Synthesis of silver oxide nanoparticles using aqueous leaf extracts of Viscum orientale Willd, Coleus amboinicus Lour and evaluation of their antibacterial activity

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Abstract

The objective of this study was to develop a straightforward biological synthesis of silver oxide nanoparticles (Ag_2O NPs) using aqueous leaf extract of Viscum orientale Willd and Coleus amboinicus Lour as a good lowering and capping agent . The UV–visible bands observed at 499 and 451 nm was confirmed the creation of nanosized Ag_2O particles making use of Viscum orientale Willd and Coleus amboinicus Lour plants. The formation of Ag_2O stretching frequency was confirmed by FTIR spectral studies. Scanning electron microscopy and EDAX analysis displayed Ag_2O nanoparticles have been pure and spherical shaped and had been the variety of length from 35 ± 2 nm for (Ag_2O NPs) by the usage of Viscum orientale Willd and 32 ± 2 nm for (Ag_2O NPs) Coleus amboinicus Lour vice-versa. The fashioned nanoparticles have been cubic in association and face-centered cubic in form, consistent with X-ray diffraction studies. The shaped green kind Ag_2O NPs confirmed ambitious antibacterial against each gram positive and gram negative bacteria.

Keywords: silver oxide, Viscum orientale Willd, Coleus amboinicus Lour, antibacterial activity, nanoparticles

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1 Introduction

Over the precious couple of long time, a significant amount of research activity in the chemical community has been devoted to the advancement of novel technologies and methodologies for environmentally friendly processes. The vicinity of interest is often referred to as 'green chemistry' [18]. Except several chemical and biochemical techniques that have been superior for creating metal nanoparticles, inexperienced nanotechnology additionally allows as a exquisite way within the growth of easy, nontoxic, and environmentally pleasant methods for the synthesis and introduction of silver NPs [7]. Between numerous metal NPs, Ag NPs have been regarded as a key component of the surface plasmon resonance (high absorption in the visible region), which is easily observed by UV-visible spectrophotometer. There are numerous well-known uses for silver nanoparticles in the fields of medicine, the environment, optoelectronics, textiles, optics, catalysis, sensors, and bio-diagnosis. [6].

Recently several authors have accomplished the biosynthesis of Ag NPs via microorganisms like microbe [19], fungi [17] and yeast [13] are utilized already. However, examination of the plant strategies as the potential nanofactories has keen interest in the biological production of NPs. Further, synthesis of Ag NPs by using extracts a variety of plants part like Jatropha curcas [1], Lippia citriodora (Lemon Verbena) [2], Iresine herbstii leaf [4], Tansy fruit [5], Terminalia chebula fruit [6], Piper longum leaf extracts [8], Acalypha indica leaf extracts [14].

Among them, Coleus amboinicus Lour (Lamiaceae family) habitually well-known as Indian Borage, is a soft, fleshy perennial medicinal plant that contains numerous phytochemicals such as carvacrol (monoterpenoid), caryophyllene (bicyclic sesquiterpene) and patchoulane and flavonoids (quercetin, agpigenin, luteolin, salvigenin, and genkwanin) which have enormous medicinal applications. It has largely proven effective in treating conditions like the common cold, asthma, constipation, headache, cough, fever, and skin problems [16]. Sunil Kumar et al., 2013, reported these plants can be found along the India, Chittagong, Myanmar, W. Peninsula, Sri Lanka, Malay Islands, China, New Guinea and Australia. According to his early phytochemical investigation, chloroform and alcohol extracts contained alkaloids, triterpenoids, steroids, tannins, glycosides, flavonoids, and coumarin. [3].

Moreover, best of our knowledge there was no literature discovered that described the creation of Ag_2O NPs using Viscum orientale Willd and Coleus amboinicus Lour leaf extracts. Herein, we report for the first time on the preparation of Ag_2O NPs by using leaf extract of Viscum orientale Willd and Coleus amboinicus . Using the agar diffusion method, the antimicrobial outcomes of Ag/ Viscum orientale Willd and Coleus amboinicus Lour were evaluated against two pathogenic microbes for the counting of both (Gram negative) and (Gram positive).

2 Materials and methods

2.1 Collection of plant materials

In Manapparai Taluk, Tiruchirappalli District, Tamil Nadu, India, during the month of December 2015, robust, disease-free leaves of Viscum orientale Willd and Coleus amboinicus Lour were collected for the purpose of making Ag_2O NPs. Dr. S. Soosairaj, an assistant professor at the postgraduate and research department of botany at St. Joseph's College (Autonomous), Tiruchirappalli, India, is well-known for having a comprehensive herbarium of plants that have been verified.

2.2 Preparation of leaf extract

The gathered Coleus ambonicus Lour leaves were properly cleansed in tap water before being rinsed with double-distilled water to ensure that no foreign objects remained. The 10 g of leaf was mixed with 100 ml of double distilled water in a 200 ml beaker and boiled with 10 minutes, until the colour of aqueous solutions turns from water to light green colour. The extract was then brought to room temperature and put through whatman filter paper no. 1. Filtered leaf extract was stored at 15C for further usage.

2.3 Viscum orientale Willd leaf extract:

The Viscum orientale Willd leaf extract was prepared by using the following procedure as given in section 2.2.

2.4 Ag2O NPs were created using coleus ambonicus Lour leaf extract :

10 ml of the aqueous extract of Coleus ambonicus Lour was taken in a 250 ml beaker and was added with 90 ml of 1mM of aqueous $AgNO_3$ solution. The solution was stirred well using magnetic stirrer for a room temperature till the colour of solution gradually changed from light green to brown colour indicating the formation of Ag_2O NPs. Centrifugation was used to separate the product from the liquid phase, and then it underwent three rounds of thorough cleaning with deionized water and ethanol to remove any leftover water-soluble organic compounds. Next, it was dried at room temperature using a desiccator.

2.5 Synthesis of Ag2O NPs by using viscum orientale Willd leaf extract:

Viscum orientale Willd leaf extract was used to reduce silver nitrate solution to synthesize Ag_2O NPs by following the procedure as given in section 2.3.

