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Research Article

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Antibacterial activity of silver nanoparticles synthesized from plant leaf extract of *Cycas circinalis, Ficus amplissima, Commelina benghalensis and Lippia nodiflora* leaves

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ABSTRACT

Green nanotechnology process has emerged as a powerful approach for the synthesis of silver nanoparticles. This would offer numerous benefits including eco-friendliness and compatibility for biomedical applications. We used an environment friendly biosynthetic technique for the production of the AgNP's. The reducing agents used to produce the nanoparticles were from aqueous extracts of Cycas circinalis, Ficus amplissima, Commelina benghalensis and Lippia nodiflora leaves. The antimicrobial activity of Ag nanoparticles was investigated against Escherichia coli, Klebsiella pneumoniae, Vibrio cholorae, Salmonella typhi and Shigella sonnei by disc diffusion method. These results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems. AgNP's are a promising candidate for development of future antibacterial therapies because of its wide spectrum of activity.

Keywords: antibacterial activity, green technology, biosynthetic, disc diffusion method.

INTRODUCTION

Silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of applications in areas such as catalysis, antimicrobials, optics, and biomaterial production [1]. Silver is a safe and effective bactericidal metal because it is non-toxic to animal cells and highly toxic to bacteria [2,3]. Silver nanoparticles (AgNPs) are one of the most commonly used nanomaterials and it is known to have antioxidant and antimicrobial properties [4]. At nanoscale, silver exhibits remarkably unusual biological, physical and chemical properties.

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new and effective antimicrobial reagents. Such problems and needs have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad spectrum activity and far lower propensity to induce microbial resistance than antibiotics.

Green technology approach emphasizes the usage of natural organisms that has offered a reliable, simple, nontoxic and eco-friendly method [5]. Therefore, researchers in the yester years have turned to biological systems for nanoparticles synthesis [6-8]. Synthesis of nanoparticles by biological methods, using microorganisms, enzyme and plant or plant extract, has been suggested as possible eco-friendly alternatives to chemical and physical methods [9-11].

Silver nanoparticles also have inhibitory and bactericidal effects. It can be expected that the large specific area and high fraction of surface atoms of silver nanoparticles will lead to high antimicrobial activity as compared with bulk silver metal [12-14]. Furthermore, nanosilver can be modified for a better efficiency to facilitate its applications.

In this study we report the synthesis of silver nanoparticles using *Cycas circinalis, Ficus amplissima, Commelina benghalensis* & *Lippia nodiflora* leaves [15,16]. These nanoparticles were evaluated for antimicrobial activity against Escherichia coli, Klebsiella pneumoniae, Vibrio cholorae, Salmonella typhi and Shigella sonnei. The results suggest that Ag nanoparticles can be used as effective growth inhibitors in various microorganisms.

EXPERIMENTAL SECTION

Silver nitrate ($AgNO_3$, 99.99%) and hydrogen peroxide (H_2O_2) were purchased from Sigma Aldrich, Germany. The extracts were obtained from the plant leaves of *Cycas circinalis, Ficus amplissima, Commelina benghalensis* & *Lippia Nodiflora* which have been collected from the forest near kallanai, South India. Bacteria such as Escherichia coli, Klebsiella pneumoniae, Vibrio cholorae, Salmonella typhi and Shigella sonnei were collected from Institute of Biotechnology, Hyderabad. Deionized water was used throughout the experiment.

Extraction method and condition

The collected fresh leaves of the plants were washed in fast flowing water. The wet leaves were left to dry for 30 min. 25 grams of leaves were taken for study by using digital weighing machine. Leaves were boiled in distilled water for 3 min and left in room temperature. The extract of the leaves were obtained using Whatman filter paper and kept in refrigerator (4°C) for further use. The same method and conditions were maintained to obtain the leaf extracts of all the three plants.

Synthesis of silver nanoparticles

Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 3 ml of Plant extract was added to 60 ml of 10^{-3} M $AgNO_3$ solution and the reaction was left to take place at ambient conditions. The reaction has been experimented for 3 h in the shade of sun light at room temperature.

