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Green Synthesis of Silver Nanoparticles Using *Cymbopogan Citratus* (Dc) Stapf. Extract and Its Antibacterial Activity

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ABSTRACT

Background: There is an increasing commercial demand for nanoparticles due to their wide applicability in different fields such as catalysis, photonics, electronics, biological labelling, biosensing, drug delivery, antibacterial, and antiviral and gene therapy. Physicochemical methods of nanoparticle synthesis causes environment hazards, so biological approach has emerged as eco-friendly and alternative method. *Cymbopogan* citratus (DC) stapf. is commonly known as lemon grass belongs to a family Poaceae. It is a native aromatic herb from India and is also cultivated in other tropical and subtropical countries. **Objective:** The main objective of the study is synthesis and characterization of silver nanoparticles in lemon grass leaves and to study its antibacterial activity. **Results:** The silver nanoparticles were formed after 3 hours of incubation at 37ºC using aqueous solution of 5 mM silver nitrate ($AgNO_3$) and synthesized silver nanoparticles were characterized by UV-vis, XRD, SEM,EDS and FTIR. The antibacterial activity of synthesized silver nanoparticles was investigated by disc diffusion method. **Conclusion:** In the present study silver nanoparticles was synthesized by green route and significant antibacterial activity was also observed.

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INTRODUCTION

Synthesis of nanoparticles in recent years has caught the attention of many researchers and it is a challenge to current scenario. Silver nanoparticles have unique catalytic, optical, electrical and antimicrobial properties (Wang, 2000). Silver is a nontoxic inorganic antimicrobial agent.

Various methods are available for synthesis of silver nanoparticles such as chemical reduction (Kim *et al*., 2004; Song *et al*., 2009) and hydrothermal (Yang *et al*., 2010) sonochemical (Zhu *et al*., 2010) and thermal decomposition (Lee *et al*., 2010). Above all methods involve the usage of hazardous reagents for synthesis of silver nanoparticles. In view of environmental pollution control and awareness, there is an urgent need to develop an ecofriendly method for the synthesis of nanomaterials.

Many of the reports published involve the biological synthesis of nanomaterials using bacteria, fungi and plant extracts. Silver nanoparticles have been synthesized using various plant extracts such as *Cinnamon camphora*, *Eucalyptus camaldulensis* (Emad *et al*., 2009) *Geranium* (Shankar *et al*., 2003), *Azadirachta indica* (Shankar *et al*., 2004), *Aloe vera* (Chandran *et.al*., 2006), *Tamarindus indica* (Ankamwar *et al*., 2005), *Acalypha indica* (Krishnaraj *et al*., 2010) and *Pongamia pinnata* (Rajesh *et al*., 2010).

Cymbopogon citratus has short underground stems, simple, alternate, linear, leaves with 5.0-7.0 cm. long and 0.5-1.5 cm. wide. Apex acute leaves are sheathed with parallel venation. They are grown abundantly in Philippines, Srilanka and also in high altitudes of India. The leaves of lemon grass are used in many homes to cope mainly with cough, cold and fever. It helps to boost the immune system, reduces uric acid, cholesterol, excess fats, and alleviates indigestion and gastroenteritis. Recently the essential oil of lemon grass has been the subject of scientific studies because of its effect on cancer cells. The oil also has a promising anticancer activity and cause loss in tumor cell viability by activating the apoptotic process. The major component of lemon grass oil is citral which has excellent anticancer properties. Since silver nanoparticles have great potential for cancer

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management, the synthesis of silver nanoparticles using plants with anticancer potentials has been started recently. With all these evidences, in the present investigation an attempt is made to synthesize silver nanoparticles by using aqueous leaf extract of lemongrass and evaluated its potential against some of the pathogenic bacteria.

MATERIALS AND METHODS

2.1 Plant material and preparation of the extract:

Fresh *Cymbopogan citratus* leaves (Fig.1) were collected from the Mother Teresa Women's University campus, Kodaikanal (Tamil Nadu) India, and it is documented in the Kodaikanal (Horticultural Research Station). The chemicals needed for the present investigation were purchased from Sigma-Aldrich, India.

