Biofabrication of silver nanoparticles using leaf extract of *Rhynchosia beddomei* Baker: spectral characterization and their biological activities

S. Soneya¹ · K. V. Saritha¹

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Abstract

The present study successfully investigated the biofabrication, spectral characterization, and biological activities of silver nanoparticles (AgNPs) using leaf aqueous extract of *Rhynchosia beddomei* Baker, important crop wild relatives of beans family (*Phaseolus*). Biofabrication of RB-AgNPs (*R. beddomei* leaf synthesized AgNPs) was confirmed by color change of the reaction solution from light yellow to dark brown. UV–Vis absorption spectrum of colloidal solution of RB-AgNPs showed characteristic surface plasmon resonance peak at 431 nm. XRD pattern showed that biofabricated RB-AgNPs were crystalline in nature with FCC lattice and the crystallite size was found to be 9.36 nm. TEM micrographs showed that the biofabricated RB-AgNPs were mainly spherical in shape with 20–80 nm in size. DLS analysis revealed that average hydrodynamic radius of RB-AgNPs was 46.5 ± 8.5 nm. Further RB-AgNPs showed very good stability with zeta potential value of -27.6 mV and polydispersity index of 0.203. FTIR analysis revealed that flavonoids and proteins take part in the bioreduction and stabilization of RB-AgNPs respectively. Biofabricated RB-AgNPs showed effective antioxidant activity against DPPH ($IC_{50} = 69.45$ $\mu\text{g/mL}$) and H_2O_2 ($IC_{50} = 87.43$ $\mu\text{g/mL}$). RB-AgNPs showed significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. RB-AgNPs were further proved to possess potent cytotoxic activity against different carcinomas including human cell lines of colon carcinoma (COLO205), prostate carcinoma (PC3) and breast cancer (MCF7). Thus RB-AgNPs showed multiple biological functions (3-in-1 system) including antioxidant, antimicrobial and anticancer activities.

✉ K. V. Saritha, kvsarithasvu@gmail.com | ¹Department of Biotechnology, Sri Venkateswara University, Tirupati, A.P., India.

Graphic abstract

Keywords *Rhynchosia beddomei* Baker · Silver nanoparticles · Biofabrication · TEM · DLS · COLO205

1 Introduction

Silver nanoparticles (AgNPs) have been extensively applied in every field of science due to their unique physico-chemical properties. Applications of AgNPs have revolutionized the medicinal industry owing to their optical, thermal, catalytic, electronic and optoelectronic properties [1–3]. The unique physico-chemical properties such as surface plasmon resonance (SPR), large surface area to volume ratio and spatial confinement are mainly size and shape dependant [1, 2]. Hence the synthesis of AgNPs with controlled size and shape has gain importance. Different physical and chemical methods including laser ablation [4], UV-irradiation assisted [5], γ -radiation assisted [6], electron beam irradiation [7] ultrasound assisted [8], solvothermal [9], thermal decomposition [10] lithographic [11], electrochemical [12], photochemical [13], sonochemical [14], polyaniline [15], polyol [16], polyvinylpyrrolidone assisted [17] and chemical reduction methods [18] have been successfully employed for the synthesis of AgNPs. Chemical and physical methods are time taking, laborious, and not eco-friendly. Further chemical and physical methods cause health hazards like genotoxicity, carcinogenicity,

mutagenic and cytotoxicities due to exposure to different toxic irradiations and chemicals [19]. Hence there is a need to develop the green procedures to synthesize clean, non-toxic and biocompatible AgNPs. Different scientists around the globe developed simple and cost effective methods for the synthesis of AgNPs using different biological entities like bacteria, fungi, yeast and plants. Biofabricated AgNPs are clean, nontoxic and biocompatible. Biofabrication of AgNPs using yeast, bacteria and fungi involve tedious culturing methods and laborious procedures, and also time taking. Biofabrication of AgNPs using plants and their extracts involves simple steps, little duration, low-cost and ecofriendly approaches. Different plants including *Mimosa elengi* [20], *Cassia fistula* [21], *Helicteres isora* [22], *Panax ginseng* [23], *Andrographis paniculata* [24], *Indigofera barberi* [25], *Clerodendrum phlomidis* [26], *Rubus glaucus* [27], *Oxalis scandens* [28], *Spondias dulcis* [29] and *Mussaenda erythrophylla* [30] have been reported for the synthesis of AgNPs with different biological activities.

