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ORIGINAL RESEARCH

Eco-friendly approach in synthesis of silver nanoparticles and evaluation of optical, surface morphological and antimicrobial properties

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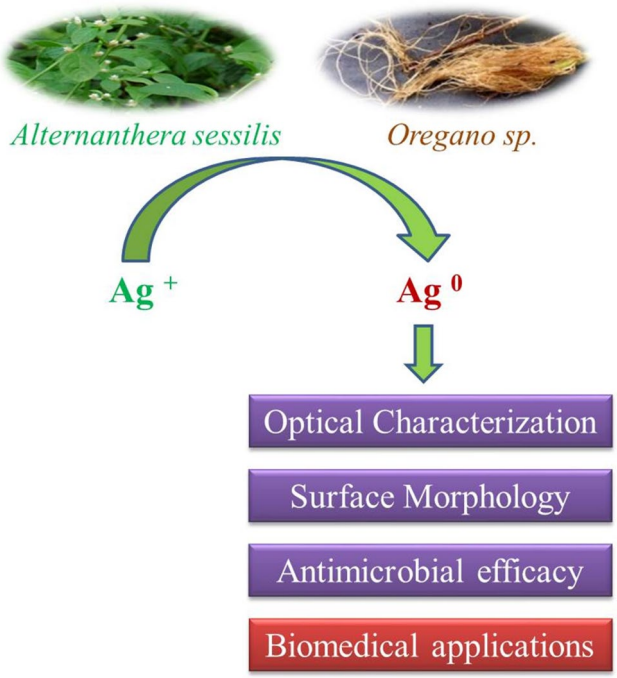
Abstract
Silver nanoparticles (Ag NPs) were synthesized using *Alternanthera sessilis* leaf *and Oregano* root extract in an eco-friendly fashion and their significant physicochemical and optochemical properties were ascertained for nano-defined characteristics. The UV–visible spectrum showed a single and distinct absorbance peak at 433 nm (*Alternanthera sessilis*) and 425 nm (*Oregano*), typical SPR (surface plasmon resonance) for silver. Structural studies revealed nano-crystal with face centre cubic (FCC) symmetry with monodispersed nature. SEM studies showed spherical-shaped particles and the purity determined from EDX spectrum. The synthesized Ag NPs showed that antibacterial activity was studied. There was a significant inhibitory effect toward clinically important pathogens viz. *B. subtilis*, *S. aureus*, *P. aeruginosa,* and *E. coli* exposed to Ag NPs at different concentrations.

Graphic abstract



Keywords Green synthesis · Silver nanoparticles · *Alternanthera sessilis* · *Oregano* · Antimicrobial activity

Extended author information available on the last page of the article

Introduction

Manoevering of particles at nanoscale tends to gain unique properties for use in various applications, viz., nano-generators, drug delivery system, and medical imaging [1–4]. Their efficiency to improve solubility, half-life, and sustained release of drugs find its application more appropriate in drug delivery [5]. In the current situation, the nano-biotechnology is one among the most energetic platform, explore the contemporary substantial discipline where the plants and various plant products find an imperious use in the fabrication of nanoparticles [6]. As vast applications of nanomaterials in various fields such as electronic, magnetic, optoelectronics, and information storage are well established. Researchers have found the remarkable application of nanomaterial in the field of medicine such as antimicrobial activity. The existing drug of choice is now becoming a major threat across the globe leading to the emergence of drug-resistant microbes or superbugs challenging the survival of humans. Therefore, to counteract the situation, researchers are on the verge of finding an alternate drug [7]. One such strategy is by the use of metals such as copper, zinc, titanium, magnesium, gold, etc., which, in their nano-form, are considered to exhibit remarkable physical, chemical, and biological properties. To counterbalance the situation, antimicrobial properties of the metallic nanoparticles have been explored to contain resistant strains [8]. Amongst them, silver nanoparticles are found to exhibit unique properties such as conductivity, chemical stability, and catalytic and biological (antibacterial, antiviral, antifungal, and anti-inflammatory) activities [9]. Despite various other methods of synthesizing nanoparticles, the eco-benign approach has been the most sought after. Besides, microbial synthesis, plant product/phytochemical-mediated synthesis of nanoparticles has drawn much attention because of its availability, reproducibility, and reliability [10–17]. This study reports on the eco-friendly green syntheses of silver nanoparticles (Ag NPs) using two plant extracts such as *Alternanthera sessilis* leaf and *Oregano* root extracts as a reduction agent. In our study, the role of the extracts in reducing Ag^+ to Ag^0 has been investigated through spectroscopic and microscopic analyses emphasizing the antibacterial efficacy. The schematic representation of Ag NPs extracted from plants, as shown in Fig. 1.

