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 (Ag₂O 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 Ag₂O particles making use of Viscum orientale Willd and Coleus amboinicus Lour plants. The formation of Ag₂O stretching frequency was confirmed by FTIR spectral studies. Scanning electron microscopy and EDAX analysis displayed Ag₂O nanoparticles have been pure and spherical shaped and had been the variety of length from 35±2 nm for (Ag₂O NPs) by the usage of Viscum orientale Willd and 32±2 nm for (Ag₂O 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 Ag₂O 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, apigenin, 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\textsubscript{2}O NPs using Viscum orientale Willd and Coleus amboinicus Lour leaf extracts. Herein, we report for the first time on the preparation of Ag\textsubscript{2}O 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$_2$O 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 15°C 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 Ag$_2$O 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$_2$O 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 Ag2O 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 Ag2O 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 aeruginosa 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 Ag2O NPs was suspended in sterile Dimethyl sulphoxide (DMSO) and constantly stirred until a uniform suspension (100 µl/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_{2}O 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_{2}O 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_{2}O NPs. [Figure 1].

![Figure 1](image_url)

**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$_2$O NPs were clearly visible between 400 and 450 nm.

![UV-visible spectra of Ag$_2$O nanoparticles synthesized from V. orientale Willd leaf extract](image)

**Figure 2**

UV-visible spectra of Ag$_2$O 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$_2$O NPs in the solution. The broadening of peaks suggests monodispersed...
3.3 FT-IR spectroscopy studies

The biomolecules that covered the Ag$_2$O NPs were identified using FT-IR spectroscopic studies. FT-IR spectrum of created Ag$_2$O 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$_2$O NPs in addition to the XRD analysis conducted.

3.4 Morphological analysis

Figure 5 and 6 showed the FESEM images of the synthesized Ag$_2$O NPs. Images show that NPs formed and were covered with their biological components. The Ag$_2$O NPs were found to be...
polydisperse and spherical in shape by means of the FESEM pictures. The size of the particles obtained as $35 \pm 2$ nm for Ag$_2$O NPS synthesized by using V. orientale Willd while it where $32 \pm 2$ for the synthesized from C. ambonicus Lour.

3.5 Elemental analysis

Figure 7 shows the EDAX spectrum of Ag$_2$O 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$_2$O NPs. The synthesised sample's atomic weight percentage is Ag = 40.71, O = 9.72 for Ag$_2$O NPs synthesis from V. orientale Willd whereas Ag = 64.20, O = 7.51 synthesized from C. ambonicus Lour leaf extract.
3.6 X-ray diffraction studies

The X-ray diffractometer The (110) and (111) of Ag$_2$O 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$_2$O (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$_2$O, 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 Ag₂O nanoparticles synthesized by using C. ambonicus Lour leaf extract

FIGURE 7
EDAX spectrum of Ag₂O nanoparticles 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$_2$O 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$_2$O NPs against several bacterial strains based on the variable diameters (in mm) of zone of inhibitions.(Figure 9 &10). Our results showed that Ag$_2$O 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$_2$O
NPs have shown a great amount of activity. Ag$_2$O NPs’ antibacterial activity greatly increases when the dosage level is raised. Therefore, we further proceeds only with the results of antibacterial activity of Ag$_2$O NPs. Our process for creating Ag$_2$O NPs was straightforward, economical, and environmentally benign.

**Figure 9**

Zone of inhibition of Ag$_2$O NPs synthesized from V. orientale Willd, S. aureus, B. subtilis, E. coli and P. aeruginosa
**Figure 10**

Zone of inhibition of Ag$_2$O 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
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$_2$O NPs. V. orientale Willd and C. ambonicus Lour leaf extracts were prepared and successfully employed for the development of Ag$_2$O NPs with spherical shapes. Powder XRD study showed the face-centred cubic lattice of both Ag$_2$O 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 biomolecules found in the nanoparticles were confirmed by FTIR analysis. The creation of Ag$_2$O NPs is supported by UV-visible research. The antibacterial activity of Ag$_2$O NPs was well demonstrated by the clear zone of inhibition against S. aureus, B.subtilis, E.coli and P. aeruginosa. The synthesis of Ag$_2$O NPs using this straightforward, environmentally friendly, affordable, and greener process could be useful for biotechnological, biomedical, and environmental applications.

References