Phytosynthesized AgNPs were proved to possess different applications. Malaria and arbovirus vectors have been successfully controlled using *Mimosa elengi* [20]. Phytosynthesized AgNPs have been reported to possess

antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus* species [21–27]. Phytosynthesized AgNPs using *Indigofera barberi* [25], *Clerodendrum phlomidis* [26] and *Rubus glaucus* [27] showed effective antioxidant activity by quenching free radicals such as DPPH (1, 1-diphenyl-2-picrylhydrazyl), BHT (butylated hydroxytoluene), H_2O_2 (hydrogen peroxide) and NO (nitric oxide) radicals. *Panax ginseng* leaf synthesized AgNPs showed antiproliferative activity against human lung adeno carcinoma (A549), human hepatic cancer (HepG2) and human breast cancer (MCF7) [23]. Further Biosynthesized AgNPs were successfully proved to possess different biomedical applications such as anti-inflammatory activity, treatment of obesity and cardiovascular diseases, drug delivery vehicles and imaging facilitator [28].

The genus *Rhynchosia*, commonly known as snout bean, consists about 200 species, and occurs in both temperate and tropical regions. *R. beddomei* is an important crop wild relatives (CWR) of beans family (*Phaseolus*) that are economically important both for human and animal consumption due to their high protein content. *R. beddomei* is a perennial flowering plant classified in the family *Fabaceae*, tribe *Phaseoleae*, sub-tribe *Cajaninae* and subfamily *Papilionoideae* [31]. *R. beddomei* is an important, indigenous, a rare forestry species, and deciduous shrub of tribal medicinal plant. The vernacular names of *R. beddomei* in Telugu are Adavivuluva, Adavi-Kandi, Vendichettu, and Vendaku [32]. The preliminary phytochemical investigation revealed the presence of polyphenols, flavonoids, terpenoids, alkaloids, saponins, steroids and glycosides [33]. *R. beddomei* plants possess antimicrobial activity used for wound healing, treatment of rheumatic pains, cuts and boils by Adivasi tribal people of Eastern Ghats, India [34].

In the present study, we report the successful synthesis of AgNPs using aqueous leaf extract of a rare and endemic plant *R. beddomei*. The biofabricated RB-AgNPs (*R. beddomei* synthesized AgNPs) were analyzed by Ultraviolet–visible (UV–Vis), Transmission electron microscopy (TEM), X-ray diffraction (XRD), Selected area electron diffraction (SAED), Fourier transform infrared (FTIR), and Dynamic light scattering (DLS) techniques. RB-AgNPs were evaluated for their antioxidant activity by free radical scavenging assays including DPPH and H_2O_2 radical scavenging activities (RSA). Antimicrobial activity of the RB-AgNPs was evaluated against both Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumonia* and *Escherichia coli*) bacteria. Further cytotoxicity of the RB-AgNPs was checked against different cancer cells including human colon carcinoma (COLO205), prostate carcinoma (PC3) and breast cancer (MCF7).

2 Materials and methods

2.1 Plant material collection and authentication

Fresh and healthy leaves of the *R. beddomei* were collected from Tirumala hills, Eastern Ghats, Andhra Pradesh, India. The leaves were authenticated with taxonomist and the voucher specimens (SVUTY-RB01-05) were deposited in the herbarium, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

2.2 Preparation of leaf aqueous extract of *R. beddomei*

Fresh and healthy leaves of the *R. beddomei* plant material were rinsed under running tap water to remove the dust. Then the leaves were surface sterilized using double distilled water (DDW) for 3 times and then the leaves were completely dried in the shade for 5 days and ground into fine powder. Ten grams of *R. beddomei* leaf powder was taken into 100 mL of DDW, mix well and then boiled at 50–60 °C in microwave oven for 30 min and then allowed to cool down the solution at room temperature for 2 h. After incubation, the filtration of extract was carried out. The obtained leaf extract of *R. beddomei* was stored in an airtight container and used for the biofabrication of RB-AgNPs (*R. beddomei* leaf synthesized AgNPs) and used for further experiment.

2.3 Biofabrication of RB-AgNPs

10 mL of leaf extract of *R. beddomei* was added into 90 mL of 1 mM $AgNO_3$ solution and then heated for 20 min at 50 °C in a microwave oven and then the experimental solution was allowed to incubate in the dark at room temperature for 24 h. After incubation, initially the reduction of silver (Ag^+) ions into AgNPs by the *R. beddomei* leaf extract was visually observed as the reaction solution was turned from light yellow to dark brown color. Dark brown color indicates the formation of RB-AgNPs by the reduction of silver ions (Ag^+) using leaf extract of *R. beddomei*.

