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Green Synthesis of Silver Nanoparticles using Cinnamon (Cinnamomum cassia), Characterization and Antibacterial Activity

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Abstract: ***Silver nanoparticles were synthesized using a queous extract cinnamonberks (Cinnamomum cassia) as a green method without using chemical reducer and stabilizers has been investigated. Different heating techniques were appliedused for sample preparations, conventional heating, microwave heating and room temperature. The samples from a prepared silver nanoparticle were characterized by UV-Vis spectroscopy and transmission electron microscopy. The synthesized nanoparticles showed antibacterial activities.***

Keywords: sliver nanoparticles, cinommon, green synthesis,antibacterial Activity

1. Introduction

Different techniques are used for synthesizing metallic and nonmetallic nanoparticles of different sizes and shapes such as physical and chemical techniques.The physicaltechnique includes laserablation(1, 2)and high energy irradiation (3, 4). Tools used for these techniques are very costly. For chemical techniques including chemical reduction(5) and photochemical reduction(6, 7), reduction agents such as sodium citrate and sodium borohydride are used which are toxic and flammable. Their disposal also raises environmental issues. Hence it is an essential to develop environmentally friendly techniques for synthesis of nanoparticles. The biological and green techniques for synthesis nanoparticles arenontoxic, faster than other techniques and potentially eliminate the environmental issues. These techniques are simple and less expensive and nanoparticles of different sizes and shapes can be produced in large scales.Lately green synthesis has emerged as an attractive alternative to hazardous and expensive physical and chemical methods for producing nanoparticles. Green synthesis involves using plantsand plantextracts such as Hibiscus cannabinus leaf(8), curcumin(9), and Henna(10)have been successfully usedfor synthesizing metal nanoparticles. Extracts from plants usually contain sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins which are excellent reducing agents useful in the synthesis of silver and gold nanoparticles. They are most probably responsible for the conversion of Ag^+ to Ag^o which happens during the formation of enol/keto form of those materials(11).It has been proposed that the interaction of these bioreduction of metal ions provide a more flexible control over the size and morphology of nanoparticles for example, by changing the medium pH and reaction temperatures (12-15). An extend review on silver nanoparticle preparation using green synthesis methodsis presented by Sharma*et.al*(16).

Silver nanoparticles (Ag NP)have recently attracted enormous interest because of their applications in manyfields, such as optics, optoelectronic, medical diagnostic imaging, and medical therapy and as a catalysis. Silver nanoparticles are reported to have anti- fungal, anti-microbial anti-bacteria and, anti- inflammatory activities. Silver nanoparticles have been investigated for antibacterial activity various stains of Gram positive and Gram negative bacteria such as *S aureus*. *M. luteus*, *E. coli* and *P. areruginosa* (17). An overview of recent trends in synthesizing nanoparticles prepared via biological entities and their applications is given by L. Wang *et.al*(18).

In this study wesynthesized AgNPS by using cinnamon berks. For optimizing of AgNPS production three different methods were used, microwave heatingmethod at different time intervals, boilingmethod,and room temperature method. The nanoparticleswere confirmed by UV-Vis spectrometry, Transmission Electron microscopy (TEM). The stability of nanoparticles were studied over four weeks using a UV-Vis spectrometer (Shimadzu UV-1800). Anti- bacterial activities of Ag NPS were investigated.

2. Materials

Sliver Nitrate was obtained from Sigma Aldrich chemicals, Germany. Cinnamon berks were obtained from local shops and E coli was obtained from ATCC, USA. The water used for this experiment was double distilled. All glassware was washed with distilled water.

Cinnamon berks were obtained from local shop and grained with a blender and sieved. A 2.5g of grained Cinnamon was dissolved in 100ml of double distilled water. Then this solution was boiled for 5 minutes and filtered twice usingWhatman No. 1 filter paper.