Disc diffusion method

The Agar diffusion test or the Kirby-Bauer disk-diffusion method is used as a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. It is usually used for antimicrobial susceptibility testing [17] and this method is recommended by National Committee for Clinical Laboratory Standards (NCCLS). The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures. Inoculum preparation is the first step for Disc diffusion methods. It is done by growth method [18-20].

The plates were prepared by pouring 20 ml of molten media into sterile petri plates. The plates were allowed to solidify for 10 min and suspension was swabbed uniformly and the inoculums were allowed to dry for 10 min. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. On the bacterial strain, $AgNO_3$ was used as a control and on the other hand, the synthesized nanoparticles were maintained on the bacterial strain. The control zones were subtracted from the test zones. The resulting zone diameters and hence the results have been tabulated.

Inoculation of test plates

Optimally, within 15 min after adjusting the turbidity of the inoculums suspension, a sterile cotton swab is dipped into the adjusted suspension [21]. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculums from the swab. The dried surface of a Mueller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As a final step, the rim of the agar is swabbed. The lid may be left ajar for 3 to 5 min, but not more than 15 min, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks [22].

Reading plates

After 16 to 18 h of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculums were correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth [23,24]. If individual colonies are apparent, the inoculums were too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc [25]. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The Petri plate is held a few inches above a black, non-reflecting background and illuminated with reflected light.

The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored [26,27].

Characterization technique

The morphology of samples was examined by Field Emission Scanning Electron Microscopy (FESEM, Phillips and Holland). The size of the particles were estimated from the FESEM images. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

RESULTS AND DISCUSSION

FESEM analysis of silver nanoparticles

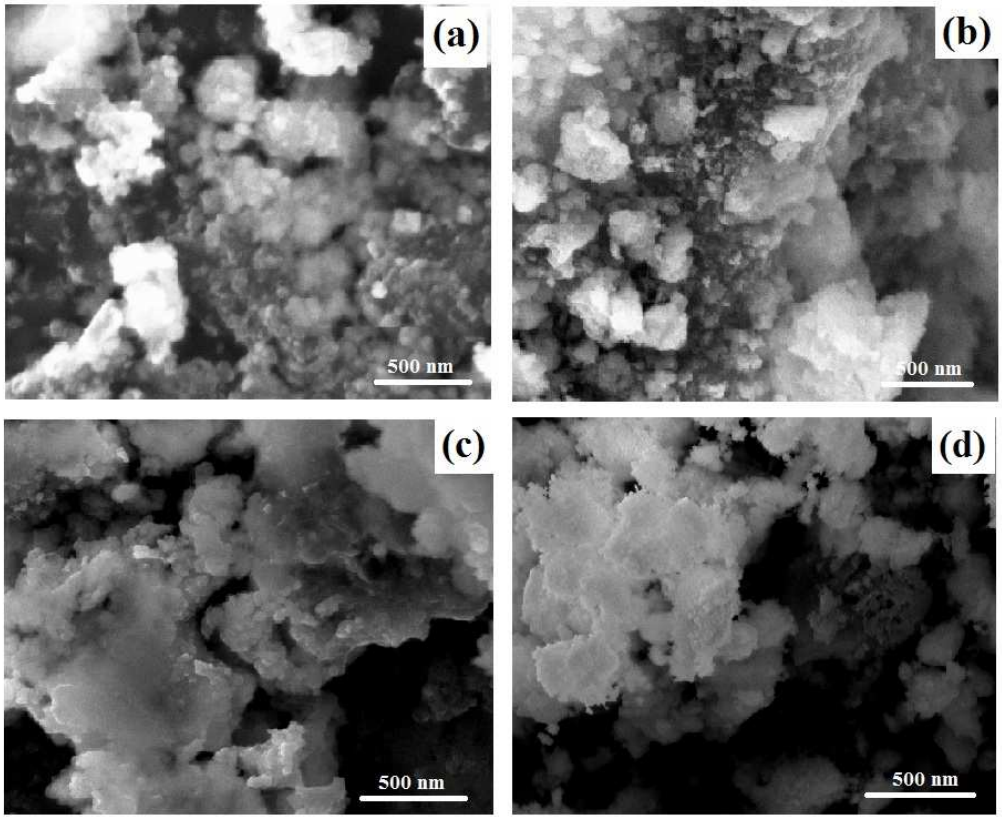

Fig. 1: FESEM image of *(a) Cycas circinalis, (b) Ficus amplissima, (c) Commelina benghalensis* and *(d) Lippia nodiflora*

