Nature-friendly synthesis and characterization of silver nanoparticles using leaves extract of Hyptis suaveolens and to evaluate its antibacterial and antioxidant potential

Aabha Bhave*, Sumita Dasgupta, Murtaza Hajoori, Sagar Desai
Biotechnology department of Bhagwan Mahavir college of science and technology, Surat, Gujarat, India

ABSTRACT: ***Silver nanoparticles (Ag NPs) are gaining importance for their application in various fields, especially for its antimicrobial activity. In the present investigation, biosynthesis of AgNPs from Hyptis suaveolens leaves extract was given. Ag NPs were generated by reaction of silver nitrate ($AgNO_3$) solution with plant extracts. Phyto-chemicals present in plants act as reducing and capping agent. Synthesized nanoparticles were characterized by visual, UV-vis spectroscopy, Fourier Transform Infrared Spectroscopy, Dynamic light scattering (DLS) and Transmission Electron Microscope (TEM). Particle size obtained via TEM analysis was more accurate than obtained via DLS. Silver nanoparticles were showing antibacterial activity against 11 test organisms and also showing impressive antioxidant potential. This green route for synthesis of nanoparticles was found to be eco-friendly and cost effective as it overcomes the drawbacks of chemical and physical methods. This method can be use for large scale production of nanoparticles.***

Key words: silver nanoparticles, Hyptis suaveolens, UV- vis Spectroscopy, DLS, TEM, FTIR, antimicrobial, antioxidant, Green synthesis.

INTRODUCTION

Nanotechnology is growing great sense of excitement in life sciences especially biomedical devices and biotechnology. Nanoparticles are building blocks of nanotechnology as they play major role in their application. Working with these extremely small structures is very much interesting due to its unique properties. Nanoparticles can be defined as sub nano sized colloidal structures with particles size between 1 and 100 nanometres (nm) [1]

Noble metal nanoparticles have been gaining a lot of significance in the past few years due to their applicability in the field of physics, chemistry, medicine, biology and material science. [2] The silver nanoparticles have a large area of interest as they have a large number of applications: nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions, antibacterial materials, chemically stable materials and good electrical conductors. [3-4] Chemical, physical, and biological methods have been developed to synthesis nanoparticles but chemical and physical methods are involved in the production of toxic byproducts which are hazardous moreover the methods are very expensive. [5-6] Currently biomimetic methods are emerging for generation of nanoparticles.[7] Biomimetic is a technique in which nanoparticles are synthesized using biological materials as they possess advantages over chemical and physical methods. The morphology of silver nanoparticles is determined by reducing precursor. These reducing and/or stabilizing precursors can be induced by bacteria, fungi, yeasts, algae, or plants as a whole or their products. The use of plant and plant extract in nanoparticle synthesis is considered advantageous over microbial based system because it reduces the elaborate process of maintaining cell cultures. [8]

Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as contain natural capping agents [9]. Among various plants, we have chosen *Hyptis suaveolens* leaves extract for the present study since it is known for its medicinal properties. Phytochemical screening indicated the presence of chemicals such as alkaloids, flavonoids, phenols, saponins, terpenes, and sterols [10].

In this present study, we attempted to ratify the reduction of water soluble silver ion using *Hyptis suaveolens* leaf extract to silver nanoparticles by green synthesis method, its characterization and its application.

MATERIALS AND METHODS

Materials

$AgNO_3$ was purchased from sigma Chemicals, India and used as received without further purification. Deionized water was used throughout the experiments for preparing the aqueous solutions and washing. Healthy leaves of *Hyptis suaveolens* collected from Bhestan area of Surat, Gujarat. Plant was botanically identified and authenticated by experts.

Preparation of Leaf Extract

A 20gm of fresh leave were washed thoroughly three times with DDW for 15min in order to remove any dust particles then air dried and cut into fine pieces and added to 100ml of boiled deionized water at 100˚C for 5min for the release of intra-cellular material into solution. After boiling, the mixture was cooled and filtered to get the extract. The filtrate is used as reducing and stabilizing agent for 1mM of $AgNO_3$.

Synthesis of Nanoparticles Using Leaf Extract

45mL of 1mM $AgNO_3$ was added to 5mL of Leaf extracts separately to make up a final solution of 50mL. A change in the color from whitish yellow to brown indicates the formation of Ag NPs within 1-24 h.