2.6 Characterization techniques

Using a UV-Visible spectrophotometer (Perkin Elmer -Lambda 35), the spectrum response of Ag_2O NPs was studied. The Jasco 6300 spectrometer's Perkin Elmer mode was used to obtain Fourier-Transform Infrared Spectroscopy (FTIR) data in the 400–4000 cm-1 range. The exterior morphology of the silver nanoparticles and binding energy of the element was inspected using FE-SEM (Hitachi SU6600, Japan) and EDAX (EMAX, Horiba 8121-H, Japan). X-ray diffraction metre - Cu K radiation (Rigaku, Miniflex-600, Japan) was used to perform powder X-ray diffraction (XRD).

2.7 Bacterial colonies and test sample preparation:

The Hi-Media Chemical Company in India provided all of the chemical reagents utilised in this investigation, all of which were of analytical grade. The test strains, Staphylococcus aureus MRSA 21, Bacillus subtilis PC 1219, Escherichia coli U 746, and Pseudomonas aeroginosa NCTC 8203, were taken from the Microbial type culture collection and the American type culture collection, respectively.Each of these strains was put into a tube with 4–5 ml of nutrient broth, and the tube was cultured at 35 C until the turbidity of 0.5 Mc Farland standards was reached. A sample of 0.001 M Ag_2O NPs was suspended in sterile Dimethyl sulphoxide (DMSO) and constantly stirred until a uniform suspension (100μl/ml.) was formed. Final concentrations of 25, 50, 75, and 100 μl/ml were achieved by serially diluting the test substances in DMSO. In visible light (2325-2500 lux), the prepared samples were photoactivated for 24 hours.

2.8 Antibacterial assay

Disc diffusion was employed to assess antibacterial activity against the test bacterium on Muller-Hinton agar, according to the Clinical and Laboratory Standards Institute (CLSI). The medium (MHA) plates were streaked with bacteria 2-3 times, rotating the plate each time at an angle of 60°, to ensure an even dispersion of inoculums.The Clinical and Laboratory Standards Institute (CLSI) states that disc diffusion against the test microorganisms on Muller-Hinton agar was used to measure antibacterial activity. Discs (6 mm Hi-Media) loaded with 2μ test samples

were placed on the bacteria seeded plates using sterile forceps after inoculation. The plates were then incubated at 37 °C for 24 hours. Measurements and records were made of the inhibitory zone surrounding the discs. To examine the effectiveness of the test sample, gentamycin (Hi-Media) was employed as a positive control against gramme negative and gramme positive bacteria, respectively. The experiment was performed twice, with DMSO serving as the negative control. We investigated whether DMSO without the Ag_2O NPs plays an active role as a biocide or not. Biocidal abilities are absent from DMSO.

3 Results and discussion

3.1 Visible observation

Ag_2O NPs solutions have a dark brown or dark reddish tint, according to studies in the literature. In the C. ambonicus Lour plant extract, the colour was light green prior to the addition of $AgNO_3$, but after being treated with AgNO3, the colour changed to dark brown, indicating the synthesis of Ag_2O NPs. [Figure 1].

FIGURE 1

Colour change of plant extract before and after addition of $AgNO_3$ solution

In a similar manner, the $AgNO_3$ treatment caused the hue of the V. orientale Willd plant extract to shift to dark brown. The quantum confinement property of nanoparticles, which depends on their size and influences their optical quality, is responsible for this colour change.

3.2 UV- visible spectroscopy studies

Due to surface plasmon resonance (SPR), which is the interaction between electromagnetic radiation and the electrons in the conduction band surrounding the nanoparticles, it was simple to identify and characterise the nanoparticle production using UV-visible spectroscopy [15]. In the visible region, Ag_2O NPs were clearly visible between 400 and 450 nm.

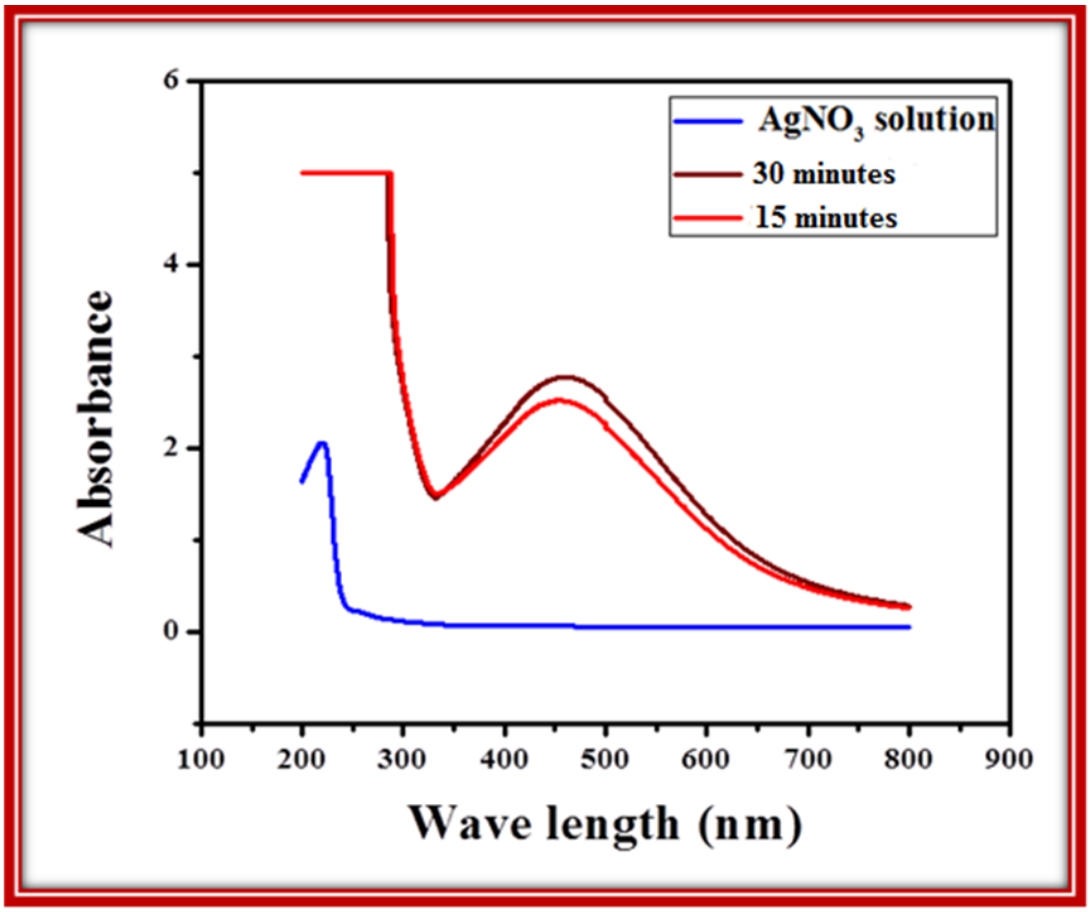

FIGURE 2

UV-visible spectra of Ag_2O nanoparticles synthesized from V. orientale Willd leaf extract