The morphological characterization of synthesized silver nanoparticles from leaf extracts of *Cycas circinalis, Ficus amplissima, Commelina benghalensis* and *Lippia nodiflora* was done by FESEM (**fig. 1**). FESEM image shows size, shape and distribution of nanoparticles. The silver nanoparticles were in spherical shape and different particle sizes for *Cycas circinalis* with 64 nm, *Ficus amplissima* with 73 nm, *Commelina benghalensis* with 84 nm and *Lippia nodiflora* with 76 nm. The larger silver particles may be due to the aggregation of the smaller ones, due to the FESEM measurements.

Comparing all the other plant leaves, the extract of *Cycas circinalis* is effective in antibacterial activity due to its smaller nanoparticles.

Possible mechanism of formation of silver nanoparticles

Many components influence the synthesis of AgNPs such as plant source, organic compound in plant extract. Organic compounds like alkaloids, polyphenols, proteins and even some pigments are present in plant extracts. In plants caffeic acid is formed from 4-hydroxy cinnamic acid and is transformed to ferulic acid by the release of hydrogen [28]. Hydrogen is an important factor for the reduction of silver ions leading to the formation of AgNPs.

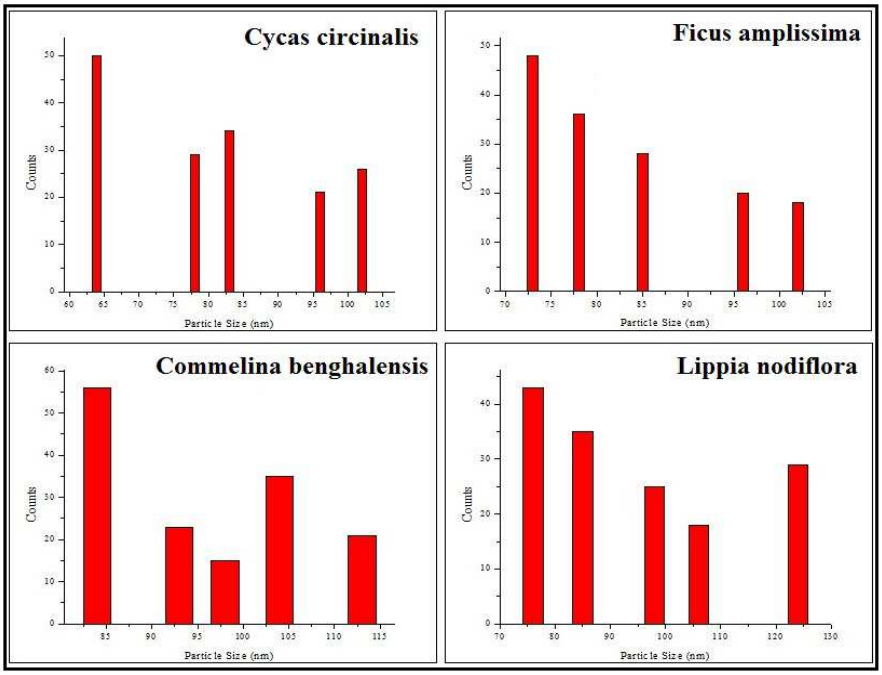

Fig. 2: Size distribution histograms of (*a) Cycas circinalis, (b) Ficus amplissima, (c) Commelina benghalensis* and *(d) Lippia nodiflora*

Antibacterial activity study of silver nanoparticles (AgNPs)

Plates 1, 2, 3 and 4 show the antimicrobial activity of synthesized Ag nanoparticles against five different bacteria such as *Escherichia coli, Klebsiella pneumoniae, Vibrio cholorae, Salmonella typhi* and *Shigella sonnei. F*As it showed a clear inhibition zone (Table 1), the synthesized Ag nanoparticles were highly effective in their activity against *Escherichia coli, Klebsiella pneumoniae, Vibrio cholorae, Salmonella typhi and Shigella sonnei* than antibiotics.

