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GREEN SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

Mallika Pathak[1*], Malti Sharma[1], Himanshu Ojha[2], Rekha Kumari[3], Navneet Sharma[2], Bani Roy[1], Gaurav Jain[4]
[1]Department of Chemistry, Miranda House, University of Delhi, Delhi, India
[2]Division of Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences, Delhi, India
[3]Department of Zoology, Miranda House, University of Delhi, Delhi, India
[4]Department of Pharmaceutics, Jamia Hamdard, Delhi 110062
[*]Corresponding author: mallika.pathak@mirandahouse.ac.in

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Abstract

Metallic nanoparticles have gained the interest of researchers worldwide due to their unique antibacterial, antimicrobial and anti-inflammatory properties. There is a constant need for the sustainable green synthesis of the metallic nanoparticles with less involvement of the toxic chemicals. In this background, our group has synthesised the silver nanoparticles from the aqueous extracts of clove and cinnamon through green method. The aqueous spice extracts were used for the reduction of silver nitrate solution. The synthesised silver nanoparticles were characterised by the UV-Visible spectroscopy, dynamic light scattering (DLS) and transmission electron microscopy (TEM). Antibacterial properties of the nanoparticles were evaluated on the *Escherichia coli* and *Staphylococcus aureus* strains using the Kirby-Bauer antibiotic testing method. UV-Vis spectroscopy confirms the size of the nanoparticles to be around 30-60 nm which is further confirmed by the DLS and TEM techniques. Further, the antibacterial activity analysis showed that the bacterial samples (*S. aureus* and *E. coli*) treated with the synthesised silver nanoparticles showed minimum inhibitory concentration in the range of 25-30 µM. The study presents an environment friendly method to synthesise metallic nanoparticles showing good antibacterial activity. This work would help other research groups working in the field of biological application of green synthesis mediated metallic nanoparticles.

Keywords: Nanoparticles, green synthesis, clove, cinnamon and antimicrobial

INTRODUCTION

Metal nanoparticles have been the focus of research in the recent years owing to their unique optical, electronic, mechanical, magnetic, chemical and antimicrobial properties [1, 2]. These properties have been ascribed to their high surface to volume ratio. Due to these properties metal nanoparticles like silver have found applications in the catalysis, photonics, biological and electronics [3, 4]. A number of methods have been developed to synthesize silver nanoparticles (AgNP) over the past two decades involving the reduction of silver nitrate, $AgNO_3$ [5,6]. However, the chemical and physical methods are expensive and pose potential environmental hazards [7] due to their use of toxic chemicals. Green synthesis of AgNP is an alternative, cost-effective, efficient and eco-friendly technique compared to the conventional chemical methods.

Synthesis of AgNP is of particular interest in the medical field because they show good antimicrobial and anti-inflammatory properties and have been used for disease diagnosis and treatment of burn infections [8, 9]. However, strict implementation of safety guidelines issued by various regulatory agencies worldwide means that environment-friendly processes for nanoparticle synthesis without using toxic chemicals need to be developed. A variety of microorganisms and their products have been used for the synthesis of AgNPs [10, 11]. But, in these methods, cultures of microorganisms are needed to be maintained often. Therefore, use of plants and their parts are easy, cost effective and reproducible agents for synthesis of AgNPs [12].

In the present study, we have prepared AgNP from the aqueous extracts of clove (*Eugenia caryophyllus*) and cinnamon (*Cinnamomum zeylancium*) through green synthesis. The aqueous extracts of these spices were used as reducing agents for silver salt solutions in a single-pot process. The synthesised nanoparticles were further subjected to characterisation techniques like UV-Visible spectroscopy, particle size distribution and Transmission Electron Microscope (TEM) analysis.

EXPERIMENTAL

Materials and Methods

Materials: The spices were procured from local market. Silver nitrate of 99.8% purity was procured from Fisher Scientific. The water used for the synthesis was 18 Mega ohm MilliQ grade water derived from Millipore water system (Elix 3, Millipore Corp USA).

Preparation of Spice Extract

To prepare the extract, the spices were washed thoroughly with MilliQ water and then oven dried. The dried spices were finely powdered using mortar and pestle. 1% to 5% extracts of spice powder were prepared by boiling for 10 min. in Milli Q water. The

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extracts were filtered with Whatman filter paper Number 1 and the residual material was discarded. The filtered extracts were stored in the refrigerator at 4 ºC for use as reducing agent in the synthesis of AgNP.

