



Antimicrobial and Antioxidant Potential of *Vernonia Cinerea* Extract Coated AuNPs

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Received: 4 May 2021 / Accepted: 18 August 2021 / Published online: 7 September 2021
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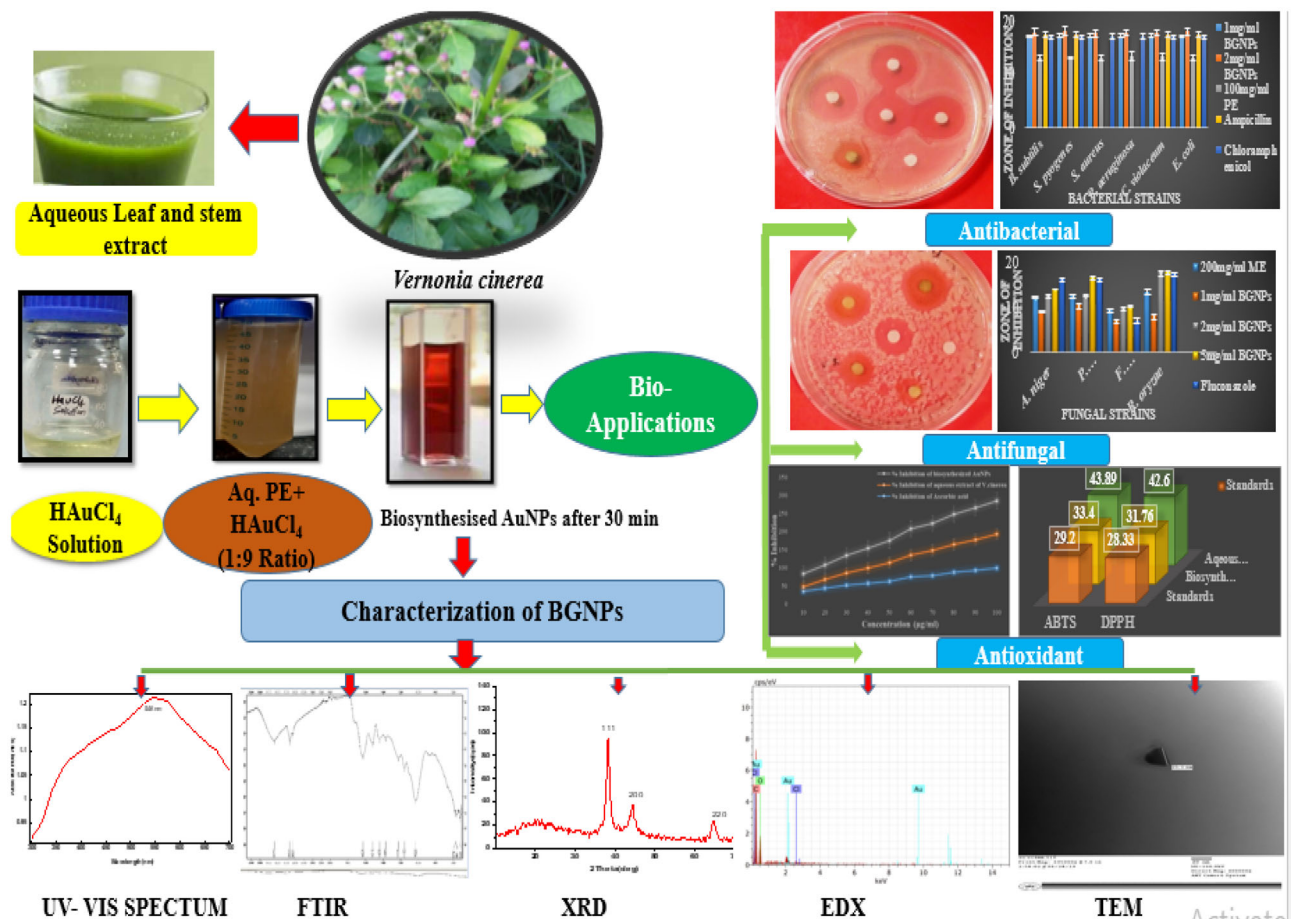
Abstract Green synthesis of nanoparticles is an important tool to reduce the harmful effects associated with traditional methods. In the present investigation, we have synthesised gold nanoparticles (AuNPs) using aqueous extract prepared from fresh aerial parts (leaf and stem) of *Vernonia cinerea* as bioreducing agent. The visual indication of change in colour from pale yellow to brown to ruby-red indicated the successful formation of the AuNPs. Characterization of nanoparticles was carried out by UV–visible spectroscopy, X-ray crystallography (XRD), Transmission electron microscopy (TEM) and Energy dispersive X-ray analysis (EDX). UV–Vis spectra showed a specific peak at

546 nm which was the initial confirmation of the biosynthesized AuNPs. TEM images showed spherical and triangular shape of AuNPs with an average size of 25 nm. From FTIR spectrum, different functional groups were identified that could be responsible for the formation, stabilization, and capping of biosynthesized AuNPs. Aqueous plant extract and biosynthesized AuNPs were separately tested for their antimicrobial activity against six bacterial strains and four fungal strains. Biosynthesized AuNPs (2 mg/ml) showed significantly high zone of inhibition against the selected bacterial strains as compared to the aqueous plant extract. Maximum zone of inhibition (18.2 mm) was observed with AuNPs against *Streptococcus pyogenes* whereas comparatively less value (12.5 mm) was recorded with the plant extract. Interestingly, the inhibitory activity observed against bacterial strains was even better than ampicillin. Antifungal activity recorded with AuNPs (5 mg/ml) was maximum (17.4 mm) against *R. oryzae* and it was higher than positive control (17.00 mm) and plant extract (13.2 mm). The present study clearly showed that AuNPs coated with *Vernonia cinerea* extract were as good as positive control in inhibiting bacterial and fungal growth. In addition, these AuNPs also showed good antioxidant potential which was comparable to ascorbic acid.

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Graphical abstract



Keywords Antifungal activity · Antimicrobial activity · Gold nanoparticles · *Vernonia cinerea*

Introduction

Nanotechnology is a developing branch of science wherein the particles extend in nanosize and display various unique properties compared to the parent material [1–3]. It is an important emerging area of research focussing on developing synthetic and biological methods of engineering nanoparticles. Noble metal-based nanomaterials have been investigated extensively for their applications in different areas of science and technology [4–6]. Among these, AuNPs are preferred over others as they are inactive and not effectively oxidized when presented to oxygen or profoundly corrosive conditions [7–9]. AuNPs are also stable with high consistency and known for biomedical applications like drug delivery, imaging, photo-thermal treatment, and pathogen detection in food and clinical samples [10–12].