2.4 Characterization of RB-AgNPs

After color change, the colloidal solution of RB-AgNPs was subjected to UV–Vis analysis for the confirmation of production of AgNPs using UV–Vis double beam spectrometry (Perkin-Elmer Ltd). UV–Vis spectrum was recorded between 200 and 800 nm. Colloidal solution of biofabricated RB-AgNPs was centrifuged at 20,000 rpm for 15–20 min. The pellet was washed with DDW and centrifugation process was repeated for three times at the same rpm for same

duration. Finally, the pellet obtained was air dried. This pure powder form of RB-AgNPs was used for further characterization using different spectrometer techniques. Functional group analysis of RB-AgNPs was carried out using FTIR (BRUKER, Alpha interferometer, Germany). FTIR spectrum was recorded between 500 and 4000 cm^{-1} wave number with the resolution of 4 cm^{-1} . XRD analysis was carried out using Cu K α radiation source on an Ultima IV X-ray powder diffractometer (Rigaku Ltd, Tokyo, Japan). TEM analysis of biofabricated RB-AgNPs was carried out using FEI Tecnai F12 (Philips Optics Ltd, Holland) operated at 100 kV. TEM micrographs were recorded at different resolutions and different nanoscale ranges. Crystal structure analysis and SAED pattern was also recorded using TEM. Particle size distribution, average hydrodynamic size, polydispersity index (PI), and zeta potential value were carried out using DLS technique.

2.5 Biological properties of RB-AgNPs

2.5.1 In vitro assessment of antimicrobial activity of RB-AgNPs

Antimicrobial activity of the biofabricated RB-AgNPs was checked against both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli* and *K. pneumonia*) bacteria by disc diffusion method using 24 h active cultures [22]. 200 μL of bacterial inoculum was spread on Nutrient agar (NA) plates. Each NA plate consists of four sterile paper discs. First three discs were impregnated respectively with streptomycin (12.5 $\mu\text{g}/\text{mL}$), 25 $\mu\text{g}/\text{mL}$ of RB-AgNPs, 50 $\mu\text{g}/\text{mL}$ of RB-AgNPs and final disc was impregnated with combination of streptomycin (12.5 $\mu\text{g}/\text{mL}$) and RB-AgNPs (12.5 $\mu\text{g}/\text{mL}$). NA plates were incubated at 37 °C for 24 h and observed for zone of inhibition (ZOI).

2.5.2 In vitro assessment of antioxidant activity of RB-AgNPs

In vitro antioxidant activity of the biofabricated RB-AgNPs was checked by DPPH and H_2O_2 RSA [22]. Ascorbic acid was used as standard antioxidant for both assays. 1 mL of methanolic solution of test samples at different concentrations (25, 50, 75 and 100 $\mu\text{g}/\text{mL}$) was added to 2 mL of methanolic solution containing DPPH radical (1 mM/L). Then the samples were incubated for 60 min at room temperature and then the absorbance was recorded at 517 nm. Ascorbic acid was used as standard antioxidant. In vitro antioxidant activity of the RB-AgNPs was further confirmed by H_2O_2 RSA [22]. 1 mL of different concentrations of test samples (25, 50, 75 and 100 $\mu\text{g}/\text{mL}$) were added to 2 mL of H_2O_2 solution (40 mM) prepared in phosphate buffer (pH 7.4) and then incubated for 20 min. After

incubation, the absorbance was measured at 230 nm. RSA was calculated using % Inhibition = [(Absorbance of control – absorbance of test sample)/Absorbance of control] $\times$ 100.

