GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *CENTELLA ASIATICA* (L.) LEAF EXTRACT AND THEIR ANTIMICROBIAL ACTIVITY*

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Abstract

Silver nanoparticles (AgNPs) exhibit unique properties due to their size, distribution, and morphology, which contribute to their prominence in the expanding domain of nanotechnology. In this research, leaf extract from *Centella asiatica* L. was used to synthesize silver nanoparticles utilizing a green synthesis. The synthesized nanoparticles were characterized using a variety of experimental techniques, such as X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), and UV-Vis Spectroscopy. The resulting silver nanoparticles were evaluated for their antimicrobial activity against six microorganisms: *Aspergillus flavous, Bacillus subtilis, Candida albicons, Escherichia coli, Klebsiella pneumonia, and Pseudomonas flurorescens*. This research leads to comprehensive and valuable results, due to their primary physicochemical characteristics and some of their potential medical uses.

Keywords: Green synthesis, XRD, SEM, FTIR, UV-Vis and Antimicrobial activity

Introduction

Nowadays, silver nanoparticles (AgNPs) have attracted a great deal of attention due to the excellent physicochemical properties and consequently their significance range of application in the fields of physics, chemistry, materials science, renewable energies, environmental remediation and biomedicine (Jamil Ahmed, et al. 2015). Silver nanoparticles exhibits good conductivity, high chemical stability, catalytic activity and antimicrobial activities (Song et al. 2009). In medicine, silver nanoparticles have widely applications including skin ointments and creams to avoid infection of burns and open wounds, resistance against microbes, drug delivery, antibiotics and immune chromatography, tissue/tumour image, anticancer activities and identification of pathogens in clinical specimens (Durán et al. 2005). Nanoparticles can be synthesized using many strategies including chemical reduction, physical vapor condensation and biological approaches. But physical and chemicals used are toxic, flammable and lead to non-ecofriendly (Verma et al. 2016). Biological method of nanoparticles synthesis using microorganisms, enzymes and plant or plant extract have advantages over other methods, because these approaches are cost-effective, one step in nature, environment friendly, cleaner synthesis route and often results in more stable materials (Logeswari et al. 2015). Plant extracts are rich bio-reducing agents that can reduce silver ions to silver nanoparticles. In the present research, the silver nanoparticles were synthesized by the reduction of $AgNO_3$ solution using medicinal plant *Centella asiatica*. *Centella asiatica* (L.) Urban., (Family Apiaceae), commonly known as Asiatic pennywort or gotu kola, is a well-known herb that has been used in both medicinal and culinary practices of many countries. It contains vitamins especially vitamin B, vitamin C, vitamin G and some amino acids etc. This plant was recommended for the treatment of various skin conditions such as leprosy, lupus, asthma, ulcers, wound healing, diarrhea, memory improvement, disease of the female genitourinary tract, antibacterial, antifungal, and anti-cancer (Chatterjee TK et al.1992). Some basic principles of "green synthesis" can be explained by several components like minimization of waste, reduction of pollution and the use of safer (or nontoxic) solvent as well as renewable feedstock.

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Experimental Procedures

Preparation of *Centella asiatica* (L.) Leaf Extract

Fresh leaves of *Centella asiatica* L. were collected from the Dagon University Campus. Firstly, 20 g of leaves were properly washed in both tap water and distilled water. Next, they were crushed and ground with a pestle and mortar by adding distilled water dropwise. After mixing the paste with 150 ml of distilled water, it was heated on a hot plate at 80 ˚C for 15 minutes. Then, it is set to cool down, and the extract solution is filtered by filter paper until no debris is present.

Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was analytically pure and used straight out of the container without any additional processing. First, 1mM of silver nitrate solution were dissolved in 100 ml of distilled water and stirred for 15 minutes. Then, these solutions were mixed with two separate volumes of leaf extract solution (10 ml and 30 ml) at 60 ˚C, 70 ˚C, and 80˚C for 15 minutes. During stirring, the yellowish color of the solution gradually changed to dark brown upon the addition of the leaf extract. The silver solution was considered ready once the dark brown color change was observed and the time recorded at 80˚C. The mixture solution was then centrifuged and washed three times with deionized water to remove the by-product. Finally, the silver nanoparticles were formed when these precipitated particles were heat treated at 300˚C for 1 h. Figure 1 showed the synthesis procedure of green synthesis of silver nanoparticles.