Biological entities (microorganisms, yeast and plants) are significant assets for synthesis of AuNPs [13, 14]. Plant-based synthesis of AuNPs is preferred as it is safe, simple, and less tedious as compared with other biological extracts [15–17]. Plants are easily available and possess a large variety of active agents like phenols, flavonoids, terpenoids, ketones, aldehydes and saponins that promotes the reduction of AuNPs [18–20]. Moreover, synthesis of AuNPs using plant extract is environmentally benign, less lethal and more economical approach that connect plants with nanotechnology. Antimicrobial impact of AuNPs is of great interest as their interaction with surface-exposed functional groups present on the bacterial cell surface may lead to its inactivation and destruction [18, 21, 22]. AuNPs are highly effective antibacterial entities due to their less-toxic property, polyvalent effect, and photo-thermal activity [23–26]. Thus, they are one of the most promising tools to combat microbial resistance via multiple approaches [22, 27–29].

V. cinerea is widely distributed throughout India with immense value in various traditional system of medicine [30–32]. The plant has been investigated for varied pharmacological activities to validate its traditional claims, and

has been scientifically reported to possess antimicrobial, anti-inflammatory, antidiabetic, renoprotective, anticancer and antiviral activities [32–35]. Looking at the vast medicinal properties possessed by the plant, authors have attempted for the first time to synthesize AuNPs coated with extract of *V. cinerea* prepared from aerial vegetative parts and evaluate them for their antimicrobial and antioxidant properties.

Materials and Methods

Preparation of Plant Extract and Biosynthesis of Gold Nanoparticles

Fresh vegetative aerial parts (stem and leaves) of *V. cinerea* were collected from Bhiwani district of Haryana, India. After repeated washing, aerial parts were crushed in a pestle and mortar. 10 g of crushed plant material was taken in a 200 ml volumetric flask, and 100 ml of double distilled (DD) water was added. After 10 min., the plant extract was filtered through eight layered muslin cloth and then with Whatman filter paper No-1 [30]. Plant extract was dried using a rotary evaporator and stored in an airtight container at 4 °C [30, 35]. AuNPs were synthesized at room temperature (25 ± 2 °C) using aqueous plant extract of *V. cinerea* and aqueous solution of Tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) procured from Sigma-Aldrich. To 5 ml of aqueous plant extract, 45 ml of HAuCl_4 (1 mM) solution was added. Biosynthesis of AuNPs was completed within 5 min. of initiation of the reaction (Fig. 1). The change in colour from pale yellow to brown to ruby-red indicated the reduction of Au^+ to AuNPs [17, 36].

The solution was centrifuged at $10,000 \times g$ for 15 min. at room temperature. Pellet obtained was washed three

times with 5 ml DD water and lyophilized using 120,890-d, Alpha 1–2 LD plus, Martin Christ Freeze Dryer, Germany. The possible mechanism of formation of biosynthesized AuNPs is given in Fig. 2.

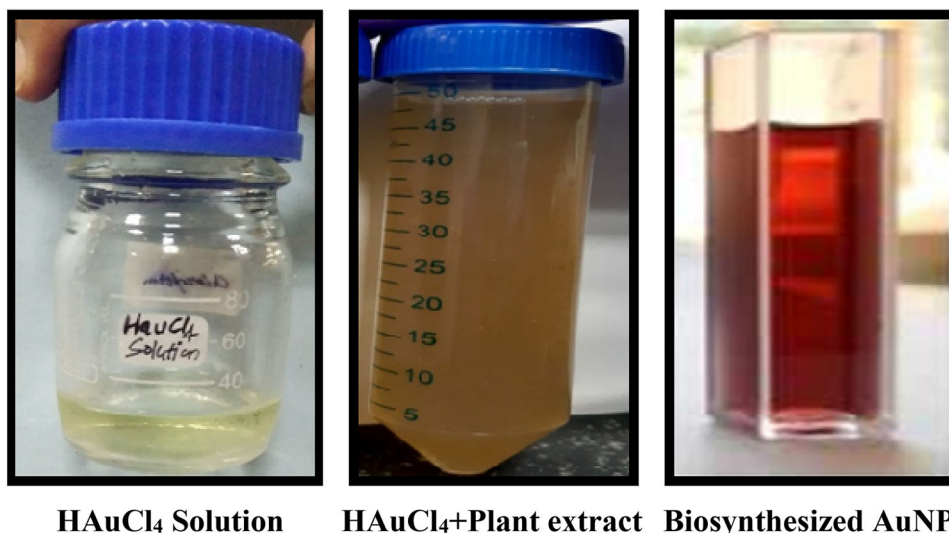
Instrumental Analysis

Absorption spectrum of biosynthesized AuNPs was recorded using UV–Vis (Thermo Fisher Scientific, Germany). FTIR spectrum was obtained using thermo scientificTM NicoletTM IS 50 spectrometer to identify the functional groups. XRD measurements were carried out using MAXima_X XRD-7000 Shimadzu, Tokyo, Japan. TEM (Tecnai, G 20 (FEI) at 200 kV) was carried out to find out the shape and size of AuNPs. TEM images were taken at various magnification levels. The composition of elements present in the AuNPs was assessed with the help of EDX using Bruker X-flash detector (Bruker, Bremen, Germany).

Microorganisms Used

For antimicrobial assessment of plant extract and AuNPs coated with plant extract, six bacterial strains namely *Bacillus subtilis* (MTCC-2057), *Chromobacterium violaceum* (MTCC-2656), *Escherichia coli* (MTCC-41), *Pseudomonas aeruginosa* (MTCC-2453), *Staphylococcus aureus* (MTCC-96), and *Streptococcus pyogenes I* (MTCC-890) and four fungal strains viz. *Aspergillus niger* (MTCC- 3002) *Fusarium oxysporum* (MTCC- 7392), *Rhizopus oryzae* (MTCC-262), and *Penicillium expansum* (MTCC- 2818) were used. Active cultures of microorganisms for experiments were prepared as per the method of Mc Farland [37] with slight modification.