Characterization of synthesized silver nanoparticles
Characterization of *Hyptis suaveolens* silver nanoparticles was carried out using the following parameters.

• **Colour change**: The primary confirmation of the synthesized silver nanoparticles is done by visual basis. The colour change of *Hyptis suaveolens* extract and silver nitrate solution with respect to time was observed [11].

• **UV visible spectroscopy**: UV visible spectral analysis characterizes the formation and completion of silver nanoparticles. The reduction of silver ions were monitored by measuring UV-Vis spectrum of reaction medium from the wavelength of 200-800 nm by using distilled water as blank.

• **Fourier Transform Infrared Spectroscopy:** FTIR spectra of the aqueous leaf extract and AgNPs samples were analyzed by FTIR spectroscopy (Shimadzu, Japan). The FTIR analysis was performed with KBr pellets and recorded in the range of 400–4,000 cm^{-1}. The various modes of vibrations were identified and assigned to determine the different functional groups present in the samples.

• **Dynamic light scattering (DLS):** Particle size measurements of AgNPs mixture were conducted with a zetasizer (Nano-ZS90, Malvern, UK) in disposable cuvettes and average hydrodynamic diameter was determined by taking an arithmetic average of 10 runs.

• **Transmission Electron Microscope (TEM):** For TEM measurements, a drop of synthesized AgNPs was placed on the carbon coated copper grids and kept overnight under vacuum desiccation before loading them on to a specimen holder. TEM micrographs of the sample were taken using the JEOL JSM 100cx TEM instrument operated at an accelerating voltage of 200kv.

Antibacterial Study of Nanoparticles
Antibacterial activities of silver nanoparticles were evaluated by the agar well diffusion method. Agar well-diffusion method was followed to determine the antibacterial activity. Nutrient agar (NA) was spread with overnight old - broth culture of respective bacteria. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of silver nanoparticles was prepared at a concentration of 1 mg/ml. About 100 µl of concentrations of silver nanoparticles were added with sterile pipette into the wells and allowed to diffuse at room temperature. Negative Control experiments comprising inoculums with sterile double distilled water were set up, while as Positive control Silver nitrate solution was taken. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured [12-13].

DPPH Free Radical Scavenging Assay

The DPPH antioxidant assay was determined [14]. Briefly, 1mM DPPH in 99.5% Ethanol. To 0.5ml of DPPH redical solution, Add 2 ml of the prepared solution of silver nanoparticles and the reaction mixture is vortexed for 10s and allow to stand at room temperature for 30 min. The absorbance is recorded at 517 nm by using UV- Spectrophotometer. Compare with the 75% ethanol which act as control solution. Ascorbic acid is used as reference antioxidant compound.

The percentage of DPPH radical scavenging activity is expressed as:

DPPH scavenging effect (%) = [1- (Test sample absorbance/blank sample absorbance)] ×100(%).

RESULTS AND DISCUSSION

Visual examination: On the addition of *Hyptis suaveolens* leaves extract to silver nitrate solution, entire mixture color changes from whitish yellow to brown color indicates the formation of silver nanoparticles. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range [15]. (Figure 1)

Figure: 1 A: silver nitrate solution with plant extract.
B: synthesized silver nanoparticles.

UV visible spectral analysis: UV Visible spectral analysis characterizes the formation and completion of silver nanoparticles. Silver nanoparticles exhibit Plasmon absorption band in the visible region. The metal nanoparticles have free electrons which gives the surface Plasmon resonance absorption band due to the combined vibrations of electrons of metal nanoparticles in resonance with light wave. Silver nanoparticles are known to exhibit UV-Visible absorption in the range of 400-500 nm [15].

The absorption bands of silver nanoparticles were observed around 436 nm. (Figure 2)

Figure: 2 UV- Vis spectroscopy analysis of silver nanoparticles synthesized by leaf extract (peak detection at 436nm)

