Biogenic synthesis of silver nanoparticles using *Priva cordifolia* leaf extract (PC@AgNPs) a potent antioxidant, antibacterial and catalytic activity

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Abstract

A nanoparticle preparation (PC@AgNPs), synthesized biogenically using *Priva cordifolia* leaf extract was found to possess quenching property of stable DPPH and ABTS in dose dependent manner. The PC@AgNPs showed well-established physicochemical stability with respect to temperature, P^H and salinity. It showed a broad bactericidal effect against 10 pathogenic bacteria, notably *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri* and *Vibrio parahaemolyticus*. Its non-toxicity up to 500 µg mL⁻¹ concentration to peripheral blood mononuclear cells was recorded. However, its toxic effect against human lung carcinoma cells and human breast adenocarcinoma cell lines at a dose of 500 and 300 µg mL⁻¹ respectively was clearly evident. The catalytic efficiency of PC@AgNPs was demonstrated in methylene blue and methyl orange dye degradation. The studies have suggested possible applications of PC@AgNPs in future biomedical, pharmaceutical, and environmental applications.

Keywords *Priva cordifolia* · GCMS analysis · PC@AgNPs · Antioxidant · Antibacterial activity · Cytotoxicity

1 Introduction

Nowadays, green approach for nanoparticle synthesis becomes a safe and tuning protocol to increases or decreases the efficacy of the biological function which is applied through microbiology, biotechnology and Nanoscience using nanotechnology. Nanoparticle synthesizing using inorganic material hugely and also by microbes and plants. The nanotechnology defines the material of 1–100 nm in size which is having tremendous physicochemical and functional properties different from bulk material which is used for multi-domains of science

including packaging, coating, cosmetics, electronics, medicine and in biotechnology also etc. [1, 2].

Many types of nanoparticles were reported by researches including Au, Ag, Pd and Pt synthesized through physical, chemical, and biological methods (supplementary Table 1) and also can be synthesizable using stabilizing agents, thermal decomposition in an organic solvents, photoreduction, and radiation methods. Mainly these methods are costly involves toxic and hazardous chemicals which create an environmental and biological risk [3] but, chemical methods popular due to its yield efficacy [4]. To synthesize silver nanoparticles (AgNPs) a required chemical reducing agent to convert Ag to

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AgNPs but, requires hazardous chemicals giving less bio-compatible AgNPs for the biological function. In a physical method, requires high energy and pressure for the reaction [5] and have their own limitations like expensive, unsuitable for ecosystem [6].

Nanoparticles are synthesized employing bacteria [7, 8], enzyme [9], fungi [10] and plant extracts [11–13]. Synthesizing from biological protocol gained tremendous momentum having nontoxic and environmentally safe. Among many metals, synthesizing silver nanoparticle proved its tremendous applications in medicine and industries due to its microbial effect such as antifungal, antibacterial, antiparasitic, and larvicidal properties. Due to its may unrevealed benefits to human, need to process and tune the synthesis and access its properties of silver nanoparticles [14–16] including in the field of degenerative disease and cancer biology [17]. Many unrevealed plant components and its characteristics induce focused research scientists to look into that using the antioxidant character as basic property linked to many other properties such as anti-mutagenic, anti-carcinogenic, and anti-allergic [18]. The property of the antioxidant due to the redox potential of the phytoconstituents [19], which involved in the quenching singlet and triplet oxygen, decompose peroxidized or neutralizing the generated free radicals. Therefore, it is hypothesized that higher nanoparticles (NPs) activity depends on the antioxidant property which is come from adsorbing antioxidant molecule from the plant extract onto the surface of the nanoparticles to drive this property was exportable.

The silver nanoparticles are known to possess the inhibitory effect on various bacterial strains involved in causing minor to major health risk globally [20]. Therefore, in medicines, silver and silver nanoparticles will be advantageous especially in skin ointments and creams containing silver to prevent infection of burns and open wounds [21]. In the green synthesis of NPs using plant has advantageous such as easy availability, cost-effectiveness, safety, broad availability of metabolites, as well as the extract act both as reducing and capping agents. The phytochemicals adsorbed on NPs will have increased stability, agglomeration, enhanced activity and broad biological properties.

Priva cordifolia (L.f). Druce is an ethno medicinal plant indicated as useful for the treatment of wounds [22], diarrhea (<https://www.giz.de/expertise/downloads/giz2014-en-aids.pdf>) and migraine (<http://opendata.keystone-foundation.org/priva-cordifolia-l-f-druce>). *P. cordifolia* leaf extract have biological macromolecules viz, polyphenols, flavonoids, steroids, saponins and Phytosterol were responsible for reducing silver nitrate and acting as a capping agent to synthesize PC@AgNPs within 10 min of reaction time without any hazardous bi-product chemicals. Lawrence et al. [23] have reported the plant *P.*

cordifolia assisted synthesis of silver nanoparticles employing water extract of the plants leaves without any detailed on the characteristics or data on application potential of the nanoparticles.

