Antimicrobial and Anticancer Activity Studies on Green Synthesized Silver Oxide Nanoparticles from the Medicinal Plant Cyathea nilgiriensis Holttum

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ABSTRACT
Objectives: To synthesize silver oxide nanoparticles from the medicinal plant Cyathea nilgiriensis Holttum and to evaluate the percentage of inhibition of silver oxide nanoparticles against some selective bacterial species (three gram positive and three gram negative) and two fungal species. Anticancer activity was performed for Ag2O nanoparticles using Trypan Blue Assay Method using Daltons lymphoma ascites cells. Methods: Silver oxide nanoparticles were synthesized by chemical precipitation method using the extract of the medicinal plant Cyathea nilgiriensis Holttum and Silver nitrate. Particle size Analyzer (PSA), X-ray Diffraction Analysis (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope Analysis (SEM) and Energy Dispersive X-Ray (EDX) studies studies were employed to confirm the size distribution, composition and crystallographic structure of the Silver oxide nanoparticles. Results: Particle size Analyzer and X-ray diffraction results prove the size and crystalline nature of Ag2O nanoparticles. Fourier transform infrared spectroscopy studies have proved that biomolecules have acted as capping and stabilizing agents in the synthesis of Ag2O nanoparticles. Scanning electron microscope analysis has exhibited morphological appearance of Ag2O nanoparticles and Energy Dispersive X-Ray analysis has proved the presence of both silver and oxygen in the synthesized Ag2O nanoparticles. On comparing the zone of inhibition of three-gram positive bacterial species, M. luteus (20 mm) has shown larger zone of inhibition (20 mm) and when comparing the zone of inhibition of three-gram negative bacterial species, K. pneumoniae has shown larger zone of inhibition (21 mm). The increase of concentration from 10 µg to 200 µg of Ag2O nanoparticles increases the anticancer efficiency of the biosynthesized Ag2O nanoparticles. Conclusion: The Instrumentation results have proved the characteristic nature of the synthesized Ag2O nanoparticles. Now it’s clear that greener synthesized nanoparticles has proved to be better antimicrobial and anticancer agents. Key words: Cyathea nilgiriensis Holttum, Ag2O nanoparticles, Green chemistry, Characterization, antimicrobial and anticancer activity.

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DOI: 10.5530/ijpi.2020.2.27

INTRODUCTION

Metallic Nano particles obtained using plant extract could be reducing and capping agents. Due to the unique properties silver oxide nanoparticle it is used in the fields such as sensing, optoelectronic, drug delivery, oxidation catalysis, sensors, fuel cells, photovoltaic cells, all-optical switching devices, optical data storage systems and in diagnostic biological probes. The syntheses of nanoparticles through chemical methods are expensive, time consuming and are not ecofriendly. Hence, Greener methods of synthesis of Ag2O nanoparticles have attracted many researchers wing to their cost-effectiveness and non-toxic nature. The green synthesis techniques make use of nontoxic solvents such as water, biological extract, biological systems and microwave irradiation. The use of plant leaf extracts, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications. Green synthesis does not require high pressure, energy, temperature and toxic chemicals. Silver has been recognized to have inhibitory effect on microbes present in medical and industrial process. The topical ointments having silver and silver nanoparticles are medicinally useful to prevent infections against burns and open wounds. Thus synthesis of nanoparticles by greener methods gain importance. In the present study, the medicinal plant Cyathea nilgiriensis plant extract has been used to produce silver oxide nanoparticles. Based on the literature survey it is evident that the systematic study on the bio-synthesis and characterization of Ag2O nanoparticles using Cyathea nilgiriensis Holttum extract has been carried out for the first time.

MATERIALS AND METHODS

Plant materials collection and preparation
The pure and analar-grade Silver nitrate [AgNO3] was used for this study. The pure and shade-dried leaves of Cyathea nilgiriensis (2 g) were powdered and subjected to extraction using deionized water. The extract obtained was filtered through Whatman No. 1 filter paper and stored in a refrigerator for further use.

Synthesis of Ag2O nanoparticles
Exactly about 0.1 M of AgNO3 was dissolved in50 mL of double-distilled water along with10 mL of Cyathea nilgiriensis aqueous extract under under magnetic stirring at 80°C for 4 hr. The complex formed was ultra-centrifuged at 10,000 rpm for 10 min, rinsed with water and further
centrifuged at 5,000 rpm for 10 min. The complex residue was dried in an oven at 40°C for 8 h and was then calcined in a muffle furnace at 600°C to obtain biosynthesized Ag₂O nanoparticles.

