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Green Synthesis of Silver Nanoparticles using *Calendula Officinalis* and its Anti-bacterial Studies

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Abstract

Green synthesis of Silver nanoparticles was carried out using *Calendula officinalis* (marigold flower) extract without use of any organic solvent and heat. The biosynthesis of Ag nanoparticles was confirmed by UV-Vis Spectrophotometer and XRD. The surface morphology and size of the prepared nanoparticles were confirmed by TEM. The anti-bacterial properties of the prepared NPs were tested against *E. coli* bacteria by growth inhibitory zone plate well method.

Keywords: Green synthesis, Ag Nanoparticles, Anti-bacterial properties, incubator

1. Introduction

In this era of science and technology, one of the major threat earth is facing is toxication of various hazardous chemicals leading to increasing fatal rate of living species. The main cause for such toxication is the use of harmful chemicals for synthesis of various drugs and their improper and untreated disposal. To overcome the problem one of the measure that can be taken is the switching of

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conventional methods of synthesis to the better, efficient green method of synthesis which involves minimizing the use of toxic chemicals, organic solvents, heat, complexity and thereby increasing the use of water based solvents and bio based compounds for synthesis of nanomaterials. The incorporation of biological materials to nanotechnology which is itself an area of scientific interest which become an emerging and very promising field for the last few years[1][2]. The use of metal based nanoparticles has gained much importance due to their potential applications in biomedical, optical and electronics and other different applications [3].

Among various metal based nanoparticles which have found a wide range of applications, the silver nanoparticles find several applications. Silver nanoparticle's ease to interact with other particles is because of its very small size and having large surface area to volume ratio. Silver nanoparticles had already been known for treatment of various diseases. It also has its conductive applications, optical applications, anti-microbial applications in cosmetic industry, as disinfectant etc[4]. Nanoparticles of silver are now used in toothpastes, soaps and face creams, food packaging, clothing, household appliances, disinfectants and wound dressing for its excellent anti-bacterial properties [5].

Synthesis of silver nanoparticles is of prime importance as silver nanoparticles have worldwide applications in almost every area of interest and their use can't be restricted thinking of its hazardous source of synthesis, so there is a need to develop a facile, cost-effective and green route for the synthesis of nanoparticles[6][7].In this study, we are interested in the green route for their synthesis of silver nanoparticles and analyzing the efficiency of synthesized silver nanoparticles for antibacterial activity thereby suppressing the growth of E.*coli*[8]which is a gram negative bacteria. It is well known for causing some of the most common diseases like Gastroenteritis, Urinary tract infections, Neonatal meningitis [9].

2. Experimental

2.1 Reagents

Silver nitrate was purchased from Sigma-Aldrich and was used without purification. The solution was prepared in distilled water and the plant extract was prepared in spring water. Marigold Flowers were purchased from a local flower merchant in Gurgaon (Haryana).

2.2 Preparation of silver nanoparticles

The preparation of silver nanoparticles was carried out by modifying the method of preparation of Sreeram*etal.* [10].2mM $AgNO_3$ solution was prepared by dissolving 0.0339g $AgNO_3$ in 100ml distilled water. To prepare the plant extract, *Calendula officinalis* (marigold flower petals) were first dried under sun and then crushed and grinded in mortar to obtain a powder like substance. The dried and grinded petals are then boiled in spring water for 20 minutes to get the extract. 3ml of this prepared plant extract was added to 5ml of $AgNO_3$ solution. This mixture was transferred to a test tube and kept in incubator at 37^oC for about 2 hours. The brown colored solution turned to yellowish brown color showing Tyndall effect in presence of light.

2.3Characterization

The synthesized silver nanoparticles were characterized spectrophotometrically using double beam UV-visible Spectrometer. The samples were analyzed in UV spectrophotometer(Shimadzu UV-1601)by diluting it two times with distilled water where distilled water was used as blank. XRD pattern was recorded using X-ray diffractometer (Shimadzu (XRD-6000). The data was collected under experimental condition $10^o<2\Theta<70^o$.The surface morphology and size of prepared silver nanoparticles were studied by TEM.TEM were performed on a JEOL 3010 instrument operated at an accelerating voltage of 300 kV.

2.4 Antibacterial Studies

Growth Inhibitory zone plate well method was used to study the antibacterial activity of prepared Ag nanoparticle tested against *E.*

coli. Gram-negative bacteria. Broth is used to prepare the bacteria culture and the same thing is incubated for 24 hours at a temperature of 37 °C using an incubator. Culture broth spreading is carried out to prepare a lawn of bacterial culture. The culture contains the test organism on solid nutrient agar plates with the aid of sterilized glass spreader. Then the plates were kept standing for 8-10 minutes to let the culture gets absorbed. After the absorption of bacterial culture, wells of 5mm diameter were made using petri dishes by punching the head of sterile micropipette tips into the gar plates and it is filled with 50 µL of Ag Nanoparticles in sterile water. The petri dishes were incubated for around 24 hours at a temperature of 37^{o}C using incubator and the antimicrobial effect of Ag nanoparticles calculated using inhibition zone.