Fig. 1: *Cymbopogan citrates.*

2.2. Synthesis of silver nanoparticles:

The fresh, disease free lemon grass leaves were collected and washed thoroughly 2-3 times with tap water and subsequently with sterile water. The washed leaves were dried at 40°C in hot air oven and powdered. The leaf powder was then used for extraction. About 2 grams of dried leaf powder were ground to fine paste with 20 ml of distilled water using mortar and pestle. It was centrifuged at 10,000 rpm for 10 minutes and supernatant was taken for synthesis of silver nanoparticles. During green synthesis of silver nanoparticles, ten ml of the plant leaf extract was added to 90 ml of aqueous solution of 5 mM $AgNO_3$ for reduction of silver nitrate into silver ions ($Ag+$) and maintained at room temperature (37 ° C) in the incubator under static condition for 4 hours. During incubation, the colourless silver nitrate solution is changed from pale yellow to ruby red and finally dark brown colour.

2.3. UV-visible spectroscopy analysis:

The colour change in reaction mixture (Silver nitrate solution + leaf extract) was recorded through visual observation. The bioreduced silver nanoparticle solution was filtered through Whatmann No.1 filter paper and the reduction of pure silver ions was observed by monitoring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the sample, compared with 1ml of distilled water used as blank.

2.4. X-ray Diffraction (XRD) Measurements:

The completely bioreduced sample was concentrated in an oven at 50º C and the concentrated solution was then centrifuged at 12000 rpm for 15 minutes. The obtained pellet was washed and redispersed in deionized water. The dried mixture of AgNPs was collected to determine the formation of AgNPs by XRD (Germany) operated at a voltage of 30 kV and a current of 30 mA with CuKα radiation in a θ- 2θ configuration.

2.5. Determination of crystalline size:

Average crystallite size of silver was calculated using the Scherrer's formula,

$$D = k\lambda / \beta\cos\theta$$

D = Average crystallite size: K - Constant: λ- X-ray Wavelength: β- Angular FWHM of the XRD peak at the diffraction angle: θ- Diffraction angle.

2.6. FTIR (Fourier Transform Infrared Spectroscopy) analysis:

The earlier centrifuged and redispersed AgNPs obtained was used for FTIR analysis. For this, the dried powder was obtained by lyophilizing the purified suspension. The resulting lyophilized powder was mixed with KCl procured from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in FTIR for the analysis of the nanoparticles (Perkin-Elmer).

2.7. SEM & EDS analysis of silver nanoparticles:

To gain further insight into the features of the silver nanoparticles, analysis of the sample was performed using SEM and EDS techniques. A scanning electron microscope (JEOL 6380A; Tokyo, Japan) was used to record the micrograph images of synthesized Ag-NPs. Energy-dispersive x-ray (EDS) spectroscopy analysis was carried out for the confirmation of elemental silver.

2.8. Antibacterial Assay:

Antibacterial activity of the silver nanoparticles was assessed using the standard agar disc diffusion method with 6 mm diameter Whatmann No.1 filter paper discs. In this method, 25 μl, 50 μl, and 100 μl of synthesized silver nanoparticles were mixed with 1 ml of ethanol and applied to sterile paper discs and plant extract without nano particles was used as a control. Bacteria such as *Psuedomonas aeruginosa, P. mirabilis, Escherichia coli, Shigella flexaneri, S. somenesis* and *Klebsiella pneumonia* were used for the antimicrobial test. All cultures were grown overnight at 37ºC ± 0.5°C, pH 7.4 in a shaker incubator (190-220 rpm). Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (50 µl) of each culture were spread on to LB agar plates. Sterile paper discs (containing various concentrations of AgNPs) along with the plant extract containing discs were placed in each plate. Antimicrobial activities of the synthesized AgNPs were determined, using the agar disc diffusion assay method.

