraction of synthesized particles. The presence of peaks at 2θ = 38.2, 44.4, 64.6 confirms that the phase of the nanocrystal is silver as the peak positions are the same as in the standard file (JCPDF card: 87-0720, Cu target) of bulk silver, which is a cubic system having a face centred lattice. The average particle size, corresponding to the highest intensity peak (111) of the particles, was calculated according to Debye Scherer’s formula given below:

\[
D (\text{Å}) = \frac{K \lambda}{\beta \cos \theta} \quad \text{(Eq.1)}
\]

where \(K\) is constant equal to 0.90, \(\lambda\) is the X-ray wavelength (1.54Å), \(\beta\) is the full-width at maximum (FWHM) expressed in radians, and \(\theta\) is the
Bragg angle (degree) corresponding to the (111) peak. The calculated particle size from Eq.1 is 33.2 nm which is in close agreement with the average particle size as determined by TEM images. These results were in accordance with the earlier study[21].

**HR–TEM analysis**

TEM images of synthesized silver nanoparticles are shown in Fig. 4. The particles are seen to be isolated and less aggregated. The average particle size calculated using Image J analysis was 30.2±5 nm, which also agree with the XRD particle size. The inset of Fig. 4 shows selected area electron diffraction pattern (SAED) of silver nanoparticles acquired from. The presence of clear rings confirms that the synthesized silver nanoparticles are highly polycrystalline. Silver nanoparticles usually exhibit absorption peak at 3 keV energy[22].

**Antibacterial activity of silver (Ag) nanoparticles**

Silver nanoparticles were considered to be one of the effective antimicrobial agents. AgNPs interacts with the protein present in the cell wall and it rupture the cell wall thereby it demolishes the microorganisms. The antimicrobial activity of AgNPs synthesized from aqueous seed extract of *Embelia ribes* was investigated against (*B. subtilis*, *E. coli*, *S. aureus*, *E. aerogens* and *A. tumefaciens*) by well...
diffusion method. Fig. 5 shows the zone inhibition of AgNPs against different bacterial species after 24 h incubation. Synthesized AgNPs possess significant antibacterial activity against \textit{B. subtilis} among different organisms tested. Synthesized silver nanoparticles exhibited 14 mm ZOI compared to other microorganisms. Recently, synthesized silver nanoparticles exhibited higher incubation zone of 19 mm than standard amikacin drug. Silver nanoparticles when treated with bacterial cells they uptake silver ions which inhibit respiratory enzymes, generate reactive oxygen species (ROS) which in turn damages the cell.

Cytotoxicity assay

The study investigated the cytotoxic studies of silver nanoparticles seed extract of \textit{Embelia ribes} in MCF-7 cells (different concentration of 1100 µg and 10 ng -100 ng/mL), by using MTT Assay. Viability of MCF-7 cell lines was found to decrease with increase in concentration of silver nanoparticles. The inhibition of cell proliferation in MCF-7 cell in dose depended activity after 24 h of treatment of MCF-7 cell line was elaborated in Fig.6. The MCF-7 cell proliferation was considerably lower, when compared to untreated (MCF-7) cell. After 24 h treatment, lower concentration (1 µg and 10 ng/mL) killed more than 60% of cancer cells, but higher concentrations showed lower cytotoxic effects on 24 h treatment of cells. The maximum inhibition of cell proliferations was obtained at the concentration of (10 ng/mL) after 24 h. The synthesized silver nanoparticles concentration at 10 mg exhibited 93% cell viability and 1 µg concentration showed 77% cell viability. Similarly, anticancer effect of silver nanocomposite on MG-63 line exhibited IC\textsubscript{50} value around 54.57% at 62.5 µg/mL concentration as reported by Bhati & Malik.

Half & Arber have highlighted the urgent need to examine reliable sources of natural substances as anticancer agents for further application in cancer therapy. Here, we explored the seed extract of one of the common herb, \textit{Embelia ribes} which is widely distributed in India for synthesizing silver nanoparticles and studied its activity on cancer cells. Extensively lower cytotoxic activities were also reported in the treatment of MDA-MB-231 cell lines\textsuperscript{27}. Similarly, silver nanoparticles synthesized from \textit{Emericella nidulans} EV4 possess inhibitory activity against \textit{P. aeruginosa} NCIM 5029 compared to standard amikacin drug\textsuperscript{22}. Furthermore, the above study indicates the lower concentration showing significant inhibition of cell proliferation. The finding revealed that the MTT reductions were observed in viable cells following the treatment of \textit{Embelia ribes} seed extract was due to cell death. The present studies on the active compound for proper consideration of chemotherapeutic agent as well as their possible development as showed potential anticancer drugs.

Conclusion

In this study, we have reported a simple method to synthesize AgNPs from aqueous seed extract of berries of \textit{Embelia ribes} as reducing agent. The biosynthesised AgNPs was characterized using UV-Vis spectroscopy, FTIR, XRD and HRTEM. From the characterisation, it was confirmed that the phytochemical present in the extract acted as reducing agent and the synthesised AgNPs has roughly spherical in shape with average particle size of approximately 30 nm. The biosynthesised AgNPs exhibits excellent antibacterial activity and significant anticancer activity against MCF-7 cell lines. This biosynthesis method can be adapted for synthesis of other noble metal nanoparticles for effective medical applications. The
cytotoxic activity of synthesized nanoparticles in MCF-7 cell lines exhibited 93% cell viability at 10 ng concentration. Antibacterial activity of AgNPs was also in comparable range with the standard drugs. Thus, from the above study, it can be concluded that the synthesized AgNPs can be used as a potent antibacterial and cytotoxic drug in pharmaceutical industrial applications.

References