Fig. 1 Biosynthesis of AuNPs coated with *V. cinerea* extract



HAuCl₄ Solution

HAuCl₄+Plant extract

Biosynthesized AuNPs

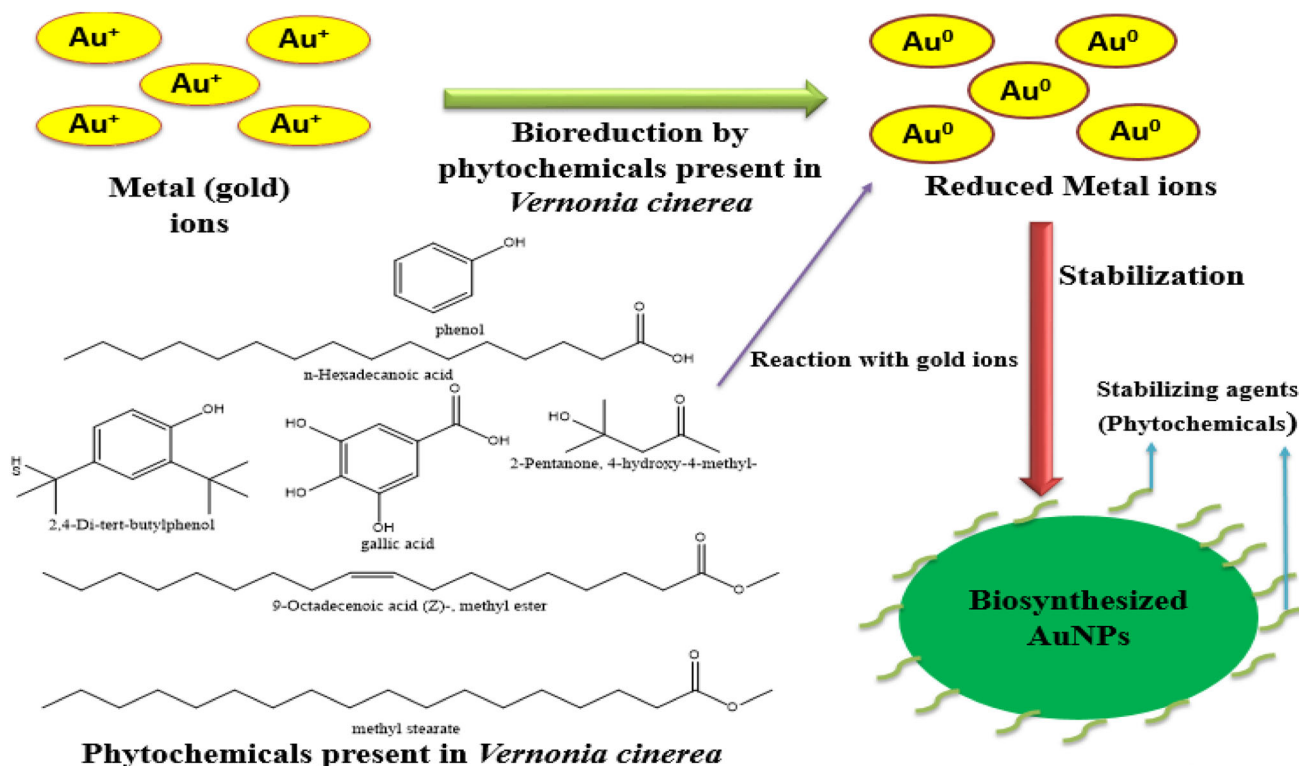


Fig. 2 Phytoconstituents present in *V. cinerea* extract that may be responsible for the reduction of gold ion

Antimicrobial Studies

Antimicrobial activity of the plant extract and biosynthesized AuNPs was assessed by disc diffusion assay [38, 39]. 6 mm sterilized discs were kept over the nutrient agar media (Hi Media) already spreaded with 100 μl different bacterial inoculum. Petriplates containing Czapekdox and Potato dextrose agar spreaded with 20 μl culture of fungal strains were used for antifungal studies.

Plant extract was reconstituted in DMSO to obtain 100 mg/ml working concentration for antimicrobial studies [35]. 10 μl plant extract and AuNPs (of different concentrations) were loaded on the discs. Petriplates were then incubated at 37 $^{\circ}\text{C}$ (in case of antibacterial activity), and 28 $^{\circ}\text{C}$ (in case of antifungal activity). The diameter of zone of inhibition (ZOI) was measured and noted. For antibacterial assay, ampicillin and chloramphenicol (0.1 mg/ml) were taken as positive control, while fluconazole (0.1 mg/ml) was the positive control for antifungal studies. DMSO acted as negative control in both cases. The assay was repeated thrice, and means value was recorded and represented graphically.

Antioxidant Studies

Antioxidant potential of the plant extract and biosynthesized AuNPs was measured using DPPH (3 mM, 1, 1-

diphenyl-2-picryl-hydrazyl) assay [40] and ABTS (7 mM, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay [41]. Ascorbic acid was used as standard in both the assays. Absorbance was measured at 517 nm in the case of DPPH assay and 745 nm in ABTS assay by UV–Vis spectrophotometer (Shimadzu 1800, Japan). Experiments were performed in triplicates and mean value was recorded. Antioxidant potential was calculated using the formula given below:

$$\% \text{ Inhibition} = \frac{\text{Ab. Control} - (\text{Ab. Sample}/\text{Ab. Control})}{\text{Ab. Control}} \times 100$$

where % Inhibition = % Antioxidant activity; Ab. control = Absorbance of pure DPPH/ABTS; Ab. sample = Absorbance of the DPPH/ABTS + Plant extract/AuNPs.

Statistical Analysis

Statistical analysis was performed using the SPSS version 24.0. Graphs were plotted using Microsoft Excel 2013 and Origin Pro-2020. Data are presented as means \pm SD, and *P* values less than 0.05 were taken as statistically significant.

Characterization of Biosynthesised AuNPs

Following techniques were used for the characterization:

UV–Visible Spectroscopy

After synthesis of AuNPs, the solution was centrifuged at 14,000 rpm for 20 min. at ambient temperature (25 ± 2 °C) to concentrate the AuNPs. The unbound capping (large aggregates) agents were removed by repeating washing (four times) with DD water and finally with ethanol. Biosynthesised AuNPs were analysed by UV–visible spectrophotometer at the absorption range of 300–700 nm. DD water was taken as blank.

Fourier Transform Infra-red (FTIR) Analysis

FTIR spectrum was recorded in the range of 400–4000 cm^{-1} at the resolution of 4 cm^{-1} .

X-ray Diffraction (XRD)

XRD was performed at 40 kV voltage, and the electric current used was 20 mA with a Cu-K α ($\lambda = 1.54$ Å). The radiation's source was from 30° to 75° from the region of 2 θ from the colloidal solution.

Transmission Electron Microscopy (TEM)

The size and shape of AuNPs synthesized using extract of *V. cinerea* were determined by TEM. A small drop of ethanol suspended with AuNPs was placed on the Cu grid and dried at room temperature (25 ± 2 °C). Images were taken at various magnification levels. TEM was performed by availing sophisticated analytical instrument facility (SAIF) at Panjab University, Chandigarh.