Figure 2 shows the UV visible spectrum measured at different time intervals from 0, 15, 30 minutes. Strong SPR bands were seen at 449 nm and 451 nm, respectively, in Figures 2 and 3, which show UV visible ranges between 340 and 740 nm. This SPR band indicates the presence of spherical Ag_2O NPs in the solution. The broadening of peaks suggests monodispersed

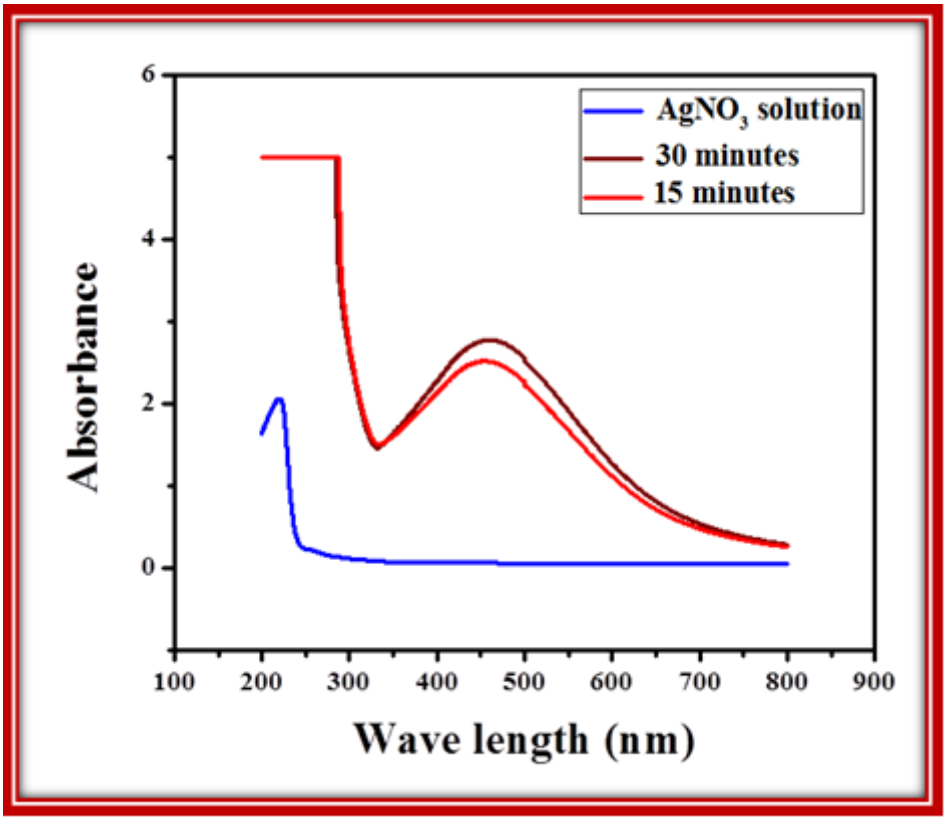

FIGURE 3

UV-visible spectra of Ag2O nanoparticles synthesized from C. ambonicus Lour leaf extract

nanoparticles. Ag_2O NPs were created at 24 hours utilising the Lippla cltriodora leaf extract, according to results that were identical. [15].

3.3 FT-IR spectroscopy studies

The biomolecules that covered the Ag_2O NPs were identified using FT-IR spectroscopic studies. FT-IR spectrum of created Ag_2O NPs are shown in Figure 3. FTIR result reveals that absorption bands at 3396, 2851 and 2362 cm^{-1} which are associated with N-H, CH_3, aliphatic C-C group and the deformation of N-H group and the aliphatic group at 1619 and 1384,1318 cm^{-1} respectively [10]. As shown in the Figure. 3 the intense peak appeared in the range of 525 and 520 cm^{-1} which where correspond to the stretching vibration of Ag-O group [21]. This further verified the compound as Ag_2O NPs in addition to the XRD analysis conducted.

3.4 Morphological analysis

Figure 5 and 6 showed the FESEM images of the synthesized Ag_2O NPs. Images show that NPs formed and were covered with their biological components. The Ag_2O NPs were found to be

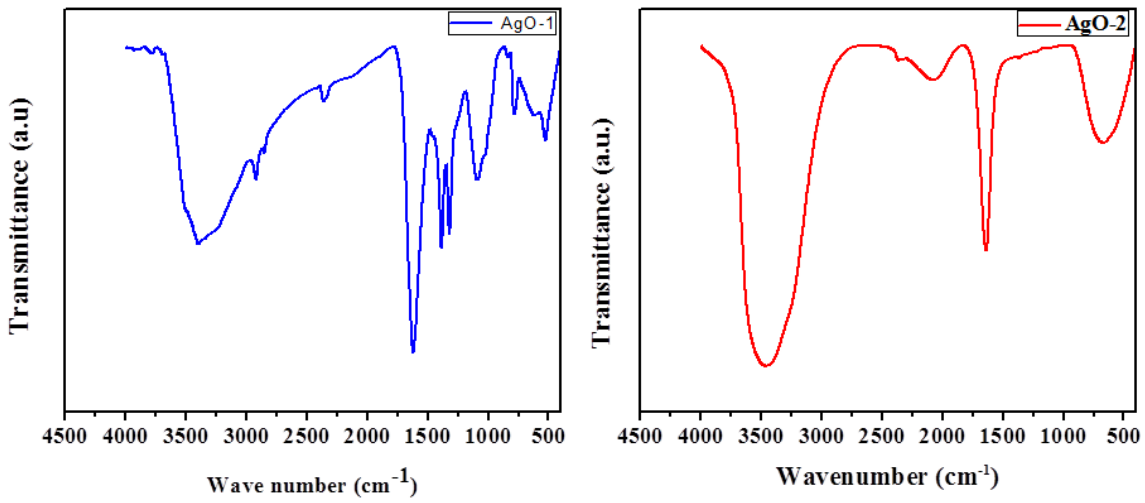

FIGURE 4

FTIR spectrum of Ag2O nanoparticles synthesized from V. orientale Willd and C. ambonicus Lour leaf extract

polydispersed and spherical in shape by means of the FESEM pictures. The size of the particles obtained as 35±2 nm for Ag_2O NPS synthesized by using V. orientale Willd while it where 32±2 for the synthesized from C. ambonicus Lour.