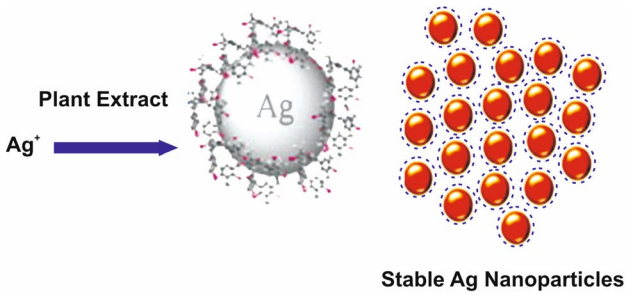


Fig. 1 Schematic representation of the synthesis of silver nanoparticles

Materials and methods

Materials

Alternanthera sessilis leaves were procured from a local market in periyakulam, and silver nitrate ($AgNO_3 \geq 99.8\%$; AR grade) was purchased from Sigma-Aldrich and used without further purification. All the glass wares used were cleaned in chromic acid and autoclaved.

Preparation of the extracts of *Alternanthera sessilis* and *Oregano* root

50 g of fresh *Alternanthera sessilis* leaves and *Oregano* root were thoroughly washed in running water followed by distilled water to remove any dust particles. They were initially dried on an absorbent paper and chopped into small pieces using a pair of scissors. Prior to surface cleansing, *Alternanthera sessilis* leaves and *Oregano* roots were blended separately in a mixer grinder for less than a minute with 10 mL of distilled water. The blending was checked for paste-like consistency and collected in two separate Erlenmeyer flasks. To the pastes, 100 mL of double distilled water was added and kept in a shaking incubator at 80 °C for 10 min. Both the mixtures were brought down to room temperature and subjected to filtration using syringe filter (pore size 0.2 µm) and the filtrate maintained at 4 °C.

Synthesis of Ag NPs by *Alternanthera sessilis* leaf and *Oregano* root extracts

To 100 mL of 1 mM silver nitrate taken in two separate flasks, 5–10 mL of *Alternanthera sessilis* leaf and *Oregano* extracts were added under the stirring condition for 20 min at 60 °C. Reaction pertaining to nanoparticle synthesis with respect to time was observed. Furthermore, separation and purification of the colloids were performed using repeated washing and centrifugation. Finally, the particles were dried and stored in airtight containers for further experiment.

Characterization Techniques

UV–Vis Schimadzu 1800 spectrophotometer was used to record the absorbance spectrum (200–700 nm) of the synthesized silver nanoparticles operated at a resolution of

1 nm. The phase purity of synthesized silver nanoparticles was determined using a Philips X'pert Pro diffractometer (Schimadzu) aided with CuKβ radiation. FT-IR spectrum was recorded on JASCO 4400 in the spectral range of 4000–400 cm^{-1} with sample pelleted using potassium bromide (1:100). Electron microscopic studies (Carl Zeiss MA15) were performed on the sample sputtered with gold to analyze the surface properties and assembly characteristics. The composition of the synthesized silver nanoparticles was determined using X-ray Energy Dispersion Spectroscopy (Inca, Oxford Instruments, Buckinghamshire, UK).

Antibacterial activity

The antimicrobial activity of phytochemically synthesized silver colloids was determined using agar diffusion method using clinically important pathogens containing Gram-positive and Gram-negative test strains. The pure cultures of the strains at 1×10^8 CFU/mL were swabbed uniformly onto the Mueller–Hinton agar (MHA) medium using sterile swabs. Four hollow blocks of medium (dia 6 mm) were cut from the MHA plates using 100 µL sterile pipette tips. Ag NPs synthesized from two different extracts at a concentration of 25, 50, and 75 µL was added to the wells using a sterile micropipette. Amoxicillin antibiotic (25 µL) was used as a positive control. The culture-inoculated plates treated with Ag NPs and antibiotic were incubated for 18–24 h at 37 °C for the observation of any inhibition zone.

