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Biogenic Synthesis, Antibacterial and Antioxidant Studies of Prepared Silver Nano Particles Using Root Extract of *Saussurea lappa*

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Abstract: Green synthesis of metal nanoparticles using plants and their extracts is a reminiscent approach in present scenario for the environmental benevolence.Present paper reports the green synthesis of silver nano particles using the aqueous root extract of *S. lappa*. Characterization was done for the synthesized silver nano particles by SEM, UV-vis spectrophotometer, EDX. Scanning electron microscopy studies revealed that cube shaped nanoparticles of size around 2µm-500nm were formed. UV-vis spectrum showed peak at 450-490 nm. Antioxidant activity of synthesized silver nanoparticles was done by DPPH assay and it showed the higher percentage of inhibition. Antimicrobial activity of the synthesized silver nano particles using root extract of *S. lappa*was studied against *Escherichia .Coli* and *Bacillus Cereus*which showed the higher degree of inhibition. Thus results confirmed that the root extract of *S. lappa*has good potential to be used for synthesizing silver nano particles for antioxidant and antibacterial activity.

Key words:Antibacterial Activity, Antioxidant, DPPH Method, *Saussurea lappa* (*S. lappa*), Silver Nanoparticles

1. INTRODUCTION:

With the advancement of technology and science there are constant changes in the system of drug delivery, drug formulations, drug administration etc.Nanotechnology has been used widely in the field of biotechnology, medical science, agriculture due to the unique physicochemical properties of the nanoparticles. Among all metals, nanoparticles of silver, gold, palladium have been extensively used in different fields such as industry, electronics, and bio-medicals etc. [1]-[3]. Silver nanoparticles (AgNPs) exhibits promising bio activities like anti-oxidant, antifungal, anti-inflammatory, anticancer and antibacterial due to its good catalytic and conductivity activities. It has been reported in research papers that Silver nano particles are quite helpful to prevent replication of HIV and AIDS virus [4].Conventional methods for the synthesis of silver nano particles like photochemical, electrochemical, chemical reductions heat evaporation etc. have many disadvantages. These processes are usually slow in nature, involved high cost, usage of chemical reducing agents,volatile solvents, intense physico-chemical conditions pose threat to the environment. Along with this formed silver nano particles are quite unstable thus to attain stability they require the addition of separate capping agent. Thus there is a growing need to develop non-toxic, ecofriendly, clean procedure for the synthesis of nanoparticles. This resulted exploration of Mother Nature by the researchers enthusiastically.

In the series many biological methods had been developed for the synthesis of nano particles under green nanotechnology using bacteria, nucleic acids, fungi, proteinsetc. Green synthesis of nano particles possess some inherent features such as fast process, safe methodology, ecofriendly nature, use of natural resources, benignancy. Another advantageous features of green synthesis are controlled and well define size of the nanoparticles without any pollutants. Synthesis of nano particles using plant is expedient since they possess bioactive compounds.

Nature has blessed the human being by medicinal plants for the treatment of various human ailments. Being rich in bio components, from past two thousand years plants had been used as a major source of drugformulation[5]. As per the recent reports of WHO, more than 80% of people in the developing countries prefer to take herbal medicines for general ailments [6]. Among various such herbs, *Saussurea lappa* (*S. lappa*)has been mentioned in Veda's and being used since long time.

Plant morphology:

*S. lappa*is well distributed all over the world. In India it found abundantly in Himalayan region[8]-[10]. It is a perennial plant with robust, erect stem of height 1-2m [6] which is stout and fibrous in nature [5]. Plant has long winged radical leaves. Flowers are of purple or black in color [6]. Root has a characteristic odor [5].In Ayurveda, the extract of this plant which is pungent and bitter taste is widely used as cough and cold preventive, for the treatment of arthritis, stomachache, altitude sickness etc. It is also found to be potentially effective as anti-cancer, antidiabetic, anti-inflammatory, antibacterial, antioxidant, antipyretic etc. and to treat the disorders of blood, heart, lungs, skin etc. [7], [11]–[13]. Usage of *S. lappa*in traditional medicine can be credited due to the presence many bioactive components such as tannins, saponins, flavonoids, lipids etc.These compounds are found to be very effective for treating various ailments. For example Saussurine relieves smooth muscle spasms of the bronchi and gastro intestinal tract, four flavonoids[9] have antibacterial function [14] whereas chlorogenic acid [15] prevents oxidization and removes free radicals. Presence of these compounds imparts antioxidant, radical scavenging property to the plant. Hence the main objective of the proposed work is to synthesize the silver nano particles using root extract of *S. lappa*. After characterization prepared silver nano particles of root extract of *S. lappa*will be study for its antioxidant activity by DPPH assay.