Energy-Dispersive X-ray (EDX) Spectroscopy

The composition of elements present in biosynthesized AuNPs was assessed with the help of EDX analysis. The beam energy of electron was set at 15 keV and used both for imaging as well as analysis of EDX. EDX spectroscopy was also carried out at SAIF, Panjab University, Chandigarh.

Results and Discussion

Biosynthesised nanoparticles are gaining great attention worldwide as an important tool to reduce the undesirable effects associated with the traditional methods of

nanoparticle synthesis. Biosynthesis of metallic nanoparticles is cost effective, single step eco-friendly bio-reduction method requiring relatively low energy to initiate the reaction. Plants being rich source of chemical compounds are important bioresource that can be utilized to reduce and stabilize the metallic nanoparticles. The phytoconstituents manage reduction of chloroauric acid to make zero-valent Au, which afterward prompt the clump of Au molecules to nanosize which are finally stabilized by the phytochemicals to produce AuNPs [17, 21]. In the present study, *V. cinerea* (Asteraceae) was selected as it exhibits a wide range of pharmacological effects including antimicrobial, analgesic, hypoglycaemic and antidiabetic activity [30, 33, 35]. AuNPs coated with *V. cinerea* plant extract were compared with aqueous plant extract for their antimicrobial and antioxidant properties. However, we should have compared these activities with gold nanoparticles also and this remains as one of the limitations of the present work.

UV–Visible Spectral Analysis

Aqueous extract of *V. cinerea* changed colour on warming and addition of aqueous solution of Tetrachloroauric acid from yellow to brown to ruby-red. AuNPs (< 30 nm diameter) showed ruby-red colour because of the narrow surface plasmon resonance [7, 17]. When UV–visible spectra of biosynthesised AuNPs was analysed, a strong peak was observed at 546 nm (Fig. 3a). Similar observations were also made by other workers from different parts of the world [21, 36].

Fourier Transforms Infra-red Analysis

From the FTIR spectra of AuNPs coated with extract of *V. cinerea*, a specific peak was observed at 1644 cm^{-1} (Fig. 3b). Additional peaks were also observed at different positions which may be because of the presence of different functional groups present in the plant extract. Functional groups play significant role in the capping and reduction of Au ion to AuNPs [7, 17]. Specific peaks from IR spectra in the range of 100–1600 cm^{-1} and 3200–3300 cm^{-1} indicated the presence of various functional groups. These functional groups are reported to help in the reduction of Au ions to AuNPs [7].

X-ray Diffraction Analysis

XRD spectrum showed peaks of 2 theta values ranging from 20° to 70° (Fig. 4a). When XRD spectrum of AuNPs was compared with the standard Au solution, it showed AuNPs to have crystalline form. Presence of strong peaks at 2 θ values of 38.21°, 44.44° and 64.61° correspond to

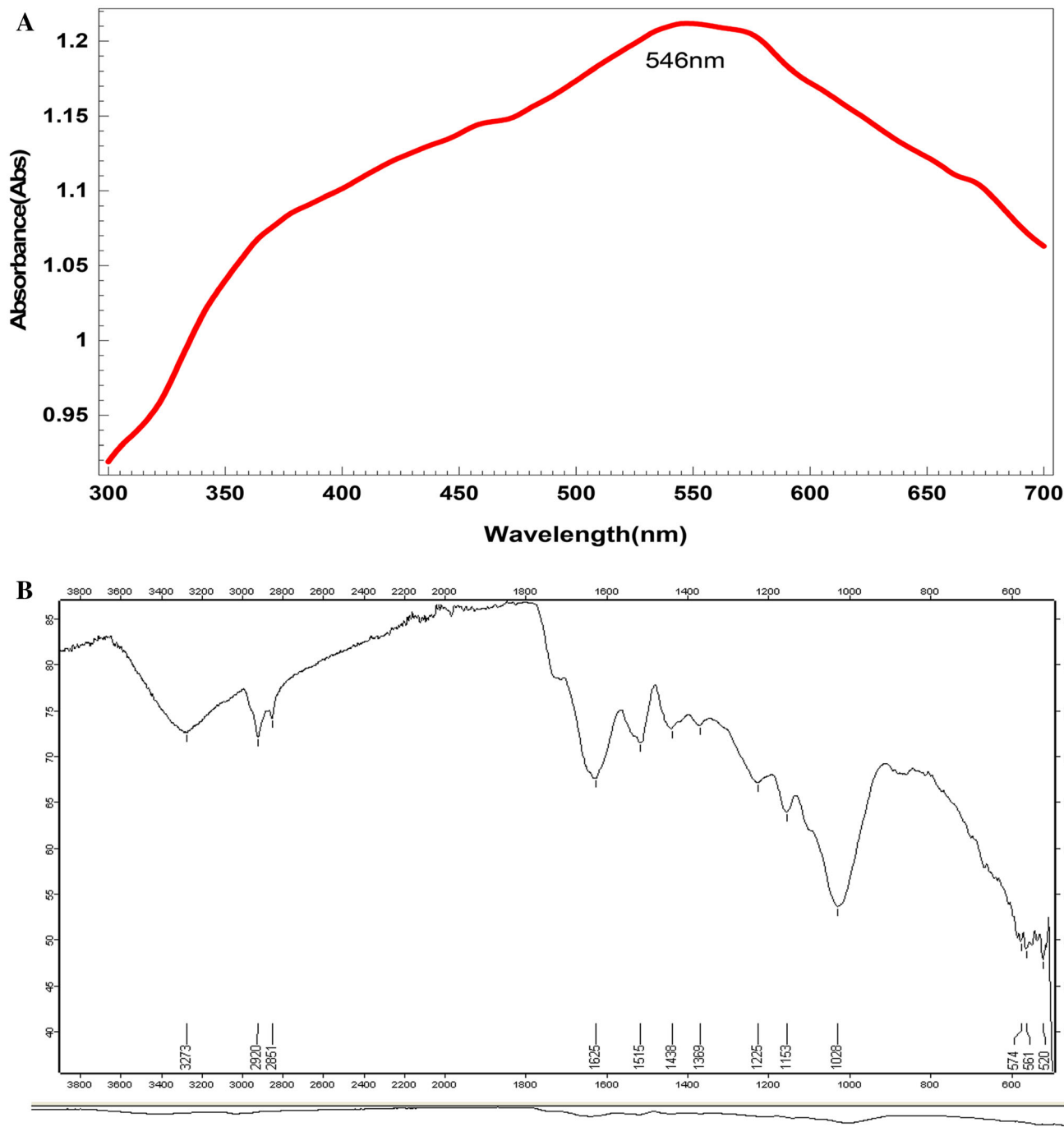


Fig. 3 a UV–VIS spectrum of biosynthesised AuNPs of *V. cinerea*. b FTIR spectrum of biosynthesized AuNPs of *V. cinerea*

(111), (200) and (220) set of planes for face centred cubic and crystalline nature [7, 24, 42].