3.5 Elemental analysis

Figure 7 shows the EDAX spectrum of Ag_2O synthesized by using V. orientale Willd and C. ambonicus Lour respectively. EDAX profile source strong silver signal along with a weak oxygen, carbon, and chloride peak were observed, which may originate from the biomolecules that were found to the surface of Ag_2O NPs. The synthesised sample's atomic weight percentage is Ag = 40.71, O = 9.72 for Ag_2O NPs synthesis from V. orientale Willd whereas Ag = 64.20, O = 7.51 synthesized from C. ambonicus Lour leaf extract.

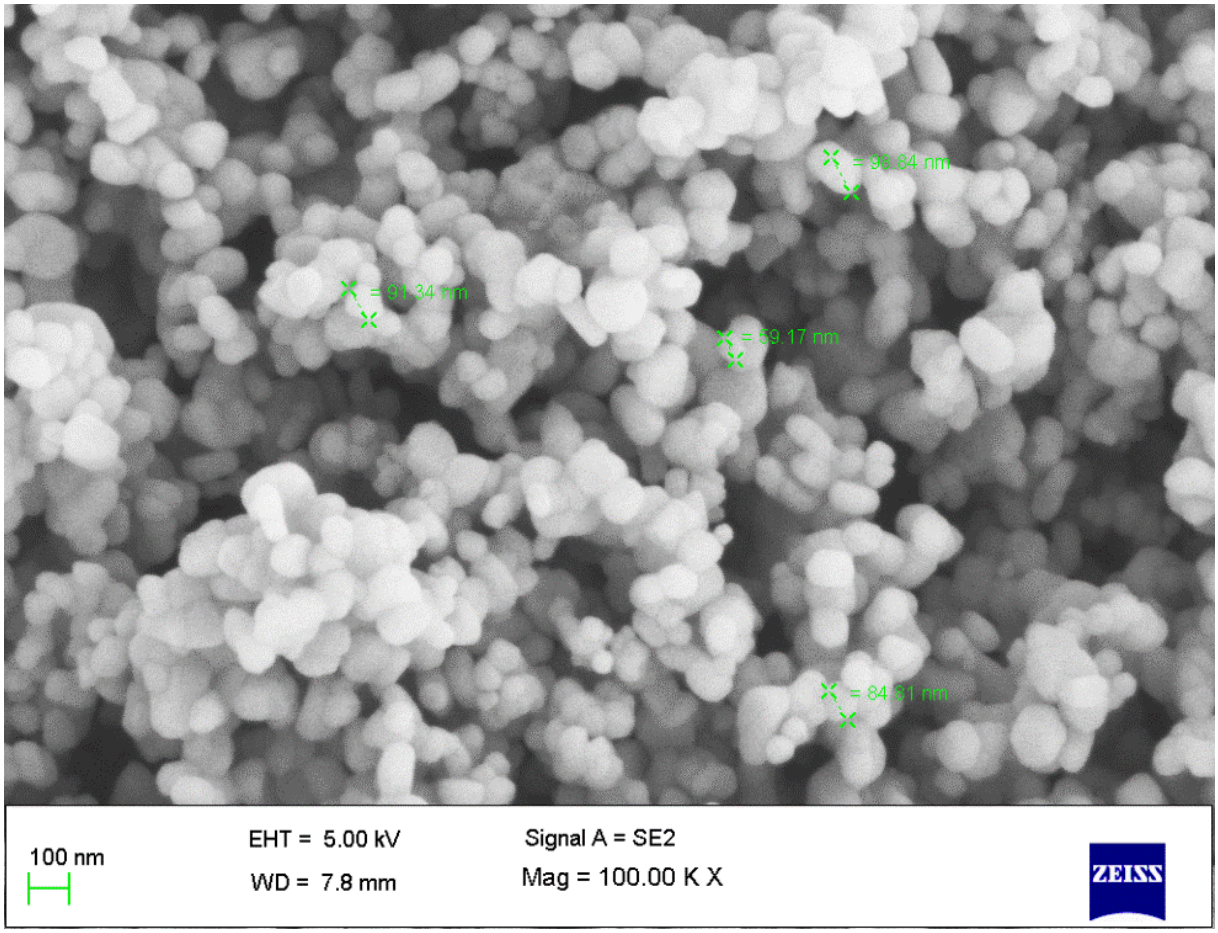

FIGURE 5

SEM of image Ag2O nanoparticles synthesized by using V. orientale Willd leaf extract

3.6 X-ray diffraction studies

The X-ray diffractometer The (110) and (111) of Ag_2O are shown by the two strong peaks in Figure 8 at 27.94 and 32.27, respectively. Aside from that, the (211), (221) and (222) planes of face-center cubic silver can each be indexed to the diffraction peaks at 46.34, 54.92, and 67.48, respectively. These peaks corroborate with the standard Ag_2O (JCPDS 76-1393) [9, 20]. A bacterial pellet may correspond to three further unidentified peaks at 57.58, 74.62, and 76.86. The nanoparticles that were produced through biosynthesis were Ag_2O, according to the XRD results.

3.7 Antibacterial activity

Silver ions' inhibitory action has long been recognised and used as a useful therapeutic agent for wound infection prevention. The literature claims that silver nanoparticles can kill bacteria through a number of different ways.