Green synthesis of Silver Nanoparticles

40 mL of 1mM silver nitrate aqueous solution taken in a conical flask was placed on the hot plate with magnetic stirrer at a temperature of 60ºC. After around 3 minutes, 1 mL of 1% extract of clove was added to the silver nitrate solution. The progress in conversion of silver ions to AgNP was monitored by observing the colour change and UV-Vis spectroscopy. The time taken for conversion to AgNP was also noted. Similar procedure was applied for the preparation of AgNP using 1% cinnamon aqueous extract but the volumes of spice extracts was varied as per requirement.

Characterization techniques

Conversion of silver ions to AgNP in the aqueous solution was followed by observing the colour change and Tyndall effect and by measuring the UV-Visible spectra of the diluted sample on a Cary 100 (BIO UV-Vis spectrophotometer, Varian, Australia). Evaluation of size and polydispersity of the particles was carried out using a Zetasizer analyzer (Zetasizer 3600) at 25 °C with a scattering angle of 90° (Nanosizer/Zetasizer ZS Malvern Instruments, Worcestershire, UK). Morphological details of the AgNP were analyzed by transmission electron microscopy TEM (Morgagni 268D, FEI, Holla). Samples were mounted on copper grid, dried under vacuum and scanned at an accelerating voltage of 15kV before observation.

Study of antibacterial property in synthesized nanoparticles

Antibacterial activity of AgNP synthesized using spice extracts was studied by Kirby-Bauer antibiotic testing method [13,14] against Gram-negative (*Escherichia coli* ATCC 82 strain) and Gram-positive (*Staphylococcus aureus*) microorganisms procured from National Centre for Disease Control (NCDC), Department of Microbiology, New Delhi. The stock culture was maintained on Muller Hilton agar media (MHA) and the slant were incubated at 31^{o}C for 24 hrs and then stored in refrigerator at 4^{o}C. Sub culturing of strain was done after 10 days. Sterile paper discs of Whatman filter paper (5 mm in diameter) impregnated with synthesised AgNP were then placed on the inoculated agar and incubated at 37°C for 24 hours. After incubation, the diameter of the growth inhibition zones was measured. Erythromycin (15 µg/disc) and Vancomycin (30 µ/disc) were used as the positive standards control. All tests were conducted in triplicate.

RESULTS AND DISCUSSION

Optical observation

Spice mediated synthesis of nanoparticles is due to the presence of amino acids, essential oils, carbohydrate derivatives, resins, glycosides, phenylpropanoids etc. present in the spices as their phytochemicals. Studies have indicated that biomolecules not only play a role in reducing the ions to the nanosize, but also play an important role in the capping of nanoparticles [15]. We have prepared silver nanoparticles using aqueous extracts of spices such as clove and cinnamon by adding certain volume of spice extracts to silver nitrate solution with stirring at 60 °C till the colour change from light yellow to dark orangish yellow was observed which indicates the formation of AgNP (Fig. 1) [16]. The colour change arises due to the excitation of Surface Plasmon Resonance (SPR) in the AgNP. However, there was a slight difference in colour of AgNP obtained using clove and cinnamon which may be due to the variation in the size of the nanoparticles obtained as according to TEM the shape is spherical for both. Also, the slight changes in the colour could also be due to the difference in the relative activity in reduction of silver nitrate ions to metal nanoparticles [17]. Tyndall effect was also observed in the synthesised nanoparticles solution which was not found in $AgNO_3$ solution.

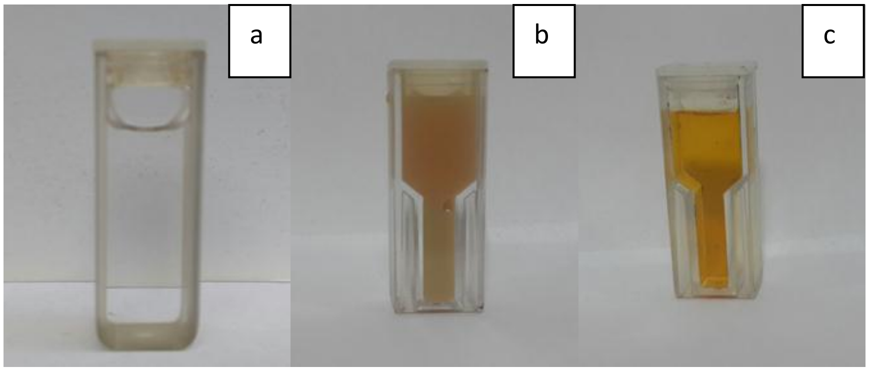

Figure 1: (a) $AgNO_3$ solution and AgNP prepared (b) using clove (b) using cinnamon

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Characterization by UV-Vis Spectroscopy