Synthesizes of silver nanoparticles

In this experiment a 0.0421g of $AgNO_3$ was dissolved in 100 ml of distilled water to prepare a 1 mM $AgNO_3$ solution. 1ml of cinnamon was mixed into 50 ml of an AgNO3 solution. The solutions were divided to three parts one part left in room temperate over 24 hours. Thesecond part was stirred and boiled for 2 minutes and the third part was divided to three parts, each part heatedby microwave oven for 10 sec., 20 sec. and 40sec. In all cases the reduction took place as indicated by a yellowish colour of the solution due to excitation of surface plasmon vibration of silver nanoparticles. These solutionswere found to be stable for a month with nocolourchanges and showed little or no precipitation.

3. Results

Initial characterization of the silver nanoparticles were carried out using UV-Vis spectrometer(Shimadzu UV-1800). The identity of the silver nanoparticles was confirmed by recording the absorption spectra over 200 -700 nm after 30 minutes. It is well known that the metal nanoparticles exhibit distinctive optical properties due to combined oscillation of conduction band electrons in resonance with the incident wavelength, which is known as the surface plasmon resonance (SPR) band. Fig.[1] Shows UV-Vis absorption spectra of silver nanoparticles for different heating techniques used for preparation, conventional heating, microwave heating and room temperature reaction. The observed plasma resonance peaksaround 433nm and is an indication of the existence of silver nanoparticles in all solutions. The sharp peaks and symmetrical nature of the graph indicates the formation of spherical nanoparticles, which was confirmed by TEM images. The increase of SPR absorption peaks may be due to differences in concentration of produced nanoparticles for each method. It can be seen from Fig.1, the peak absorbance of AgNPs produced by microwave radiation is higher than that of thermal heating followed by room temperature reaction. The stability of the samples was monitored over two weeks by recoding the absorption spectra. During this period, the absorption band was constantlyobserved around 433nm, confirming thepreservation ofnanoparticles in the solution. The increase of theabsorption and broadening of the SPR band were observed over the time Fig. [2.a]. This may be considered as indication of the increase of the concentration and sizes of nanoparticles. Fig.[2.b] show the variation of the SPR peak at 423 nm as a function of time, the formation of Ag nanoparticles increased up to five days, after that time the graph plateaued with a very slight variation. This indicates the stability of concentration and sizes of nanoparticles [8]. The stability may arise froma competition between electrostatic repulsion force and a weak Van-der Waals attraction force(19).Fig.3 shows UV-Vis absorption spectra of silvernanoparticles for different time exposuresto microwave radiation for the same powerand for same concentration of the reagent precursor.It can be seenfrom the absorption spectra that there wasa change in the peak position to longer wavelength (red shifted) withthe exposure time. It was observed that as the absorption peak intensity increases over time the curves became broader. This indicates that bigger-sized nanoparticles will be produced, leading to the shift in of absorption peak wavelength.

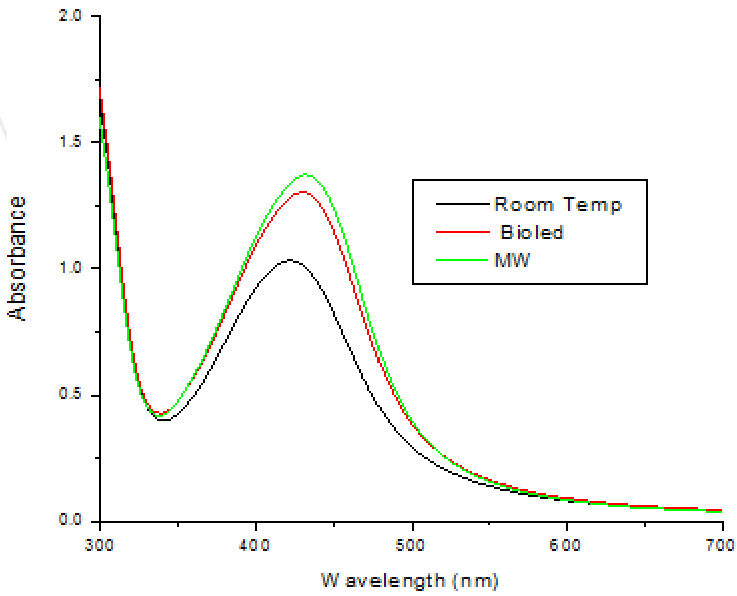


Figure 1: Absorption spectra of synthesized Ag NPs with different heating techniques.