**Instrumentation for Characterization of Nanomaterial**

The size of the nanoparticle was measured using the particle size analyzer (Nanophox, Sympatec, Germany). The biosynthesized Ag₂O nanoparticles were analyzed for IR characteristics using a Nicolet 520P FT-IR spectrometer set to be in the range of 500 – 4,000 cm⁻¹. A powder XRD analysis was carried out on a PAN analytical X-ray diffractometer operated at 40 kV with a current of 30 mA under Cu-Kα radiation of a 2θ range of 10–80°. The SEM images were recorded using a JEOL JSM 6390 system to identify the crystallographic details of Ag₂O nanoparticles. To confirm the presence of the constituent elements – silver and oxygen, an energy dispersive spectroscopic (JED 2300, JEOL) study was done on Ag₂O nanoparticles.

**Antimicrobial activity**

The antimicrobial activity was studied applying the disc diffusion method.²⁴ Ciprofloxacin and fluconazole were taken as standards for antimicrobial activity. A panel of 6 common pathogenic bacteria consisting of three gram-positive type S. aureus (NCIM 2079), B. subtilis (NCIM 2063), M. luteus (MCIM2169) and three gram-negative type E. coli (NCIM 2065), S. paratyphi (NCIM 2501), K. pneumonia (NCIM2707) and 2 fungal strains C. albicans (MTCC 3100) and A. niger (MTCC 1344) was used. These microbial strains were obtained from the Kovai Medical College and Hospital (KMC), Coimbatore, Tamil Nadu, India.

**Anticancer activity**

The in vitro cytotoxicity potentials of the biosynthesized Ag₂O nanoparticles were evaluated using Trypan blue dye assay method.²⁷,²⁸ The cells were aspirated from the peritoneal cavity of tumor bearing mice. The cells were washed three times using PBS and the viability of the cells was checked using trypan blue. Different concentrations (10, 20, 50, 100 and 200 µg) of Ag₂O nanoparticles were prepared. In a test tube, 100µl of Ag₂O nanoparticles was mixed with 800µl of phosphate buffer saline and 100µl (1×10⁶ in 1ml) of Dalton’s Lymphoma Ascites (DLA) was added. All the test tubes were incubated at 37°C for 3 hr. About 100µl of trypan blue dye was added to each of the test tubes. Dead cells got stained by the trypan blue color while live cells did not absorb the dye. The numbers of stained and unstained cells were measured using hemocytometer. Percentage of cytotoxicity was calculated by the given formula.

\[
\text{Cytotoxicity} (%) = \left( \frac{N_d}{N_d + N_l} \right) \times 100
\]

Where \( N_d \) - No of dead cells

\( N_l \) - No of live cells

**RESULTS**

**Particle Size Analyzer**

The average size of the nanoparticles and the statistical distribution of the size were determined using the particle size analyzer. The results are shown in Figure 2. The particle size of silver oxide nanoparticles is found to be below 100nm.

**Fourier Transform Infrared Spectroscopy Analysis**

Figure 3 shows the FT-IR spectrum of bio-synthesized silver oxide nanoparticles. The peak around 574 cm⁻¹ corresponds to Ag₂O vibrations.¹⁹⁻²¹ Other bands can be attributed to silver nitrate and the phytochemical constituents of the leaf extract. The O-H stretch appears in the spectrum at 3449 cm⁻¹. The band 1629 cm⁻¹ corresponds to the carbonyl group of flavonoids. Strong band at 1383 cm⁻¹ corresponds to phenolic stretching.
vibrations. Strong band at 823 cm$^{-1}$ corresponds to aromatic C-H bending. The results of FT-IR analysis indicated that phenolic type of compound has favored and stabilized the formation of metal nanoparticles minimizing the agglomeration. Figure 1 shows the scheme of the nanoparticle synthesis.

X-ray diffractometer

Figure 4 shows the X-ray diffractometer (XRD) spectrum of the biosynthesized silver oxide nanoparticles. It shows intense band peaks at 27.94, 32.27, 46.34, 54.92 and 67.48 which corresponds to (110), (111), (211), (220) and (222) planes of face centered cubic silver respectively. All the obtained peaks are similar to standard pure Ag$_2$O. The average size of the particles was determined by applying the Debye-Scherrer’s formula.