One of the most useful methods for characterization of metal nanoparticles is UV-Vis spectroscopy due to their intense Surface Plasmon Resonances (SPRs). Sharp SPR in the range of 350-600 nm confirms the presence of silver nanoparticles [18,19]. Size and shape are the various factors on which position of SPR depends. UV-Vis absorption spectra (Fig. 2) of synthesised AgNP obtained using clove and cinnamon extracts showed SP bands differing in their λ_{max} and SP band intensities. This indicates a clear influence of the nature of natural reducing agent i.e. spices on both λ_{max} and SP band intensities. From the Figure 2 it is clear that AgNP prepared using clove and cinnamon extracts showed single and intense SPR absorption band at 416 and 430 nm. The Plasmon band position for cinnamon is broader and less intense while SPR for clove is sharper and is towards lower wavelength indicating the particle size to be smaller than cinnamon. The SPR band in the range of 400-500 nm give preliminary indication of the size to be small (around 20-60 nm). This also strongly suggests that the AgNP were spherical [20].

Stability of prepared AgNP was also observed for one month using UV-Vis spectroscopy and it was found that the position of SPR band did not change even after one month depicting good stability of AgNP using spices as reducing agent. The UV-Vis spectra also revealed that the formation of AgNP occurred rapidly within the first 20-25 minutes. Furthermore, a graph was plotted between absorbance and time by collecting UV-Vis spectra after every 24 hrs. A straight line parallel to time axis confirms that synthesized AgNP remained stable in solution even after one month of completion of reaction (Fig. 3). The frequency and width of the SPR band depend on the size and shape of the metal nanoparticles. [21].

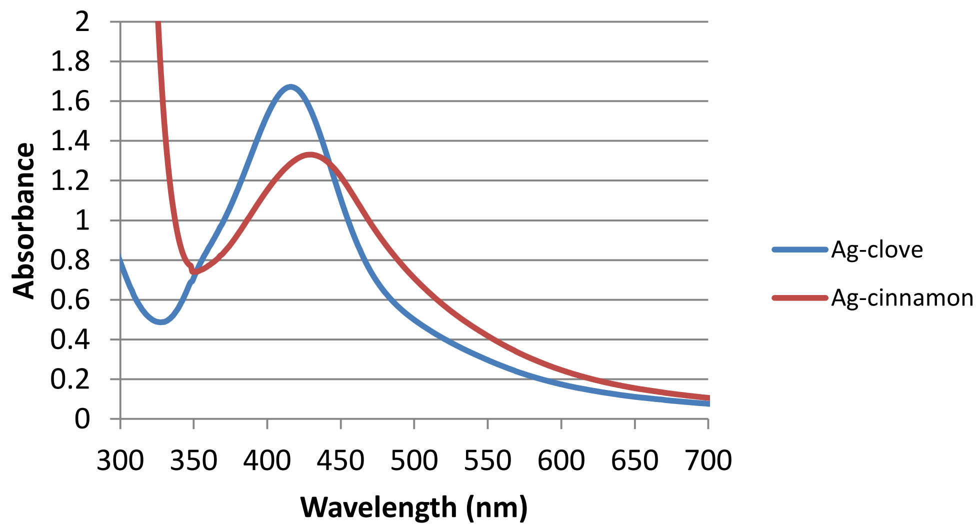

Figure 2: UV-Vis Spectrum: Plasmon Resonance of AgNP synthesised using clove and cinnamon

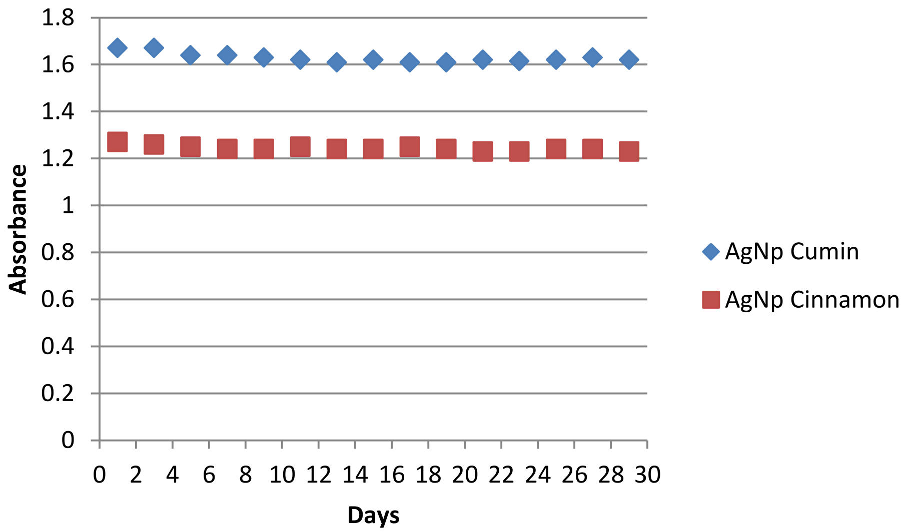

Figure 3: Stability of AgNP formed using clove and cinnamon in terms of absorbance values over 30 days

Effect of temperature in the preparation of NP was also noted and it was found that as temperature increases, rate of reaction also increases.