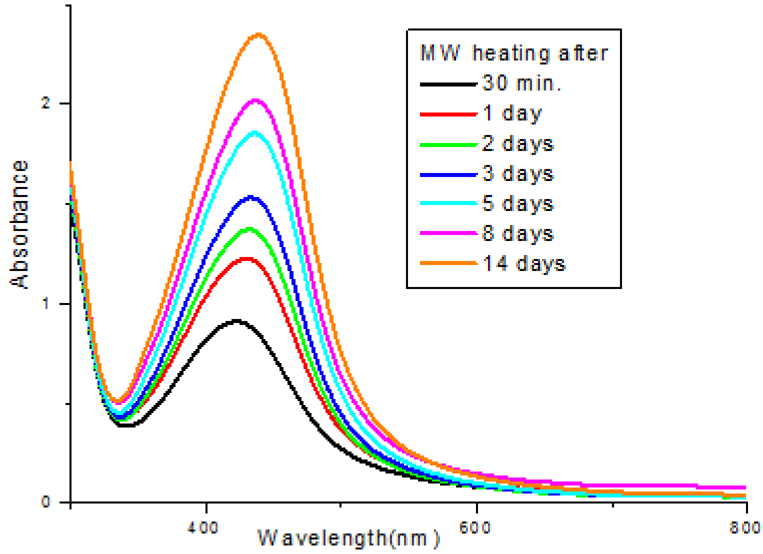


Figure 2 (a): Absorption spectra of synthesized colloidal Ag NPs with different heating technique

