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Green Synthesis of Silver Nanoparticles from the leaf Extract of *Quisqualis Indica* L. for Enhanced Antibacterial activity

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ABSTRACT

Green synthesis of nanoparticles using plant source has been given much important. In the present study, silver nanoparticles (AgNPs) were synthesized using the aqueous extract of *Quisqualis indica*. The synthesized nanoparticles were characterized by UV-visible spectroscope, FT-IR and TEM analysis. The particle size of the synthesized AgNPs was 40 nm as confirmed by TEM. The qualitative assessment of reducing potential of the leaf extract of *Quisqualis indica* indicated the presence of reducing agents. Synthesized AgNPs exhibited significant antimicrobial activity against human bacterial pathogens like *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, and pseudomonas aeruginosa.*

Introduction

The metal nanoparticles are finding rich applications in optoelectronics, nanodevices, nano electronics, and nano sensors (Ramyadevi et al., 2012). Among various metal nanoparticles, silver nanoparticles have attracted more attention because of their antimicrobial activity (Bozanic et al., 2011). Nanoparticles of noble metals have unique physical and chemical attributes that differ from the bulk substance (Feldheim 2002). The extremely small and large surface area relative to their volume makes them useful for applications in nonlinear optics, spectrally selective coating for solar energy absorption, optical receptors, catalysis in a chemical reaction, bio labelling, water purification and as antibacterials (Sherly 2013). The production of metal based nanoparticles by physical and chemical methods are not eco friendly. Hence biological and biomimetic method of synthesis of nanoparticles are given much attention. In this approach green synthesis of metal nanoparticles has received an importance, as this approach does not involve any hazardous material. Synthesis of inorganic nanoparticles by biological synthesis makes them more biocompatible and environmentally caring (Govindaraju et al., 2010). Plant extract mediated synthesis of metal nanoparticles has an edge over microbial mediated biosynthesis of nanoparticles because the green synthesis of nanoparticles takes place extracellular. Further, this process is quick and suitable for large scale synthesis (Charusheela Ramteke et al., 2013). The present study focuses on the synthesis of silver nanoparticles using the leaf extract of Quisqualis indica. Also attempts were made to characterize the synthesized nanoparticles using UV-Visible spectroscopy, FTIR, and TEM study. The inherent antibacterial potential of synthesized silver nanoparticles and aqueous extract of Quisqualis indica were explored. In the present work we present a rapid method for nanoparticles production using the leaf extracts of Quisqualis indica.

2. Materials and Methods

2.1. Collection of plant material

To prepare the *Quisqualis indica* leaf extract, the leaves were washed thoroughly with tap water, and repeated twice with distilled water. Subsequently, they were left in the shade to dry out and finally grounded using an electric grinder.

2.2. Preparation of plant material

To prepare the aqueous extract of *Quisqualis indica*, the leaves were cut into small pieces and 10 g was weighed, put into a 250 ml flask. Deionised water (50 ml) was added and the solution was placed on a stirrer in a dark place at room temperature for 24 h. The solution was separated by filtration with Whatman No.1filter paper and then centrifuged at 5500 rpm for 10 min. The upper phase was retained for synthesis of AgNPs.

2.3. Reagents and chemicals

Distilled water and AgNO3, Nutrient agar Muller-Hinton media, Sabouraud Dextrose Agar (SDA), DMSO, Tween 20, DPPH

2.4. Preparation of 1Mm silver nitrate aqueous solution

An accurately weighed 0.017g of silver nitrate was dissolved in 100ml distilled water and stored in amber colour bottle until further use.

2.5. Synthesis of silver nanoparticles

The aqueous leaf extract of *Quisqualis indica* and ninety millilitre of silver nitrate solution (1mM: 0.169 gram of silver nitrate dissolved in 1000 ml (double distilled water) was added and mixed well. The mixture was kept at room temperature (36±1ºC) undisturbed for 24 hrs the bio reduced silver nitrate solution became brown in colour indicating the synthesis of silver nanoparticles.

2.6. Characterization of synthesized silver nanoparticles

The synthesized nanoparticles were characterized by using UV- visible spectrophotometer, FTIR and Transmission Electron Microscope (TEM). The UV- visible analysis was performed in the absorption wavelength of 200 to 1000 nm. The synthesized silver nanoparticles were studied using FTIR (Perkin-Elmer FT-IR spectrometer). Transmission Electron Microscopy was used to observe the size, shape and morphology of the synthesized nanoparticles. Samples for TEM observation were prepared by casting a drop of the silver nanoparticles on a carbon coated copper grid and the excess solution was removed by tissue paper and allowed to air dry at room temperature for overnight.