(a) leaves

(b) Leaf extract solution

(c) Silver nitrate solution

(d) Silver solution

(e) Silver nanoparticles

Figure 1. Synthesis procedure of green synthesis of silver nanoparticles

Characterization Techniques

The green synthesized silver nanoparticles will be characterized by XRD, SEM, FTIR and UV-Vis spectroscopy for structural, morphological, chemical and optical properties. The antimicrobial activity will be studied with these silver nanoparticles using *Centella asiatica* (L.) leaf extract.

Results and Discussion

XRD Analysis of Silver Nanoparticles from Centella asiatica (L.) Leaf Extract

The silver nanoparticles were obtained by green synthesis and XRD technique was used to examine the phase analysis, particle structure and crystallographic investigation and lattice parameters. Sample was scanned from 10˚ to 80˚ in diffraction angle 2θ with a step-size of 0.02˚. The XRD profiles of silver nanoparticles synthesized from different leaf extract concentrations (10 ml and 30 ml) at 300˚C are shown in Figure 2. As observed, the peaks at 2θ values of 38.1°, 44.4°, 64.5°, and 77° correspond to the (111), (200), (220), and (311) crystal planes, respectively, according to JCPDS No. 04–0783. According to the XRD pattern, the growth direction of silver nanoparticles was mostly in the (111) plane. A few additional peaks (especially at 35.25° and 40.21°) were appeared, which are weaker than the silver peaks. These peaks are due to the crystallization of organic compounds that are responsible for covering and stabilizing silver nanoparticles. A similar result has been reported in the investigation of silver nanoparticles synthesized by the green method (Kumar et al. 2009). The crystallite size was calculated from the XRD peak broadening of the (111), (200), (220) and (311) peaks using Scherrer's formula. The average crystallite sizes of silver particles were in the range of 55.05 nm for 10 ml of extract solution and 54.33 nm for 30 ml **o**f extract solution.