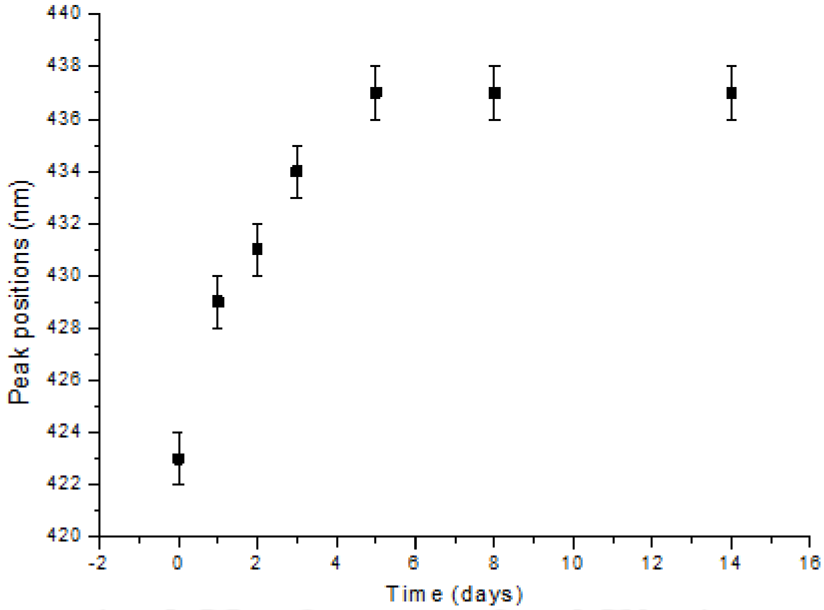


Figure 2 (b): Variation of the SPR absorption peak with respect of the time

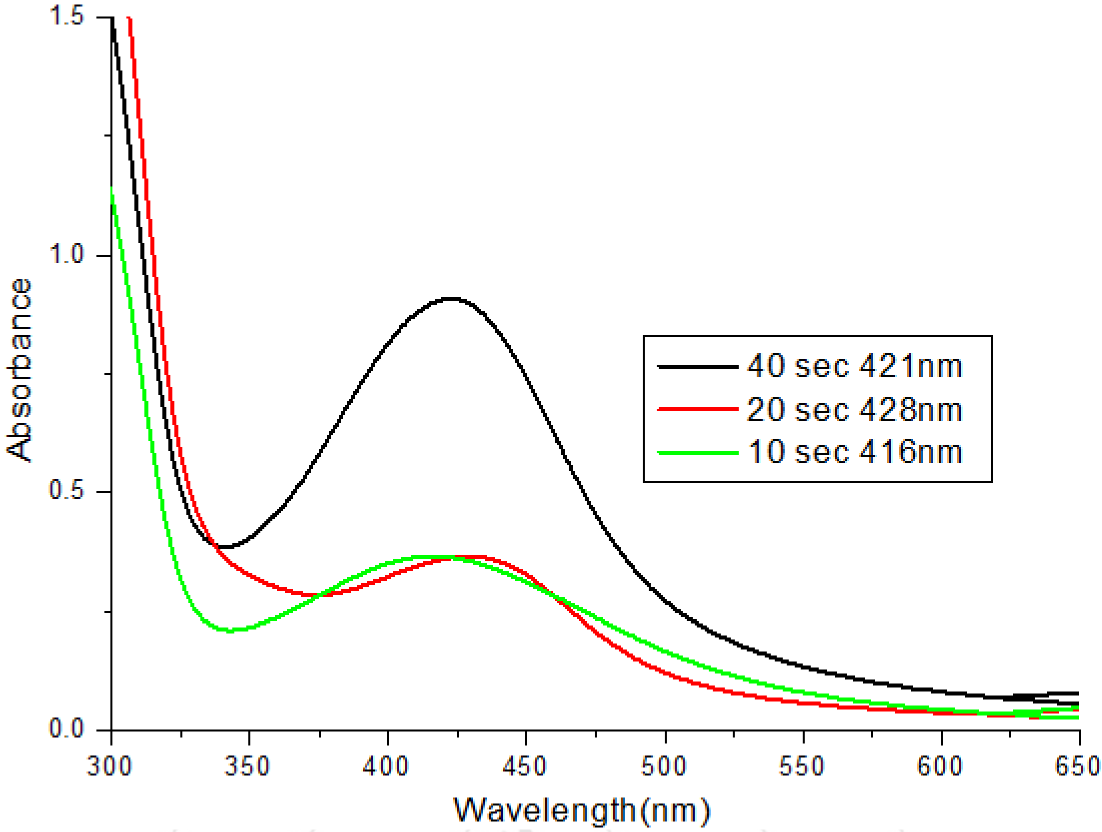
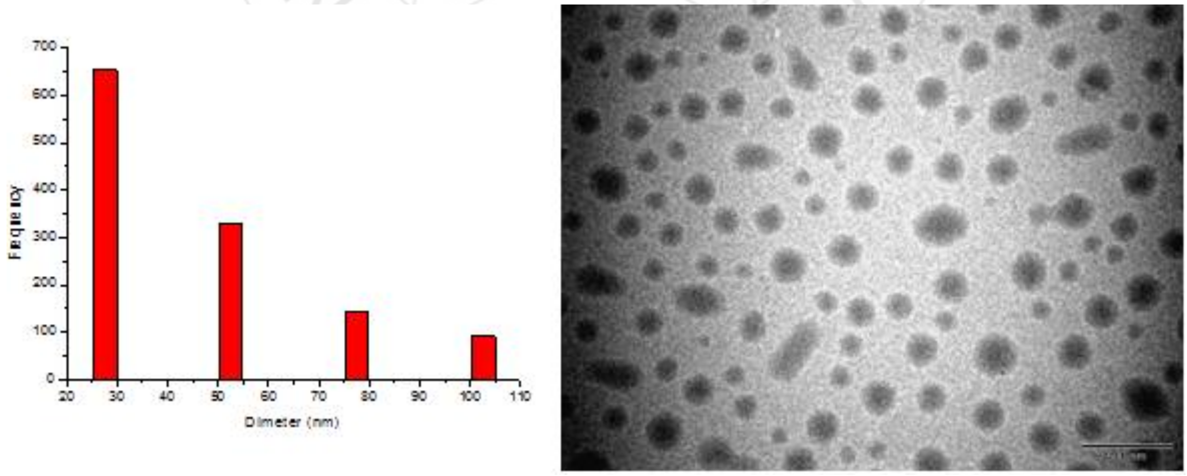


Figure 3: Absorption spectra of colloidalAgNPs for microwave heating at different time of exposure

Transmission electron microscopy (TEM)was performed for determine shapes and sizes of synthesized nanoparticles. The samples were prepared by dispersing a few drops of the solution on a copper grid and left in room temperature to dry. Fig.4 shows image of sliver nanoparticles for all three methods of preparation. The TEM study reveals the size and shapes ofmost nanoparticlesare nearly spherical. The size distribution of nanoparticles was analyzed using ImageJ 1.5j software. This was performed by converting pixels on the TEM images into nanometers by applying the scale of the image. Fig.4 shows the size distribution histogram of AgNP's for all three methods of preparation. It can be seen that the microwave heating has more narrow distribution with a high yield of size 2nm compared with room temperature with a high yield of size 6nmand room temperature has lower distribution compared with thermal heating with a high yield of size 19nm.