Results and discussion

Structural analysis

The typical XRD pattern of silver nanoparticles synthesized using a leaf and root extracts of *Alternanthera sessilis* and *Oregano* root, respectively, is shown in Fig. 2a, b. Both ASL- and OR-mediated synthesis showed diffraction peaks at $2\theta = 38.2°$, 44°, 64.4°, and 77.2° corresponding to (111), (200), (220), and (311) that can be assigned to face centered cubic symmetry (fcc) [18]. These diffraction patterns were compared and found closely associated to JCPDS No. 04-0783 [19]. The robust intensity of diffraction at 38° indicated silver crystal's preferential orientation along (111) plane. In addition, we could observe a peak at $2\theta = 46.3°$ which might have been associated with bio-organic phase crystallization [20]. The mean crystallite size of the silver nanoparticles was calculated from Scherrer's formula [21]:

$$D = \frac{0.9\lambda}{\beta \cos\theta},$$

where D denotes mean crystallite size of the nanoparticle, λ denotes wavelength of X-ray, β accounts for full width at half maximum intensity (FWHM), and θ represent Bragg's angle. Accordingly, the mean crystal size of the ASL- and OR-mediated nanoparticle synthesis worked out to 19 nm and 10 nm.

Optical studies

A dark brown suspension of colloids was formed after the addition of the extracts to the reaction mixture. This dark brown color may be due to the collective vibrations of the charged particles present on the surface of nanoparticles