FTIR spectroscopy: FTIR measurement were performed to identify the biomolecules responsible for capping and reducing the silver nanoparticles present in the leaf extract of *Hyptis suaveolens.* The bonds at 1243, 1137, 1043, 1021 cm^{-1} are due to ether linkage and suggest the presence of flavanones adsorbed on the surface of metal nanoparticles [16].Peak at 2927 cm^{-1}represents C-H, which might represent the Flavonoid compound [17]. The Phenolic groups participating in ion replacement response are placed in the 1315–1037 and 1456–1600 cm-1 regions for the plant extract [18]. The FTIR spectrum of the leaf extract, which shows peak at 3522, 3474, 3405, 3382, 3341, 3289 cm^{-1} corresponds to the O-H stretching of hydroxyl group [19]. By comparing FTIR spectrum of plant extract to its derived Ag NPs, absence of bands in the range of 3000-3500 nm in the synthesized nanoparticles indicates the decrease in the concentration of the sample after complete reaction. The decrease in O-H concentration can be attributed to its reaction with Ag+ ions added to the solution for nanoparticle synthesis. When Ag+ ions come in contact with –O–H groups, the white colloidal particles of AgOH are formed which turns to brown colored Ag nanoparticles [20]. (Figure 3-4)

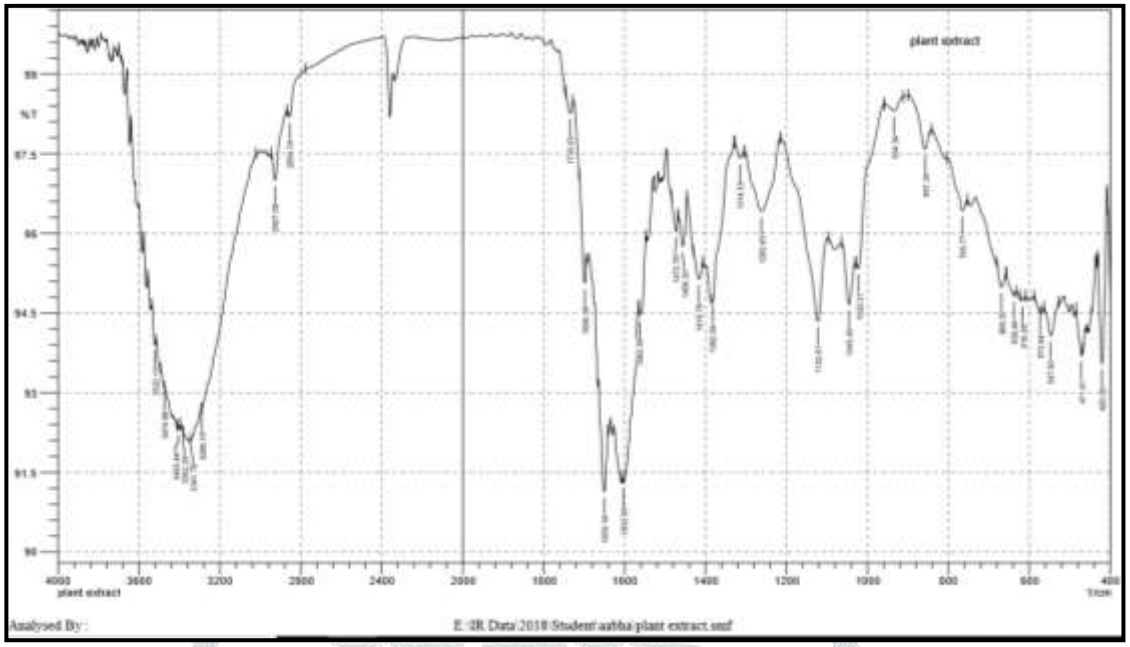

Figure: 3 FTIR analysis of plant extract.