Energy-Dispersive X-ray Analysis

EDX spectrum of the AuNPs showed a very strong signal in the region of Au which confirmed the successful formation of AuNPs. A very clear and specific peak (Fig. 4b)

was observed at around 2.40 keV, which indicated the presence of gold nanoparticles [21, 24]. Some weak signals for carbon, oxygen, and chlorine atoms were also recorded. This could probably be due to the emission of x-rays from biological entities like proteins or enzymes which may be responsible for the formation and capping of AuNPs [17, 21, 43]. Percentage of elements present in the gold solution is shown in supplementary Table 1.

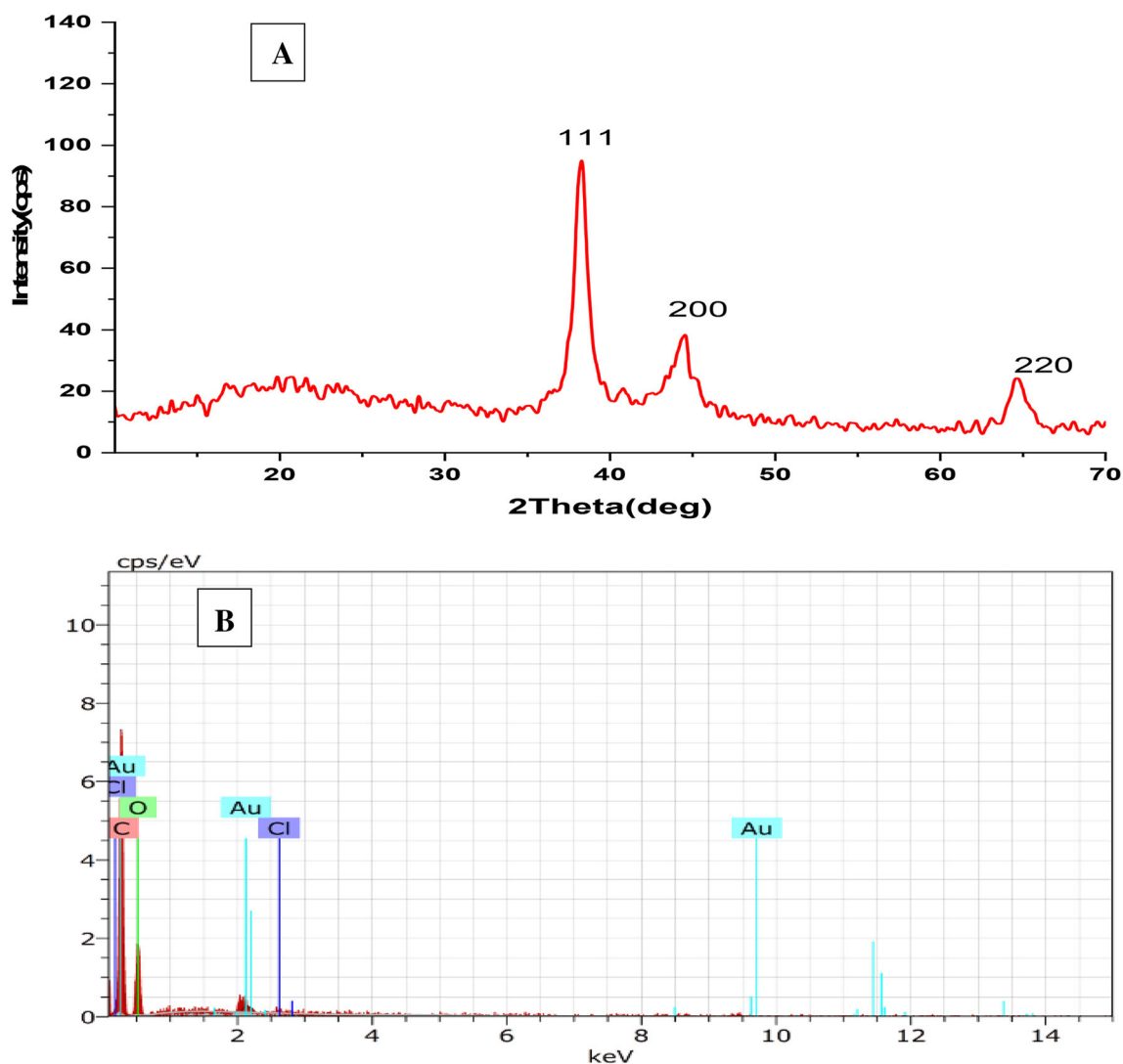


Fig. 4 a X-ray crystallography of biosynthesized AuNPs of *V. cinerea*. b EDX spectrum of biosynthesized AuNPs of *V. cinerea*

Transmission Electron Microscopy

Size and morphology of biosynthesized AuNPs was deduced from images obtained from TEM analysis. AuNPs were dispersive in nature; there was no aggregation which indicated the formation of stabilized AuNPs (Fig. 5). AuNPs were spherical and triangular in shape having an average size 25 nm.

Antimicrobial Studies

Antimicrobial potential of biosynthesized AuNPs was assessed against six bacterial and four fungal strains and also compared with the aqueous plant extract of *V. cinerea* and positive control (Figs. 6, 7, 8 and 9). Of the six bacterial strains, strong inhibitory activity was obtained against *S. pyogenes* with ZOI of 12.5 mm and 18.2 mm

with plant extract and biosynthesized AuNPs (2 mg/ml), respectively. The ZOI obtained with positive control i.e. ampicillin and chloramphenicol was 17.7 mm and 17.1 mm, respectively. *P. aeruginosa* was found to be resistant to ampicillin. However, strong inhibitory activity (ZOI of 17.8 mm) against this bacterial strain was recorded with biosynthesized AuNPs. Interestingly, inhibitory activity obtained with biosynthesized AuNPs was higher than that obtained with the positive control i.e., ampicillin and chloramphenicol. This clearly showed the enhanced antibacterial potential of AuNPs of *V. cinerea* as compared to the plant extract. Among fungal strains, maximum ZOI (17.4 mm) was obtained with AuNPs (5 mg/ml) against *R. oryzae* which was more than ZOI obtained with the positive control i.e. flucanazole (17.00 mm) and plant extract (13.20 mm). This again showed the enhanced antifungal potential of AuNPs coated with plant extract. The