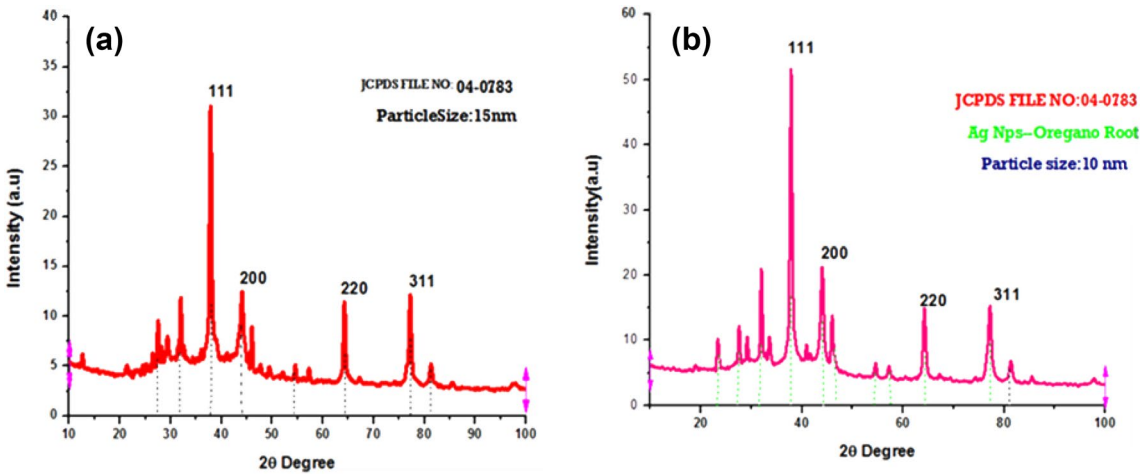


Fig. 2 XRD pattern of plant extracted from **a** ASL and **b** OR of Ag NPs

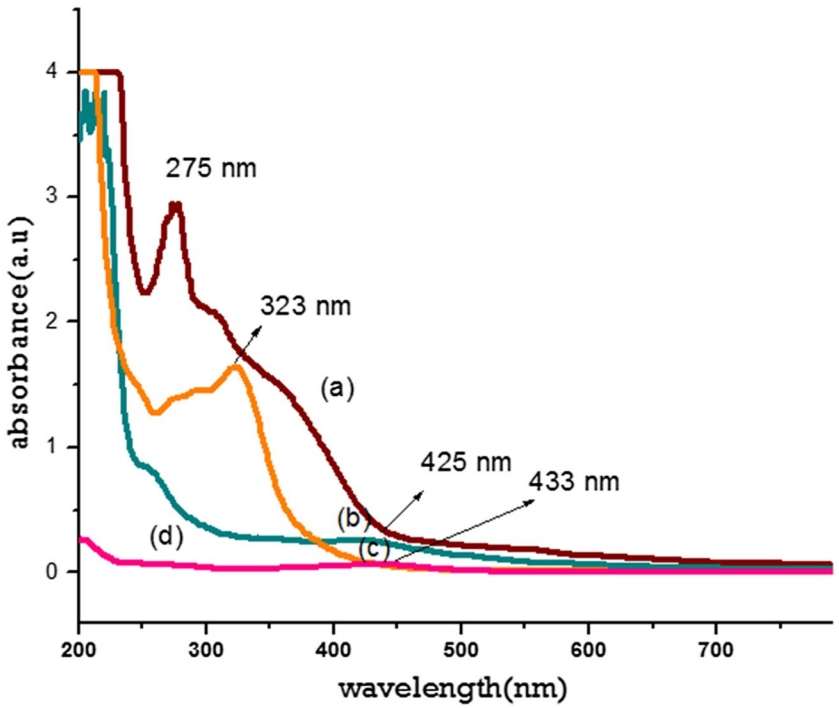


Fig. 3 UV absorption spectra of a ASL, b synthesis Ag NPs from ASL extract, and c OR and d synthesis Ag NPs from OR extract

and the resonance [22–24]. A sharp and a narrow distinct peak at $\lambda_{max} = 433$ nm in the visible region clearly elucidate the formation of Ag NPs in a short duration (10–15 min) after the addition of ASL extract as in Fig. 3a, b [25]. Similarly, UV–visible spectra recorded an absorbance of 425 nm within 10 min of addition of OR extract as in Fig. 3c, d.

SEM and EDX analysis

The morphology and the elemental composition of Ag NPs were observed using SEM and EDX studies as shown in Figs. 4 and 5. Electron micrograph revealed spherical-shaped particles with a smooth surface and closely arranged [26]. The size of Ag NPs synthesized using ASL and OR extract was around 23.44 nm and 17.58 nm, respectively, which is in good agreement with the crystallite size as inferred by XRD. The elemental composition as demonstrated from EDX spectra revealed a strong signal for silver. In addition, carbon, oxygen, and nitrogen signals could also be detected. Carbon signal might have resulted from the grid, oxides during sample preparation, and nitrogen might have been a phytochemical moiety responsible for capping nanoparticle as shown in inset Figs. 4 and 5.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy exhibited the possible biomolecular interaction in the formation of nanoparticles using ASL and OR (Fig. 6a, d. The FT-IR spectra of OR Ag NPs showed prominent peaks at 3452 cm^{-1}, 2072 cm^{-1}, 1634 cm^{-1}, and 642 cm^{-1} attributing N–H asymmetric stretching assigned to Amide group, C=C-stretching vibration denoting Alkyne group, N–H bend indicating primary amine group, and C–Br stretching vibration indicating the Alkyl halide group. A peak residing at 655 cm^{-1} represented a key component responsible for the reduction and capping of the extract with that of metal by their intermolecular interaction [27]. The sharp absorption peak at 664 cm^{-1} can be attributed due to C–Cl stretching for halogen compounds, the alcohol and phenols stretching of C–O bond occur at 1074 cm^{-1}, the band at 1615 cm^{-1} exist due to C=O stretch of tertiary amides, and the broad peak at 3426 cm^{-1} is the characteristic O–H stretching for alcohols and phenols. In addition, the functional biomolecules in ASL extract were hydroxyl, carboxylic, phenol, and amine groups involved in silver ion reduction. Thus, biological molecules enforce the dual role of formation and stabilization of Ag NPs in the aqueous medium [28].

Antimicrobial assay

The antimicrobial activity of ASL- and OR-mediated Ag NPs is elaborated in Figs. 7 and 8. There was a significant inhibition of growth of bacteria tested. ASL-mediated Ag NPs showed the maximum inhibition zone against *Staphylococcus aureus* (11 mm) followed by *Pseudomonas aeruginosa* (10 mm), *Escherichia coli* (9 mm), and *Bacillus subtilis* (4 mm). OR-mediated Ag NPs susceptibility pattern showed a maximum zone toward *Pseudomonas*