RESULTS AND DISCUSSION

UV-Visible spectra analysis:

The synthesized Ag nanoparticles using lemongrass plant extracts confirmed by visual observation. The colour was changed from pale yellow to ruby red and finally to dark yellowish brown due to reduction of silver ions during the reaction. The dark yellowish-brown colour indicates the formation of silver nanoparticles in the reaction mixture (fig.2). It is well known that silver nanoparticles exhibits yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations (Chandan Singh *et al.*, 2011).

UV–Vis spectroscopy is an important technique to establish the formation and stability of metal nanoparticles in aqueous solution (Philip *et al.*, 2011). Silver nanoparticles were detected by UV-Vis spectrophotometer in the range of 350- 620 nm. The maximum absorption spectra of silver nanoparticles formed in the reaction mixture has increasingly sharp absorbance maximum peak at 510 nm (fig.3). The absorption spectrum of the yellowish-brown silver nanoparticles showed the surface Plasmon resonance band at 510 nm indicating the presence of spherical silver nanoparticles (Ahmad, 2003). Our results were in agreement with findings of Shalaka A. Masurka *et al.*, 2011 who observed the surface Plasmon resonance band at 430 nm indicating the presence of silver nanoparticles.

Fig. 2: Photographs of (a) $AgNO_3$ (b) Leaf extract (c) Leaf extract + $AgNO_3$ after 4 h of reaction.

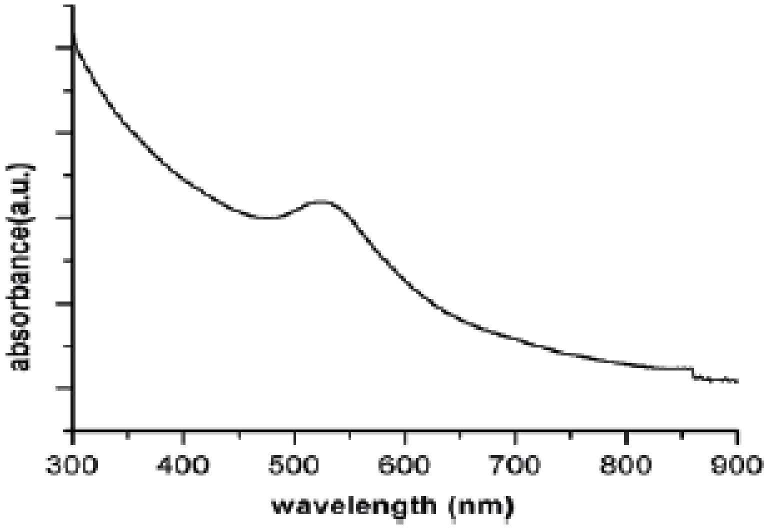

Fig. 3: UV-vis spectra of reduction of Ag ions to Ag nanoparticles.

XRD:

The XRD patterns of lemon grass dried leaf powder (control) is shown in Fig. 4a which does not show any peak. The XRD pattern of synthesized silver nanoparticles showed eight intense peaks in the whole spectrum of 2θ values ranging from 27.80 to 77.40 (Fig.4b). The peaks observed in the spectrum at 2θ values of 38.126°, 44.26°, 46.19° and 64.46° corresponds to 111, 200, 220, and 311 planes for silver, respectively. This result clearly indicates that the silver nanoparticles formed by the reduction of Ag+ ions using lemon grass leaf extract are crystalline in nature (Joerger *et al*., 2000).

The average estimated particle sizes of the samples were calculated using the Debye-Scherrer formula. An average size of the particles synthesized by lemon grass leaf extract was 34 nm (Table 1.).