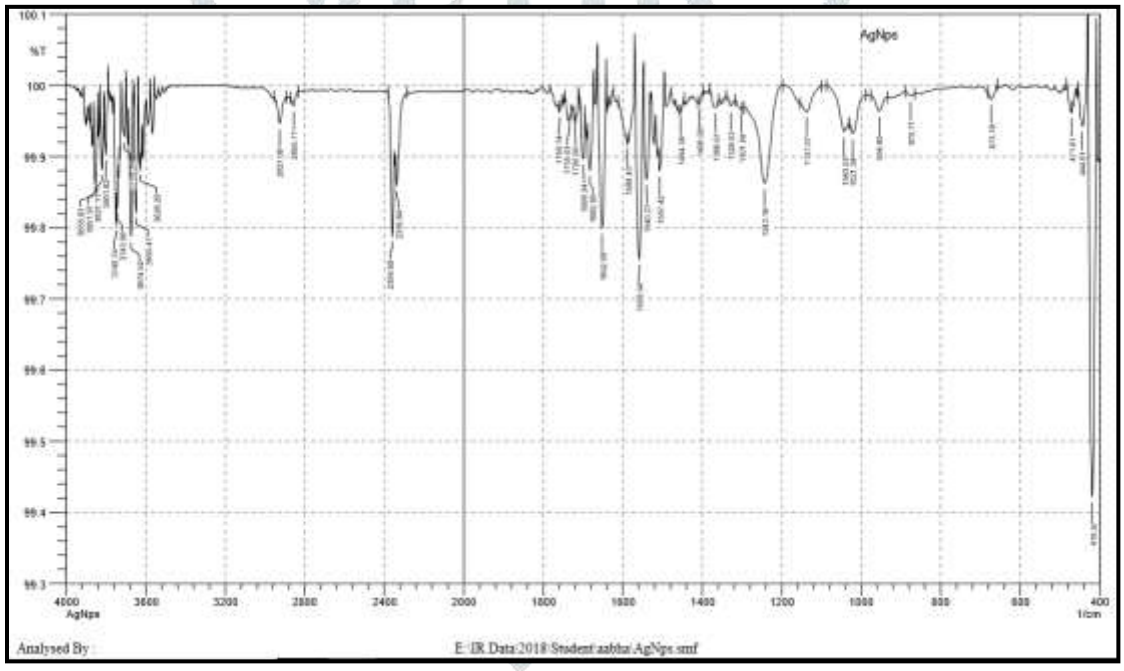

Figure: 4 FTIR analysis of silver nanoparticles synthesized using leaf extract.

Particle size measurement: The particle size is one of the most important parameter for characterization of nanoparticles. The average particle size of silver nanoparticles was found to be 116.9 nm. Particle size
analysis showed the presence of nanoparticles with polydispersity indices PDI value 0.173. According to manual of Malvern zetasizer, Intercept refers to the intersection of the correlation curve on the y-axis of the correlogram. It is usually scaled such that an ideal signal will give a value of 1, and a good system will give intercepts in excess of 0.6, and greater than 0.9 for the best systems. In this study, intercept found to be 0.933. (Figure 5)

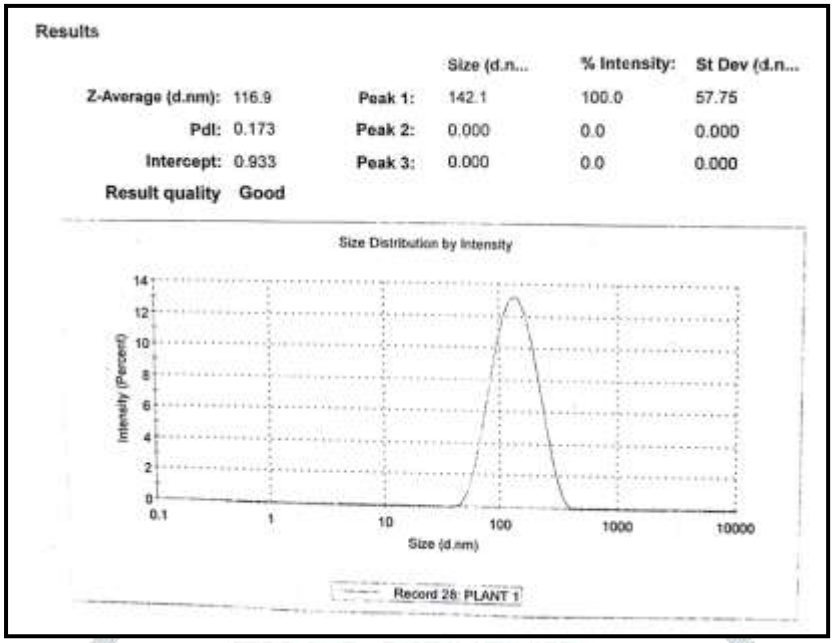

Figure: 5 Particle size analysis of silver nanoparticles synthesized by leaf extract.

