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Research article

ANTIBACTERIAL EFFECT OF SILVER NANOPARTICLES SYNTHESIZED USING *CURCUMA AROMATICA* LEAF EXTRACT

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ABSTRACT: This article reports an ecofriendly approach for the synthesis of Ag nanoparticles (Ag NPs) using aqueous leaf extract of *Curcuma aromatica*. The synthesized Ag NPs were characterized using UV-visible spectroscopy, FTIR, XRD and TEM analyses. UV–Visible spectra of the aqueous medium containing Ag NPs showed a gradual decrease of the absorbance peak observed at 442 nm. The biomolecules responsible for the reduction of Ag NPs were analyzed by FTIR. XRD result confirmed the presence of AgNPs with FCC crystal structure. The calculated crystalline size using Scherrer formula is in the range from 20 to 80 nm. TEM analysis ascertained spherical nature of the Ag NPs. The synthesized Ag NPs exhibited good antibacterial performance against gram-positive and gram-negative bacteria strains, which was studied using standard disc diffusion method. The synthesis of Ag NPs by this method is rapid, cost effective and can be used for various applications.
Key words: *Curcuma aromatica*, Curcumin, Ag NPs, Green synthesis.

INTRODUCTION

Nanotechnology is the fascinating field of modern research and nanoparticles usually ranging from 1 to 100 nm dimension. Nanomaterial has different capabilities in the field of materials science and engineering, especially metal nanoparticles has gaining importance in areas such as mechanics, optics, biomedical sciences, chemical industry, electronics, space industries, drug-gene delivery, energy science, catalysis (Schmid, G. 1992, Hoffman, A. J et al, 1992) optoelectronic devices (Colvin, V. L et al, 1994, Wang, Y., & Herron, N. 1991) photo electrochemical applications, (Mansur, H. S, 1995) and nonlinear optical devices. (Wang, Y. 1991, Yoffe, AD (1993). Silver nanoparticles have important for their extensive applications in areas such as integrated circuits, (S. Kotthaus et al, 1997) sensors, biolabelling, filters, (G. Cao, 2004) antimicrobial deodorant fibres, (W. Zhang and G. Wang, 2003) cell electrodes, (Krishnaraj, C et al, 2011) and antimicrobials. (Elumalai, E. K et al, 2010, Klaus-Joerger, T et al, 2001) Antimicrobial properties of silver nanoparticles (Ag NPs) caused the use of these nanometals in different fields of medicine, various industries, animal husbandry, packaging, accessories, cosmetics, health and military. The Ag NPs show potential antimicrobial effects against infectious organisms such as *Escherichia coli, Bacillus subtilis, Vibria cholera, Pseudomonas aeruginosa, Syphillis typhus* and *Staphylococcus aureus* (Cho, K. H et al, 2005, N.Dura'n et al, 2007). Nanoparticles have been synthesized by physical and chemical method for a long time, but these methods are not compatible with environment and recent developments show the critical role of bio-organisms in the synthesis of metal nanoparticles, Therefore, biosynthesis of clean, biocompatible, nontoxic and eco-friendly nanoparticles produced both in-vitro and in-vivo deserves many advantage (Sharma, V. K et al, 2009, Narayanan, K. B., & Sakthivel, N. (2010). The plant extracts itself acts as a reducing and capping agent for nanoparticle synthesis, limiting particle size and barring agglomeration, resulting in the formation of desired nanoparticles Zayed, M. F et al, 2012). K. Shameli *et al* have found that *Curcuma longa* tuber powder exhibited as best reducing and capping agent in the formation of Ag NPs.

Recently B. Ajitha et al. have studied the synthesis of Ag NPs with *Plectranthus amboinicus* leaf extract and observed anti bacterial properties against multi-drug resistant pathogens. G Sathishkumar et al. have studied the synthesis of Ag NPs with *Morinda citrifolia L.* leaf extract and observed anti-bacterial properties against multi-drug resistant pathogens. G Sathishkumar et al. have studied the synthesis of Ag NPs with *Neem mistletoe* leaf extract and their cytotoxicity effect against human breast cancer cell line (MCF-7). From the above observations it is concluded that colloidal Ag NPs can be established as anti-microbial and anti-cancer agents.