(a)

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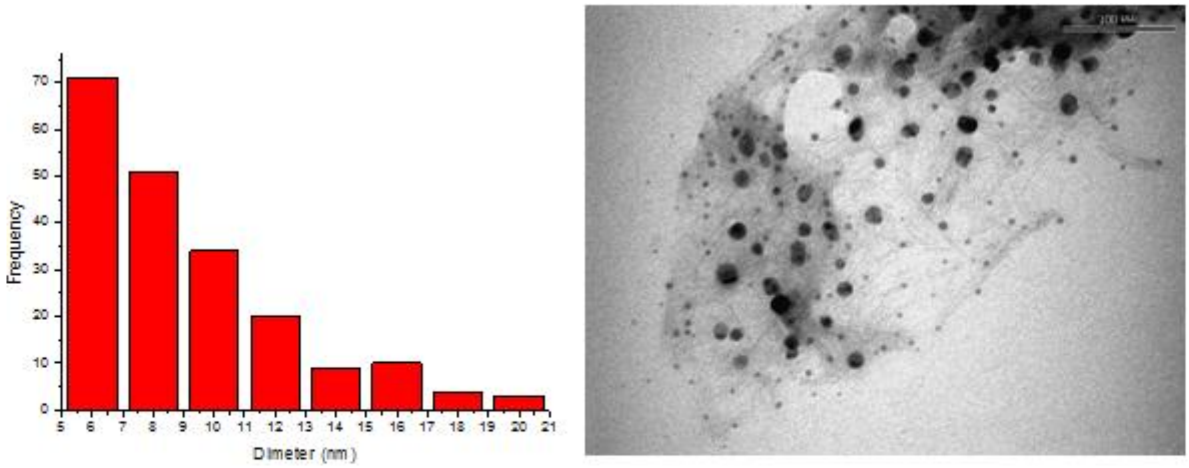
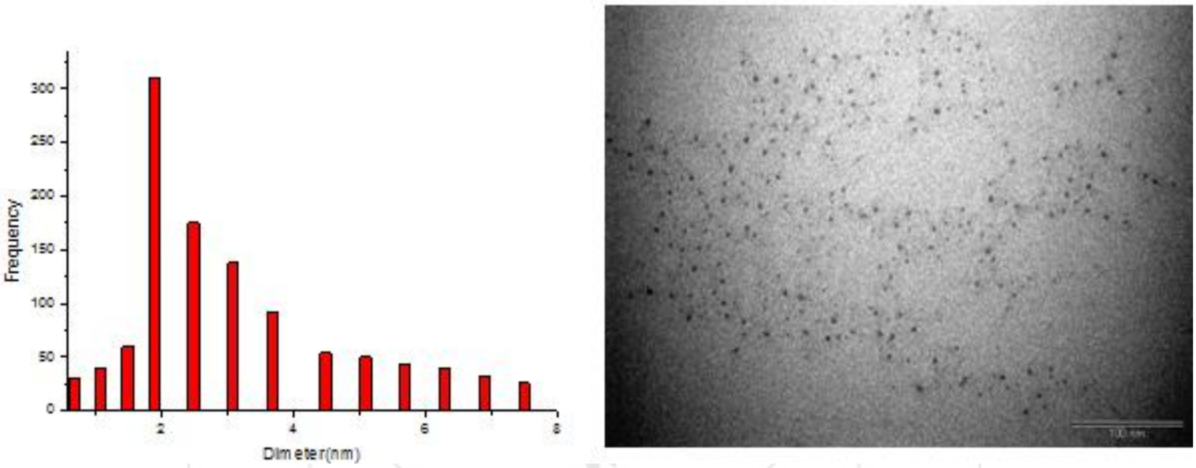