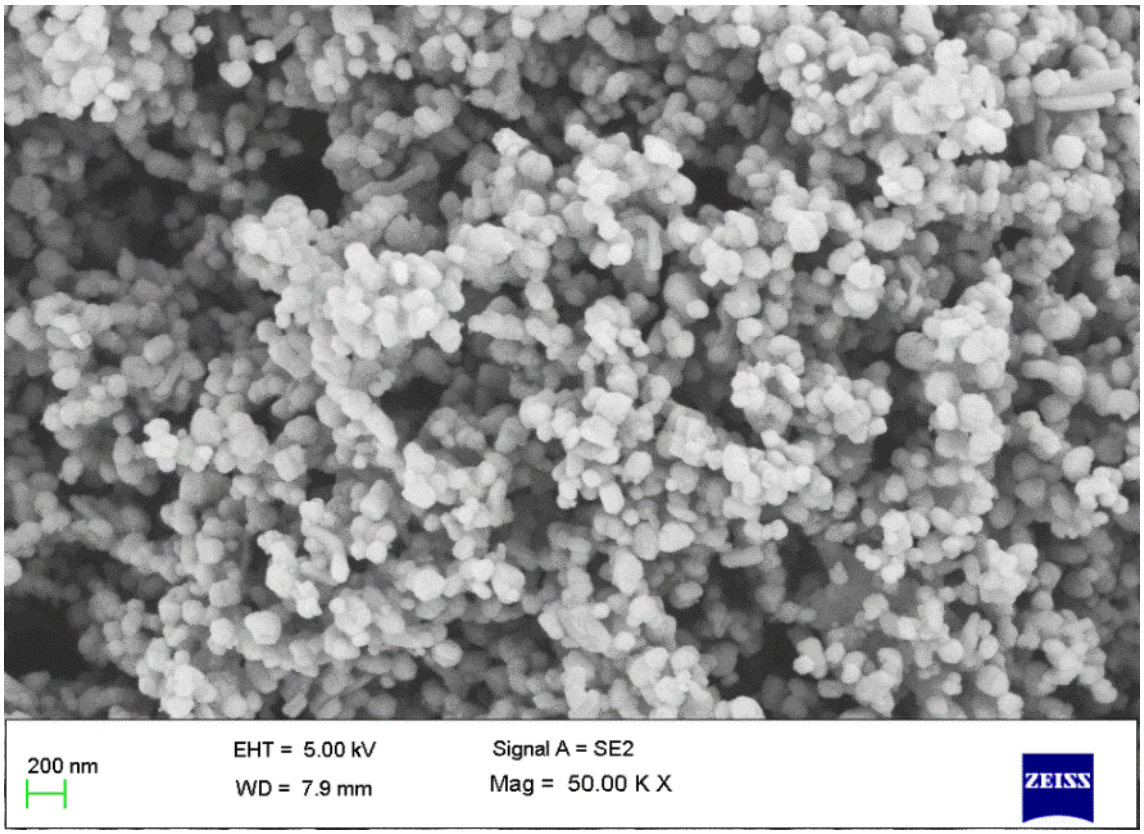

FIGURE 6

FESEM image of Ag_2O nanoparticles synthesized by using C. ambonicus Lour leaf extract

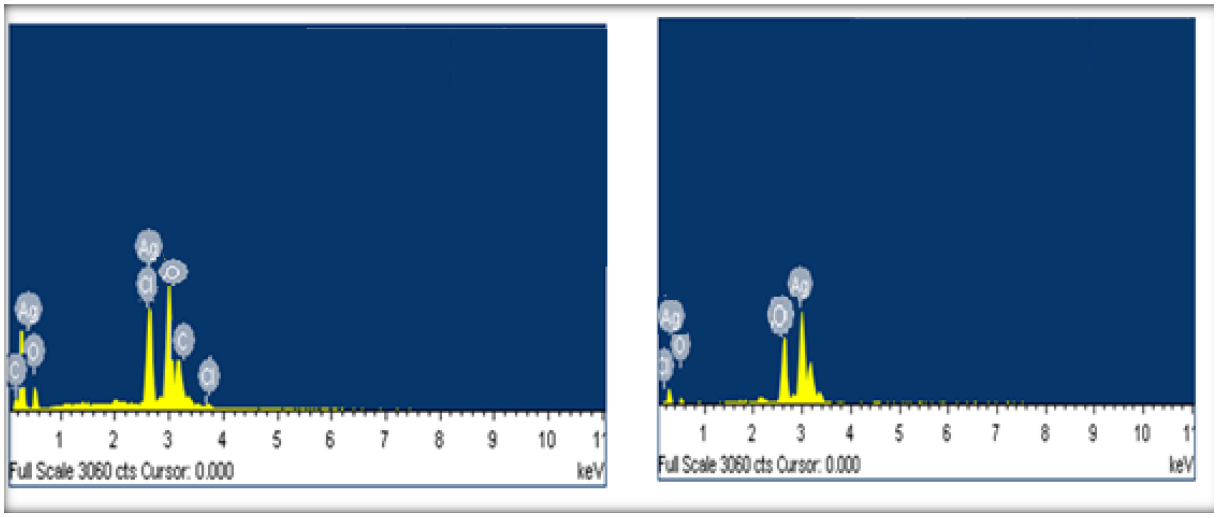

FIGURE 7

EDAX spectrum of Ag_2O nanoparticles synthesized from V. orientale Willd and C. ambonicus Lour leaf extract