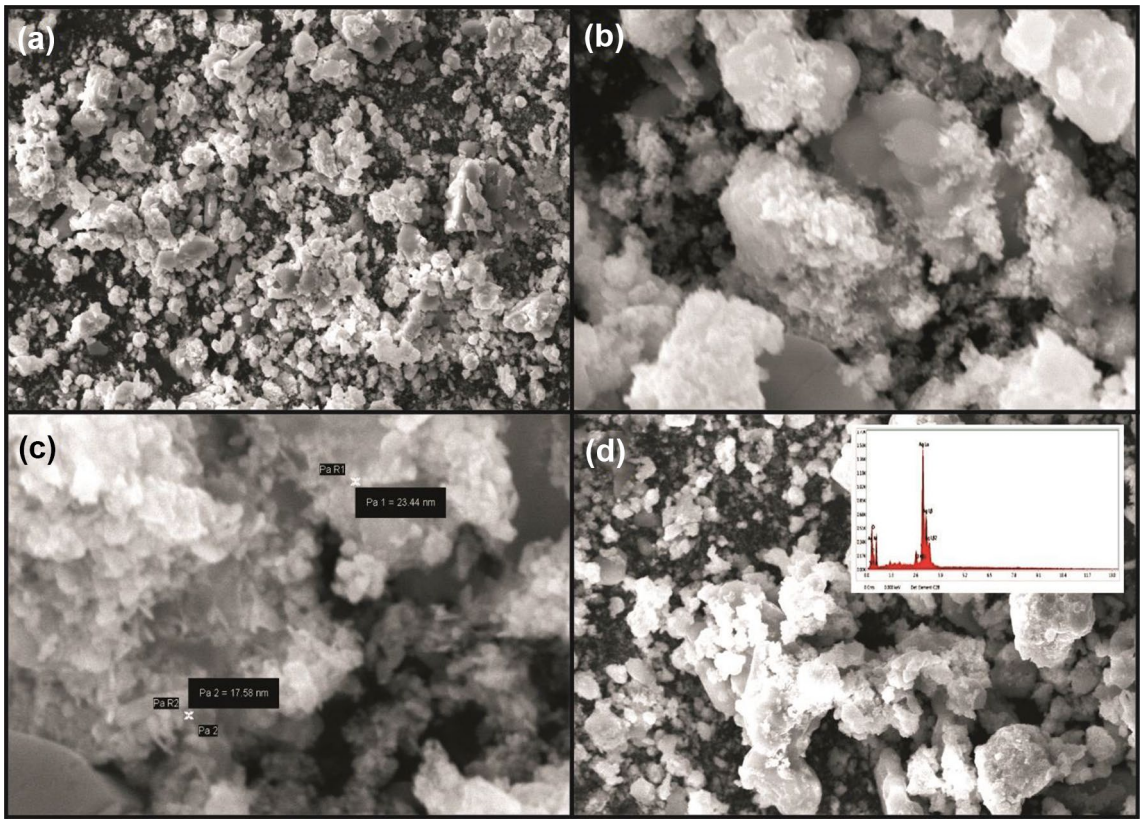


Fig. 4 SEM with EDX images of plants extracted from ASL of Ag NPs

aeruginosa (6 mm) followed by *Escherichia coli* (5 mm), *Bacillus subtilis* (4 mm), and *Staphylococcus aureus* (3 mm).

Antibacterial mechanism exhibited by metal nanoparticles depends on the degree of susceptibility of microbes. The nanoparticles when encountered with the microbe adhere to the bacterial surface via electrostatic interaction. Their significantly smaller size helps gain entry into the bacterial cell via the transmembrane proteins and by the influence of proton motive force. It has a greater affinity towards sulfur groups present in proteins to form thiols [29] and on phosphates forming complexes resulting in DNA damage. It was demonstrated that Ag NPs' interaction with cysteine residues results in the generation of ROS by inhibiting electrons at terminal oxidase, thereby inducing bacterial cell death. The difference in susceptibility pattern toward Ag NPs exposed to Gram-positive and Gram-negative strains relies on their cell wall make up. Gram-positive strains possess a thick cell wall made of peptidoglycan that helps to prevent intrusion of foreign body selectively, whereas the Gram-negative strain lacks such component that falls easy prey to an antimicrobial agent, in this case, Ag NPs, which sustains severe damage leading to cell death [30–33].

Conclusions

The present work highlights the most simple and economical approach in the synthesis of Ag NPs using plant extracts of *Alternanthera sessilis* (leaf) and *Oregano* (root) as reducing agents. Spectroscopic and Microscopic analyses revealed typical nano-characteristics of silver. FT-IR results confirmed the contribution of phytochemicals, viz., terpenes, flavonoids, and proteins for effective synthesis. There was a significant bactericidal activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli* as evidenced from agar well-diffusion method. This biosynthesized Ag NPs represented a promising antimicrobial with potential biomedical applications. Therefore, this green chemistry approach towards the synthesis of Ag NPs has been the most sought-after method in terms of economic viability.