2.7. Assessment of antibacterial activity

The antibacterial activity of green synthesized AgNPs was tested against four bacterial isolates using Agar well diffusion method (Ahmad and Beg, 2001). Nutrient Agar Plates were inoculated with 100 µl of standardized culture ($1.5x10^8$ CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Wells of 8 mm size are made in the agar plates containing the bacterial lawn. From the synthesized AgNPs, 25 µl , 50 µl , 75 µl , and 100 µl volume were poured into the wells made in the bacterial culture plates. Standard chemical (AgNO3) solution was used as a negative control. The plates thus prepared were left at room temperature for ten minutes for allowing the diffusion of the extract into the agar bacterial lawn. After incubation for 24 hr at 37ºC, the plates were observed. The zone of inhibition was measured and expressed in millimetres. The antibacterial activity was expressed in terms of the diameter of the zone of inhibition and <9 mm zone was considered as inactive; 9-12 mm as partially active; while 13-18 mm as active and >18 mm as very active (Mariselvam *et al.,* 2012).

2.8. Minimum inhibitory concentration (MIC) of AgNPs

The minimum inhibitory concentration at which a silver nanoparticle exhibits the antimicrobial activity was determined by using a 96 wells titre plate. Each well in the plate was filled with 250 µl of nutrient broth and inoculated with different microorganisms selected for the assay. Different concentrations (5, 10, 15, 20, 25, 30, 35, and 40) of AgNPs were added in to the wells and incubated at 37ºC for 24 h. The growth rate of each bacterium was determined by the turbidity method using ELISA reader at 600 nm wavelength.

2.9. Mechanism of action of AgNPs on microorganisms using the simple staining method

One ml of 24 h bacterial culture was mixed with 1 ml of synthesized silver nanoparticles incubated at 37ºC for 24 h. After incubation the bacterial cultures were Gram stained and the colony characteristics were observed using a light microscope.

3. Results and discussion

3.1. Synthesis of AgNPs

Formation of AgNPs by the reduction of AgNO3 during treatment with the leaf extract of *Quisqualis indica* is evident from the change in colour of the reaction mixture. The change in colour of the reaction mixture after 2 hours is which indicated the formation of AgNPs. This reaction indicates that silver ions in reaction mixture had been converted to elemental silver having the size of nanometric range.

3.2. UV-Visible spectrum of synthesized AgNPs

The UV-vis absorption spectrum of silver nanoparticles using aqueous extract of quisqualis indica was evidenced by the visual colour change of solution from pale pink to deep brown due to excitation of surface Plasmon vibrations in AgNPs. The surface Plasmon resonance of AgNPs showed a peak centered near 430 nm at UV-vis spectra which corresponds to the absorbance of AgNPs (Fig 1). This peak indicates the reduction of silver nitrate into AgNPs. It is suggested the spherical size of the nanoparticles as per Mie theory. The spherical size of the AgNPs was further confirmed by TEM study. The periodic observation of prepared AgNPs during the storage period did not show any variation in the absorption spectrum. This indicated the constancy of particle size even during storage.

3.3. FTIR spectrum of synthesized AgNPs

FTIR analysis is characterizing the surface chemistry of nanoparticles. Organic functional groups like OH, C=O linked to the surface of nanoparticles are found by FTIR. The FTIR spectra of aqueous leaf extract of *Quisqualis indica* are shown in (Fig 2). The synthesis of AgNPs, the solution containing nanoparticles was centrifuged at 8000 rpm for 10 min to separate AgNPs from other composition of solution and the deposit was prepared for FTIR analysis. The FTIR spectra of the aqueous extract of *Quisqualis indica* displayed peak in the range of 600-700 cm^{-1}, which demonstrate the alkyl halides band especially the C-CI bond. The strong band 1640 cm-1 (C=O) established the formation of silver nanoparticles. The peaks in the range of 3000-3500 cm-1 were assigned as –OH stretching in alcohols and phenolic compounds with strong hydrogen bonds.

3.4. TEM microscope of synthesized AgNPs

The TEM image of AgNPs (Fig 3), confirmed that the silver nanoparticles are spherical in shape and the particle size was 26 nm.

3.5. Antibacterial activity of synthesized AgNPs

The antibacterial potential of silver is well known. The synthesized AgNPs were tested for their antibacterial action using four types of human bacterial pathogens (Table 1). The bacterial growth inhibitory potential of AgNPs was compared with standard antibiotics. At a 100 µl dose the inhibitory action of AgNPs was high. The maximum inhibition was observed for *Escherichia coli* (16 mm), *Staphylococcus aureus* (24 mm), *Klebsiella pneumoniae* (20 mm) and *Pseudomonas aeruginosa* (24 mm) respectively.