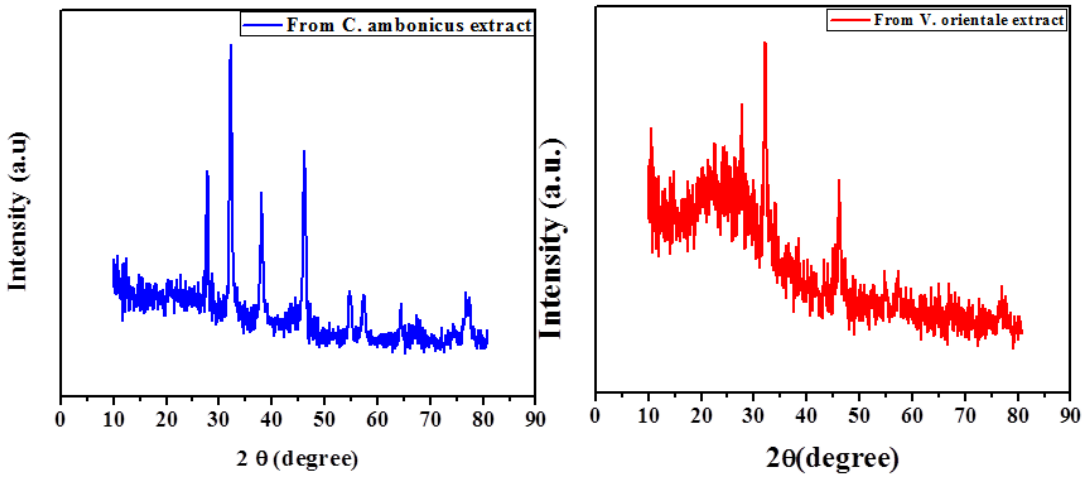

FIGURE 8

XRD pattern of Ag2O NPs synthesized from V. orientale Willd and C. ambonicus Lour leaf extract

(i) Inhibiting cell permeability and destroying the cell wall

(ii) Free radical protection

(iii) Significant enzymes are inactivated by thiol-based reactions,

(iv) Silver nanoparticles interactions with DNA, disruption of DNA replication and translation, and inhibition of signal transmission and bacterial growth via dephosphorylating tyrosine residues on peptides [11]. Studies on the antibacterial activity of Ag_2O NPs have shown that they have a considerable antibacterial effect on both Gramme positive and Gramme negative pathogens. Larger inhibition zones were seen in the disc diffusion studies compared to Gentamycin (Positive Control), which is similar to the outcomes reported by [12]. We were able to verify the bactericidal effectiveness of the biosynthesized Ag_2O NPs against several bacterial strains based on the variable diameters (in mm) of zone of inhibitions.(Figure 9 &10). Our results showed that Ag_2O NPs synthesized from V. orientale Willd and C. ambonicus Lour possess discrete antibacterial activity at different concentrations of 25-100 μg/mL (Figure 11 & 12). From 14 to 30, the zone of inhibition is present. There is no doubt that these eco-friendly Ag_2O

NPs have shown a great amount of activity. Ag_2O NPs' antibacterial activity greatly increases when the dosage level is raised. Therefore, we further proceeds only with the results of antibacterial activity of Ag_2O NPs. Our process for creating Ag_2O NPs was straightforward, economical, and environmentally benign.

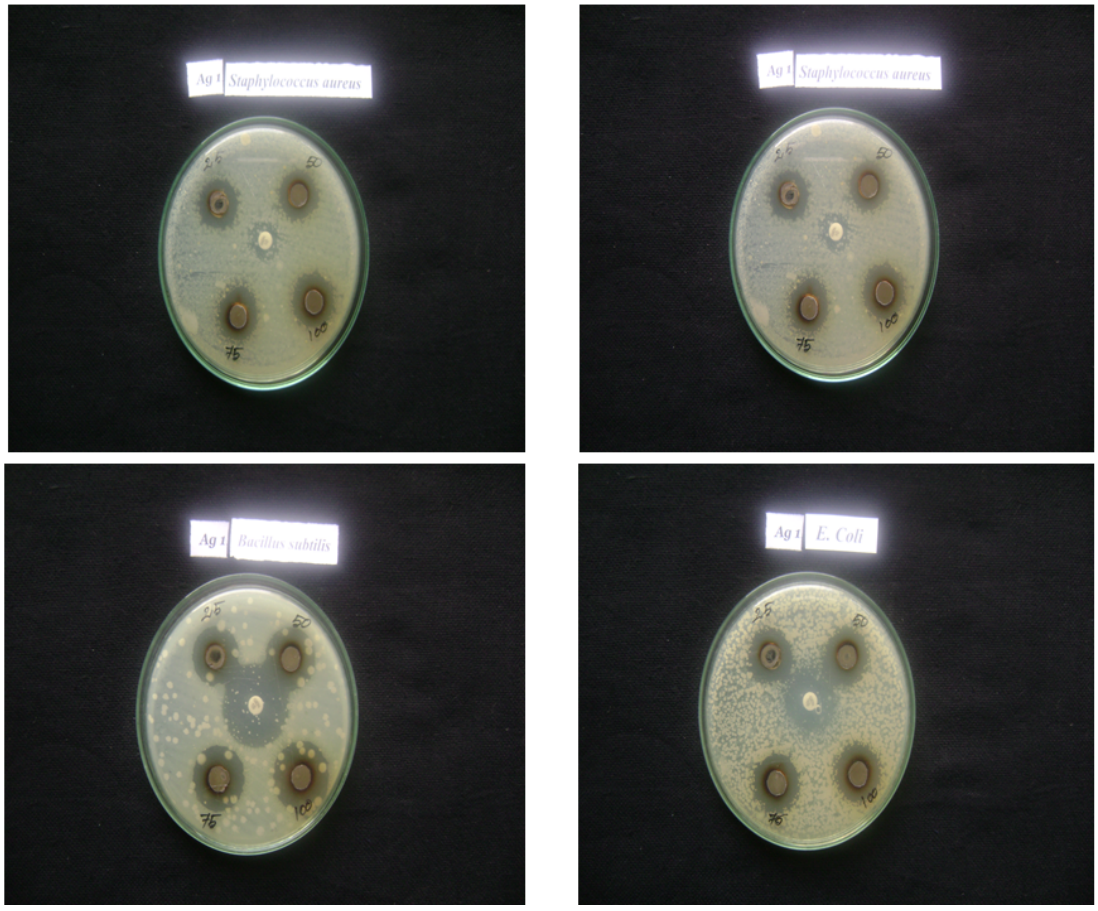

FIGURE 9

Zone of inhibition of Ag_2O NPs synthesized from V. orientale Willd S. aureus, B. subtilis, E. coli and P. aeruginosa