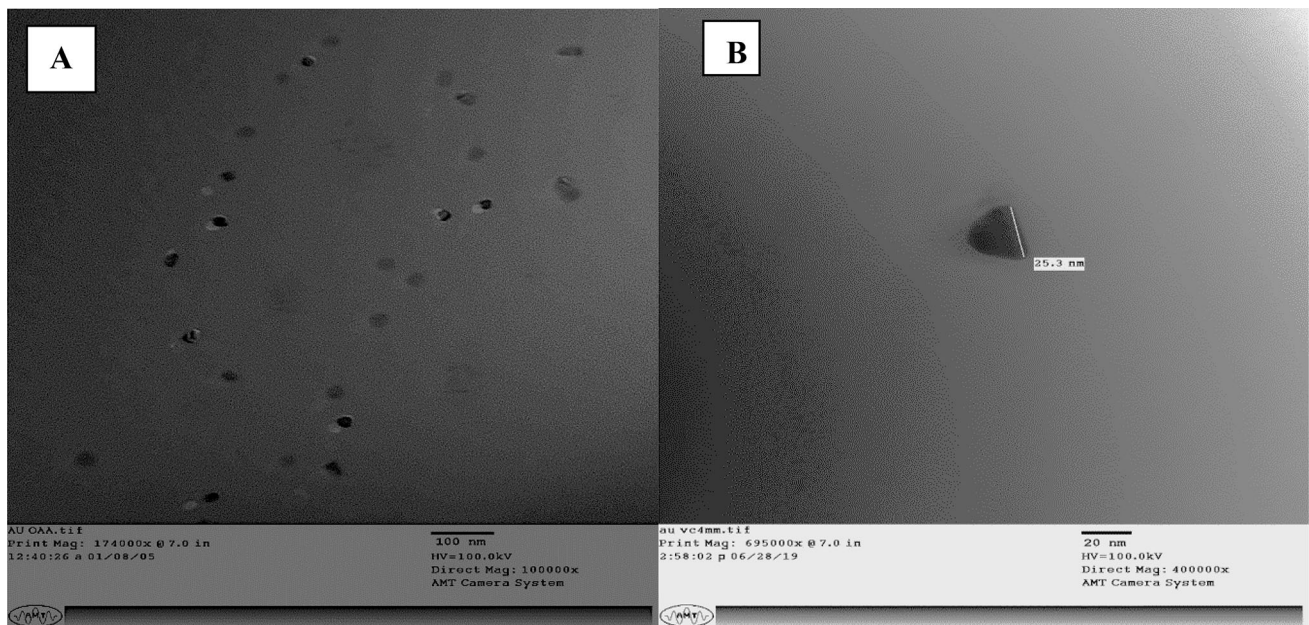
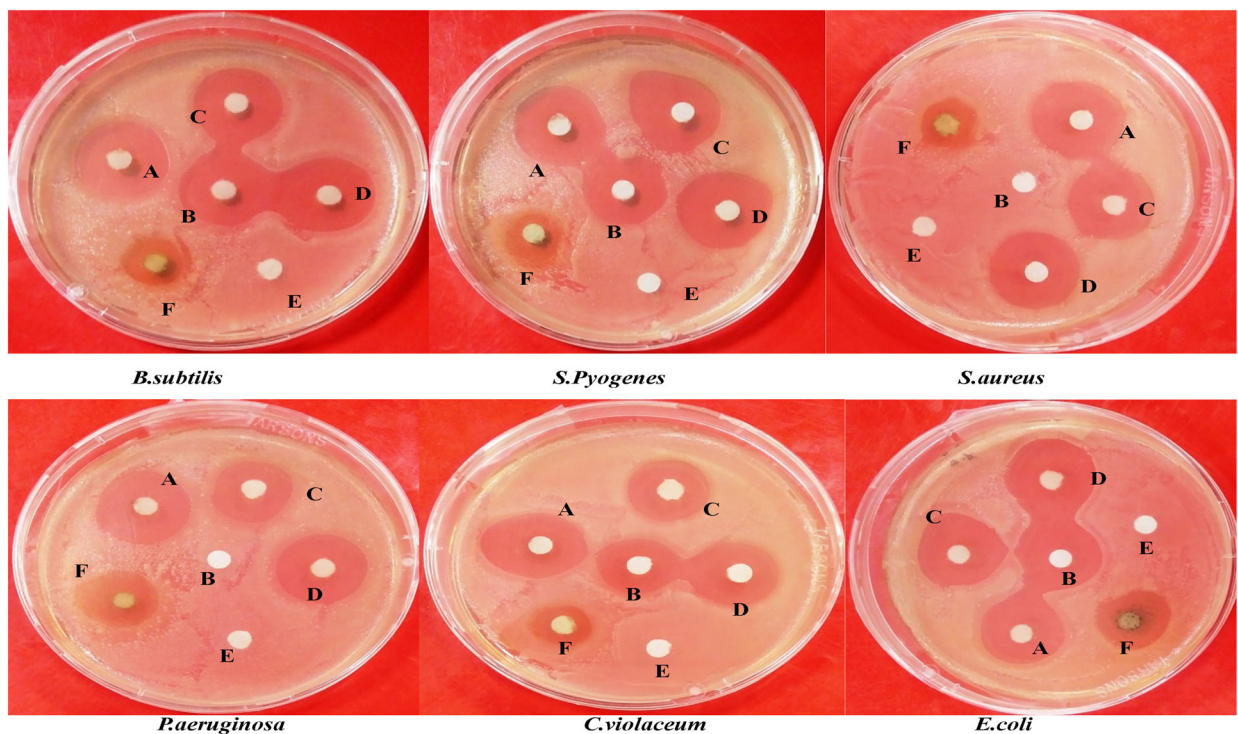


Fig. 5 TEM images of biosynthesized AuNPs of *V. cinerea* **a** at 100 nm, **b** at 20 nm