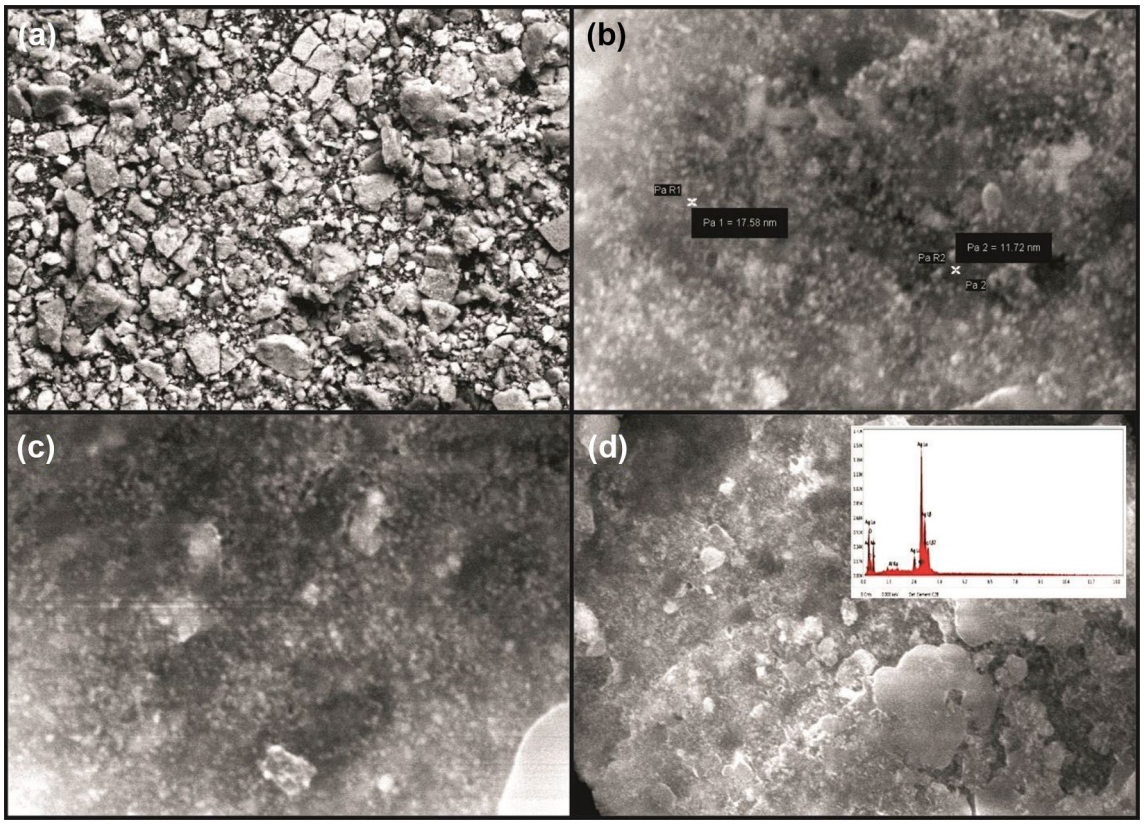


Fig. 5 SEM with EDX images of plants extracted from OR of Ag NPs

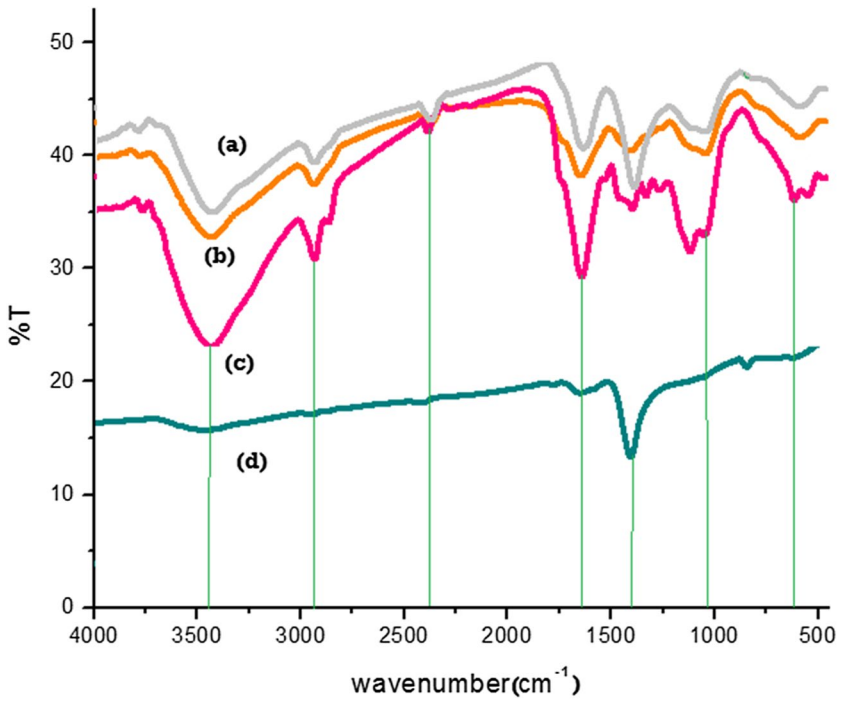


Fig. 6 a OR-mediated silver nanoparticles, b OR extract, c ASL extract-mediated silver nanoparticles, and d ASL extract