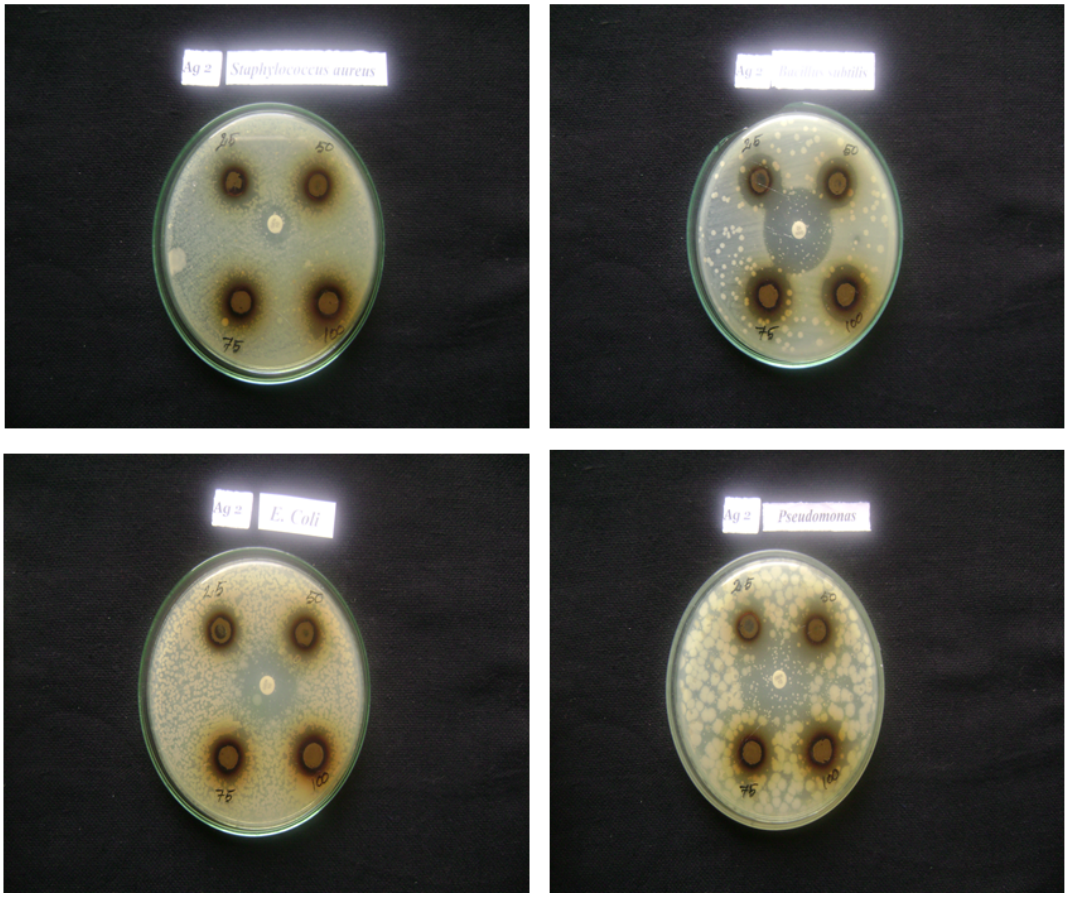

FIGURE 10

Zone of inhibition of Ag_2O NPs synthesized from C. amboinicus Lour leaf extracts against S. aureus, B. subtilis, E. coli and P. aeruginosa