Standard antibiotic disc $AgNo_3$ was used as control. Bacterial membrane proteins and DNA makes preferential sites for silver nanoparticles interaction as they possess sulphur and phosphorus compounds and silver have higher affinity to react with these compounds [29-31]. Antibacterial effect of silver nanoparticles obeyed a dual action mechanism of antimicrobial activity, (i.e.) the bactericidal effect of Ag+ and membrane disrupting effect of the polymer sub-units [32,33].

Table 1: Antimicrobial activities (zone of inhibition in cm) of synthesized silver nanoparticles against five different common pathogens

Treatments	Zone of inhibition (cm)				
	Escherichia coli	*Klebsiella pneumoniae*	*Vibrio cholorae*	*Salmonella typhi*	*Shigella sonnei*
A	0.0	0.0	0.0	0.0	0.0
B	**1.6**	**1.8**	0.0	**1.9**	**1.8**
C	0.0	**1.2**	0.0	**1.5**	**1.2**
D	**1.3**	0.0	**1.7**	**1.8**	**1.7**
E	0.0	0.0	0.0	**1.3**	**1.6**

Where,
A) Antibiotics as control ($AgNO_3$),
B) AgNPs from Plant extract of *Cycas circinalis,*
C) AgNPs from Plant extract of *Ficus amplissima,*
D) AgNPs from Plant extract of *Commelina benghalensis and*
E) AgNPs from Plant extract of *Lippia nodiflora.*

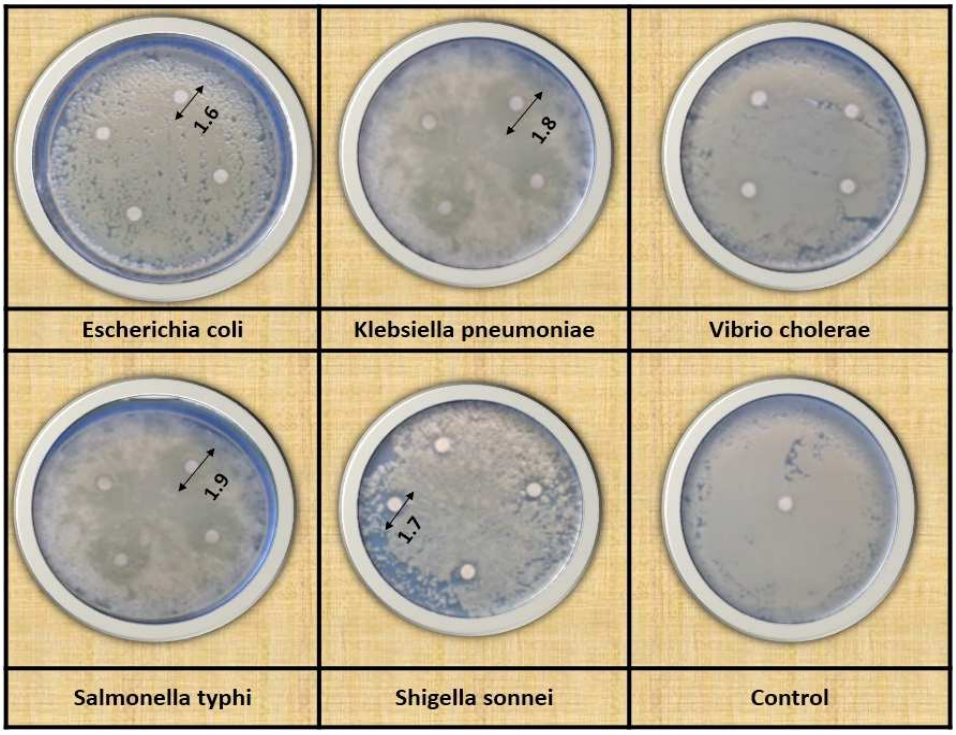

Plate 1: Antibacterial activity of *Cycas circinalis* (AgNPs)