2.5.3 In vitro assessment of anticancer activity of RB-AgNPs

Different human cancer cell lines including colon cancer (COLO205), prostate cancer (PC3), and breast cancer (MCF-7) cell lines were obtained from National centre for cellular science (NCCS), Pune, India. Cells were cultured either in RPMI-1640 medium (PC3) or Dulbecco's Modified Eagle's (DME) medium (COLO205 and MCF-7) supplemented with 10% FBS, 2 mM glutamine, 1 mM NaHCO_3 , 100 $\mu\text{g}/\text{mL}$ streptomycin and 100 units/mL penicillin. All the cells were maintained in culture at 37 °C in 5% CO_2 chamber. MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay was employed to determine the cytotoxic activity of RB-AgNPs against human cancer cell lines including COLO205, PC3 and MCF-7 [35]. Briefly, the cells (5×10^3) were seeded in each well containing 100 μL of medium in 96 well plates. After overnight incubation in 5% CO_2 chamber, exactly 100 μL of media with different concentrations of RB-AgNPs (25, 50, 75, 100, 150 and 200 $\mu\text{g}/\text{mL}$) were added to the cell suspension. The cell viability was assessed after 24 h by adding 10 μL of MTT (5 mg/mL in PBS pH-7.4) per well and incubated at 37 °C in 5% CO_2 chamber for additional 3 h. Then the medium was discarded and the formazan blue formed in the cells was dissolved in 100 μL of DMSO. The intensity of color formation was measured at 570 nm in a spectrophotometer. The percent inhibition of cell viability was determined and the IC_{50} values were calculated.

Fig. 1 **a** Reaction mixture containing leaf aqueous extract of *R. beddomei* and AgNO_3 solution, and **b** dark brown solution indicates the formation of RB-AgNPs

Fig. 2 Schematic diagram represent **a** plant mediated synthesis of silver nanoparticles and **(b)** mechanism of formation of silver nanoparticles through plant synthesis approach

3 Results and discussion

3.1 Biosynthesis of RB-AgNPs

In the present study we report the biofabrication of RB-AgNPs using leaf aqueous extract of *R. beddomei*. The leaf aqueous extract of *R. beddomei* act as reducing agent which reduced silver ions (Ag⁺) into nanosilver/RB-AgNPs (*R. beddomei* leaf mediated synthesis of AgNPs). Initially, the biofabrication of RB-AgNPs was confirmed by observing the color change of the colloidal solution from light yellow to dark brown color (Fig. 1). Further the biofabrication was confirmed by UV–Vis analysis of colloidal solution of RB-AgNPs. The simple and easy green synthesis approach using plant extracts (Fig. 2a) and mechanism of formation of nanoparticles were clearly depicted (Fig. 2b). Plants can be considered as bio factories for the synthesis of silver or other metallic nanoparticles [36, 37]. Plants contain wide range of secondary metabolites otherwise known as phytochemicals. Phytochemicals including, flavonoids, quinines, alkaloids, coumarins, triterpenoids, steroids, glycosides, vitamins, coenzymes, proteins, amino acids, polysaccharides and sugars. Most of these compounds contain more than one hydroxyl groups. These compounds have redox potential and play an important role in the biosynthesis of metallic nanoparticles [36–38].

The synthesis of silver nanoparticles can be accomplished in two phases. The first phase involving dissociation of the metal salts (AgNO₃/AgCl) into metal ions in aqueous solution (Ag⁺). The second phase is actual bioreduction phase in which hydroxyl group of phytochemicals bind to metal ions (containing valency) and reduce it into metal nanoparticles (containing '0' valency). Bioreduction phase involves three steps. In the first step, reduction of metal ion (Ag⁺) to zero valent metal atom (Ag⁰) occurs. The second step involves nucleation and growth phase in which small adjacent reduced metal atoms are self assembled into metal nanoparticles of a particular size and shape. The final step is the capping of the nanoparticles by phytochemical which leads to stabilization of newly synthesized nanoparticle [39]. Thus the plant extracts act as both reducing and stabilizing agents for metal nanoparticles.

3.2 UV–Vis analysis of RB-AgNPs

UV–Vis analysis at 1 h of incubation shows initiation of synthesis of RB-AgNPs. After 12 h of incubation, it showed a peak which indicates the formation of RB-AgNPs. UV–Vis analysis after 24 h of incubation showed an absorption peak at 431 nm (Fig. 3) which is characteristic of AgNPs due to surface plasmon resonance (SPR) a unique interaction of light where the free electrons of the metal nanoparticles

Fig. 3 UV–Vis analysis of reaction solution containing leaf aqueous extract of *R. beddomei* and aqueous AgNO_3 solution at different time intervals. After 24 h of incubation, reaction solution showed characteristic SPR peak of RB-AgNPs at 431 nm

Fig. 4 FTIR spectrum of RB-AgNPs

undergo an oscillation with respect to the metal lattice [1, 2] in the presence of an oscillating electromagnetic field of the light. A band shift in the UV–Vis spectra signifies changes in the particle size, adsorbed species in the surface, chemical surrounding, and dielectric constant, etc. has facilitated broad applications of AgNPs due to surface conjugation with molecular probes and optical properties related with localized SPR [1–3]. The relatively narrow SPR peak observed in this study indicates small monodispersed and spherical shaped RB-AgNPs and absence of the

agglomeration processes in consonance with the particle size confirmed by particle size and TEM analysis.