Further details on the physic-chemical and biological attributes of *P. cordifolia*-assisted silver nanoparticles were reported by the present authors [24]. It was considered necessary to obtain extra information to substantiate the potential of the nanoparticle preparation in biomedical, pharmaceutical and environmental and the data obtained are presented in this article.

2 Materials and methods

2.1 Chemicals and bacterial strains

All the chemicals used in this study were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), silver nitrate, Tris-buffer, and butylated hydroxytoluene (BHT) were purchased from Merck, Mumbai, India. Dimethyl sulfoxide (DMSO) was procured from Hi-media Mumbai, India. All lyophilized bacterial strains were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India and American Type Culture Collection (ATCC). *Enterobacter faecalis*-439, *Escherichia coli*-1610, *Salmonella typhimurium*-98, *Shigella flexneri*-1457, *Vibrio cholera*-3904, *Vibrio parahaemolyticus*-451, *Pseudomonas aeruginosa*-1688, *Staphylococcus aureus*-96, *Bacillus cereus*-430, *Enterobacter aerogenes*-13048 and strains were cultured in recommended broth as per the revival procedure provided by MTCC and ATCC. Cell lines Hela (Human cervical cancer), peripheral blood mononuclear cell (PBMC) and A549 (Human lung carcinoma cells) were purchased from the National Centre for Cellular Sciences, Pune, India.

2.2 Preparation of *Priva cordifolia* leaf extract

25 g of dried leaves were ground to a fine powder and refluxed with 225 mL of methanol for 5 h. Then the extract was filtered with Whatman paper and evaporated to dry paste was used in the analysis.

2.3 Qualitative and quantification phytochemical analysis

The preliminary assessment of the extracted phytochemicals was performed by the methods of Harbor [25] to test for the presence of steroids, carbohydrates, alkaloids, flavonoids, and saponins.

2.4 Phytochemical characterization by using GCMS

The presence of phytochemicals in the plant extract was characterized by gas chromatography with mass spectra (Thermo scientific ITQ 900) system interfaced with a TG-95 MS column (5% diphenyl/95% dimethyl polysiloxane, 30 × 0.25 mm ID × 0.25). Helium gas (99.999%) was used as a carrier with pragmatic 70 eV ionizing energy in an electron ionization with a flow rate of 1 mL/min. 2 µL of the sample was injected to system with a split ratio of 10:1. The GC injector temperature was set to 250 °C, ion source temperature was kept at 200 °C. Oven temperature was preset to rise from 60 to 200 °C at 5 °C/min, and then to 280 °C at 10 °C/min and it stable for 9 min. The mass spectra of fragmented compounds were measured from 45 to 450 Da. The entire run time of programmer was 36 min. The peaks were compared with known spectra with a database of National Institute of Standards and Technology.

2.5 Synthesis and characterization of PC@AgNPs

The synthesized PC@AgNPs was confirmed by the absorption spectrum obtained using Shimadzu UV–visible spectrometer. The PC@AgNPs was detail characterized by FTIR, SEM, TEM, EDX, DLS and XRD in a previous report and same material was used for further studies [24].

2.6 Physicochemical interaction of PC@AgNPs

The physicochemical interaction of synthesized PC@AgNPs was amply studied with respect to ionic strength, pH and temperature [26]. The salinity interaction of PC@AgNPs was studied from 0.2 to 1 M at a constant pH of 7.0 and the colloidal solution was measured by UV–Vis spectroscopy. The consistency of PC@AgNPs with respect to temperature and P^H were monitored in the range of 30–90 °C and 2–10 respectively.

2.7 Colloidal stability

100 µg mL⁻¹ of synthesized PC@AgNPs was dissolved in Millipore water and the colloidal stability was monitored by using UV–Vis spectroscopy at 415 nm at different interval of time.