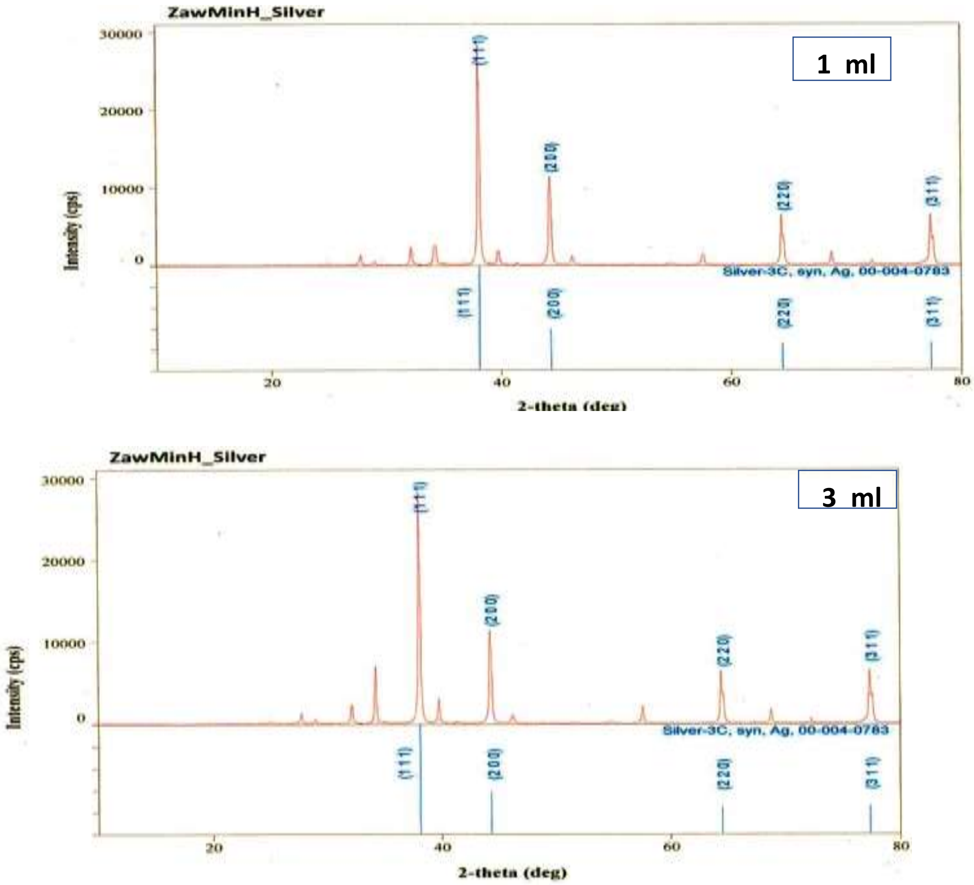

Figure 2. XRD pattern of silver nanoparticles with different leaf extract concentrations

SEM Analysis of Silver Nanoparticles from *Centella asiatica* L. Leaf Extract

The microstructural properties of silver nanoparticles synthesized using the green synthesis method was examined by scanning electron microscopy (SEM). Figure 3 shows the SEM images of silver nanoparticles synthesized with 10 ml and 30 ml leaf extract solutions. The grain sizes were calculated by using image J software. For the 10 ml extract solution, the average grain size of the silver nanoparticles was calculated to be 46.30 nm. The nanoparticles exhibited a relatively uniform distribution, though some larger particles were present. The overall morphology showed slight roughness, with pores scattered throughout. In contrast, the nanoparticles synthesized with the 30 ml extract solution exhibited a smaller average grain size of 30.98 nm**.** The nanoparticles displayed a more uniform size distribution, with fewer pores and a denser, smoother structure compared to the 10 ml extract solution. No visible cracks were observed, indicating enhanced structural stability. These results suggest that increasing the leaf extract concentration leads to a reduction in both grain size and pore size**,** resulting in more uniform nanoparticles with improved structural integrity.

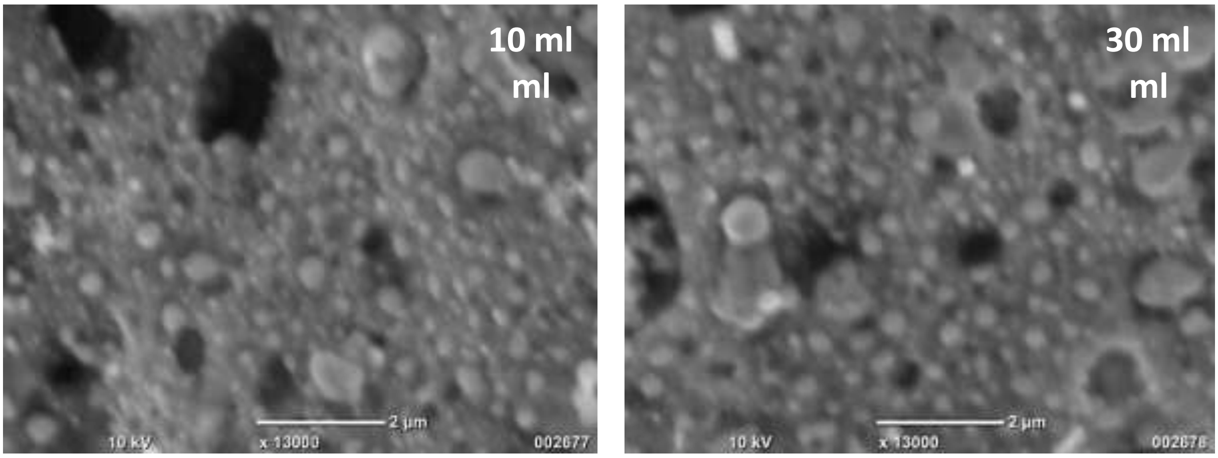

Figure 3. SEM image of silver nanoparticles with different leaf extract concentrations