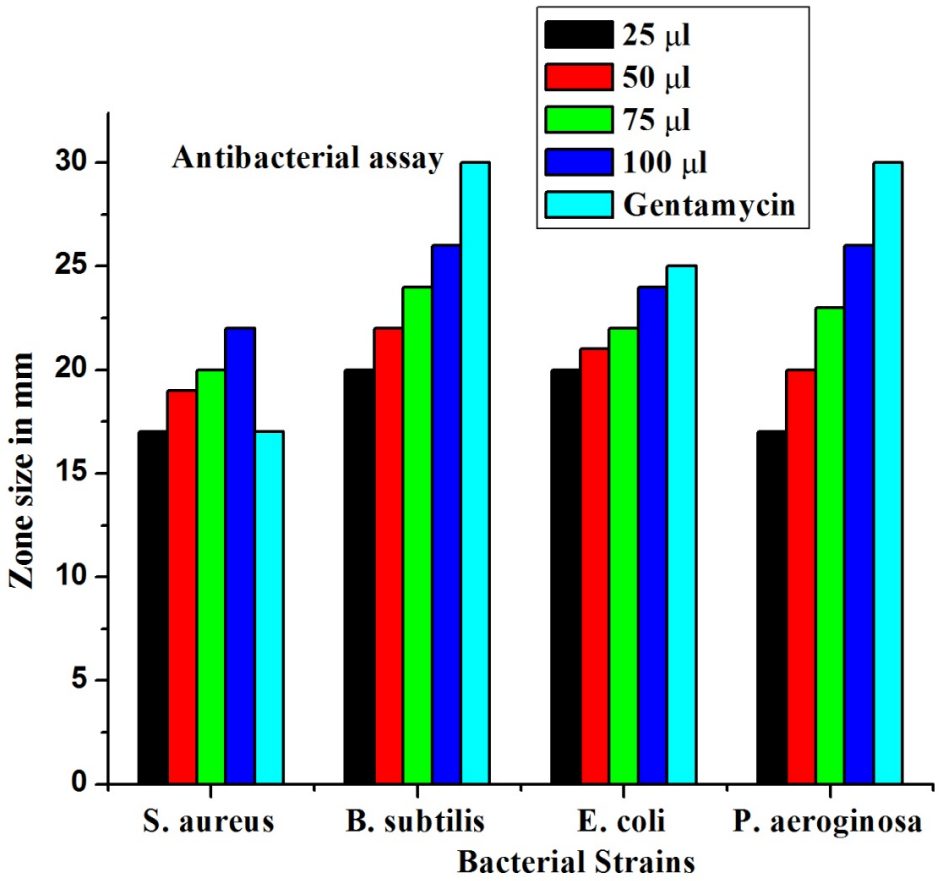

FIGURE 11

A bar diagram for the antibacterial activity

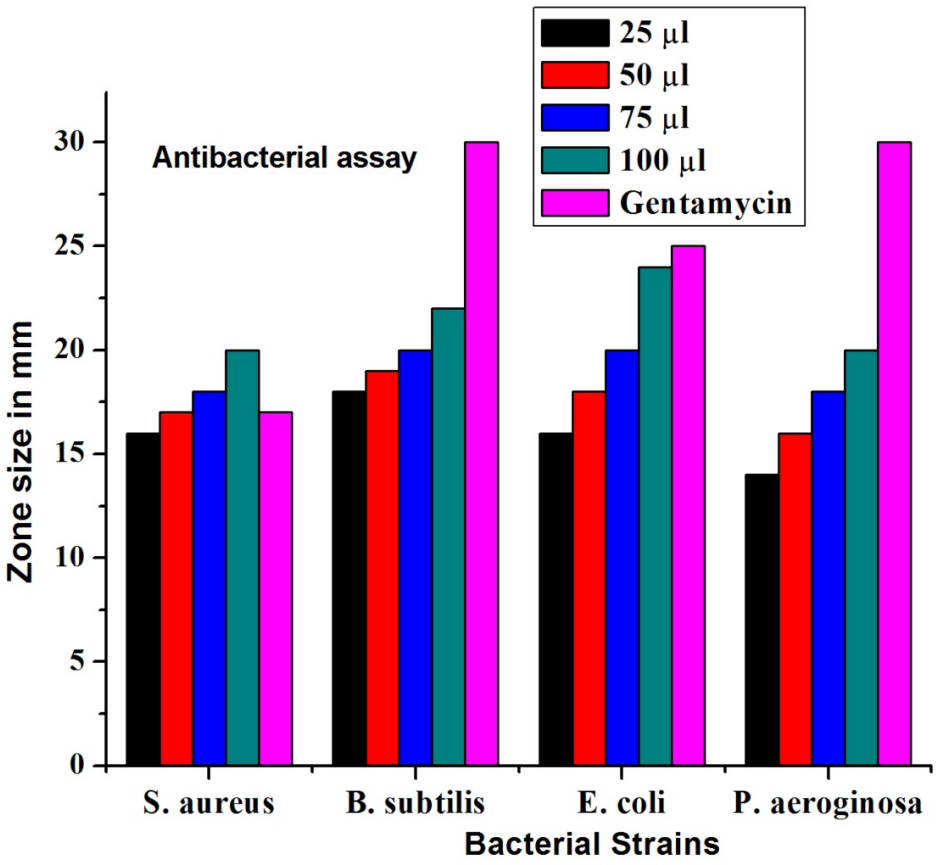

FIGURE 12

A bar diagram for the antibacterial activity

4 Conclusion

A noteworthy addition to green synthesis and nanotechnology is made in this study by the straightforward and biological synthesis of Ag_2O NPs. V. orientale Willd and C. ambonicus Lour leaf extracts were prepared and successfully employed for the development of Ag_2O NPs with spherical shapes. Powder XRD study showed the face-centred cubic lattice of both Ag_2O NPs. The average crystal of silver oxide nanoparticles are 34 ± 2 and 32 ± 2 nm synthesized from V. orientale Willd and C. ambonicus Lour plant extracts respectively. The biomoities found in the nanoparticles were confirmed by FTIR analysis. The creation of Ag_2O NPs is supported by UV-visible research. The antibacterial activity of Ag_2O NPs was well demonstrated by the clear zone of inhibition against S. aureus, B.subtilis, E.coli and P. aeruginosa. The synthesis of Ag_2O NPs using this straightforward, environmentally friendly, affordable, and greener process could be useful for biotechnological, biomedical, and environmental applications.

References

[1] H Bar, D K Bhui, G P Sahoo, P Sarkar, P De, A Sankar, and Misra. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, pages 134–139, 2009.

[2] D Cruz, P L Fale, A Mourato, P D Vaz, M L Serralheiro, and A R L Lino, 2010.

[3] P Cruz, F L De Quadros, J P Theau, A Frizzo, C Jouany, and M Duru. *Forest Ecology Management*, pages 350–358, 2010.

[4] C Dipankar, S, Murugan Colloids, and B Surface. Biointerfaces, 2012.

[5] S P Dubey, M Lahtinen, and M Sillanpaa, 2010.

[6] T Jebakumar Immanuel Edison and M Sethuraman, 2012.

[7] S Honary, H Barabadi, E Gharaeifathabad, and F Naghibi. *Digest Journal of Nanomaterials and Bio*, structures(7):999–1005, 2012.

[8] S.Justin Packia Jacob, J S Finub, A Narayanan Colloids, and B Surface, 2012.

[9] R Janardhanan, M Karuppaiah, N Hebalkar, and T N Rao, 2009.

[10] P Jiang, S S Xie, J N Yao, S J Pang, and H J Gao. *Journal of Physics D: Applied Physics*, pages 2255–2259, 2001.

[11] J S Kim, K Eunye, K N Yu, J H Kim, S J Park, and H J Lee. *Biology and Medicine*, (3):95–101, 2007.

[12] S H Kim, H S Lee, D S Ryu, S J Choi, and D S. *Lee Korean Journal of Microbiology Biotechnology*, pages 77–85, 2011.

[13] M Kowshik, S Ashtaputre, and S Kharraz. *Nanotechnology*, pages 95–100, 2003.

[14] C Krishnaraj, E G Jagan, S Rajasekar, P Selvakumar, P T Kalaichelvan, N, Mohan Colloids, and B Surface. Biointerfaces, 2010.

[15] K N Kumar, B Sangeetha, M Rajalekshmi, B Ravishankar, R Muralidhar, and B. *Yashovarma Indian Journal Natural Products Resources*, pages 260–269, 2013.

[16] K B Narayanan and N. *Sakthivel Material Characterization*, pages 1232–1238, 2010.

[17] A Nasrollahi, K H Pourshamsian, and P. *Mansourkiaee International Journal Nano Dimension*, pages 233–239, 2011.

[18] T Premkumar and K E Geckeler. *Journal of Physics and Chemistry of Solids*, pages 1451–1456, 2006.

[19] N Saifuddin, C W Wong, and A A. *NurYasumira European Journal Chemistry*, (6):61–70, 2009.

[20] W Wei, X Mao, A Luis Ortiz, and D R. *Sadoway Journal of Materials Chemistry*, pages 432–438, 2011.

[21] N L Yong, A Ahmad, and A W. *Mohammad International Journal Scientific Engineering Research*, pages 155–158, 2013.