Transmission Electron Microscopy: TEM provide the morphology and particle size distribution profile of the synthesized silver nanoparticles. The particle were spherical in shape in the range of 11~31 nm. (Figure 6)

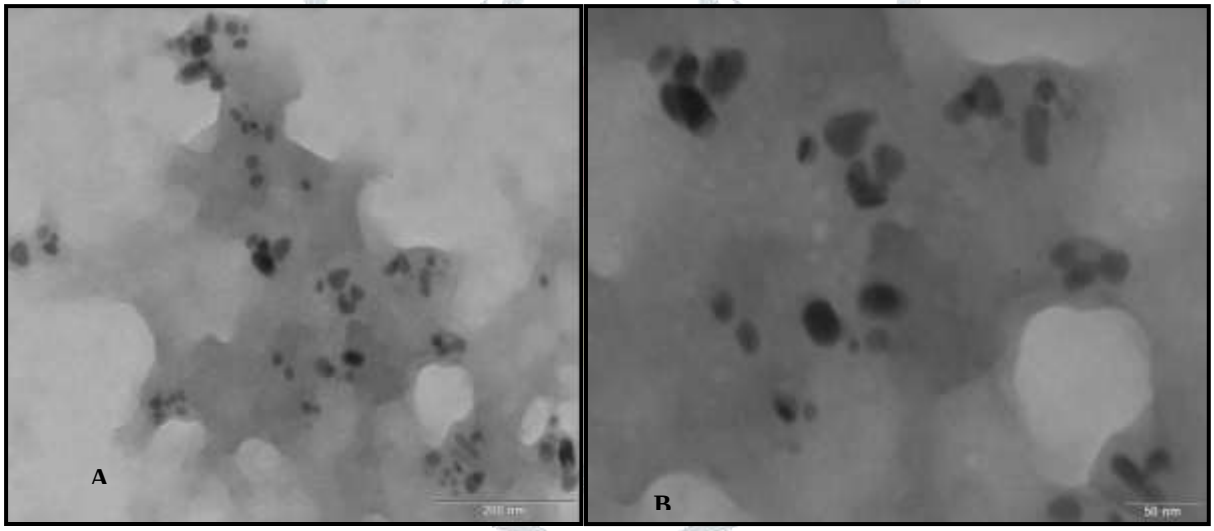

Figure: 6 A: TEM image of AgNPs at 200 nm range
B: TEM image of AgNPs at 50 nm range

Particle size of synthesized nanoparticles was determined by DLS and TEM, The size measured by TEM analysis was lower than that measured by DLS analysis and this might be due to DLS measurement is known to be more sensitive to larger particles [21]. Thus, DLS measurement may not be accurate for polydisperse samples due to its nature to respond toward larger particles [22].

Antibacterial Study of Nanoparticles

In the present investigation, the antibacterial effect of prepared silver nanoparticles is studied on different types of bacteria such as, some Gram negative bacteria *like Salmonella typhi, Salmonella Paratyphi A, Salmonella Paratyphi B, Enterobacter aerogenes, Psudomonas aeruginosa, Proteus vulgaris* and some Gram positive bacteria like *Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Staphylococcus epidermidis*. Table 1 shows Zone of inhibition in mm for each tested organism. In this study, as a positive control $AgNO_3$ salt was taken, while as negative control ultra purified water was taken and as test sample silver nanoparticles was taken.

Table: 1 Zone of inhibition in mm for each tested organism.

Sr. No.	ZOI (mm)			
	Test organisms	**Synthesized silver nanoparticles**		
		Positive control	**Negative control**	**Test**
1	*B.subtilis*	20	0	21
2	*B.megaterium*	18	0	19
3	*B.cereus*	10	0	12
4	*S.aureus*	10	0	12
5	*S. epidermidis*	15	0	16
6	*S. typhi*	12	0	13
7	*S. Paratyphi A*	19	0	20
8	*S. Paratyphi B*	10	0	16
9	*E. aerogenes*	10	0	11
10	*P. aeruginosa*	14	0	15
11	*P. vulgaris*	13	0	15

Investigations reveal that the positive charge on Ag ions is crucial for its antimicrobial activity. The antibacterial activity is probably derived through electrostatic attraction between negative charged cell membrane of microorganisms and positive charged nanoparticles. Antibacterial activity of Ag NPs on Gram Negative Bacteria depends on concentration of Ag NPs and closely associates with formation of pits in cell wall of bacteria. Accumulation of Ag NPs in pits results in permeability of cell membrane, causing cell death. Ag NPs generate free-radicals develop from surface of Ag NP, are responsible for damaging the membrane [23].