FTIR Analysis of Silver Nanoparticles from *Centella asiatica* (L.) Leaf Extract

Fourier Transform Infrared Spectroscopy (FTIR) is important tool to characterize the molecular orientation in the condition of material transition, detecting functional groups and characterizing covalent bonding information. The FTIR spectra of silver nanoparticles in case both of 10 ml and 30 ml leaf extract solution were shown in Figure 4. According to FTIR analysis, the prominent bands of absorbance were observed at around 3270.93 cm^{-1}, 3210.69 cm^{-1}, 2920.82 cm^{-1}, 2116.99 cm^{-1}, 1551.02 cm^{-1}, 1552.05 cm^{-1}, 1375.25 cm^{-1},1304.14 cm^{-1}, 1270.75 cm^{-1}, 1242.27 cm^{-1}, 1030.07 cm^{-1}, 1027.96 cm^{-1} and 1024.15 cm^{-1} for silver nanoparticles (10 ml extract solution) and silver nanoparticles (30 ml extract solution) respectively. The peak in the range of above these values were assigned as –OH stretching in alcohols with strong hydrogen bond, C=O bond of the carbonyl group and the stretching variations of amides, N-O stretching, C-O stretching of alcohol and phenol, C= C groups or from aromatic rings and alkyne bonds respectively. The presence of these peak values confirmed that the silver nanoparticles were converted by plant secondary metabolites with functional groups such as ketone, aldehyde, carboxylic acid and proteins. The presence of these groups is due to the stability of the silver nanoparticles.