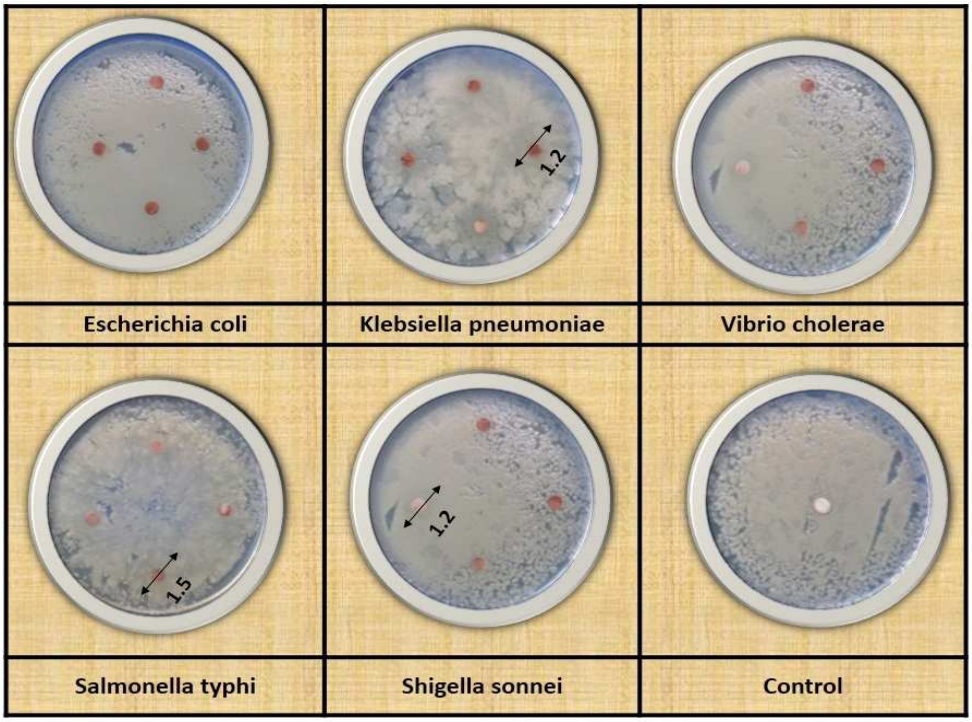

Plate 2: Antibacterial activity of *Ficus amplissima* (AgNP's)