2.8 Determination of antioxidant activity

2.8.1 DPPH radical scavenging activity

The free radical quenching property of PCE and PC@AgNPs was measured by using stable free radical chemical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in accordance with Phull [27]. Briefly, different concentration of PCE and

PC@AgNPs was mixed 2 mL of methanolic DPPH solution (40 mg^{-L}) and 1 mL of 50 mM tris HCl. Ascorbic acid and BHT were used as a positive control and distilled water as a negative control. The reaction mixture was incubated at room temperature in dark for 30 min and absorbance was recorded at 517 nm. All the experiments were carried out in triplicates. The percentage of free radical quenching property was calculated as follows.

$$\% \text{ free radical quenching} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \right] \times 100$$

2.8.2 ABTS radical scavenging activity

The free radical quenching property of PCE and PC@AgNPs was measured by using stable chemical 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in accordance with Arnao [28]. Briefly, ABTS stock solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium per sulfite in methanol and incubated in a dark at room temperature. The working solution of ABTS was prepared by diluting the ABTS stock solution to 0.7 absorbance at 734 nm by using UV–vis spectrometer. Different concentration of 50 µL of PCE and PC@AgNPs was mixed with 2.95 mL of ABTS working solution. Absorbance was recorded after 30 min of incubation in a dark at 734 nm. Ascorbic and BHT were used as a positive control. All the experiments were carried out in triplicates. The percentage of free radical quenching property was calculated as follows.

$$\% \text{ free radical quenching} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \right] \times 100$$

2.9 Antibacterial activity of PC@AgNPs

The antibacterial activity was tested by using disc diffusion method according to Ghanwate [29] with minor changes to work out the PC@AgNPs susceptibility. The bacterial cultures were prepared from the overnight culture and 1 × 10⁷ CFU mL⁻¹ cells and inoculated on to nutrient agar, then sterile disc (6 mm) was loaded with 5 µL of different serially diluted PC@AgNPs (1, 2, 3, 4, 5 and 10 mg disc⁻¹). The sterile saline water used as negative control and Streptomycin (10 µg disc⁻¹) as a positive control. The plates were inverted incubated at 37 °C for 24 h to examine the zone of inhibition (ZOI).

2.9.1 Minimum inhibitory concentration by resazurin assay

The resazurin solution was prepared by 270 mg of powder dissolved in 40 mL of sterile water. 100 µL of sterile Baird Parker broth was dispensed in 96 well [30]. 50 µL of

different concentration of plant extract (1 to 5 mg mL⁻¹) and PC@AgNPs (10, 20, 40, 60, 80 & 100 µg mL⁻¹) dissolved in DMSO was mixed with media. The tips were discarded and freshly used for each transfer. To each well had a 10 µL of resazurin indicator solution. Finally, 10 µL of bacterial suspension (5 × 10⁶ cfu mL⁻¹) was added to each well to achieve a concentration of 5 × 10⁵ cfu mL⁻¹. A broad-spectrum of antibiotic used as positive control Ciprofloxacin and Tetracycline (1 mg mL⁻¹). A column with all solutions and exception of the test compound (positive control) and a column with all solutions without the addition of bacterial culture (negative control) instead of 10 µL of nutrient broth were added. Each plate was wrapped loosely with cling film to ensure that culture media did not become dehydrated and incubate at 37 °C incubator for 24 h.

2.10 Cell membrane damage study by SEM

The *S. aureus* and *E. coli* culture were used as model organisms and the cell membrane damage was studied by treating with minimum inhibitory concentration (MIC) of PC@AgNPs for 2 h. The treated culture was centrifuge at 1000 rpm for 5 min. Finally, cells were smeared on a glass slide with help of 2.5% glutaraldehyde in phosphate buffer solution. The deposited cells treated with 30–100% ethanol in stepwise to dry and SEM images were taken after 2 days [31].

2.11 Nano-toxicity of PC@AgNPs

The Peripheral blood mononuclear cells (PBMC), Human breast adenocarcinoma cells (MCF-7) and Human lung carcinoma cells (A549) cell lines were used to examine the toxic effect of PCE and PC@AgNPs. Prior to starting the experiment all the cell lines were cultured in Dulbecco's Modified Eagle's medium (DMEM) in media, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 1 mM NaHCO₃, 100 µg mL⁻¹ streptomycin and 100 units mL⁻¹ penicillin. All the cell cultures were incubated at 37 °C in 5% CO₂ incubator.

2.11.1 Cytotoxicity by MTT assay

The cytotoxicity effect of PCE and PC@AgNPs was measured by MTT assay in accordance with Mossaman [32]. Briefly, 100 µL of culture medium was dispensed in 96 well plates and all cell lines were inoculated to the well at 1 × 10⁵ cells mL⁻¹ density. The different concentration of PCE and PC@AgNPs (0–1000 µg mL⁻¹) and 10 µL of MTT (5 mg mL⁻¹) per well were mixed and incubated at 37 °C for additional 3 h at 37 °C in 5% CO₂ incubator for 24 h. The

intensity of culture solution was measured at 540 nm with the ELISA micro plate reader (Spectra MAX Plus; Molecular Devices; supported by SOFT max PRO-5.4). The percent of cell viability was determined with reference to the control (without test compound). Assay performed in triplicates and repeated thrice.