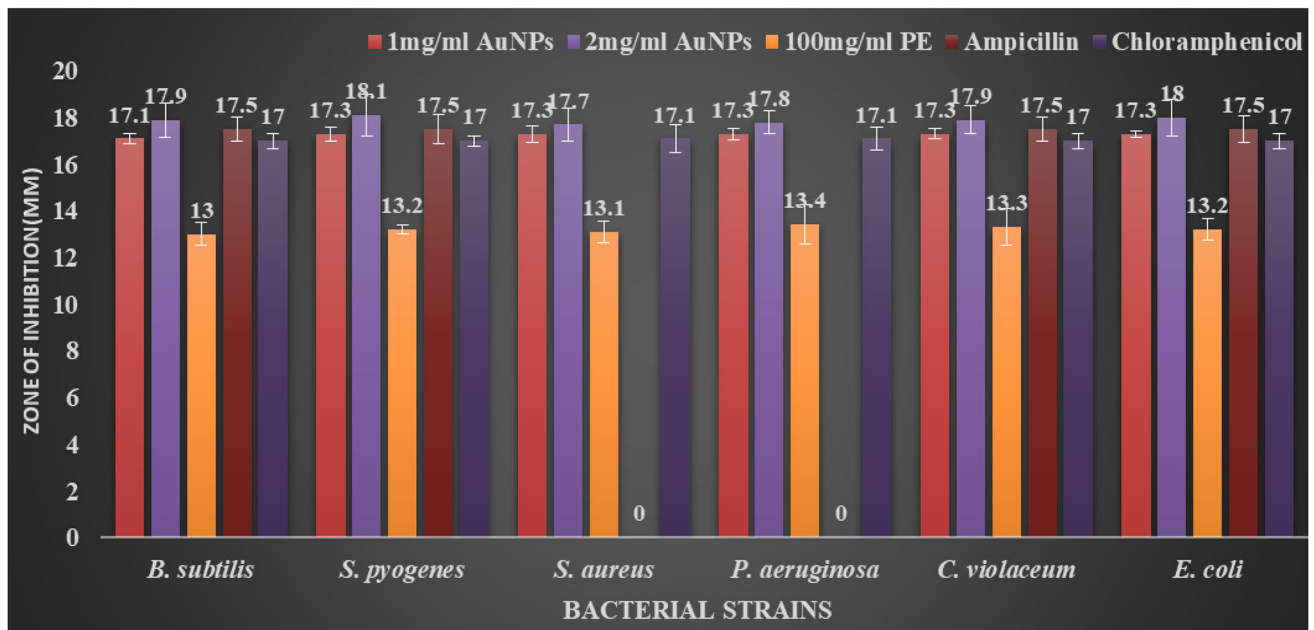
A- Chloramphenicol; B – Ampicillin; C- AuNPs (1mg/ml); D- AuNPs (2 mg/ml); Chloramphenicol; E- Negative control; F- Plant extract (100mg/ml)

Fig. 6 ZOI obtained against six bacterial strains with aqueous plant extract of *V. cinerea*, biosynthesized AuNPs and positive controls

antimicrobial activity was categorized into strong inhibitory activity when ZOI obtained was of 10–19 mm [44].

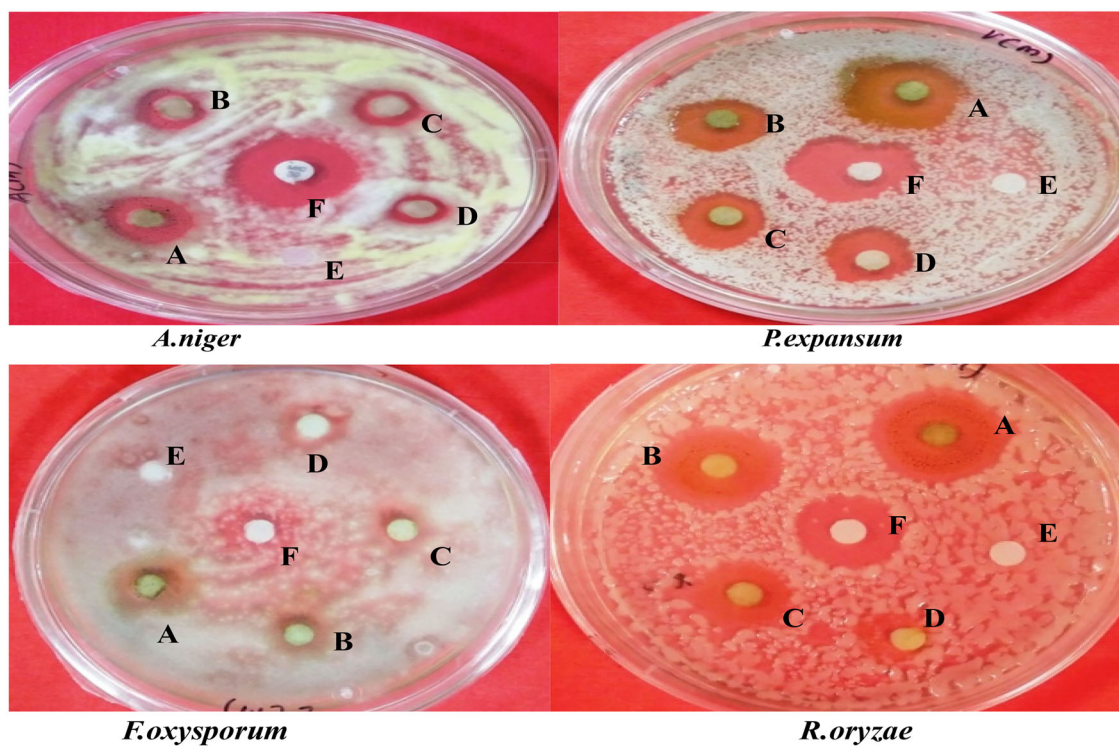
High antimicrobial activity of AuNPs coated with plant extract as compared to plant extract alone might be due to

their smaller size and high surface region which empower them to enter inside the bacterial and fungal cells [24, 45, 46]. AuNPs have the capacity to bind with the bacterial cell wall followed by penetration, thus changing