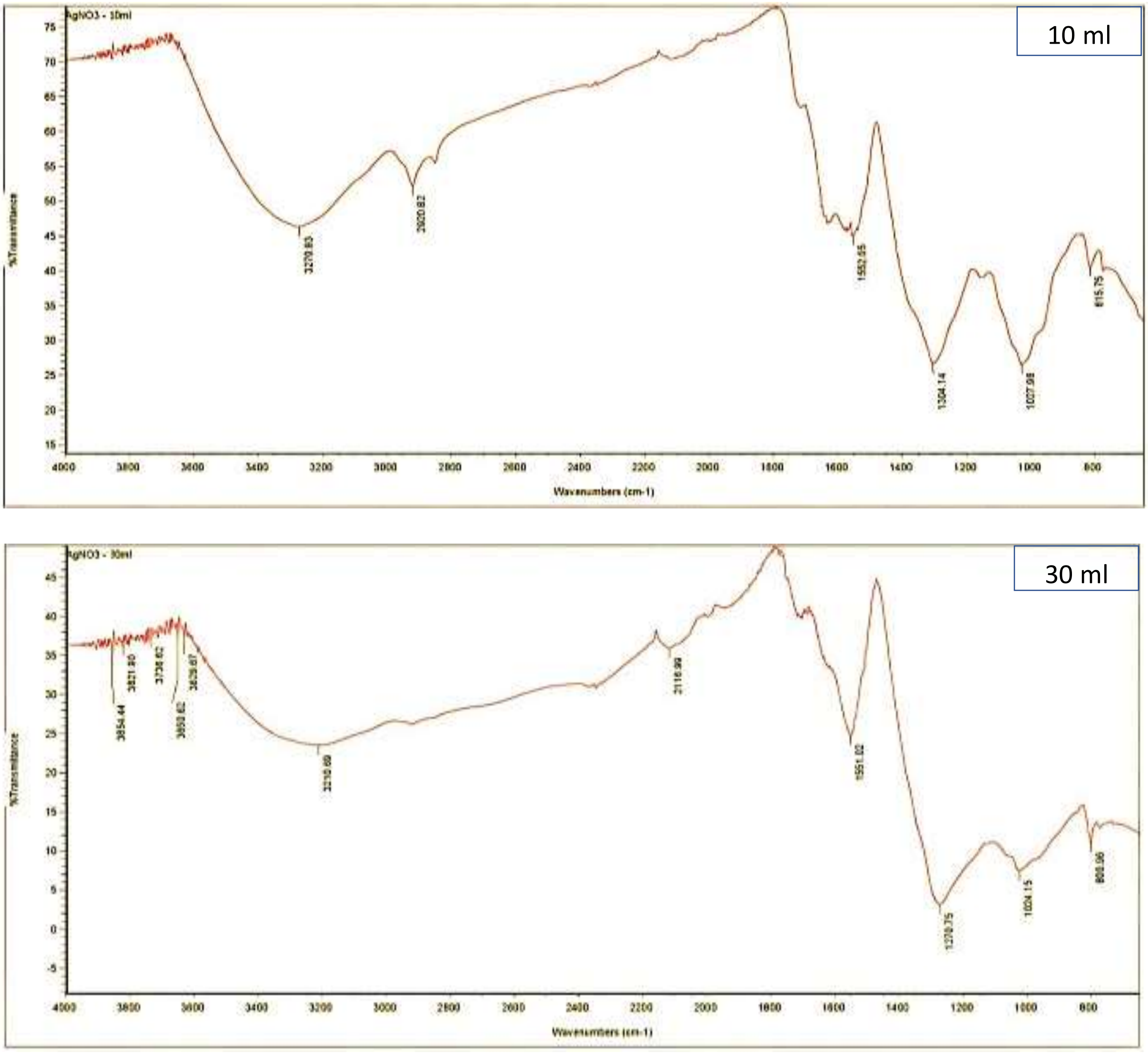

Figure 4. FTIR spectrum of silver nanoparticles with different leaf extract concentrations

UV-Vis Analysis of Silver Nanoparticles from *Centella asiatica* (L.) Leaf Extract

The optical absorption spectra for a range of samples of silver nanoparticles with different *Centella asiatica* L. leaf extract concentration was obtained in UV/ VIS/ NIR region (up to1100 nm) using a SHIMADZU U-1800 UV/ VIS Double Beam Spectrophotometer. The absorption spectra of silver nanoparticles with different *Centella asiatica* L. leaf extract concentration were shown in Figure 5. As the different leaf extracts were added to aqueous silver nitrate solution, the colour of the solution changed from faint light to yellowish brown to reddish brown and finally to colloidal brown indicating silver nanoparticles formation. The colour change is due to the Surface Plasmon Resonance phenomenon. From different literatures, it was found that the silver nanoparticles show SPR peak at around (350 nm – 550 nm). In this result, the dominant sharp band of silver nanoparticles was observed around 392 nm in case of 10 ml extract solution whereas the band for 30 ml extract solution was found around 430 nm. This blue shift in the 30 mL extract solution indicates the formation of smaller nanoparticles, as the SPR peak shifts to a lower wavelength with decreasing particle size (Zielińska et al. 2009). Additionally, the intensity of absorption peak increases with increasing more extract solution. It was observed that the silver nanoparticles were dispersed in the aqueous solution with no evidence for aggregation in UV-Vis absorption spectrum.