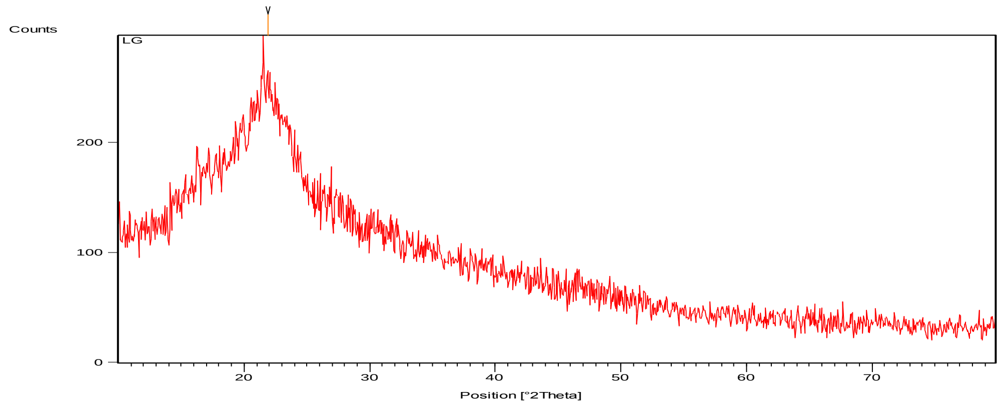

Fig. 4: a XRD patterns of Lemon grass dried leaf powder.

SEM:

The scanning electron microscopic (SEM) image showed high density silver nanoparticles synthesized by the plant extract and further confirmed the presence of Ag nanoparticles. SEM image has showed individual silver particles as well as a number of aggregates. The observed morphology of the silver nanoparticles was predominately cuboidal, and rectangular, uniform, poly dispersed and aggregated into larger irregular structure with no well-defined morphology (fig.5 a & b). Ag nanoparticles were formed with diameter of 15 to 65 nm. The SEM image of silver nanoparticles were synthesized from plant extracts are assembled onto the surface due to the interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bound to the Ag nanoparticles. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (proteins secreted by plant leaf extracts). The presence of secondary materials capping with the silver nanoparticles may be assigned to bio-organic compounds from leaf extracts (Kohler *et al*., 2001).

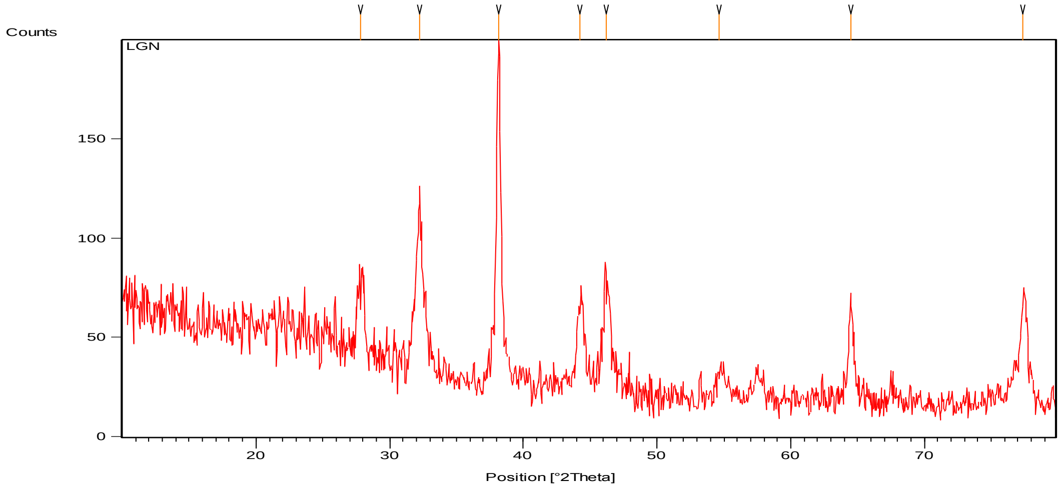

Fig. 4: b. XRD patterns of silver nanoparticles synthesized using dried leaf powder of Lemon grass.