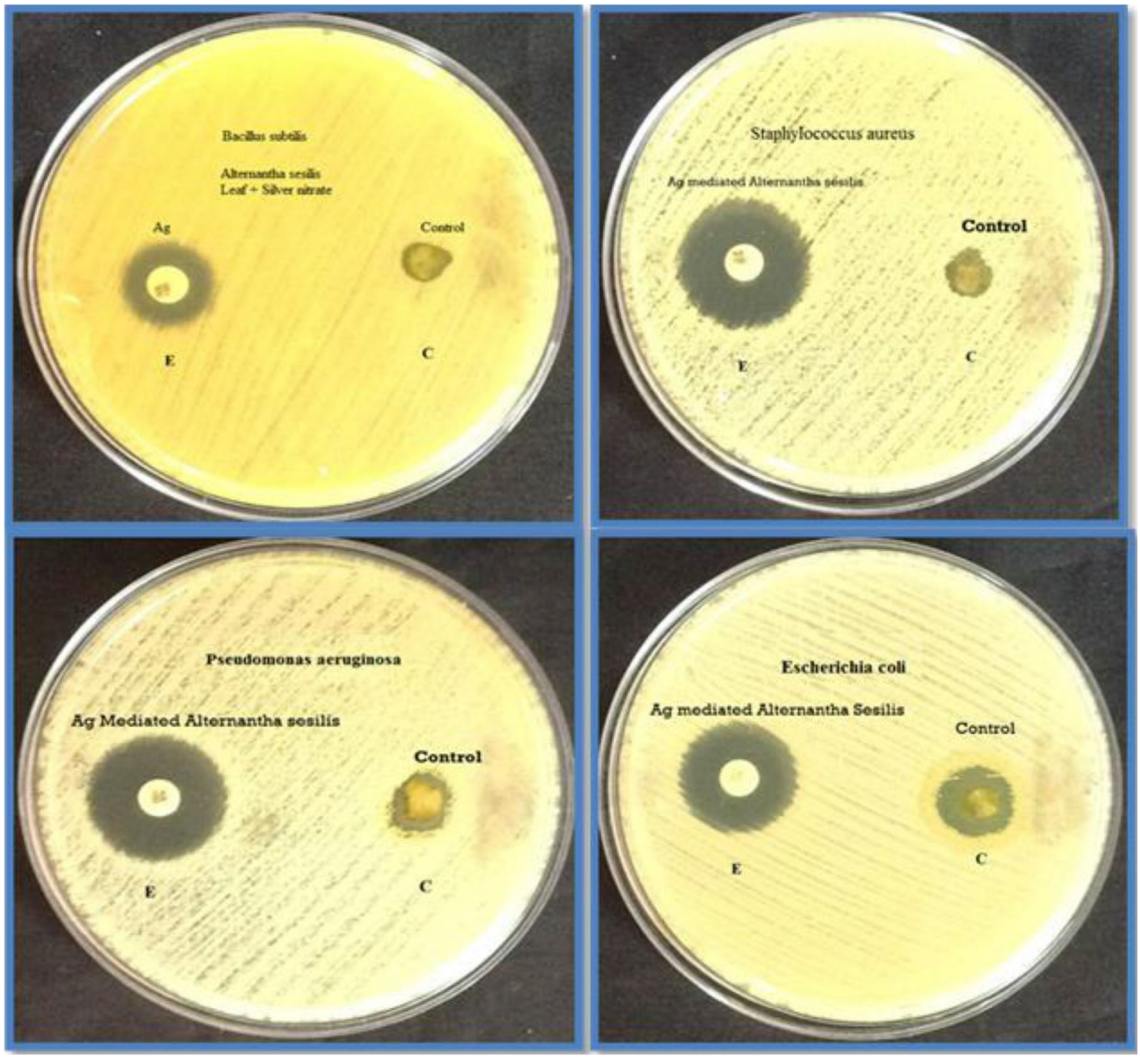


Fig. 7 Antimicrobial activity of *Alternanthera* leaf extract of silver nanoparticles

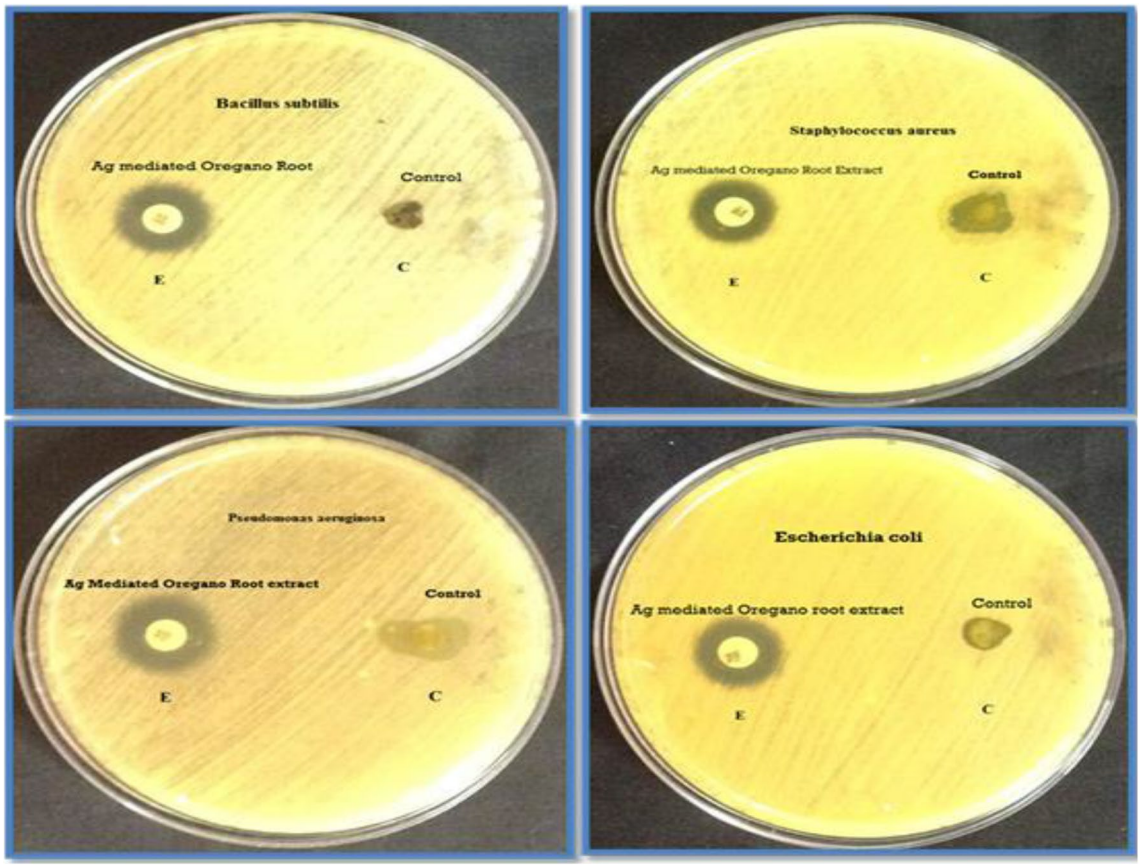


Fig. 8 Antimicrobial activity of *Oregano* root extract of silver nanoparticles

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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