2.12 Catalytic degradation of methyl orange and methylene blue

The catalytic activity of green syntheses PC@AgNPs was assessed to measure the degradation potency of methyl orange and methylene blue dyes by using sodium borohydride [33]. A stock solution (100 ppm) was prepared by dissolve the dye in Millipore water. 10 mL of dye was dispensed in a beaker, and then treated with different concentration of synthesized PC@AgNPs. 1 mL (1 g in 10 mL) of NaBH₄ was mixed with dye at room temperature. The dye degradation was measured at λ_{max} 460 and 664 for MO and MB respectively at every 5 min intervals. The percentage of dye degradation was calculated by using the formula.

$$C(\%) = [A_0 - A_t] / A_0 \times 100$$

where A₀ = Absorbance of untreated dye, A_t = Absorbance of treated dye with PC@AgNPs at different interval is time and C = percentage of dye degradation.

3 Result and discussion

3.1 Phytochemical data

The preliminary study of leaves methanolic extract of *Priva cordifolia* showed the presence of phenolics, flavonoids, steroids and saponins (Supplementary Table 2). Therefore, we have chosen the methanolic extract for the synthesis of PC@AgNPs.

3.2 GC–MS data

The data on GC–MS analysis of the methanolic extract of *Priva cordifolia* leaves was given in supplementary figures 1 and 2. The 3 predominant peaks were found in the plant extract viz, Decanedioic acid bis (10-ethoxycarbonyldecyl) ester (RT-22.85), Docosahexaenoic acid, 1,2,3-propanetriyl ester (RT-28.83) and beta-Sitosterol (RT-45.85). It clearly indicated the presence of a variety of bioactive compounds with different retention times (RT). The compounds identified by their RT, molecular weight, molecular

formulas, structure are illustrated in Supplementary Tables 3 and 4, along with their, summarizes the nature of the identified compounds and their biological activities. Therefore, we opted this extract to increase its therapeutic activity by synthesizing the PC@AgNPs.

3.3 Synthesis and characterization of PC@AgNPs

The PC@AgNPs was synthesized by adding plant methanolic extract as reducing agent to 1 mM AgNO₃. The development of the nanoparticles was evident by altering the color of the solution from the yellowish color to brown color. This clearly confirmed that the plant extract contains polyphenols, flavonoids, saponins, carotenoids and

Phytosterol were responsible for reduced and acting as a capping agent (Supplementary Table 2). The development of colloidal PC@AgNPs was confirmed by UV–Vis spectral pattern. The dispersed PC@AgNPs showed maximum absorption at 415 nm after 10 min of reaction and also retained the stability, shape, point of absorption band even after a month (Fig. 1).

3.4 Physicochemical interaction of PC@AgNPs

The biological applications of synthesized nanoparticles depend on size, surface chemistry and silver ions release. Decreases in the stability of nanoparticles are a result of self-aggregation of the materials and this reduction finally affects overall function. Due to the reduction in the stability, biological properties such as cellular uptake, bio-availability. Therefore, it is very essential to characterize the influence of factors such as pH, ionic strength and temperature on nanoparticles.

The pH effect on PC@AgNPs stability was investigated with different pH ranges from 2 to 10. The data indicated that PC@AgNPs was most stable with high pH at 10 as evidenced by the absorbance at 415 nm (Fig. 2a). The ionic strength of PC@AgNPs was evaluated by dissolved in PBS with 0.2–1 M NaCl and absorbance was measured in UV–Vis spectra. PC@AgNPs has a characteristic peak at 415 nm. The data indicated PC@AgNPs was stable up to 0.2 M and beyond this concentration broaden the peak (Fig. 2b). Finally, effect of temperature was investigated in the temperature range of 30–90 °C. PC@AgNPs was stable in the temperature range of 35–75 °C (Fig. 2c) [26].