3.3 FTIR analysis of RB-AgNPs

FTIR spectrum showed the peaks at 3287, 2928, 2352, 1608, 1386, 1006, 847 and 669 cm^{-1} (Fig. 4). The broad peak at 3287 cm^{-1} was related to O–H stretching vibrations of phenols and alcohols in the leaf extract. The peak at 2928 cm^{-1} is responsible for C–H stretching vibration [23, 40]. The strong absorption peak at 2352 cm^{-1} could

Fig. 5 XRD pattern of RB-AgNPs showed FCC crystal structure

be indexed to $-SH$ (thiol group) vibration of amino acid cysteine. This peak clearly indicates that the amino acid cysteine might be involved in the capping of RB-AgNPs and provides the stability. Further the thiol groups also involved in the bioreduction of silver ions into nanosilver [41]. The peaks at 1608 and 1386 cm^{-1} were respectively related to amide I and amide II linkage of proteins [23]. The peak at 1006 cm^{-1} could be assigned to $O-H$ deformation of phenolic groups. The peak at 847 and 669 cm^{-1} is relating to flexural vibrations out of plane bending of $C-H$ for alkenes and alkynes [23, 25]. The peaks clearly indicated the participation of proteins and polyphenols in the bioreduction of silver ions into RB-AgNPs. The proteins plausibly involved in the formation of a coat/cap around the particles and prevent the agglomeration of the particles which in turn stabilize the formulation. The stability of RB-AgNPs was further confirmed by zeta potential measurement.

3.4 XRD analysis of RB-AgNPs

XRD analysis was carried out to reveal the crystalline nature of the biofabricated RB-AgNPs. XRD pattern (Fig. 5) showed Bragg's diffraction peaks at 38.64° , 44.66° , 64.88° and 77.64° which were respectively corresponding to (111) (200) (220) and (311) planes of the face centered cubic crystal (FCC) lattice. XRD data was consisted with the JCPDS data file number 87-0720. Thus XRD pattern showed that the biofabricated RB-AgNPs were crystalline in nature with FCC lattice. Further the crystallite size was determined using Debye–Scherrer equation ($D = K\lambda/\beta_{0.5} \cos\theta$) where 'D' denotes crystallite size in nm, 'K' denotes Scherrer constant (0.95), ' λ ' denotes wavelength of source

light (0.154 nm), $\beta_{0.5}$ denotes full width half maximum length (FWHM) and ' θ ' denotes Bragg's angle in the radian. In this study value of FWHM was determined as 0.8586 based on Gaussian multiple peak fit analysis. By employing Scherrer formula, the crystallite size 'D' was calculated as 9.36 nm. Further the crystal lattice inter plane spacing was also calculated using Bragg's equation ($n\lambda = 2d\sin\theta$). Crystal lattice spacing 'd' was determined as 0.173 nm. The results of the present study are in line with previous reports. Leaf synthesized AgNPs using *Bridelia retusa* are crystalline in nature with FCC structure and the crystallite size was determined as 16.21 nm [42]. Similarly *B. retusa* fruit synthesized AgNPs are FCC crystals with crystallite size of 22.48 nm [43].

3.5 TEM analysis of RB-AgNPs

TEM analysis of the RB-AgNPs was carried out to determine their size and shape. The TEM micrographs were taken at different nanoscales such as 200 nm, 100 nm and 50 nm (Fig. 6a–c). The TEM micrographs showed that the biofabricated RB-AgNPs are mainly spherical in shape. Rhombic and rod shaped RB-AgNPs were also found. Further TEM analysis showed that the size ranges between 20 and 80 nm. SAED pattern (Fig. 6d) showed very clear circular diffraction rings of the crystal lattice with face centered cubic phase.