The present study explores the capability of using *Curcuma aromatica* leaf extract as a capable material for the synthesis of Ag NPs. *C. aromatica* belongs to Zingiberaceae family and is known as *Kasthuri mangal* in Tamil. *C. aromatica* belongs to genus of Curcuma and it consists of about 70 species of rhizomatous herbs. Curcumine is the major compound in these families. *C. aromatica* plays vital role as antimicrobial agent, anti-ageing, and so on (Al-Reza, S. M et al, 2010).

So that curcumine rich leaves of *C. aromatica* used as reducing and capping agent for the synthesis of Ag NPs. Further we investigated its antibacterial properties of Ag NPs against different microorganisms. To the best of our knowledge, this is the first report on the synthesis of Ag NPs using *C. aromatica* leaf extract.

MATERIALS AND METHODS

Collection Plant Material

The *C. aromatica* plants were collected from Science Campus, Alagappa University, Karaikudi, Tamil Nadu, India. The taxonomic identification was made by Dr. S. John Britto, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Synthesis of Ag NPs using *C. aromatica* leaf extract

Fresh *C. aromatica* leaves were cleaned in running tap water, and then by double distilled water. 10 g of leaves was added with 100 ml of double distilled water and boiled at 50–60°c for 5 min. The obtained extract was filtered using Whatman No. 1 filter paper and the filtrate was collected in 250-ml Erlenmeyer flask and stored at room temperature for further usage. Thereafter, 1 ml of *C. aromatica* leaf extract was added to 100 ml of 1 mM $AgNO_3$ solution and stirred at 100 °C for 20 min. The reduction of Ag NPs was clearly observed within 20 min. The brown solution was changed to light blackish yellow color, which indicates the formation of Ag NPs.

Characterization of silver nanoparticles

The reduction of pure silver ions was confirmed by measuring the UV–Visible spectrum of the reaction mixture by using double beam Shimadzu spectrophotometer at a resolution of 1 nm from 200 to 800 nm. Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out in the range of 400–4000 cm^{-1}. Then AgNPs were characterized by using X-ray diffractometer (XPERT-PRO) with Cu Kα radiation (1.5405 Å). The X-ray powder diffractometer was operated at 60 kV and 40 mA at a 2θ range of 20-80^o using a step size of 0.033. The nanometric particle size was analyzed by using a transition electron microscope (TEM).

Antimicrobial activity

The biocidal property of the green-synthesized Ag NPs was examined against three gram-positive (*B. subtilis, S. aureus, S. pneumoniae*) and four gram-negative bacteria (*Escherichia coli, K. pneumoniae, P. aeruginosa, S. dysenteriae, P. vulgaris, V. cholera*) by disc diffusion method. These nine bacterial strains were grown in nutrient broth at 37 °C until the bacterial suspension has reached 1.59108 CFU/ml. Approximately 20 ml of molten nutrient agar was poured into the Petri dishes and cooled. All the bacterial suspension was swapped over the medium, the disc loaded with 100 µl of Ag NPs and drug loaded discs 30 mcg were placed over the medium using sterile forceps. Plant extract (100 µl) was used as a control. The plates were then incubated for 24 h at 37°C. The inhibition zone formed around each discs was measured. Each experiment was performed for three times.

RESULT AND DISCUSSION

UV –Visible spectral analysis

Figure1 represents UV-visible adsorption spectra for colloidal Ag NPs synthesized using *C. aromatica* leaf extract. UV-visible absorption spectra of the Ag NPs were measured in the range of 190–800 nm. The strong surface plasmon resonance centered at 442 nm clearly indicates the formation of Ag NPs which were extremely stable. This wavelength corresponds to the assimilation by colloidal silver nanoparticles in the visible region (380–450 nm) due to the excitation of surface Plasmon vibrations (Njagi, E. C et al, 2010) Maximum intensity was achieved after 5 h of the reaction, which indicates the complete reduction of the Ag^+ ions. An intense brown color of the reaction mixture further supports the complete reduction of Ag^+ ions and the formation of Ag NPs.