Fig. 1 UV–Vis spectra of *P. cordifolia* leaf silver nanoparticles (PC@AgNPs). Bio-synthesized PC@AgNPs was dissolved in the Millipore water and absorbance readings measured from 300 to 1000 nm wave length ranges. The observed λ max band at 415 nm for PC@AgNPs and symmetry of relative stability after 1 month

Fig. 2 Physicochemical interaction of PC@AgNPs. **a** The UV-absorbance of PC@AgNPs suspension of various pH values. **b** The ionic strength of PC@AgNPs at different concentrations of NaCl at con-

stant pH 7.0 and at different temperature variation on PC@AgNPs at pH 7.0 was estimated (**c**)

Fig. 3 DPPH and ABTS radical scavenging activity of PCE and PC@AgNPs. **a** The data represented dose dependent DPPH radical scavenging activity with IC_{50} value of Vit-C, BHT, PCE and PC@AgNPs at the concentration of 5.02 ± 0.08 , 27.9 ± 0.10 , 49.02 ± 0.24 and

$43.63 \pm 0.18 \mu\text{g mL}^{-1}$ respectively. **b** Indicated ABTS radical scavenging activity with IC_{50} value of Vit-C, BHT, PCE and PC@AgNPs at the concentration of 5.3 ± 0.08 , 7.37 ± 0.06 , 114.40 ± 0.60 and $77.04 \pm 0.32 \mu\text{g mL}^{-1}$ respectively

Fig. 4 Antibacterial activity of PC@AgNPs against pathogenic bacteria. The disc diffusion assay was done to measure the MIC values of PC@AgNPs against disease causing pathogens. **a** Typical plate displaying concentration of PC@AgNPs correspondingly added in

tested plates (N: Negative control; Positive control: PC@AgNPs at 1 (1); 2 (2); 3 (3); 4 (4); 5 (5) and 10 (6) mg disc⁻¹). **b** *S. aureus*; **c** *B. cereus*; **d** *E. faecalis*; **e** *E. aerogens*; **f** *E. coli*; **g** *P. aeruginosa*; **h** *S. typhimurium*; **i** *S. flexneri*; **j** *V. cholera* and **k** *V. parahaemolyticus*

3.5 DPPH radical scavenging activity of analysis of PC@AgNPs

The potent antioxidant property of PCE and PC@AgNPs were estimated by DPPH radical scavenging activity. The dose dependent DPPH activity of PC@AgNPs were observed and showed DPPH radical (purple) into neutralized form (yellow) which is statistically significant ($P \leq 0.05$) represented in Fig. 3a. The IC_{50} values of PCE and PC@AgNPs are 49.02 ± 0.24 and $43.63 \pm 0.18 \mu\text{g mL}^{-1}$ respectively compared to standard ascorbic acid and BHT (IC_{50} value is 5.02 ± 0.08

and $27.90 \pm 0.10 \mu\text{g mL}^{-1}$). With increasing concentration of PC@AgNPs change in color was observed due to scavenging of DPPH radical by donating a hydrogen atom to neutralize the reactive DPPH molecule. This shows a responsible decrease in absorbance at 517 nm and it is attributed due to the functional groups of PC adhered to NPs present in the PC@AgNPs. The data indicated the scavenging activity was increased to $5.39 \pm 0.08 \mu\text{g mL}^{-1}$ after the silver was reduced by the plant extract.

The free radicle quenching of PCE and PC@AgNPs were estimated by another stable chemical ABTS assay.

Table 1 Antibacterial activity of plant extract and PC@AgNPs against commonly encounter food pathogens