Paper reports a low cost, easy and green synthesis of silver nano particles byreduction of silver ions using root extract of *S. lappa*. Novel feature of present work is surface capping of silver nano particles by bio active constituents of medicinal plant i.e. *S. lappa*. Furthermore research focused on synthesis of nanoscale particles mediated by plant metabolite can be explored.

2. EXPERIMENTAL

2.1 Materials

Reagents like Silver nitrate (99.9%), DPPH-1, 1–diphenyl-2-picrylhydrazyl (97%), Ethanol, Ascorbic acid, used in this study were procured from Sigma-Aldrich. All chemicals were of AR grade and used as they received. Roots of*Saussurea lappa* root was purchased from herbal shop of Bangalore and identified.Double distilled deionized water was used to prepare all solutions and for the complete removal of oxygen, they were purged with nitrogen gas.

2.2 Preparation of root extract of *S. lappa*

Roots of *S. lappa*were dried and ground to powder. About 10g of powdered *S. lappa*was extracted successively with double distilled water in Soxhelt apparatus for 48 hours. The root extract was filtered and dried by rota evaporator and stored for the preparation of AgNPs.

2.3. Synthesis of silver nanoparticles using root extract of *S. lappa*

Silver nanoparticles were synthesized by the standard procedure. In four different Erlenmeyer flask different concentrations of $AgNO_3$(10ml, 5ml, 3ml, 2.5 ml) was taken and to each flask 1 ml root extract of *S. lappa*was added dropwise with continuous stirring. Prepared solutions were kept in dark chamber for one hour till pale yellow color of the solution changes to dark yellow color specifying that silver nanoparticle were formed (*Figure* 1). After centrifugation at 10,000 rpm collected silver nanoparticle (AgNPs) particles washed with double distilled water and dried in hot air oven.Same procedure was repeated for the preparation of silver nanoparticle by varying the time interval (1hr, 2 hr, 3hr, 4 hr and 5hr) for the reduction of silver nitrate by root extract of *S.lappa*[16].

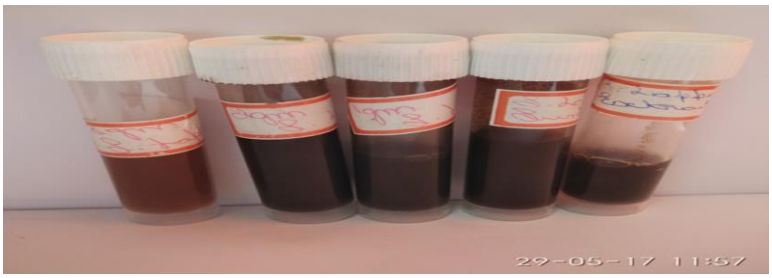

Fig 1: Synthesized Silver nanoparticles of root extract of *S.lappa* in different concentration of silver nitrate.

2.4. Characterization of synthesized AgNPs from *S. lappa*root extract:Prepared AgNPs were characterized as follows:

2.4.1. UV–Vis spectral: Biosynthesized silver nanoparticles were analyzed by using UV-Visible spectroscopy ((Spectrophotometer UV–vis Elico SL 150). UV–Vis spectral analysis was observed at a resolution of 300–700 nm.

2.4.2. Scanning Electron Microscope (SEM): Morphologyof the synthesized silver nano particles were analyzed by using Scanning Electron Microscope (SEM;JEOL JSM-6480 LV). For this thin layer of silver nanoparticle was coated on silicon plate to make them conductive. Then the samples were analyzed in the SEM at an accelerating voltage of 10 KeV.

2.4.3. Energy-dispersive X-ray spectroscopy (EDX): To confirm the presence of elemental silver in the synthesized silver nanoparticles using root extract of *S. lappa*Energy-dispersive X-ray spectroscopy (EDX; JEOL-JSM-6480 LV,) was used.

2.5. Antioxidant activity of synthesized silver nanoparticles from *S. lappa*root extract:

DPPH method was used to determine the free radical scavenging activity of the synthesized silver nanoparticles.For this 0.1 mm of DPHH solution was prepared in 95% methanol. 1ml of 0.1 mM of DPHH solution added to different concentrations of synthesized silver nanoparticles solution. At room temperature prepared solution was allowed to incubate in dark for 30 min and change in color was observed from deep purple to pale yellow. By using a UV–Vis spectrophotometer absorbance was measured at450-490 nm. Without adding silver nanoparticles control was prepared and ascorbic acid was used as a standard. Experiment was carried out in triplicate [17]. DPPH scavenging activity of the synthesized silver nanoparticles was calculated by using following formula:

$$\%\ Inhibition = \frac{(A_{control} - A_{sample})}{A_{control}}\ X\ 100$$

Where A_{sample} is the absorbance of the synthesized silver nanoparticle solution and $A_{control}$ is the absorbance of the control solution.