XRD analysis

The synthesized Ag NPs were highly crystalline with diffraction peaks corresponding to the face-centred cubic (fcc) phase of metallic silver. The X-ray diffraction (XRD) determining the crystal structure of the as-prepared Ag nanoparticles and to calculate the crystalline particle size. XRD analysis showed three distinct diffraction peaks at 38.1˚, 44.31˚, 64.45˚, and 77.42˚ which correspond to the planes 1 1 1, 2 0 0, 2 2 0, 3 1 1 of face cantered cubic crystal structure respectively (J.Spreadborough, J.W.Christian, 1959, G.I. Williams, E.A.Owen 1954). No secondary diffraction peaks corresponding to the precursor $AgNO_3$ and their side products were observed, which confirms that only metallic Ag is formed by *C. aromatica* extract treatment. The intensity of peaks reflects the high degree of crystallinity of the Ag nanoparticles. Conversely the diffraction peaks are extensive which indicates that small crystalline size is obtained (I.A. Wani et al, 2011, Ashokkumar, S et al, 2013). The above data was matched with database of Joint Committee on Powder Diffraction Standards (JCPDS) file no.89-3722.The average grain size of the silver nanoparticles formed in the bio-reduction process was determined using Scherr's formula, $d = k\lambda / \beta\cos\theta$, where 'd' is particle diameter size, k is a constant equal 1, 'λ' is wavelength of X-ray source (1.54060 Å), 'β' is the full width half maximum (FWHM) and 'θ' is the diffraction angle corresponds to lattice plane (111). The average size of particles was estimated as in the range between 20-80 nm. Fig 2 recorded XRD profile of synthesized AgNPs.

Table-1: Antibacterial activity of silver nanoparticles with various drugs against gram-positive and gram-negative bacteria

S. No	Bacterial Names	Zone of inhibition in mm				
		Control	Penicillin	SilverNPs	Rifamycins	vancomycin
1	Vibrio cholerae	0	0	1	8	4
2	Pseudomonas aeruginosa	0	0	2	7	4
3	Escherichia coli	0	6	3	5	5
4	Proteus vulgaris	0	4	2	4	6
5	Bacillus subtilis	0	0	1	4	8
6	Staphylococcus aureus	0	3	2	3	3
7	Streptococcus pneumoniae	0	4	2	5	6
8	Klebsiella pneumoniae	0	0	2	9	6
9	Shigella dysenteriae	0	4	2	4	5

FT-IR analysis

To determine the specific site of interaction of Ag ions on bio molecules FT-IR has been performed. The synthesis solution of *C. aromatica* has enclosed many molecules and some of these become adsorbed on the surface of Ag NPs. Fig. 3 shows FT-IR spectra of both plant extract and AgNPs. The bands at 3435 cm^{-1} represent the in-plane bending of the hydroxyl groups and bands at 2256 cm^{-1}, 2057 cm^{-1} and 1637cm^{-1} represent carbonyl groups (Pant, N et al, 2013), the remaining transmission peaks corresponding to presence of fatty acids, carbonyl groups, flavanones. Some of the above bands wherein absence was observed in Figure 3, it is an indicative of the curcumin based compounds act as capping and reducing agents during the course of nanoparticle synthesis.