Test microorganisms	Zone of inhibition in mm							Standard streptomycin 10 µg	Nega- tive control
	1 mg mL ⁻¹	2 mg mL ⁻¹	3 mg mL ⁻¹	4 mg mL ⁻¹	5 mg mL ⁻¹	10 mg mL ⁻¹			
Gram-positive									
<i>Staphylococcus aureus</i>	4.21 ± 0.04 ^c	4.42 ± 0.03 ^d	5.20 ± 0.01 ^c	5.80 ± 0.03 ^d	6.23 ± 0.01 ^c	9.38 ± 0.03 ^a	10.95 ± 0.01 ^e	NI	
<i>Bacillus cereus</i>	4.62 ± 0.01 ^c	4.84 ± 0.02 ^d	5.34 ± 0.01 ^c	5.82 ± 0.02 ^d	7.42 ± 0.01 ^b	8.60 ± 0.02 ^b	14.33 ± 0.03 ^a	NI	
<i>Enterobacter faecalis</i>	4.20 ± 0.02 ^c	4.36 ± 0.03 ^d	4.68 ± 0.04 ^d	4.88 ± 0.03	4.98 ± 0.02	5.20 ± 0.01 ^d	12.06 ± 0.02 ^c	NI	
Gram-negative									
<i>Eentrobacter aerogenes</i>	4.32 ± 0.02 ^c	4.64 ± 0.04 ^d	5.32 ± 0.03 ^c	5.82 ± 0.02 ^d	7.10 ± 0.03 ^b	8.22 ± 0.04 ^b	11.10 ± 0.04 ^d	NI	
<i>Escherischia coli</i>	5.82 ± 0.03 ^b	6.10 ± 0.03 ^b	6.46 ± 0.03 ^b	6.80 ± 0.03 ^c	7.80 ± 0.04 ^b	9.20 ± 0.04 ^a	13.03 ± 0.03 ^b	NI	
<i>Psuedomonas aeruginosa</i>	4.62 ± 0.01 ^c	4.88 ± 0.20 ^d	4.96 ± 0.04 ^d	5.44 ± 0.01 ^d	5.90 ± 0.02 ^d	7.60 ± 0.02 ^c	12.57 ± 0.04 ^c	NI	
<i>Salmonella typhimurium</i>	4.26 ± 0.01 ^c	5.42 ± 0.04 ^c	6.20 ± 0.03 ^b	6.64 ± 0.04 ^c	7.22 ± 0.01 ^b	8.50 ± 0.03 ^b	10.50 ± 0.01 ^e	NI	
<i>Shigella flexneri</i>	6.62 ± 0.02 ^a	7.46 ± 0.01 ^a	7.82 ± 0.01 ^a	8.10 ± 0.02 ^a	8.62 ± 0.04 ^a	9.60 ± 0.01 ^a	13.40 ± 0.03 ^b	NI	
<i>Vibrio cholera</i>	5.10 ± 0.02 ^b	5.82 ± 0.02 ^c	6.22 ± 0.01 ^b	6.46 ± 0.02 ^c	6.78 ± 0.03 ^c	8.46 ± 0.03 ^b	08.27 ± 0.01 ^f	NI	
<i>Vibrio parahaemolyticus</i>	6.21 ± 0.02 ^a	7.16 ± 0.01 ^a	7.60 ± 0.04 ^a	7.96 ± 0.04 ^b	8.42 ± 0.01 ^a	9.10 ± 0.02 ^a	12.06 ± 0.02 ^c	NI	

Values are means of three independent replicates ($n=3$). $\pm$ Indicate standard errors. Means followed by the same letter(s) within the same column are not significantly ($P \leq 0.05$) different according to Turkey's HSD

NI no inhibition

Table 2 Minimum inhibitory concentration of plant extract and PC@AgNPs against commonly encountered food pathogens

Name of pathogenic bacteria	MIC of PCE (mg mL^{-1})	MIC of PC@AgNPs ($\mu\text{g mL}^{-1}$)	Ciprofloxacin MIC value ($\mu\text{g mL}^{-1}$)	Tetracycline MIC value ($\mu\text{g mL}^{-1}$)
Gram-positive				
<i>Staphylococcus aureus</i>	1.0 ± 0.04^a	100.0 ± 2.40^c	10.0 ± 0.86^e	10.0 ± 0.76^e
<i>Bacillus cereus</i>	10.0 ± 0.74^b	40.0 ± 0.96^b	2.0 ± 0.50^a	2.0 ± 0.15^a
<i>Enterococcus faecalis</i>	15.0 ± 0.98^c	40.0 ± 1.20^b	12.0 ± 1.00^f	10.0 ± 0.70^e
Gram-negative				
<i>Enterobacter aerogenes</i>	15.0 ± 1.20^c	50.0 ± 1.85^c	10.0 ± 0.84^e	10.0 ± 0.66^e
<i>Escherichia coli</i>	15.0 ± 1.17^c	40.0 ± 1.62^b	10.0 ± 0.62^e	10.0 ± 0.80^e
<i>Pseudomonas aeruginosa</i>	10.0 ± 1.80^b	40.0 ± 1.84^b	6.0 ± 0.44^d	7.0 ± 0.56^d
<i>Salmonella typhimurium</i>	15.0 ± 1.64^c	40.0 ± 1.74^b	5.0 ± 0.38^c	6.0 ± 0.48^c
<i>Shigella flexneri</i>	10.0 ± 0.84^b	30.0 ± 1.10^a	3.0 ± 0.28^b	3.0 ± 0.29^b
<i>Vibrio cholera</i>	10.0 ± 0.74^b	40.0 ± 1.50^b	3.0 ± 0.24^b	2.0 ± 0.18^a
<i>Vibrio parahaemolyticus</i>	1.0 ± 0.02^a	30.0 ± 1.25^a	3.0 ± 0.26^b	2.0 ± 0.20^a

Values are means of three independent replicates ($n=3$). $\pm$ Indicate standard errors. Means followed by the same letter(s) within the same column are not significantly ($P \leq 0.05$) different according to Turkey's HSD

Fig. 5 The SEM images showing cell membrane damage. The after treatment of nanoparticles with *S. aureus* and *E. coli* overnight of incubation exhibited the alterations in the membranes of bacteria

(**a, b**) respectively, compared to the control (without treatment). The alteration in the membrane was showed by arrow indications

The dose dependent ABTS activity of PC@AgNPs were witnessed and showed ABTS radical (blue) into neutralized form (colorless) which is statistically significant ($P \leq 0.05$) represented in Fig. 3b. The IC_{50} values of PCE and PC@AgNPs are 114.40 ± 0.80 and $74.29 \pm 0.30 \mu\text{g mL}^{-1}$ respectively compared to standard ascorbic acid and BHT (IC_{50} value is 5.3 ± 0.04 and $7.37 \pm 0.08 \mu\text{g mL}^{-1}$).