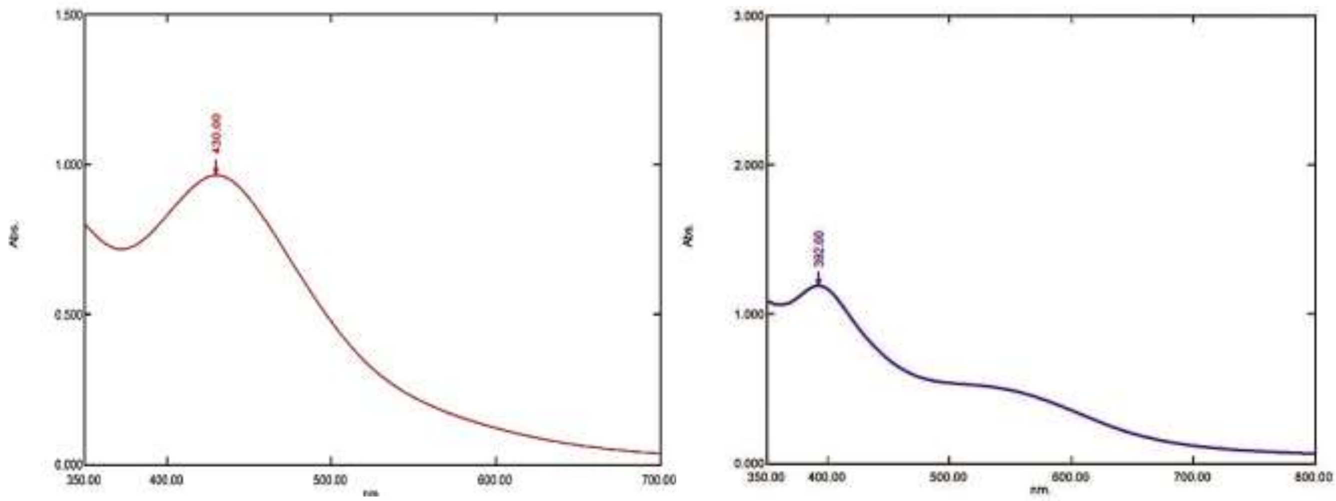

Figure 5. Absorption spectrum of silver nanoparticles with different leaf extract concentrations

Antimicrobial activity of microorganisms by paper disc diffusion assay

Isolated bacterial strains grown on nutrient agar were inoculated into 50 ml conical flasks containing 10 ml of sterile growth medium. Then, they were incubated for 72 hours at 30 °C on a reciprocal shaker starting at 200 rpm. In this research, test organisms were *Aspergillus flavous, Bacillus subtilis, Candida albicons, Escherichia coli, Pseudomonas flurorescens, Klebsiella pneumonia.* The assay medium was mixed with 0.3 ml of test organisms and then transferred into plates. After solidification, test plates were covered with paper discs that had been impregnated with broth samples. The plates were then incubated at 30 °C for 24 to 36 hours. After for 24-36 hours, the test discs' surrounding "inhibitory zones" or clear zones indicate the presence of bioactive substances that prevent test organism growth. Table 1 and Figure 6 display the widths of the inhibition zones that appeared. According to experimental results, the enhanced antimicrobial activity observed in AgNPs synthesized with a higher extract concentration is attributed to their smaller particle size, which leads to increased surface area, improved bacterial interaction, and greater silver ion release. Smaller nanoparticles maintain closer contact with bacterial membranes, facilitating deeper penetration into microbial cells and enhancing their reactivity with biomolecules, leading to increased antimicrobial effectiveness.

Table 1. Inhibitory zones of silver nanoparticles with two different volume of leaf extract solution

No	Test organisms	Inhibition zones (mm)		Diseases
		10 ml extract solution	30ml extract solution	
1	*Aspergillus flavous*	8	8	Aspergillosis, Allergic reactions
2	*Bacillus subtilis*	14	28	Food poisoning, Gastroenteritis
3	*Candida albicons*	12	16	Yeast infections, Oral thrush
4	*Escherichia coli*	12	16	Urinary tract infections, Diarrhea
5	*Klebsiella pneumonia*	12	28	Pneumonia, Urinary tract infection
6	*Pseudomonas flurorescens*	8	8	Skin infections, Respiratory infections