Morphological analysis

The SEM and EDX images of synthesized silver oxide nanoparticles are shown in Figure 5 (a), (b), (c) and (d). The results showed that Ag$_2$O nanoparticles were formed in different sizes and below 100 nm. A closer look at the image shows the presence of several nanoparticle clusters. In Figure 5 (a), particles the particles appear to be agglomerated and some individual crystals are clearly visible and are hexagonal in shape. In Figure 5 (b) and (c) some particles appear to be spherical in shape. The EDX results shown in Figure 5(d) further confirm the presence of silver and oxygen.

Antimicrobial activity of Ag$_2$O nanoparticles

In this study antimicrobial activity of Ag$_2$O nanoparticles synthesized using the extract of the medicinal plant Cyathea nilgiriensis was found to be significant. Figure 6 shows the antibacterial activity of the biosynthesized Ag$_2$O nanoparticles. Antibacterial activity was measured against three grams of positive bacteria (S. aureus, B. subtilis, M. luteus) and three gram of negative bacteria (E. coli, S. paratyphi, K. pneumoniae). Anti-fungal activity was carried out against C. albicans and A. niger. Ciprofloxacin is taken as the standard for all microbial strains. Table 1. Shows inhibitory effect of the Ag$_2$O nanoparticles. The zone of inhibition for standard ciprofloxacin for three gram positive bacteria was found to be 34 mm (S. aureus), 27 mm (B. subtilis) and 36 mm M. luteus. The zones of inhibition of Ag$_2$O nanoparticles for three gram positive bacteria are found to be 14 mm (S. aureus), 12 mm (B. subtilis) and 20 mm M. luteus. When we compare the above zone of inhibition of Ag$_2$O nanoparticles for three gram positive bacteria, M. luteus (20 mm) shows a larger zone of inhibition. The zone of inhibition of standard ciprofloxacin for three gram negative bacteria ranges from 36 mm (E coli), 35 mm (S. paratyphi) and 32 mm (K. pneumoniae). The zones of inhibition of Ag$_2$O nanoparticles for three gram negative bacteria are found to be 15 mm (E coli), 17 mm (S. paratyphi) and 21 mm (K. pneumoniae). On comparing the zone inhibition values of Ag$_2$O nanoparticles for three-gram negative bacterial species K. pneumoniae (21 mm) shows a larger zone of inhibition. Table 2. Shows antifungal results for the biosynthesized Ag$_2$O nanoparticles. The antifungal images of the Ag$_2$O nanoparticles protecting against two strains are shown in Figure 7. The zone of inhibition for the standard fluconazole of two fungal species was found to be C. albicans (35 mm) and A. niger (09 mm). The zone of inhibition of Ag$_2$O nanoparticles for two fungal species is found to be C. albicans (13 mm) and A. niger (10 mm).

Figure 4: XRD spectra of Ag$_2$Onanoparticles.

Figure 5: SEM (a, b & c) & (d) EDX spectra of Ag$_2$O nanoparticles.

Figure 6: Antibacterial images of Ag$_2$O nanoparticles a) S. aureus b) B. subtilis, c) M. luteusd) S. Paratyphi e) E. coli f) K. pneumoniae
DISCUSSION

The scheme of the possible mechanism for the formation of silver oxide nanoparticles is given in Figure 1. Suresh et al. performed Preliminary phytochemical screening for the ethanolic extract of C. nilgiriensis and confirmed the presence of Tannin, Saponnin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Carbohydrate, Protein, Anthroquinone, Polyphenol, Glycoside and Coumarin. Flavonoids are a group of polyphenolic compounds comprising anthocyanins, isoflavonoids, flavonols, chalcones, flavones and flavanones. The metal ion reduction may be due to the internal mechanism which leads to the conversion ketone to carboxylic acid in flavonoids. Some flavonoids have the ability to chelate with metal ion with their carbonyl group or p-electrons. Moreover, the functional group of water-soluble compounds such as flavonoids, terpenoids and phenolic groups present in the aqueous extracts of C. nilgiriensis appears to be responsible for the synthesis of Ag$_2$O nanoparticles and their stabilization. Johnson et al. reported the reduction of Ag ions and the stabilization of Ag nanoparticles due to the presence of flavonoids in Cyathea nilgiriensis extracts. In this work the flavonoid type of compounds could have formed complex with silver (I) ion of Silver Nitrate solution. Then this complex solution was then heated in hot air oven for 8 hr to form silver hydroxide further it was calcinated at 600°C to form stable silver oxide nanoparticles. The results of FTIR analysis confirmed that phenolic compounds in flavonoids have a stronger ability to bind with metal, indicating that phenolic group could form metal nanoparticles to prevent agglomeration and thereby stabilize the medium. This suggests that biological molecules could do dual functions of formation and stabilization of silver oxide nanoparticles in aqueous medium.