3.6 Zone of inhibition (ZOI) by PC@AgNPs against pathogenic bacteria

Currently, emergence of drug resistant microbes is a serious worldwide health issue. The PC@AgNPs shown

significant inhibition zone against bacteria compared to the standard antibiotic (Fig. 4, Table 1; Supplementary Table 5 and Fig. 3).

The high antibacterial activity showed at a concentration of 10 mg mL^{-1} against Gram-positive *S. aureus*, *B. cereus*, *E. faecalis* and Gram-negative *E. coli*, *E. aerogenes*, *S. typhimurium*, *V. cholera*, *P. aeruginosa*, *S. flexneri*, and *V. parahaemolyticus*. The PC@AgNPs having strong ZOI for *S. aureus* (9.38 ± 0.06), *E. coli* (9.20 ± 0.09), *S. flexneri* (9.60 ± 0.11) and *V. parahaemolyticus* (9.10 ± 0.13) mm clearance zone observed after overnight incubation. While no ZOI was observed for the negative control (sterile distilled water) in any case. In the resazurin assay,

Fig. 6 Cytotoxicity analysis of PC@AgNPs. The normal cell PBMC, cancerous cells (A549 and MCF-7) were treated different concentrations (0–1000 $\mu\text{g mL}^{-1}$) of PE (a) and PC@AgNPs (b) for 24 h and tested for cell viability using MTT assay. The PE indicated less toxic to PBMC up to 300 $\mu\text{g mL}^{-1}$ and showed anti-cancer activ-

ity to A549 (300 $\mu\text{g mL}^{-1}$) and MCF-7 (500 $\mu\text{g mL}^{-1}$) concentration onwards. PC@AgNPs indicated toxic above 500 $\mu\text{g mL}^{-1}$ concentrations onwards to PBMC and significantly responds to cancerous A549 (500 $\mu\text{g mL}^{-1}$) and MCF-7 (400 $\mu\text{g mL}^{-1}$)

indicated the increase of MIC concentration values after synthesis of PC@AgNPs against commonly encounter food pathogens compared to crude extract (Table 2). The antibacterial activity was dependent on particle size; shape and importantly capping agent loaded verify the important role in the bactericidal activity. The bacterial cell wall composed of thick peptidoglycan consisting of a similar type of linear polysaccharide chains cross-linked by short peptides formed into the rigid structure [35]. The plants are rich source of antioxidants containing different phytochemical involved in antioxidant activity by neutralizing reactive oxygen species or free radicals, as anti-microbials, as anti-helminthic, as anti-inflammatory, anti-diabetic, and as anti-thrombus etc. [36–38]. The high bactericidal property of cationic Ag^+ ions leading to damage the bacteria intern stops the growth of the microorganisms [36]. The possible antibacterial mechanism of metal toxicity is due to protein dysfunction, production of reactive oxygen species (ROS), depletion of antioxidants, impaired membrane function, interfere with nutrient assimilation and also be gene-toxicity. The coating of Ag^+ ions widely used in food industries medical and pharmaceutical industries [34]. The increasing of synthetic antibiotics and its improper use without knowledge, bacteria resistant and cause minor to major

health issues. This may lead to cause severe life threatening diseases worldwide. There is a need to anticipate and develop potent antimicrobial agent against multi-drug resistant microbes. The present study highlights the loading of active bactericidal PCE to synthesize PC@AgNPs and play a potent role in inhibiting the growth of both Gram-positive and Gram-negative microorganisms.

3.7 Bacterial cell damage

The bacterial cell damage was studied by SEM of *E. coli* and *S. aureus* cells treated with PC@AgNPs are presented in the Fig. 5.

The electron microscopy determined the distribution and location of the silver nanoparticles, as well as the morphology of the bacteria after treatment with silver nanoparticles. In the controlled untreated sample, the surface of bacterial cells was smooth and showed typical characters of surface of native cells, such as smooth and intact, while cells treated with NPs appeared damaged severely. Some cells showed large leakage, others mis-shapen many pits and gaps appeared in their membrane [25].

