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Green Synthesis of Silver Nanoparticles by Using Neem (Azadirachta Indica) and Amla (Phyllanthus Emblica) Leaf Extract

KEYWORDS: Silver nanoparticles, Azadirachta indica, Phyllanthus emblica

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ABSTRACT *The present study describes a novel green synthesis of silver nanoparticles by using Azadirachta indica and Phyllanthus emblica leaf extract. The green synthesized silver nanoparticles had been confirmed by UV-Vis spectrum. Based on the findings, it seems very reasonable to believe that this greener way of synthesizing silver nanoparticles is an environmentally viable technique.*

Introduction

Silver is an effective antimicrobial agent, exhibits low toxicity (Sondi and Sondi 2004). Although there are many routes available for the synthesis of silver nanoparticles, bio-inspired synthesis such as use of plant sources offers several advantages such as cost-effectiveness, eco-friendliness and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods (Sun and Xia 2002). A variety of techniques including physical and chemical methods have been developed to synthesize silver nanoparticles. The physical methods are highly expensive and chemical methods are harmful to the environment (Yang et al 2011). Therefore, there is a growing need to develop eco-friendly nanoparticles that do not use toxic chemicals in the synthesis (Saad et al 2011). The recent reports include the synthesis of nanoparticles using medicinal plants (Mukunthan and Elumalai 2011). This stands as a great application in the field of nano-medicine. Medicinal property of the extract and nano-silver could play vital role in treatment of many diseases. Most of the reported green synthesis methods using plants took more than 1 hour for the formation of colloidal silver. In the present paper, the emphasis is on a greener synthesis of Ag-NPs using an extract from the leaves of Azadirachta indica and Phyllanthus emblica. The nanoparticles obtained through this method will be referred to as 'greener' silver nanoparticles (Gr-Ag-NPs). The antibacterial properties of Gr-Ag-NPs have received most attention; they were made to interact with Pseudomonas aeruginosa and Staphylococcus aureus. We also evaluated the leaf extract for their antibacterial properties using the same experimental methods in order to compare them with Gr-Ag-NPs. The results suggested that Gr-Ag-NPs had better antibacterial properties. Thus greener synthesis of Ag-NPs which not only have environmental advantages but also showed better antibacterial properties than those plant leaf extract. We assume that some organic molecules present in the leaf extract reduce the Ag+ to Ag-NPs. Our present conclusion undoubtedly reveal that it is really possible to have a much greener way to synthesize Ag- NPs without compromising their antibacterial properties and thus plant extracts may prove to be a good alternative to obtain Ag-NPs with enhanced antibacterial properties.

Materials and methods

Plants and Chemicals

Healthy leaves of Azadirachta indica and Phyllanthus emblica were collected from the Kurukshetra University campus. The chemicals, media and reagents used in the present studies were taken from Hi Media Laboratories, CDH and Rankem etc. The chemicals were of AR grade.

Preparation of plant leaf extract, silver ion complex and green synthesis silver nanoparticles

The plant leaves of Azadirachta indica and Phyllanthus emblica were washed thrice with tap water and distilled water and kept in the room temperature for air dry. After drying the known amount of leaf samples were chopped into fine and small pieces. The chopped 25 gram of leaves added with 100 ml of distilled water and boiled up to 100℃ for 30 minutes. After the desired reaction period the desired samples were filtered through Whattman filter paper to get the leaf extract. Leaf extracts were stored at -20℃ for further study. For the preparation of 1mM silver nitrate, 0.0421gm of $AgNO_3$ was added to 100 ml of double distilled water. The solution was mixed thoroughly and stored in colored bottle in order to prevent auto oxidation of silver. For the synthesis of plant mediated silver nanoparticles, the leaf extract and 1mM silver nitrate solution were taken in 1:4 ratio respectively and kept on a water bath at 60℃ for 30 minutes until the color change was observed. This indicates the preliminary confirmation for the formation of plant mediated silver nanoparticles. UV visible spectrophotometer is the main technique to examine the silver nanoparticles in the aqueous suspension. Green synthesized silver nanoparticles from Azadirachta indica and Phyllanthus emblica were taken for UV spectra Analysis (320-750 nm).

Results

It is well known that silver nanoparticles exhibit yellowish colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. The appearances of yellowish colour in the reaction tubes suggest the formation of silver nanoparticles. Silver nitrate was used as reducing agent as silver has distinctive properties such as good conductivity,

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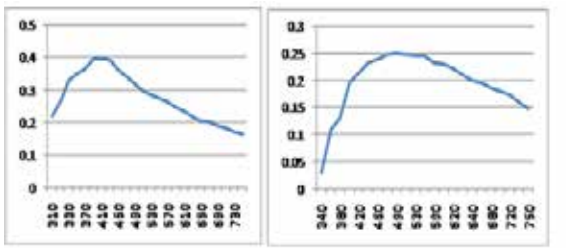
catalytic and chemical stability. The aqueous silver ions when exposed to herbal, leading to the formation of silver hydrosol. The green synthesized silver nanoparticles had been confirmed by UV-Vis spectrum of the reaction media. The UV-Vis spectrum of green synthesized silver from Azadirachta indica and Phyllanthus emblica have maximum absorbance peaks at 370 and 350 respectively as shown in Fig. 1.



(A) *Azadirachta indica* leaf extract (B) *Phyllanthus emblica* leaf extract

Fig. 1 UV-Visible absorption spectrum of green silver synthesized nanoparticles

The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles. The electron released during glycolysis (photosynthesis) for conversion of NAD to NADH led to transformation of Ag (NO_3) to form nanoparticles.

Discussion

Aqueous silver ions were reduced to silver nanoparticles after addition of Azadirachta indica and Phyllanthus emblica leaf extract followed by incubation of the mixture for studied period of time. Reduction of colloidal silver leading to change in colour has been frequently observed by several authors who successfully synthesized silver nanoparticles using different biomaterial (Khandelwal et al., 2010). This colour was primarily due to surface Plasmon resonance of deposited silver nanoparticles. In case of control experiment where leaf extract was not added to silver nitrate solution exhibited no change in colour even left for one week duration. The UV-Vis spectrum of green synthesized silver from Azadirachta indica and Phyllanthus emblica have maximum absorbance peaks at 370 and 470 respectively as shown in Fig. 1. Absorption spectra of the mixture was continuously recorded for 5 days time period, yielded no significant change in the intensity of absorption maxima suggested a stable nanoparticle formation. Dilute solution of silver nanoparticles have been used to treat various infections and burns (Raut et al., 2010). A number of theory for antimicrobial actions of colloidal silver solution have been proposed for example, alteration of permeability of cell membrane (Sondi and Sondi, 2004), release of lipopolysaccharides and membrane proteins (Amro et al., 2000), generation of free radicals responsible for the damage of membrane, dissipation of the proton motive force resulting in the collapse of the membrane potential, however, exact mechanism has not been fully deciphered.

Conclusion

The present study describes synthesis and antimicrobial activity of silver nanoparticles using Azadirachta indica and Phyllanthus emblica leaf extract. Characteristic light yellow colour appeared after incubation of the mixture. UV-Vis spectroscopy showed absorption maxima at 370 and 470 respectively. Use of Azadirachta indica and Phyllanthus emblica leaf extract offers an affordable, environment friendly technique for synthesis of large scale silver nanoparticles.

Conflict of Interest - The authors declare that they have no conflict of interest

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