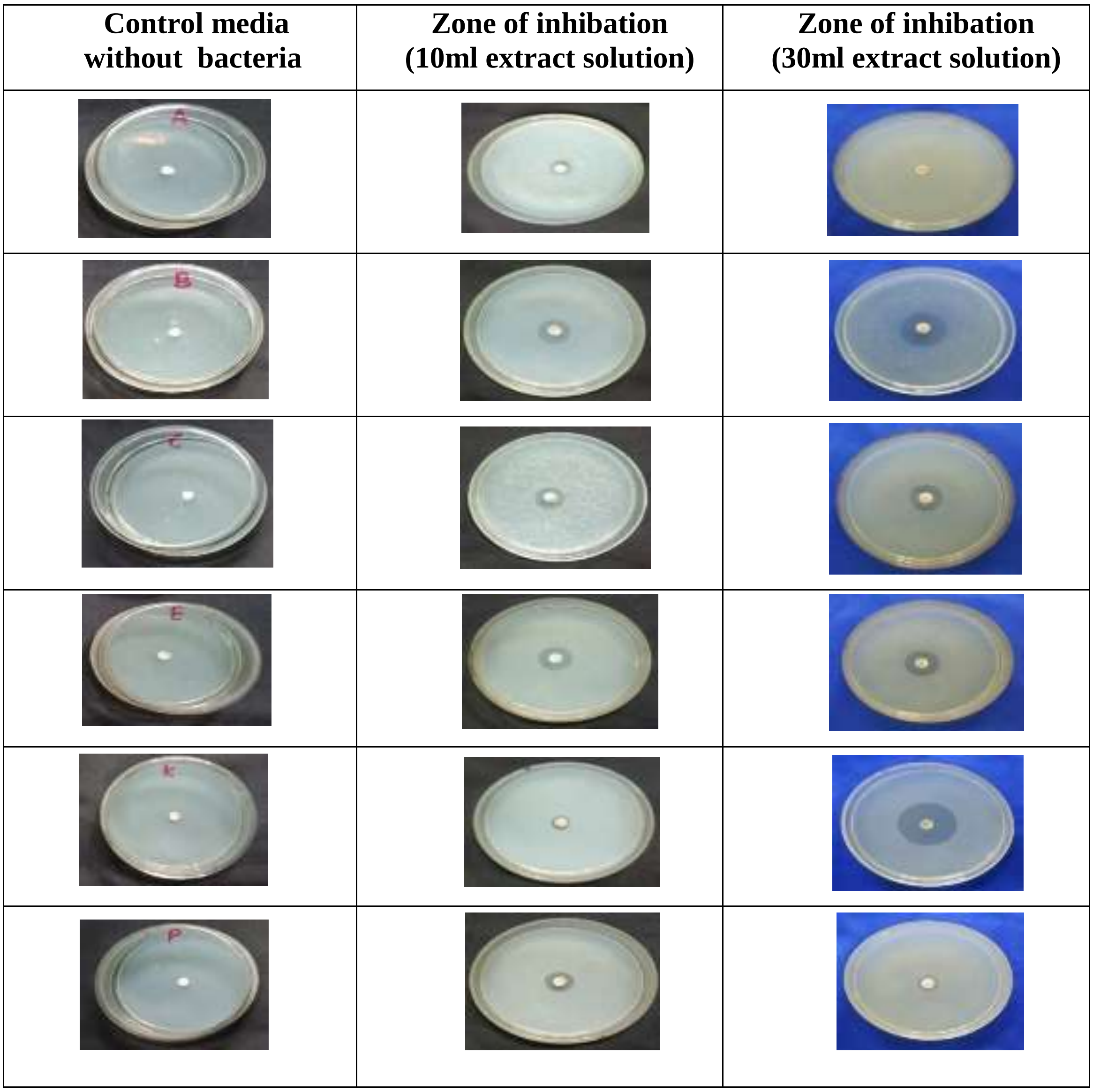

Figure 6. Comparing control and inhibitory zones against *Aspergillus flavous, Bacillus subtilis, Candida albicons, Escherichia coli, Klebsiella pneumonia, Pseudomonas flurorescens* of silver nanoparticles

Conclusion

In conclusion, the synthesis of silver nanoparticles using the leaf extract of *Centella asiatica* L. has been successfully demonstrated. XRD results showed that the average crystallite sizes of AgNPs were 55.05 nm for a 30 ml extract solution and 54.33 nm for a 10 ml extract solution in a cubic structure. According to the SEM data, the average particle sizes were found to be 30.98 nm and 46.30 nm with spherical shapes. The smaller particle size of 30.98 nm was observed with the larger extract volume, suggesting that a higher extract concentration leads to the formation of smaller nanoparticles. FTIR confirmed that plant compounds were involved in reducing and stabilizing the nanoparticles. According to UVVis analysis, there were no signs of aggregation, and the silver nanoparticles were evenly distributed throughout the aqueous solution. This indicates that the plant compounds prevented particle clumping, leading to a uniform distribution of nanoparticles in solution. Importantly, the antimicrobial results demonstrated that AgNPs synthesized with the 30 mL extract solution exhibited significantly enhanced antibacterial activity, ascribed to their smaller particle size and increased surface area, which promote stronger interactions with microbial cell membranes. From a technological perspective, these silver nanoparticles have promising applications in the biomedical field. This simple synthesis method offers several advantages, including cost-effectiveness and compatibility with medical and pharmaceutical applications, particularly in wound healing, antimicrobial coatings, and pharmaceutical formulations.

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