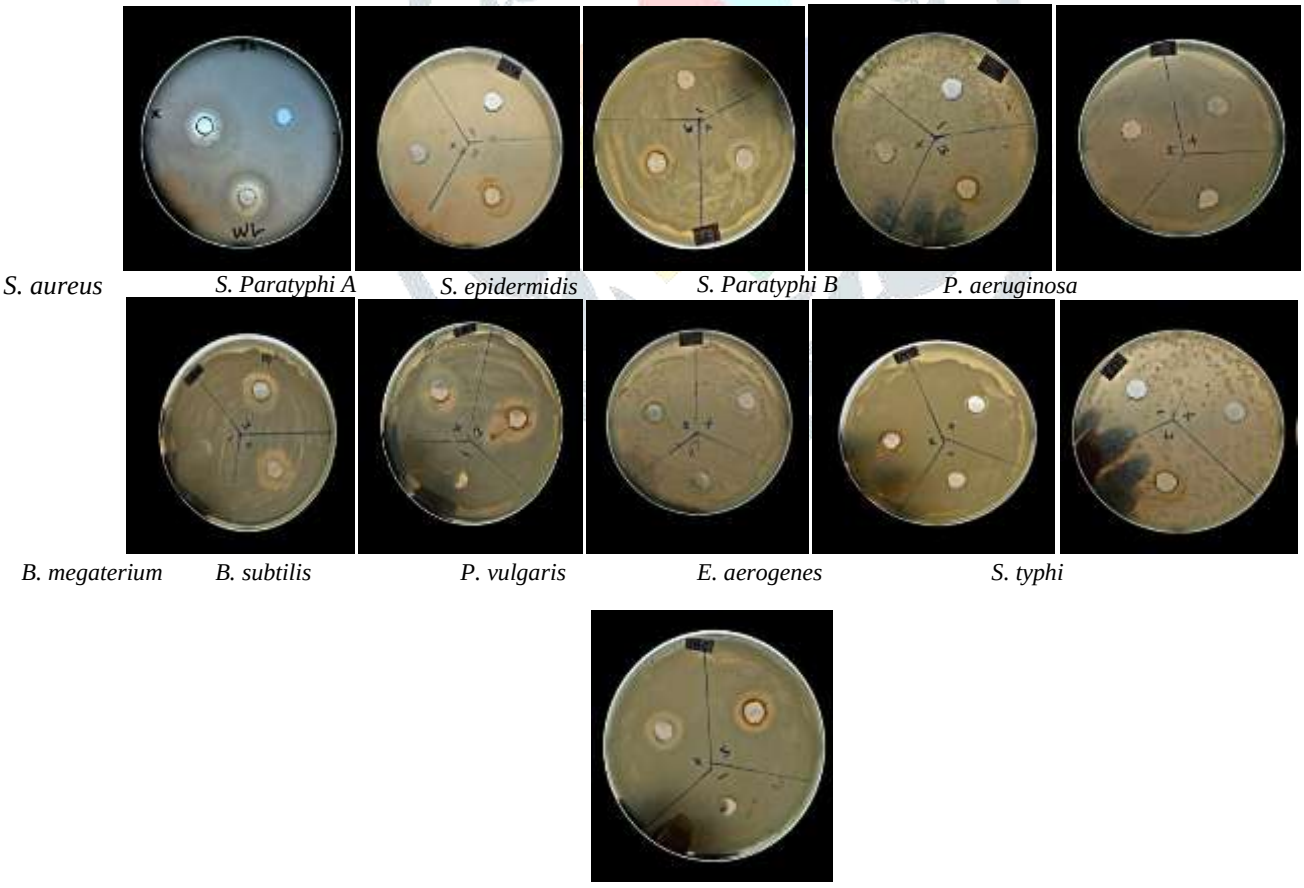

Figure: 7 Antibacterial analysis of silver nanoparticles

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of silver nanoparticles was studied by its ability to reduce the DPPH. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [24]. A freshly prepared DPPH solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant this colour disappears due to quenching of DPPH free radicals and convert them into a colorless product i.e. 2,2-diphenyl-1-hydrazine. Antioxidant mechanism performed by providing hydrogen atoms or electron [25].

In the present study AgNPs showed free radical scavenging properties in different levels. Free radical scavenging activity of AgNPs was around 86%. Silver salt does showed 20 % free radical scavenging.

CONCLUSION

Nanoparticles synthesized using leaves extract of *Hyptis suaveolens* are found to be active against 11 test organisms and also found to possess antioxidant potential. In this study, no chemical reagents were used as reducing and/or capping agent, natural compounds present in plant extract were acted as reducing and/or capping agent for nanoparticle synthesis. Hence, this method found to be nature-friendly, frugal and can be promising for future.

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