Characterization by Zeta Potential and Dynamic Light Scattering

Zeta potential is an essential characterization technique to study the stability of silver nanoparticles in aqueous solution. It is used to determine the surface potential of the silver nanoparticles. Zeta potential values more positive than +30mV or more negative than -30mV indicate stable silver nanoparticles [22]. The size distribution and zeta potential of the AgNP prepared by clove and cinnamon extracts were determined. For the obtained AgNP, zeta potential values using clove and cinnamon were found to be -16mV and -30mV respectively. This indicates the higher stability, good colloidal nature and high dispersity of AgNPs due to negative–negative repulsion for AgNP using cinnamon as compared to using clove. Particle size distribution curve reveals that AgNPs obtained are monodispersed in nature with PDI 0.3-0.5 (Tables 1 and 2) and Z-average (d.nm) ranging from 93-170.

Table 1: Size distribution analysis of AgNP prepared by cinnamon extract

Parameter	value	Peak no.	Peak diameter (nm)	Peak intensity (%)	Peak width (nm)
Z-average (d.nm)	170.3	Peak no. 1	224.6	94.9	136.0
PDI	0.385	Peak no. 2	4559	5.1	851.2
Intercept	0.745	Peak no. 3	0.000	0.0	0.00

Table 2: Size distribution analysis of AgNP prepared by clove extract

Parameter	value	Peak no.	Peak diameter (nm)	Peak intensity (%)	Peak width (nm)
Z-average(d.nm)	93.15	Peak no. 1	169.0	95.9	143.0
PDI	0.501	Peak no. 2	3970	4.1	1090
Intercept	0.762	Peak no. 3	0.000	0.0	0.00

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Transmission electron microscopic studies

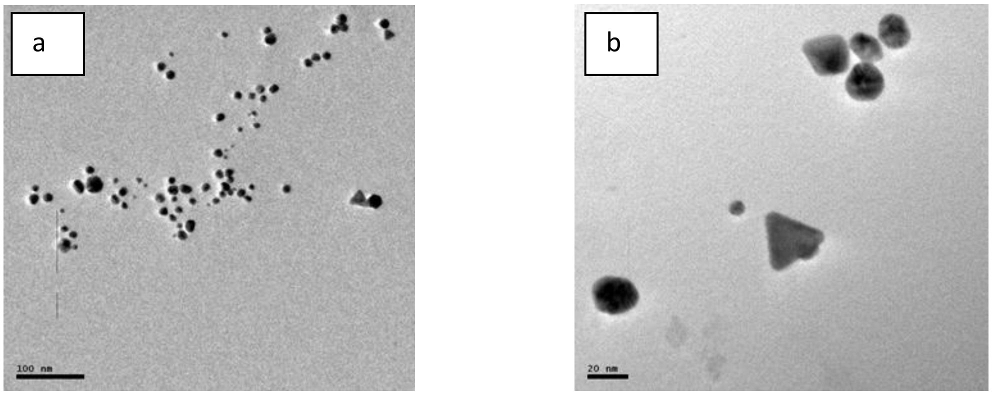

Figure 4: TEM images of AgNP prepared (a) using clove and (b) cinnamon extracts

Transmission electron microscopy (TEM) is used to understand surface morphology, size of the nanoparticles and provide information about their distribution. TEM micrograph suggest that the prepared AgNPs are mostly spherical and well dispersed (Fig. 4 (a), (b)) with particle size ranging from 10 - 15 nm using clove extract and 20 - 25 nm using cinnamon extract. It is also evident from the TEM images that AgNP prepared using clove and cinnamon did not aggregate in solution confirming the suitability of using clove and cinnamon not only as reducing agent but also as effective stabilizers.