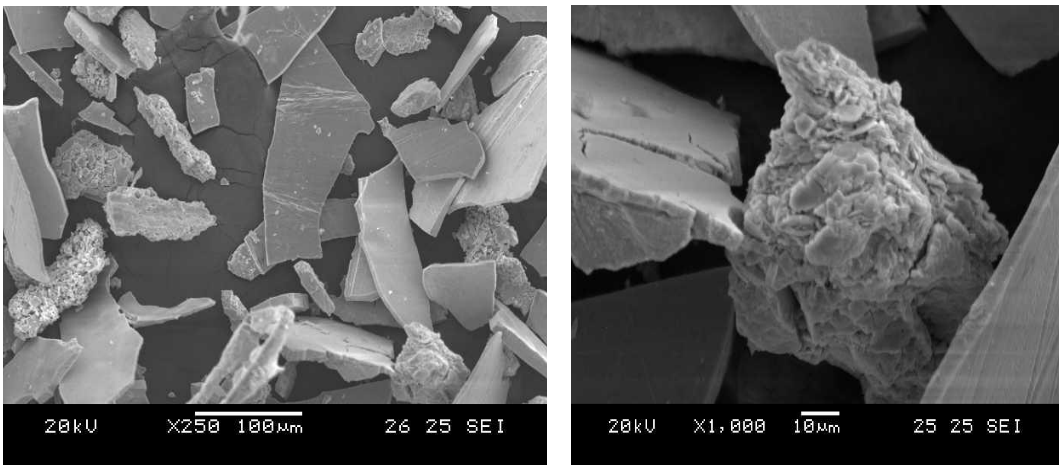

Fig. 5: SEM images of Ag Nanoparticle (a) 250000 (b) 1 lakh magnification.

EDS:

Energy dispersion Spectroscopy (EDS) analysis confirms the presence of elemental silver signal in the sample and is shown in fig: 6. EDX analysis give qualitative as well as quantitative status of elements that may be involved in formation of nanoparticle (Kude *et al.*,2013). The results were in agreement with reports of Shalaka A. Masurka *et al*., 2011 who reported that the silver content in the lemon grass extract showed strong Signals and content in the particle was found to be 69.81%.

Plant extract	θ value (degree)	d- spacing (Å)	FWHM (degree)	Intensity (CPS)	Average Particle size (nm)
Lemon grass s*ilver nanoparticles*	19.1	2.36	0.2949	100.00	33.89

FTIR:

The analysis of FTIR spectrum throws light on biomolecules bearing different bioactive compounds present in the plant extract. In the FTIR spectrum several absorption peaks were centered at 3951.12, 3846.11, 3733.64, 3414.10, 3299.85, 3194.8, 2150.29, 2150.29, 1367.05, 1223.90 cm-1 which was in the region range of 1000 - 4000 cm-1 (Fig.7). The spectrum absorption observed at 3414.10 cm-1 indicates the stretching vibrations of -NH (amide groups), C= C (alkane) groups, - C- D deuterated alkanes groups, C=O simple $_\gamma$ lactams, amide I band groups and C-H (-CH3 - band) of alkanes groups. These groups are mainly derived from heterocyclic compounds of lemon grass leaf extract and they are water soluble in nature. So it can be assumed that these different water soluble heterocyclic compounds such as alkaloids, flavonoids etc., worked as the capping ligand for the synthesis of silver nanoparticles (Kim *et al.*, 2007 and Krishnaraj *et al*., 2010). The result also shows that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. It is also evident from the differences in the peak for dried powder and silver nanoparticles that the flavonoids are responsible for the bioreduction. Flavonoids could be adsorbed on the surface of the metal nanoparticles, possibly by interaction through π-electrons of carbonyl groups in the absence of other strong ligating agents in sufficient concentrations.