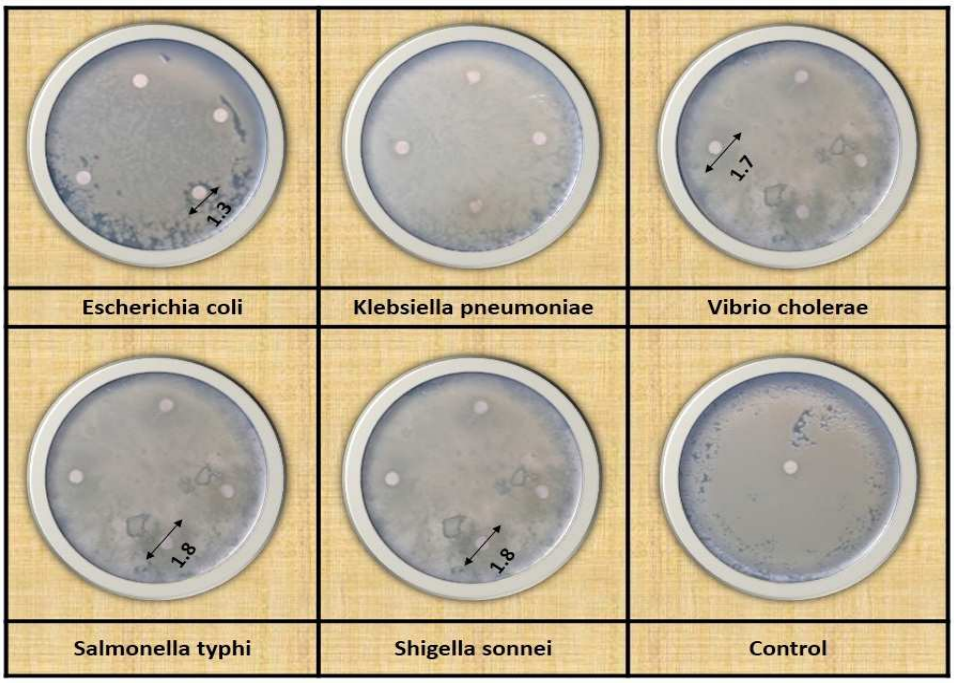

Plate 3: Antibacterial activity of *Commelina benghalensis* (AgNP's)

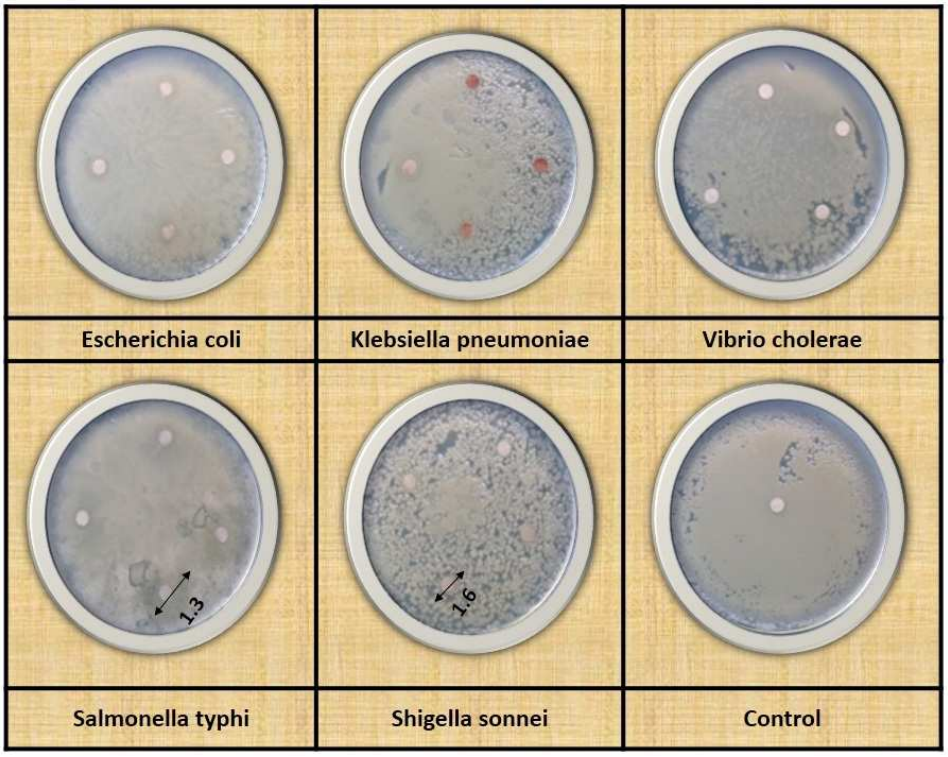

Plate 4: Antibacterial activity of *Lippia nodiflora* (AgNP's)

CONCLUSION

In conclusion, this study showed that AgNPs have potent antibacterial activities against *Escherichia coli, Klebsiella pneumoniae, Vibrio cholorae, Salmonella typhi and Shigella sonnei*. This study demonstrated the possibility of use of biologically synthesized silver nanoparticles and their incorporation in materials, providing them sterile properties. The cotton fabrics incorporated with these bio synthesized silver nanoparticles exhibited antibacterial activity against the common pathogens. Their superior antibacterial activity and environmental friendly preparation give them potential applicability in bioengineering and other fields. Prepared nanoparticles can be used as

bactericidal, electronic applications, wound healing, and water purification and in the field of medicine, which makes this method potentially exciting for the large-scale synthesis of nanoparticles. This process of synthesizing nanoparticles is eco-friendly as it is free from any solvent or toxic chemicals, as well easily amenable on and far-reaching production.

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