Antimicrobial activity evaluation

The in vitro antimicrobial activity was evaluated using the disc diffusion method with determination of inhibition zones [23]. The antimicrobial activity can be inferred from the zones of inhibition seen around the disc impregnated with AgNP made using clove and cinnamon extracts as reducing agents. AgNP made using spice extracts have shown better antimicrobial activities (Fig. 5) in general [24] both against *S. aureus* and *E. coli*. AgNPs showed best antimicrobial activity at concentration of 30 µM with an inhibition zone of 2.2 mM against *S. aureus* and 2.1 mM against *E. coli*. (Table 3). Clove showed best antimicrobial activity against *S. aureus* while cinnamon showed best antimicrobial activity against *E. coli* (Fig. 6). The method has its merits in using natural spices as reducing agents which show no toxicity or side effects against human cells.

Table 3: Average Inhibition zone of synthesised AgNPs

Bioactive agent	**Zones of Inhibition (in cm)**		
		S. aureus	***E.coli***
AgNP	1µM	Nil	Nil
	10 µM	Nil	1.3
	20 µM	Nil	1
	25 µM	Nil	Nil
	30 µM	2.2	2.1
Erythromycin	(15 µg/disc)	1.6	1.1
Vancomycin	(30 µg/disc)	1.2	Nil

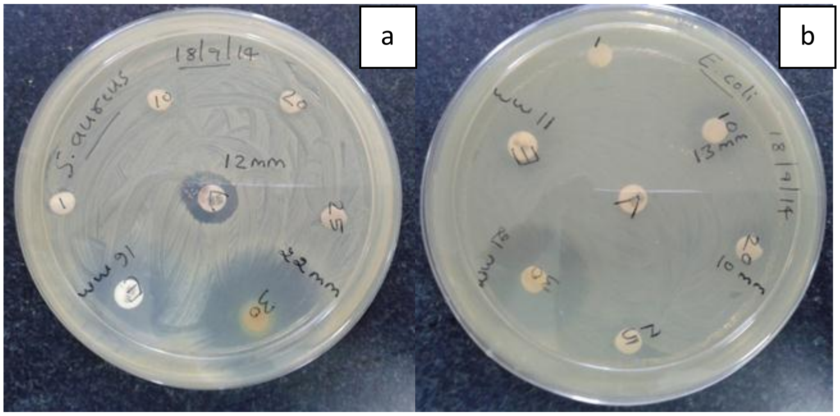

Figure 5: Antimicrobial activity of AgNPs against (a) S. *Aureus* (b) E. *Coli*

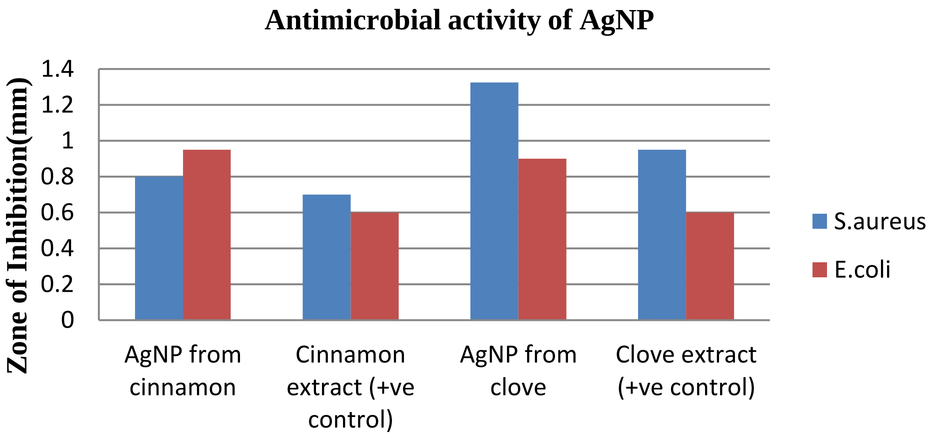

Figure 6: Comparison of antimicrobial activity of AgNP synthesized using clove and cinnamon extract against (a) *S. aureus* (b) *E. coli.* To compare the effect of AgNP, extract alone were used to study the antibacterial effect.

CONCLUSION

Silver nanoparticles (AgNPs) were synthesised with success using aqueous extracts of clove (*Eugenia caryophyllus)* and cinnamon (*Cinnamomum zeylancium)*. The synthesized AgNP were spherical in shape and monodispersed. Characterization of synthesized AgNP was done using UV-Vis spectroscopy, Zeta Potential, DLS and TEM followed by antimicrobial study. This study shows that AgNP prepared using cinnamon are more stable than obtained using clove and size of AgNP using clove is smaller than that using cinnamon. These AgNP have also shown good antibacterial properties. In future, green AgNPs can be used in catalysis, medicines and in other applications instead of using chemically synthesized AgNP so they can be used as a safer alternative to chemically synthesized AgNP. The authors in the study are working on other metal NPs using a similar method.

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