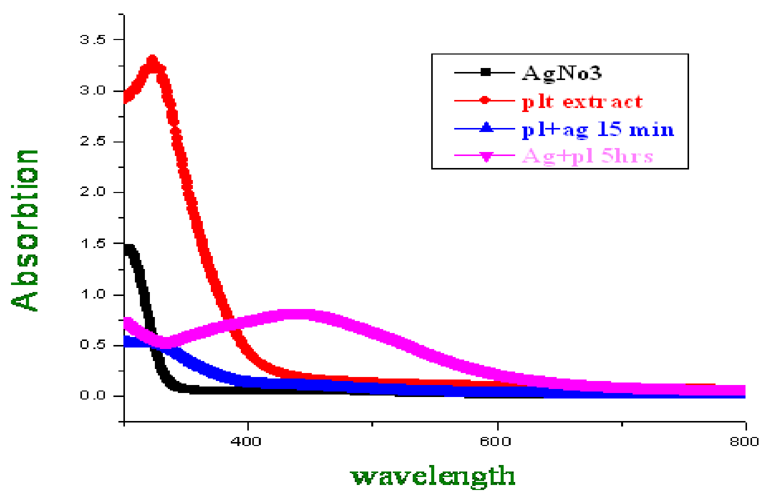

Figure-1: UV spectra of Ag NPs using the leaf extract of C.aromatica

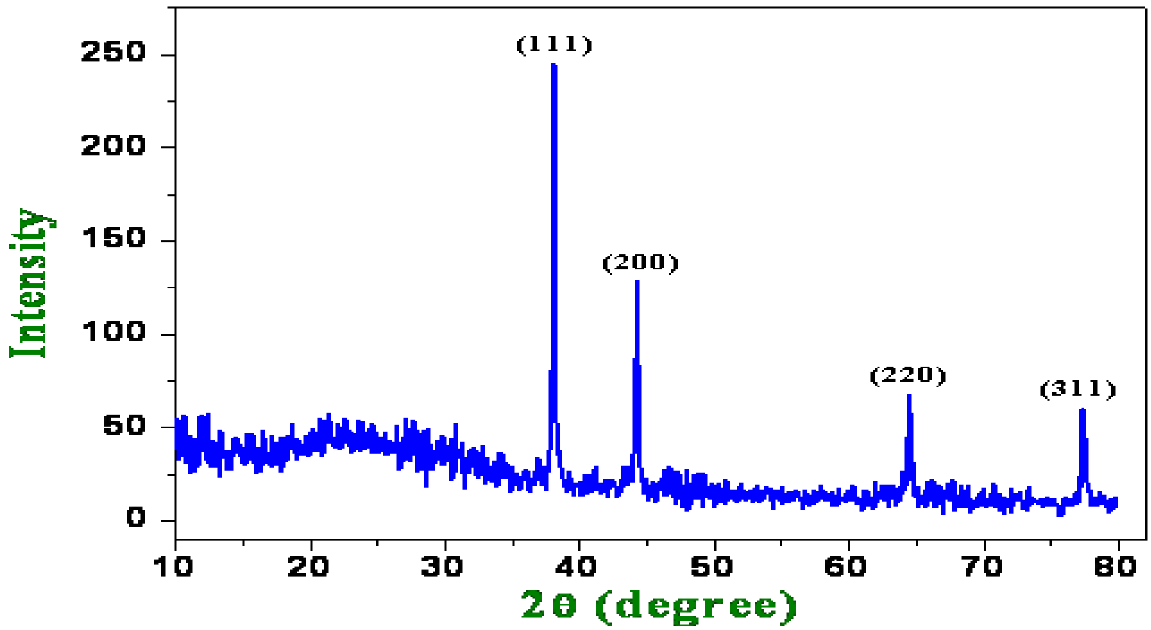

Figure-2: XRD profile of synthesized AgNPs

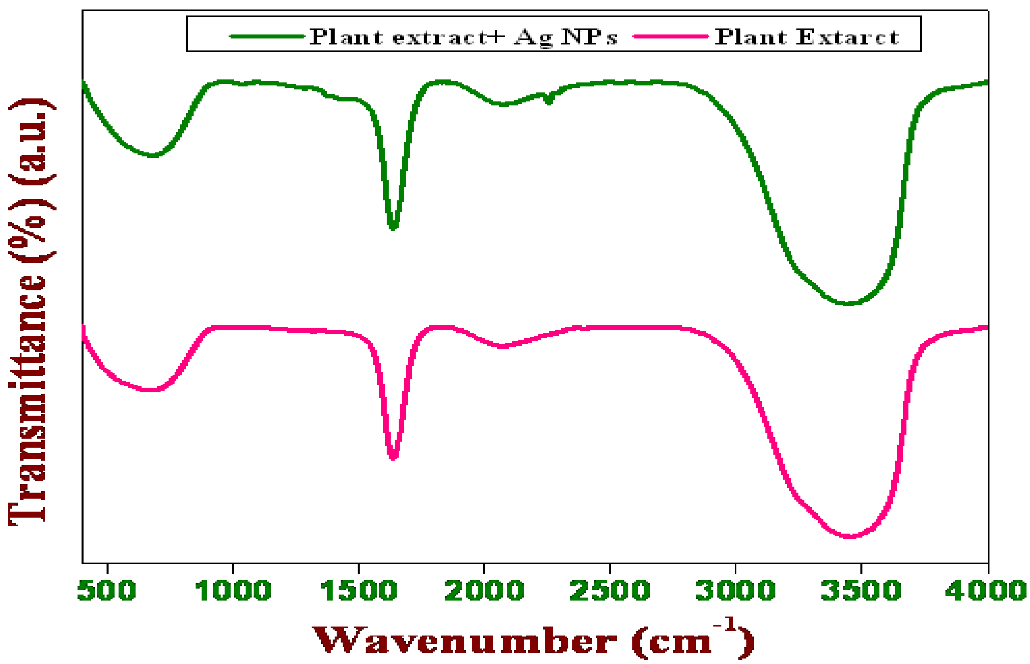

Figure-3: FTIR spectra of Ag NPs using the leaf extract of C.aromatica.

TEM analysis

A transmission electron microscopy (TEM) was engaged to analyze size, nature and morphologies of obtained Ag NPs. Fig. 4 depicts the TEM micrograph of Ag NPs. TEM analysis proved the formation of nanocrystalline silver particles which are somewhat agglomerated and average particle size was estimated between 20-80 nm. The crystallinity of Ag NPs was observed by selected area emission diffraction (SAED) and size distribution plot as shown in the Fig.4 The characteristic fringe array can be indexed as (111), (200), (220) and (311) of the pure face centered cubic (fcc) lattice structure commonly found for silver crystal, which was also confirmed by XRD results.