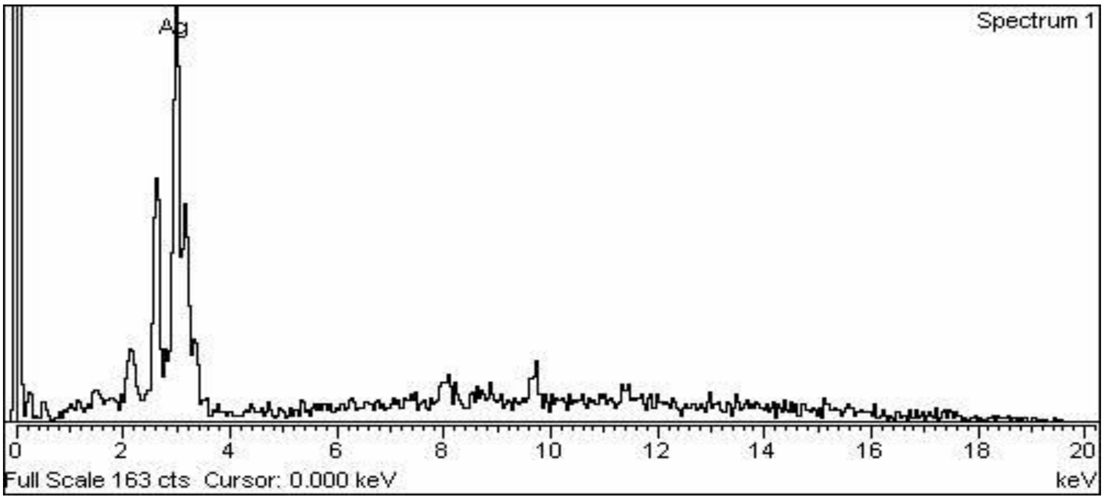

Fig. 6: EDS spectra of prepared silver nanoparticles. Strong signals from the atoms in the nanoparticles are observed in spectrum and confirm the reduction of silver ions to silver nanoparticles.

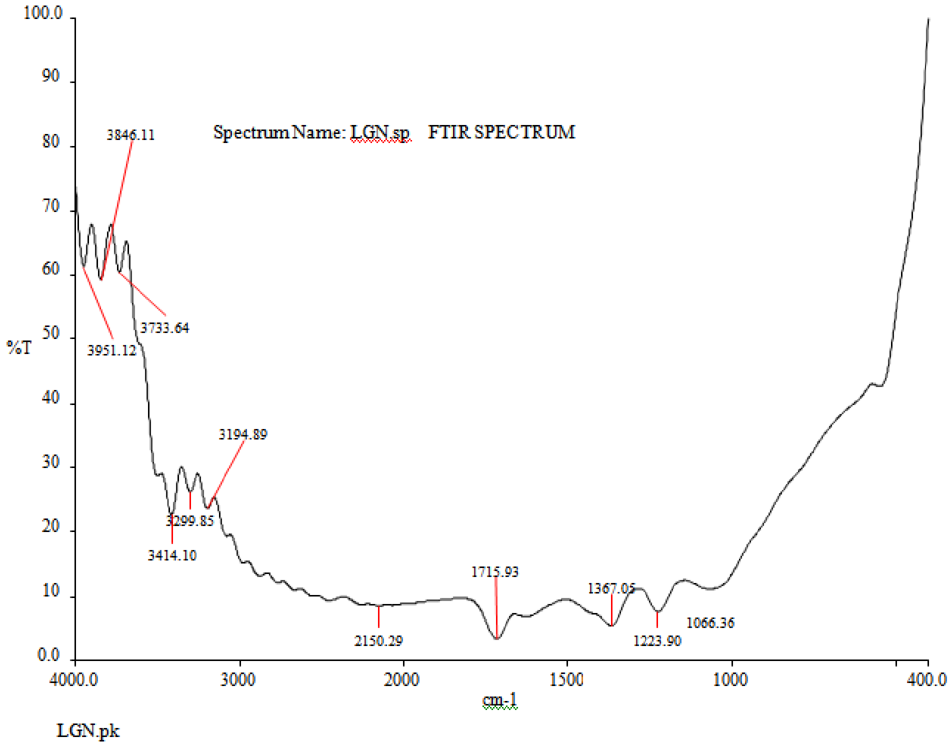

Fig. 7: Showing the FTIR spectrum of synthesized silver nanoparticles of lemon grass.