Fig. 7 Dye degradation of methyl orange (MO). **a** MO degradation capacity of PC@AgNPs at different concentration. **b** Dye degradation absorbance at 5, 10 and 15 min intervals and **c** 1 Control MO

dye. **2** MO mixed with NaBH₄ and **3–6** dye treated with different concentration of PC@AgNPs

3.8 Cytotoxicity analysis

The synthesized PC@AgNPs demonstrates no toxic effect to the non-cancerous peripheral blood mononuclear (PBMC) cells up to 500 $\mu\text{g mL}^{-1}$ (Fig. 6a). When PCE (Fig. 6a) treatment to PBMC cells in a dose dependent manner (20–1000 $\mu\text{g mL}^{-1}$) induced to kill PBMC cells at 300 $\mu\text{g mL}^{-1}$ concentration itself and started to prevent growth of lung cancer (A549) and breast cancer (MCF-7)

cells at concentrations of 300 and 500 $\mu\text{g mL}^{-1}$ respectively. Thus, a free active component of PCE was toxic to both cancerous and non-cancerous cells at higher doses. On the other hand, PC@AgNPs showed toxicity at 500, 500 and 300 $\mu\text{g mL}^{-1}$ against PBMC, A549, and MCF-7 cells respectively (Fig. 6b), indicating silver NPs with PCE active synergistic components increased the bioavailability at lower doses apart from increasing its therapeutic efficacy.

Fig. 8 Dye degradation of methyl orange (MB). **a** MB degradation capacity of PC@AgNPs at different concentration. **b** Dye degradation absorbance at 5, 10 and 15 min intervals and **c** 1 Control MB

dye. **2** MB mixed with NaBH₄ and **3–6** dye treated with different concentration of PC@AgNPs

3.9 Catalytic effect of PC@AgNPs

3.9.1 Degradation of methyl orange and methylene blue

We investigated the catalytic activity of PC@AgNPs against cationic (MB) and anionic (MO) dyes routinely used in textile, paper, printing, pharmaceutical, food

industries and research laboratories. The release of residual of untreated dye through effluent discharge and it cause potent pollution to the environment and toxic to aquatic animals. Methylene blue and methyl orange dye degradation were studied by using synthesized PC@AgNPs and monitoring the degradation capacity at every 5 min intervals. The absorbance peak of methylene blue

Table 3 Percentage degradation of dyes MO and MB in the presence of different concentration of PC@AgNPs

Volume used (mL)	Amount of NaBH ₄ added (μg)	Amount of PC@AgNPs added (μg)	Dye degradation (%)		
			After 5 min	After 10 min	After 15 min
Methyl orange (MO)					
10.0	0.0	0.0	0	0	0
10.0	100.0	0.0	0.37	2.50	7.50
10.0	100.0	250.0	13.42	95.13	97.55
10.0	100.0	500.0	47.10	95.50	97.98
10.0	100.0	750.0	71.93	95.70	98.40
10.0	100.0	1000.0	83.88	95.98	98.85
Methylene blue (MB)					
10.0	0.0	0.0	0	0	0
10.0	100.0	0.0	1.37	2.82	7.56
10.0	100.0	500.0	1.57	2.03	4.81
10.0	100.0	1000.0	2.41	5.11	4.81
10.0	100.0	1500.0	8.97	42.84	50.73
10.0	100.0	2000.0	83.38	83.42	83.55

dye in water was recorded at 664 nm and 460 nm for methylene orange. In the presence of PC@AgNPs as a catalyst, absorbance spectra of MO and MB were decreased with a varying interval of time and also the color of the dye was disappeared at higher concentration (Figs. 7, 8). 250 μg of PC@AgNPs amount is adequate to reduce the 97.50% of MO dye in 15 min time intervals and 2 mg for MB (83.55%) (Table 3). The result has shown the synthesized PC@AgNPs work more potent in the degradation of anionic dye compare to the cationic dye and excellent catalyst on the reduction of hazardous dyes [33, 39].

4 Conclusion

Of the various known methods to generate nanoparticles, the 'green' approaches using *P. cordifolia* leaf extract proved simple, but adapt to produce a functionally competent end product. The leaf extract apparently possessed appropriate reducing components to change and link silver to the right herbal factors leading to improved antibacterial properties and also possess other favorable attributes indicative of its potential for therapeutic applications, and also use in the process of degradation of hazardous dyes in an economically and ecofriendly. The present observations have provided an impetus for further detailed studies to realize, in practical terms, the said potential. However, understanding the toxicity and fate of AgNPs in ecosystems requires further investigation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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