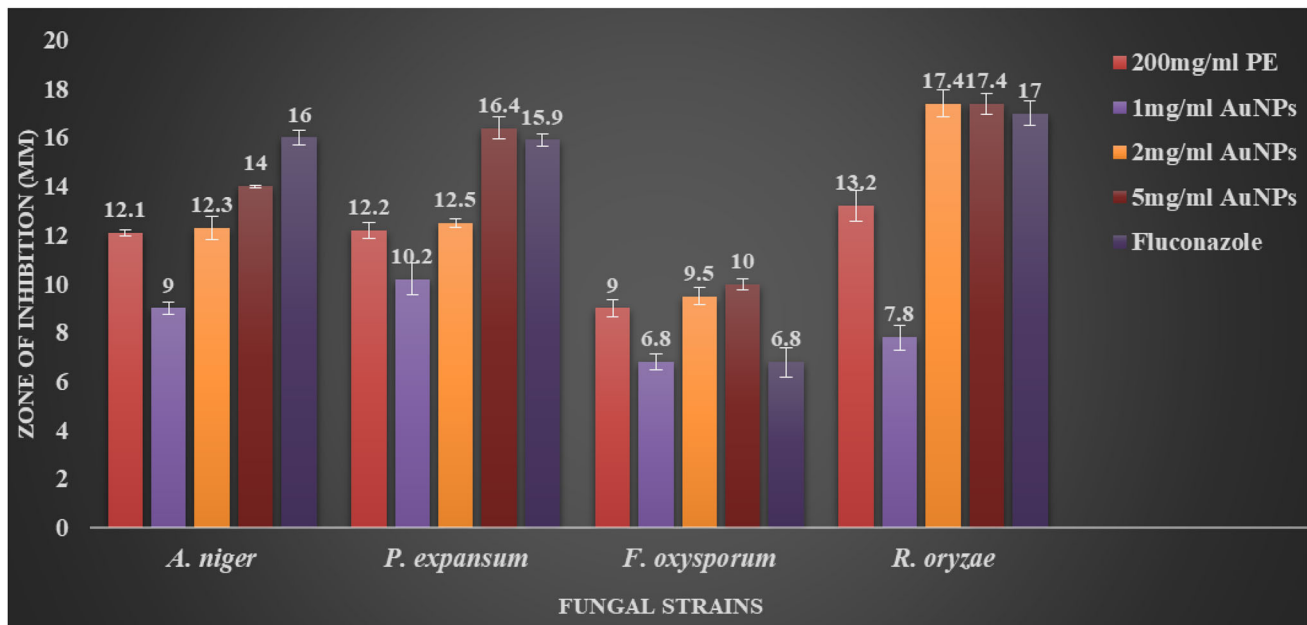
AuNPs- Biosynthesised AuNPs; PE- Plant extract

Fig. 7 Graphical representation of ZOI obtained against six bacterial strains with aqueous plant extract of *V. cinerea*, biosynthesized AuNPs and positive controls



A- AuNPs (5mg/ml); B- AuNPs (2mg/ml); C- AuNPs s (1mg/ml); D-Plant extract (200 mg/ml); E- Negative control; F-Fluconazole

Fig. 8 ZOI obtained against four fungal strains with aqueous extract of *V. cinerea*, biosynthesized AuNPs and positive control



AuNPs- Biosynthesized AuNPs; PE- Plant extract

Fig. 9 Graphical representation of ZOI obtained against four fungal strains with aqueous extract of *V. cinerea*, biosynthesized AuNPs and positive control

the permeability of the cell membrane which leads to the death of bacterial cell [38, 45, 47–51]. In compliance to our study, previous reports also showed higher antimicrobial activity of biosynthesized gold nanoparticles as compared to plant extract [47, 52, 53].

Earlier studies carried out by us on GCMS analysis of the plant extract revealed the presence of phytochemicals like hexadecanoic acid, octadecadienoic acid, phenols, flavonoids, esters, caryophyllene-oxide, etc. [35] Among these, hexadecanoic acid and octadecadienoic acid are reported to have antimicrobial, antioxidant, anticancer, and hyper-cholesterolemic properties [30, 54–56]. Octadecadienoic acid is also reported to have anti-inflammatory and antiarthritic properties [54, 55]. These phytochemicals may contribute significantly to the enhanced antimicrobial properties of biosynthesized AuNPs coated with plant extract.

Antioxidant Studies

Antioxidant potential of biosynthesized AuNPs, aqueous plant extract and standard ascorbic acid was evaluated by ABTS and DPPH assay. The results obtained are shown in (supplementary Fig. 10a–c). Percentage scavenging activity of *V. cinerea* AuNPs (in case of DPPH assay) varied from 34.29% (10 $\mu\text{g/ml}$) to 95.04% (100 $\mu\text{g/ml}$) while percentage inhibition recorded for plant extract was from 13.84% to 92.03% (supplementary Fig. 10a). In case of ABTS assay, the percentage of scavenging activity of

biosynthesized AuNPs ranged from 33.22% (10 $\mu\text{g/ml}$) to 94.01% (100 $\mu\text{g/ml}$) whereas percentage inhibition of plant extract varied from 12.84% to 93.03% (supplementary Fig. 10b). In both the assays, percentage inhibition recorded for AuNPs coated with plant extract was almost similar to ascorbic acid. IC_{50} value obtained for biosynthesized AuNPs, plant extract and ascorbic acid is graphically represented in Fig. 10c. AuNPs of *V. cinerea* showed IC_{50} value (28.78 $\mu\text{g/ml}$ in the case of DPPH assay and 30.22 $\mu\text{g/ml}$ in ABTS assay) comparable to ascorbic acid (28.33 $\mu\text{g/ml}$), thus showing high antioxidant potential of biosynthesized AuNPs. IC_{50} value of plant extract was found to be 49.93 $\mu\text{g/ml}$ (DPPH) and 40.89 $\mu\text{g/ml}$ (ABTS), respectively. The enhanced antioxidative potential of the biosynthesized AuNPs may be explained based on their high surface area to volume ratio [57]. Moreover, adsorption of the antioxidant entity on the AuNPs surface might also be the reason for their enhanced antioxidant potential. Our results are in compliance with studies carried out by other workers on *Eclipta prostrata*, *Lotus leguminosae* and other plants [47, 57].

Conclusions

Freshly collected vegetative aerial parts of *V. cinerea* were used for the synthesis of eco-friendly AuNPs for the first time in the present study. Stable gold nanoparticles were prepared by treating aqueous HAuCl_4 solution with plant

extract which acted as reducing agent. Biosynthesized AuNPs exhibited spherical and triangular shape with an average size of 25 nm. Antimicrobial potential of AuNPs was found to be much higher than the plant extract. Interestingly, the inhibitory activity of the AuNPs against microbial strains was comparable with the standards used which clearly showed vast utility of the plant in medicinal field. Likewise, the antioxidant potential of the biosynthesized AuNPs was found to be more than plant extract and ascorbic acid. Multiple surface functionalities of AuNPs allow them to be more robust and flexible when combined with biological materials. Biosynthesized AuNPs thus can be excellent tools in the future world of biomedicine. However, more studies are required on nanoformulations using isolated bioactive compounds and their assessment for various pharmacological properties.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12088-021-00976-w>.

Acknowledgements We are thankful to the Department of Botany and the CIL (central instrumentation laboratory), Maharshi Dayanand University for providing the necessary support to carry out this research work.

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