3.6 DLS analysis of RB-AgNPs

The particle size analysis of the biofabricated RB-AgNPs was carried out using DLS. The particle size analysis revealed that the biofabricated RB-AgNPs were 20–80 nm in size with average hydrodynamic radius of 46.5 nm (Fig. 7a). Polydispersity index (PI) of the RB-AgNPs was found to be 0.203. In this present study, zeta potential value of the biofabricated RB-AgNPs was found to be -27.6 mV (Fig. 7b). The electrostatic repulsion between the nanoparticles depends on the charge that present on their surface. The high negative value confirms the repulsion among the nanoparticles and thereby prevents them from agglomeration in the medium, which in turn leading to long-term stability of the RB-AgNPs [24, 25]. DLS results are in consistence with previous reports. PI, zeta potential and hydrodynamic diameter of *B. retusa* leaf synthesized AgNPs were found to be 0.283, -18.1 mV and 201.68 nm respectively [42]. *Ipomoea digitata* flower synthesized AgNPs possess average hydrodynamic diameter of 111 nm, with PI and zeta potential value of 0.197 and -25.1 mV respectively [44]. PI, zeta potential and hydrodynamic size of *Spondias dulcis* leaf synthesized AgNPs were found to be 0.187, -15.7 mV and 59.66 nm respectively [29].

Fig. 6 TEM micrograph of RB-AgNPs at different scales **a** 200 nm, **b** 100 nm, **c** 50 nm and **d** SAED pattern showed diffraction peaks

3.7 In vitro biological effects

3.7.1 In vitro assessment of antimicrobial activity of RB-AgNPs

In the present study antimicrobial activity of the RB-AgNPs was checked against both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*K. pneumonia* and *E. coli*) bacteria (Fig. 8a–d). Diameter of inhibition zones were represented in Table 1. RB-AgNPs showed effective antimicrobial activity against all the tested bacteria. RB-AgNPs showed highest inhibition against *E. coli* followed by *S. aureus*, *K. pneumonia* and *B. subtilis*. Increase in the concentration of RB-AgNPs further increases the inhibition zones. RB-AgNPs with the combination of a broad

spectrum antibiotic drug streptomycin showed synergistic inhibitory effect. RB-AgNPs formed significant inhibition zones (16–20 mm) against all the bacteria at a concentration of 25 µg/mL. Further increase in the concentration from 25 to 50 µg/mL significantly increases the inhibition zones (18–24 mm). Combination of a streptomycin and RB-AgNPs showed synergistic effect (19–27 mm). The combination of antibiotic and RB-AgNPs showed 1.5 to 2.0 fold high inhibition compared to RB-AgNPs alone. Thus the RB-AgNPs were evaluated as effective antimicrobial agents against Gram-positive and Gram-negative bacteria. Antimicrobial activity of the RB-AgNPs could be due to three reasons viz., firstly, flavonoids and polyphenols involved in the biosynthesis impart antimicrobial nature of AgNPs, secondly innate antimicrobial nature of silver imparts

Fig. 7 **a** Particle size distribution and **b** zeta potential measurement of RB-AgNPs

Fig. 8 In vitro antimicrobial activity assay of RB-AgNPs against different bacterial organisms of Gram-positive and Gram-negative **a** *Bacillus subtilis*, **b** *Staphylococcus aureus*, **c** *Klebsiella pneumonia*, and **d** *Escherichia coli*

Table 1 Antimicrobial activity of RB-AgNPs against different bacteria. Zone of inhibition values were represented in diameter (mm)

S. no.	Tested bacteria	Streptomycin	RB-AgNPs		RB-AgNPs + streptomycin
			25 µg/mL	50 µg/mL	
1.	<i>Bacillus subtilis</i>	21	16	18	19
2.	<i>Staphylococcus aureus</i>	20	20	22	27
3.	<i>Klebsiella pneumonia</i>	24	17	21	23
4.	<i>Escherichia coli</i>	22	20	24	27

antimicrobial nature to the biosynthesized AgNPs, and finally crystalline nature and small size of AgNPs [22–25].