3.6. The minimum inhibitory concentration (MIC)

The MIC of silver nanoparticles dispersed in the micro litre plate is summarized. The growth rate was calculated using the standard control. Xx µl of silver nanoparticles was the MIC for following organisms viz., *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, and pseudomonas aeruginosa* . The result of the MIC value indicates the high inhibitory potential of AgNPs on gram positive bacterial pathogens. The plant *Quisqualis indica* showed potent antibacterial activity against two bacterial strains: Gram-positive *S. Aureus* and Gram-negative *E. Coli*. Aqueous extract of *Quisqualis indica* containing Ag nanoparticles showed activity in all Ag concentrations tested all bacteria (Fig 4). Antibacterial activity was shown by an inhibition zone which was characterized by a clear zone between the wells (containing samples) and a certain distance. Formation of inhibition zones around the wells shows bacterial sensitivity to antibacterial and antibiotic ingredients (which are used as positive controls). The positive control used in the well was an ampicillion 500 mg solution and functioned as a control of the test solution by comparing the diameter of the inhibition zone formed. On the contrary, distilled water as negative control was used to determine the effect of solvents in the test solution on the growth of

S. Aureus and *E. Coli* bacteria. It was clear that it was the extract containing Ag nanoparticles that had the antibacterial activity, not the solvent. The diameter of inhibition zones formed for each concentration of the AgNO3 precursor added to the aqueous extract of *Quisqualis indica* plant in synthesizing Ag nanoparticles.