(b)



(c)

Figure 4: Transmission Electron microscopy image and corresponding particle size distribution J image softwarefor: (a) room temperature reaction, (b) conventionalboiling,(c) Microwave heating. The prepared AgNP samples were characterized by UV-Vis spectroscopy and transmission electron microscopy. The synthesized nanoparticles showed antibacterial activities

4. Antimicrobial activity

AgNPs were tested fortheir antibiotic sensitivity pattern against E.coli (l0 µg) by the Kirby–Bauer disk diffusion method. E. coli is a known gram negative bacterium that is fairly resistant to antibiotics. Commercially available Hi-media disks were used. Hichrome selective agar medium was used for the growth and the inoculated samples of E.coli were incubated for 24 hrs at 37°C in an incubator. With the help of straight wire 3-4 identical colonies were picked up and were inoculated into 5ml of nutrient broth (LB broth). The broth was incubated at 37°C for 24 hrs., so as to obtain moderate turbidity. A streak was made on a nutrient agar (LB agar) medium using a sterile spreader in all directions and rotating the plate every time.

The antibiotic disks were applied with aseptic precautions. Disks were deposited with centers at least 30 mm apart. The plates were incubated at 37°C in an incubator for 24hs. After incubation, the plates were observed for the zone of inhibition around the disks. The formation of the clear zone around the LB agar is an indication of antibacterial activity. The zone showing inhibition was measured to the nearest whole millimeter using a travelling microscope. Zone inhabitation around silver nanoparticles synthesized by different methods are shown in Fig.5. All treatments were performed in duplicates.

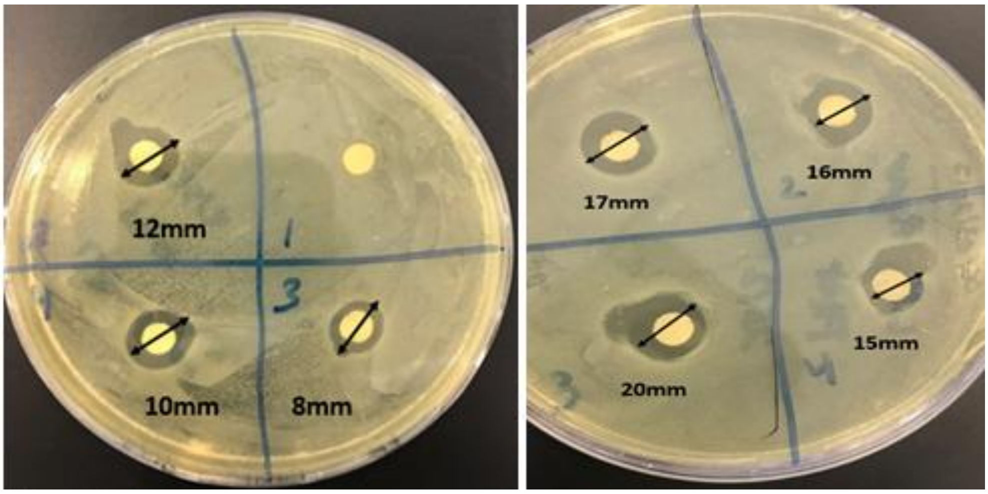


Figure 5a: Antibacterial activity of AgNPs against E. coli bacteria for : left: 1- Cinnamon solution, 2- boiling , boiled solution, 3- Microwaved solution for80s, 4- Microwaved for60s solution. Right: 1- microwaved 20s, 2- boiled solution, 3- microwaved for 40 sec, 4- Room temperature solution.

5. Conclusion

AgNPS were synthesized using aqueous extract cinnamon berks. Different heating techniques were used for preparation, conventional heating, microwave heating and room temperature. The changes in sizes were observed with increase time of exposure to microwave heating. The stability of Ag NPSwere monitored over a month using UV-Vis spectrometer. The green synthesized nanoparticles showed activity against E. coli. The green synthesized Ag nanoparticles using cinnamon is quick, stable, and safe and can be used against bacteria and in medical applications.

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