Further the possible mechanism of the antibacterial activity of AgNPs was clearly explained and represented

Fig. 9 Schematic diagram represents possible mechanism of antibacterial activity of AgNPs

with schematic diagram (Fig. 9). AgNPs damage the peptidoglycan layer of the bacterial cell wall and formed the pores or pits. Through this pores, AgNPs enter inside the bacterial cell and exhibit their action. AgNPs interact with bacterial DNA and leads to fragmentation of DNA. As AgNPs interact with bacterial DNA, replication/division of bacteria doesn't occur [45]. Further AgNPs interact with major proteins or enzymes of bacteria and leads to their denaturation [46]. AgNPs interact with ribosomal subunit complex (containing small subunit, large subunit and mRNA) and leads to destabilization (dissociation of ribosomal subunits and degradation of mRNA) [47]. AgNPs enter into matrix through mitochondrial membrane and halt electron transport chain, oxidative phosphorylation and ATP synthesis. AgNPs produced ROS and causes oxidative stress lead to death of bacteria [48]. Cytoplasmic contents such as ions or important molecules come out of the cell membrane. Leakage of important ions/molecules causes change in osmosis of cell and lead to lysis of cell. Further leakage of ions causes change in membrane electric potential or proton motive force [49]. All of these synergistic effects ultimately lead to death of bacteria. The results of the present study are in consistence with previous reports. *I. barberi* leaf synthesized AgNPs showed

antibacterial activity against both Gram-negative (*E. coli*, and *K. pneumonia*) and Gram-positive bacteria (*S. aureus* and *B. subtilis*) [25]. *Rhynchosia heynei* leaf synthesized AgNPs showed antibacterial activity against both Gram-negative (*E. coli*, and *K. pneumonia*) and Gram-positive bacteria (*S. aureus* and *B. subtilis*) [50]. Similarly *Mimusops elengi* leaf synthesized AgNPs exhibited effective antimicrobial activity against both Gram-negative and Gram-positive bacteria [20]. Leaf synthesized AgNPs using *Helicteris isora* also showed antibacterial activity against both Gram-negative and Gram-positive bacteria [22].

3.7.2 Antioxidant activity of RB-AgNPs

In vitro antioxidant activity of the biofabricated RB-AgNPs was measured by DPPH and H_2O_2 RSA (Fig. 10a, b). RSA of the RB-AgNPs was increased with increasing in concentration. RB-AgNPs exhibited maximum scavenging activity of 62.59 and 53.14% against DPPH and H_2O_2 radicals respectively at the highest concentration of 100 $\mu\text{g/mL}$ used in this assay. While RB-LE exhibited maximum RSA of 42.1 and 39.45% against DPPH and H_2O_2 radicals respectively. The IC_{50} values of RB-AgNPs against DPPH and H_2O_2 radicals were found to be 69.45 and 87.43 $\mu\text{g/mL}$ respectively.

Fig. 10 In vitro antioxidant activity assay of RB-AgNPs **a** DPPH radical scavenging activity and **b** H₂O₂ radical scavenging activity of RB-AgNPs

The IC₅₀ values of RB-LE against DPPH and H₂O₂ radicals were found to be 130.98 and 137.46 µg/mL respectively. The lower IC₅₀ value indicates the higher RSA of RB-AgNPs. It indicates that RB-AgNPs showed 1.5 to 2 fold higher RSA compared to RB-LE. Hence in this study, the biosynthesized RB-AgNPs were proved to be effective scavengers. Reactive oxygen species (ROS) like hydroxyl radicals, epoxy radicals, peroxy nitrile, superoxide and singlet oxygen causes oxidative stress which could be linked to atherosclerosis, inflammation, neurodegenerative disorders, aging and cancer etc. Antioxidants are the compounds which protect cells against various cellular damaging effects of oxidative stress caused by ROS. Scientists focus on the natural antioxidants due to side effects and high cost of synthetic antioxidants. Plant compounds such as polyphenols, flavonoids and glycosides are proved to be effective antioxidants. These compounds (polyphenols, flavonoids and glycosides) participated in the synthesis of metallic nanoparticles. These compounds contribute antioxidant property to the metallic nanoparticles [22, 25–27]. The results of the present study are in consistence with previous reports. *Rhynchosia heynei* leaf synthesized AgNPs showed in vitro antioxidant activity against DPPH and H₂O₂ radicals with maximum inhibition of 57 and 52% respectively [50]. *Vigna mungo* seed hull extract synthesized AgNPs showed significant DPPH scavenging activity with 75.53% inhibition [51]. *Punica granatum* leaf synthesized AgNPs showed DPPH scavenging activity with IC₅₀ value of 67.1 µg/mL [52]. Similar kinds of results have been reported from *I. barberi* leaf synthesized AgNPs. *I. barberi* leaf synthesized AgNPs showed inhibition of 63.45 and 60.11% respectively

Fig. 11 In vitro anticancer activity assay of RB-AgNPs against different human cancer cell lines including colon cancer (COLO205), prostate cancer (PC3) and breast cancer (MCF7) at different concentrations. Increase in the concentration of RB-AgNPs showed decrease in the cell viability of cancer cells

against DPPH and H₂O₂ radicals [25]. The excellent radical scavenging activity of AgNPs allowed them to be used as antioxidants and ingredients of antioxidant formulations in the biomedical and pharmaceutical fields.