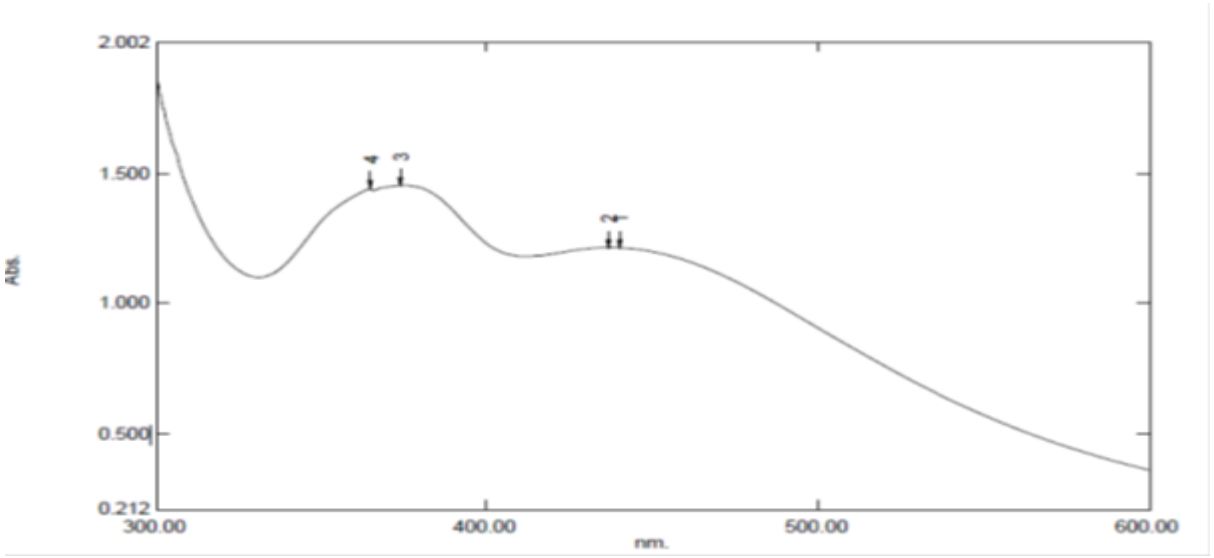

Fig 1. UV-visible spectrum of AgNPs

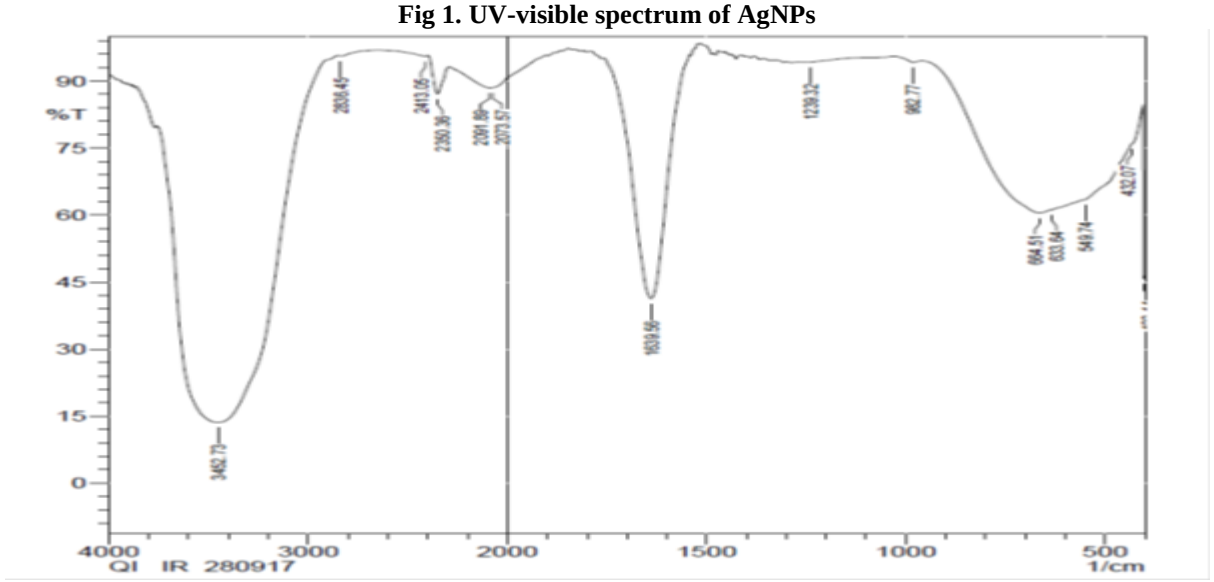

Fig 2. FTIR spectrum of AgNPs

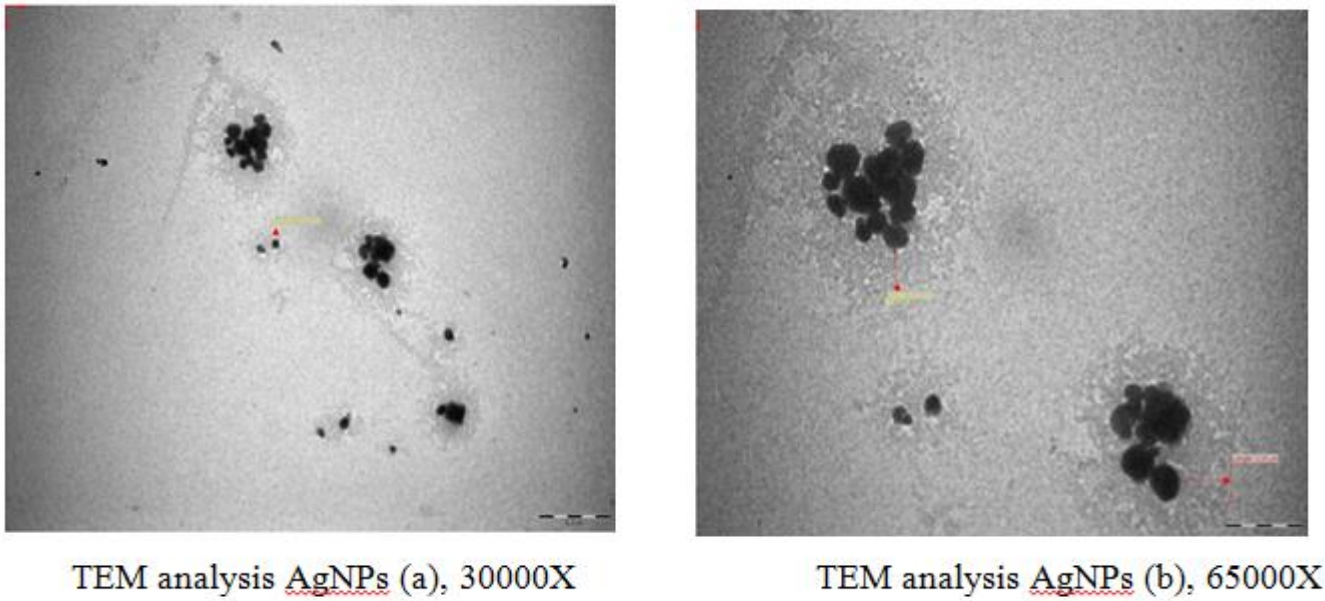

Fig 3. TEM analysis of AgNPs

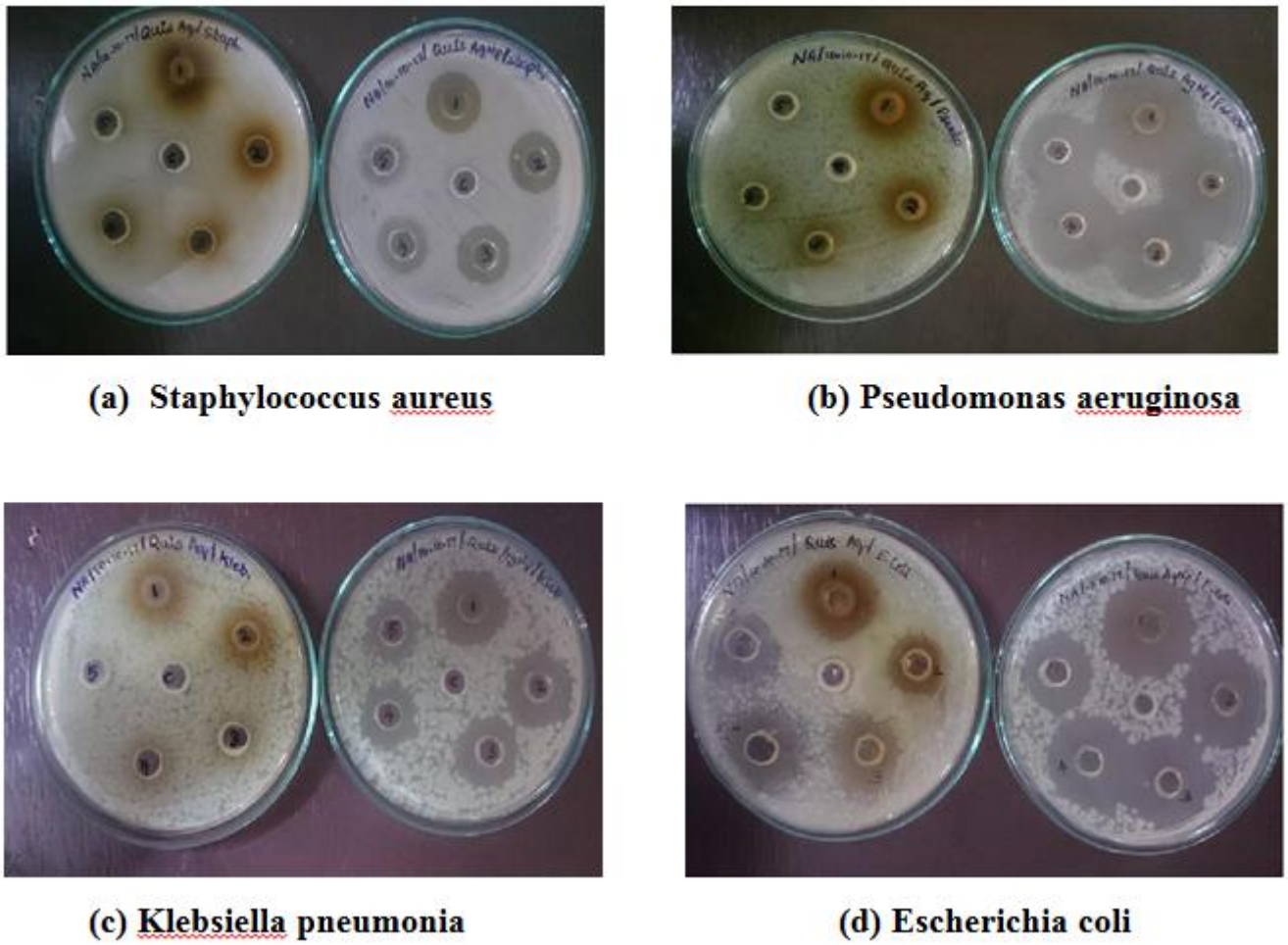

Fig 4. The inhibition zone (mm) of aqueous and AgNPs treated against (a), *Staphylococcus aureus*, (b) *Pseudomonas aeruginosa*, (c) *Klebsiella pneumonia*, (d) *Escherichia coli*.

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Table 1. Zone of inhibition antibacterial activity.

No	Microorganism	Zone of Inhibition (mm)									
		Aqueous plant extract					AgNPs				
		C1	C2	C3	C4	C5	C1	C2	C3	C4	C5
1	*Staphylococcus aureus*	16	14	11	09	-	22	18	15	13	09
2	*Pseudomonas aeruginosa*	24	21	19	17	14	29	25	22	20	13
3	*Klebsiella pneumonia*	20	17	15	11	-	23	19	18	17	16
4	*Escherichia coli*	24	22	18	13	11	28	26	23	18	14

(-) No activity

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