Antimicrobial studies:

Antibacterial activity of biogenic silver nanoparticles was evaluated by zone of inhibition using standard agar disc diffusion method. The nanoparticles showed inhibition zone against all the studied bacteria (Fig. 8). Among three different concentrations (25, 50 and 100 µl), 100 µl concentration showed maximum activity against *Klebsiella pneumoniae* (2.2 cm), *Shigella somenesis* (1.2 cm), *Pseudomonas aeruginosa* (1.5 cm), *E. coli* (1.3 cm), *P. mirabilis* (1.1 cm) and *S. flexaneri* (1.1 cm), when compared to the other two concentrations. Zone of inhibition was not found when lemon grass plant extract was used as control. The investigation made by

(Li *et al*., 2006) revealed the antibacterial effect of nanosized silver colloidal solution against *S. aureus* and *K. pneumoniae* after padding the solution on textile fabrics such as hospital masks and guage clothes.

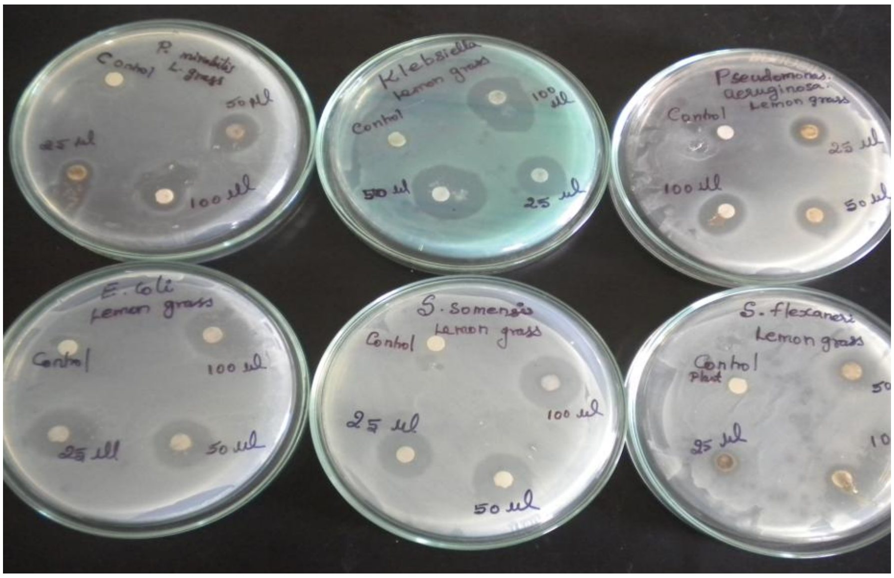

Fig. 8: Antibacterial activity of synthesised nanoparticles from lemon grass leaf extract.

Conclusion:

In this study we present a simple, low cost, clean, nontoxic and rapid approach for the bio reduction and synthesis of silver nanoparticles. This method provides an efficient green route for the synthesis of nanoparticles with tunable optical properties directed by particle size. From the point of view of nanotechnology this is a significant advancement to synthesize silver nanoparticles economically. The silver nanoparticles show distinct poly dispersity as it shows particle size between the ranges of 15 to 65 nm with an average size of 34 nm. The identification of compounds, capping agents and tapping the enzymes responsible for the reduction process may open up new avenues in the field of nanotechnology and nanoparticle stabilization. Investigation on the antibacterial effect of nanoparticles against *P. aeruginosa, P. mirabilis, E. coli, Shigella flexaneri, S. somenei* and *Klebsiella pneumonia* reveals high efficacy of silver nanoparticles as a strong antibacterial agent. This can be useful in food industries, cosmetic industries and medicines.

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