3.7.3 In vitro assessment of anticancer activity of RB-AgNPs

In the present study RB-AgNPs showed dose dependant increase in cytotoxicity against human cancer cell lines

including COLO205, PC3 and MCF7 (Fig. 11). RB-AgNPs showed the maximum inhibition of 63.6, 69.2 and 65.9% against COLO205, PC3 and MCF7 cells respectively. PC3 cells are slightly more susceptible than COLO205 and MCF7 cells. IC_{50} concentrations (inhibition of cell viability by 50%) for COLO205, PC3 and MCF7 were found to be 136.6, 118.3 and 132.4 $\mu\text{g/mL}$ respectively. Hence it was clear that the biosynthesized RB-AgNPs are potent cytotoxic agents against human cancer cell lines. The anticancer activity of the RB-AgNPs could be due to flavonoids and polyphenols present in the leaf extract of *R. beddomei*. Further the ultra small size of AgNPs has added advantage that small sized AgNPs can easily penetrate into the tumor matrix. As a result they can completely eradicate the cancer cells. There are some reports on the anticancer activity of AgNPs synthesized from different plant extracts. *Cassia fistula* flower extract synthesized AgNPs showed cytotoxic effect on MCF-7 cells with IC_{50} value of 7.19 $\mu\text{g mL}^{-1}$ [21]. *Helicteres isora* stem bark synthesized AgNPs showed effective antiproliferative activity against oral carcinoma cells with IC_{50} value of 70 $\mu\text{g mL}^{-1}$ [22]. *Clerodendrum phlomidis* leaf synthesized AgNPs exhibited anticancer activities against human colorectal adenocarcinoma (HT 29) and Ehrlich ascites carcinoma (EAC) cell lines with 84.91 and 91.84 inhibition [26]. *Rhynchosia heynei* leaf synthesized AgNPs showed effective anticancer activity against different human cell lines including COLO205 and A549 with IC_{50} values of 38.14 and 86.8 $\mu\text{g/mL}$ respectively [50]. Similarly *Ola scandens* leaf synthesized AgNPs showed anticancer activity against A549, B16 and MCF-7 with IC_{50} values between 3 and 30 μM [28]. Based on this results it is evident that biosynthesized AgNPs are cytotoxic to cancer cells and they can be employed as future anticancer agents or drug delivery systems.

4 Conclusion

The present study has successfully represented a simple, eco-friendly and efficient approach for the biofabrication of RB-AgNPs by leaf aqueous extract of *R. beddomei*. The leaf extract of RB-AgNPs act as both bioreduction and capping agent in this approach. FTIR analysis revealed that phytocompounds such as polyphenols, flavonoids and proteins involved in the bioreduction and stabilization of RB-AgNPs. The TEM micrographs showed that the biofabricated RB-AgNPs were crystalline in nature, spherical in shape, 20–80 nm in size. XRD characterization revealed that RB-AgNPs were FCC crystals with crystallite size of 9.36 nm. Average hydrodynamic radius of 46.5 nm, zeta potential measurements value of -27.6 mV and poly dispersity index (PI) of 0.203 were carried out using DLS technique. Biofabricated RB-AgNPs showed

good antioxidant activity assay with IC_{50} values of 69.45 and 87.43 $\mu\text{g/mL}$ respectively against DPPH and H_2O_2 free radicals. RB-AgNPs showed significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. RB-AgNPs were further proved to possess potent cytotoxic activity against human cancer cell lines including COLO205, PC3 and MCF7. We conclude that biofabricated RB-AgNPs were proved their excellent biomedical importance by exhibiting significant bactericidal, free radical scavenging and anticancer activity properties.

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Author's contribution All the work was done by S. Soneya under the guidance of K. V. Saritha. Manuscript was written and corrected by K. V. Saritha.

Compliance with ethical standards

Conflict of interest All the authors declared that they are no personal/financial conflict of interest.

Ethics statement No animals were used in this present study.

Consent for publication All the authors approved and consent for publication.

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