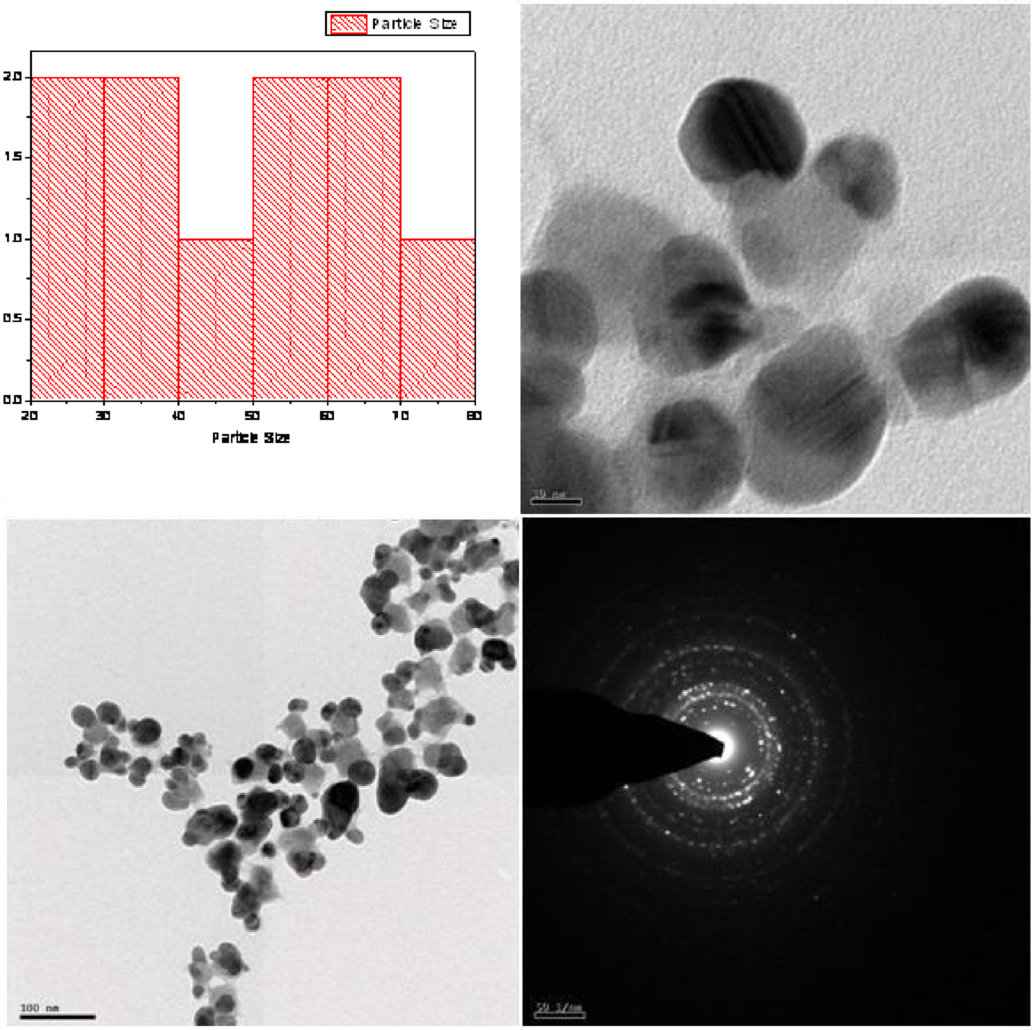

Figure-4: TEM images of Ag NPs using the leaf extract of C.aromatica

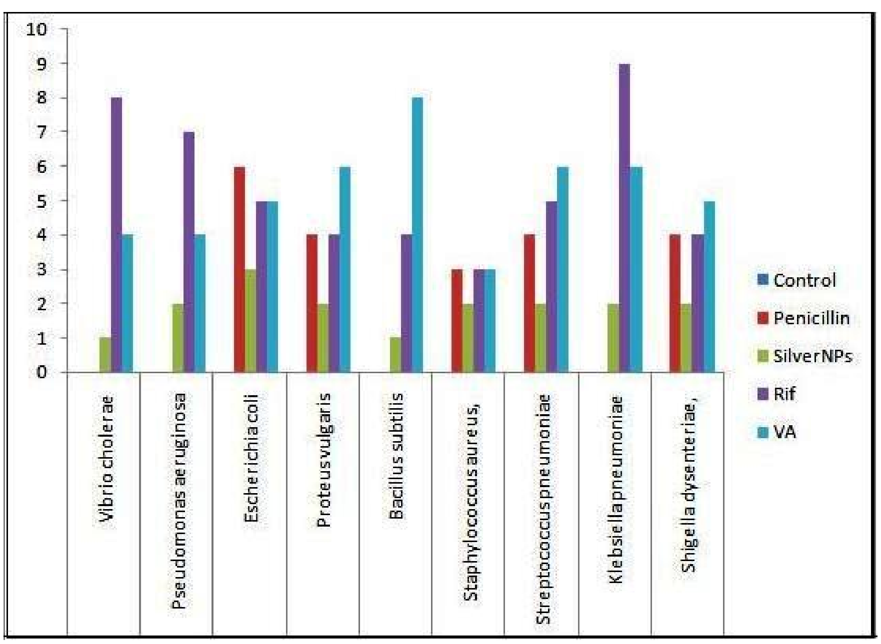

Figure-5: Antibacterial activity of AgNPs with different drugs against gram-positive and gram-negative bacteria.