From the above result it’s clear that C.albicans (13 mm) presents a larger zone of inhibition compared to those of the other fungal species A. niger (10 mm) for the synthesized Ag$_2$O nanoparticles. Thus, nanoparticles can be used as antifungal agents and can help to overcome hurdles of fungal diseases in human beings.

Anticancer activity

Several in vitro studies have indicated that silver nanoparticles can enter cells by endocytosis. Figure 8. [a-f] shows anticancer images of Ag$_2$O nanoparticles at different concentrations. DLA (Daltons lymphoma ascites) bearing mice cell lines is used for finding cancer activity using different concentrations (10, 20, 50, 100 and 200µg) of Ag$_2$O nanoparticles. The cyclophosphamide drug is taken as a standard for the DLA cell line. Figure 8a shows a control image which has no inhibition. The cyclophosphamide drug is taken as a standard for the DLA cell line. Figure 8a shows a control image which shows no inhibition. Figure 8b-f shows clear that Ag$_2$O nanoparticles have increasing percentage of inhibition over higher concentration of the test sample. Table 3 shows the anticancer activity of Ag$_2$O nanoparticles.
role and finally lead to the death of pathogens. Interestingly, the inhibition zone was different according to the type of pathogens and the concentrations of Ag₂O nanoparticles employed. From the antimicrobial result its clear that by increasing the concentration of Ag₂O nanoparticles in the discs, the growth inhibition has also been increased consistently because of proper diffusion of nanoparticles in the agar medium. Another important impact is due to the small size of nanoparticle than the pore size of bacteria it crosses the cell membrane of the bacteria and inhibits its growth. The antifungal activity of Ag₂O is probably derived, through the electrostatic attraction between negatively charged cell membrane of microorganism and positively charged nanoparticles and the orientation of Ag₂O nanoparticles. Ag₂O nanoparticles synthesized using green method was found to have good antifungal activity against the tested fungi. Thus, nanoparticles can be used as potential antifungal agents and help overcome the hurdles in fungal disease management posed by development of resistance to conventional fungicides.

From the Anticancer activity report it’s clear that the increase of concentration from 10 µg to 200 µg increases the inhibition. At 200 µg the maximum percentage of inhibition was observed for Ag₂O nanoparticles. So it is concluded that green synthesized silver oxide nanoparticles have shown good antimicrobial and anticancer behavior at different concentrations.

CONCLUSION

Ag₂O nanoparticles have been successfully synthesized via novel greener procedure using only silver nitrate and the aqueous extract of the leaves of Cyathea nilgiriensis Holttum. The biosynthesis of Ag₂O nanoparticles was done via hydroxide precipitation following calcination at 600°C. The Ag₂O nanoparticles were analyzed through PSA, XRD, FT-IR, SEM and EDX to prove the characteristics of the nanoparticles. The PSA result confirmed the produced Ag₂O nanoparticles are below 100 nm in size. Biosynthesized Ag₂O nanoparticles were found to have significant antimicrobial and anticancer activities. The Ag₂O nanoparticle can be studied further to be the composite of a drug for commercial biomedical applications.

ACKNOWLEDGEMENT

The authors are extremely grateful to the SNS College of technology, Coimbatore, Tamil Nadu, India for providing financial support (faculty research seed money) and research facilities to accomplish this study. The authors are extremely grateful to the SNS College of Technology, Coimbatore, Tamil Nadu, India for providing financial support (faculty research seed money) and research facilities to accomplish this study. The authors are extremely grateful to the SNS College of Technology, Coimbatore, Tamil Nadu, India for providing financial support (faculty research seed money) and research facilities to accomplish this study.

CONFLICT OF INTEREST

There is no conflict of interest with this research.

ABBREVIATIONS


REFERENCES


Article History: Submission Date : 27-03-2020; Revised Date : 16-04-2020; Acceptance Date : 09-05-2020.