3. Results and discussions

3.1 Formation of silver nanoparticle

A colourless clear solution appeared at room temperature which turned to brownish colour on heating and gradual further heating for next 2 hours resulted into a greyish black colour solution, the UV scan for the solution was done which gives the absorption maximum at 420 nm(Surface Plasmon Resonance) which confirms the formation of silver nanoparticles (Figure 1).The XRD patterns of the synthesized silver nanoparticles suchas (111), (200) and (220) are in good agreement with the literature results obtained for silver nanoparticles (Figure 2) [11].

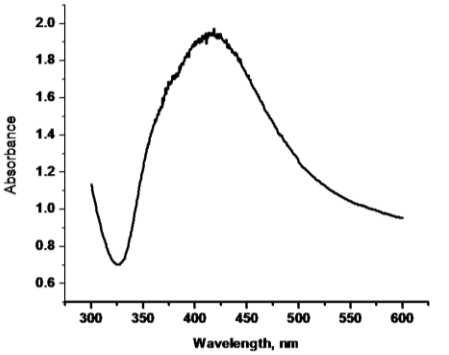


Figure 1: UV-Vis absorption of biosynthesized Ag Nanoparticles

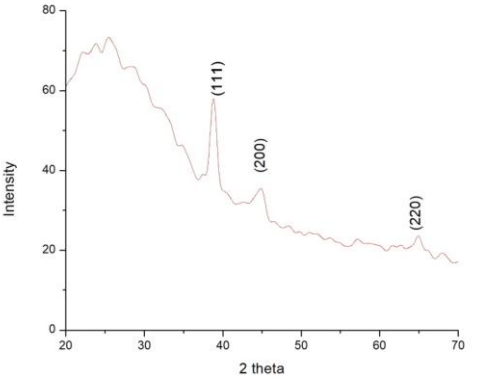


Figure2: XRD pattern of biosynthesizedAg Nanoparticles

3.2 TEM analysis

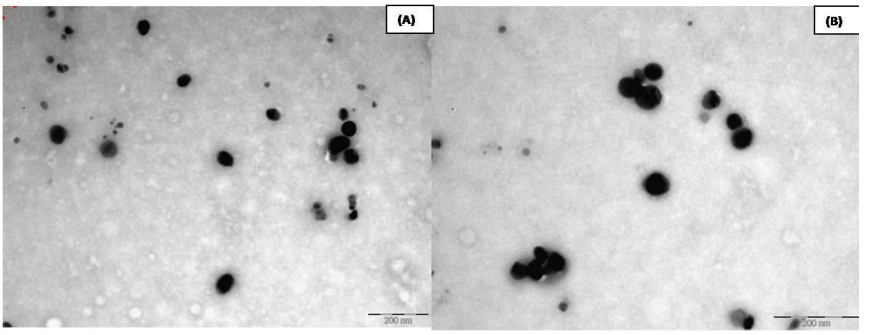


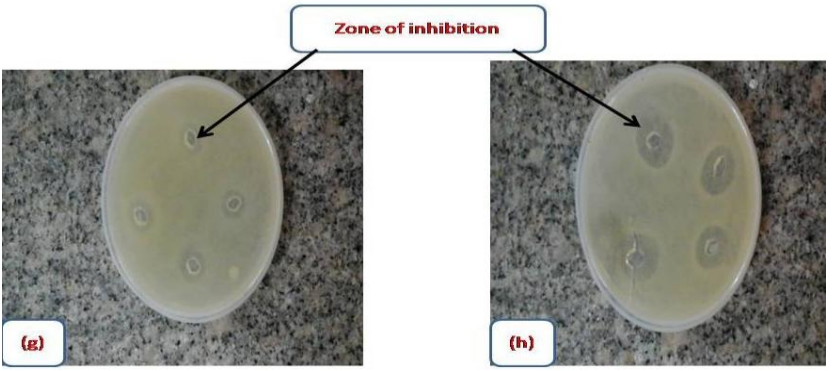
Figure 3: TEM images of Silver naoparticles (A and B) through green chemistry route

TEM images for sample were taken and the partcle size was found between in the range of 5 - 20 nm.The shape of nanoparticles was found to be spherical.TEM also shows the narrow size distribution of these particles with low agglomoration.

3.3Antibacterial properties

Antibacterial materials protect against the growth of various bacteria in personal care products and play a vital role in ensuring that cosmetics are free from bacteria during the storage and after they are opened. Nanomaterials may serve as effective disinfectants

and efficient antibacterial for cosmetic products. The biosynthesized Ag nanoparticles were analyzed for anti-bacterial activity against E. coli (50 µL of Ag nanoparticle). It was observed the zone of inhibition was wider in case of nano-sized silver (20mm) than the normal silver (5mm), which is clearly depicted by Figure 4.



(A) (B)

Figure 4: (A) Antibacterial activity of bulk silver showing lower zone of inhibition (5mm), (B) Antibacterial activity of Ag nanoparticle showing higher zone of inhibition (20mm).

4. Conclusion

The syntheses of silver nanoparticles were carried out through a green method using marigold flower extract. The synthesized Ag nanoparticles were confirmed by the surface Plasmon resonance peak around 420nm using UV-Vis Spectrophotomer and the nanoparticles are found to be around 5-20nm with spherical shape which is confirmed by TEM analysis. The study also includes the antibacterial property of the silver nanoparticles synthesized using marigold flower extract which showed greater zone of inhibition for E-coli bacteria such as 20mm compared to the lower zone of inhibition shown by the bulk silver (5mm).

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