Antimicrobial activity

The antibacterial activity was performed against three gram-positive and six gram-negative bacterial pathogens by using green-synthesized Ag NPs furthermore their comparison between drugs like *Penicillin, vancomycin, Rifamycins* also performed. Table 1 represents ZOI of silver nanoparticles with various drugs against gram-positive and gram-negative bacteria. Ag NPs produce the significant effects against both the gram-positive and gram-negative bacteria. *Escherichia coli* exhibit most significant effect for silver nanoparticles at the same time all the pathogen having some least significance towards Ag NPs. Capability of Ag NPs to attach by electrostatic interaction between the negatively charged bacterial cell and the positively charged nanoparticles this disrupts the reliability of the bacterial membrane and consequently cell death takes place due to the structural change (Sondi, I., & Salopek-Sondi, B. 2004). Ag NPs interact with thiol groups of bacterial protein which induced the deactivation of the protein synthesis and DNA replication (Feng, Q. L et al, 2000). Ag NPs prying with the bacterial cell membrane and their binding with mesosome will thereby reduce the mesosomal function and increase the production of ROS generation, which leads to cell death. Hence, Ag NPs could be used in pharmacology industry to develop drugs for gram-positive and gram-negative bacterial diseases. Figure 5 and 6 represent Antibacterial activity of Ag NPs with different drugs against gram-positive and gram-negative bacteria.

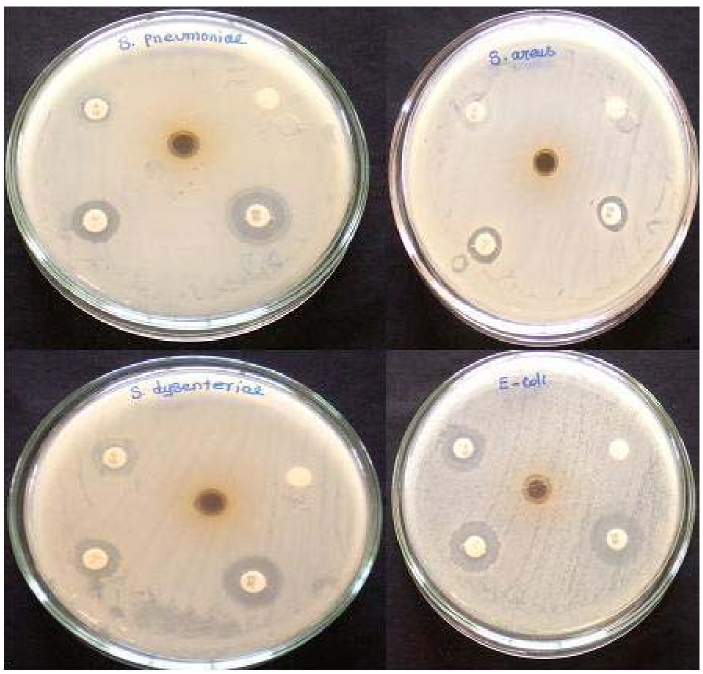

Figure-6: Disc diffusion assay of prepared AgNPs.

CONCLUSION

The synthesis of silver nanoparticles using aqueous extracts of *C. aromatica* has been demonstrated. Curcumin based compound acting as both the reducing and stabilizing agents. Particles are mostly spherical in shape. The nanoparticles were characterized by UV- visible, XRD, FTIR and TEM analyses. These silver nanoparticles were of high purity, making them potentially useful for biological applications. The TEM image substantiated that the particles are spherical shaped with the average size between 20-80nm. The antibacterial activity of Ag NPs has significant effects against both the gram-positive and gram-negative bacteria. This green synthesis is rapid, facile, convenient, less time consuming and environmentally safe. We propose this green synthesized Ag NPs potentially useful for biological application.

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