2.6.In vitro antibacterial screening

In vitro antibacterial studies for the sythesised silver nanoparticles were carried out by agar well disc diffusion method against *Escherichia coli* and Bacillus bacteria. Penicillin, Erythromycin, Streptomycin and Chloramphenicol were used as positive control. Different concentration of prepared silver nanoparticles solution (12.5, 25, 50 and 100 lg/mL) was poured on the 6- mm sterile disc containing bacterial culture. All discs were incubated at 37^0C for 24 hrs.Antimicrobial activity of silver nanoparticles were analyzed by measuring the zone of inhibition in diameters (millimeter). The microbial test were carried out in microbiology lab of Maharanis College of Science following the standard method [18].

3. RESULTS AND DISCUSSION:

The current study reports the synthesis of Silver nanoparticles using root extracts of *S. lappa*. Formation of Silver nanoparticles was observed by UV-Visible spectrophotometer. Antioxidant activity of the synthesized silver nanoparticles were studied by DPPH method and antibacterial activity by disc diffusion method.

3.1 Synthesis of silver nano particles using root extract of *S. lappa*

After the addition of the 1 ml root extract of *S. lappa*with different concentrations of silver nitrate solution (1:10; 1:5; 1:3; 1:2.5) visual change in colour from pale yellow to the dark yellow colour specifies the reduction of silver ions into AgNPs (*Figure* 1).

3.2 Characterization of synthesized silver nano particles from root extract of *S. lappa*

3.2.1. UV-vis spectroscopy:

In the Uv-spectral data a band at 450-490 nm was observed for the prepared silver nanoparticles which was absent for the root extract and $AgNO_3$ which is shown in *Figure* 2. The formation of this Surface Plasmon Resonance (SPR) band observed at 450-490nm which corresponds to the absorbance of Silver nanoparticles is in well agreement with previous reports [19]. The Uv-visible spectrum of silver nanoparticles prepared from different concentration of $AgNO_3$indicates that the rate of formation of nanoparticles by natural nucleation increased by increasing the concentration of $AgNO_3$. This data indicates that the formation of Silver nanoparticles started at very first hour of addition and number of nano particles increased upon increasing the time of incubation. However, at the24th hour no much significant changes were indicated when compared to 5th hour data. This further inferenced that formed Silver nanoparticles were due to completely reduction Ag^+ ion to Ag^0at fifth hour. Root extract of *S.lappa* contains many phytoconstituents such as Sesquiterpenoid, Costunolide, and Dihydrocostunolidewhich are main constituents and might have helped in reduction of silver ions to silver. These formed nanoparticles were stabilized due to capping by suitable capping agents. Compounds such as amine, carbonyl, and hydroxyl functional groups are also present in root extract of *S. lappa*. They act as capping agents and there by helpful in stabilizing the Silver nanoparticles.

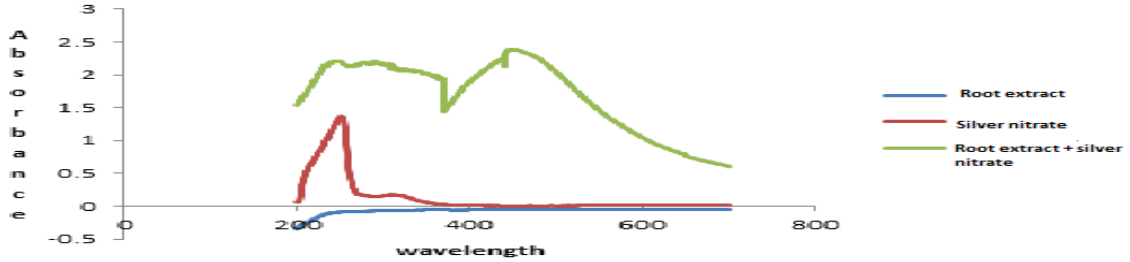

Fig 2: U.V –Visible spectra of root extract of S.lappa, Silver nitrate, synthesized silver nanoparticles.

3.2.1.1 Analysis of UV-Visible spectra of synthesized silver nanoparticles using various concentration of silver nitrate by root extract of S.lappa

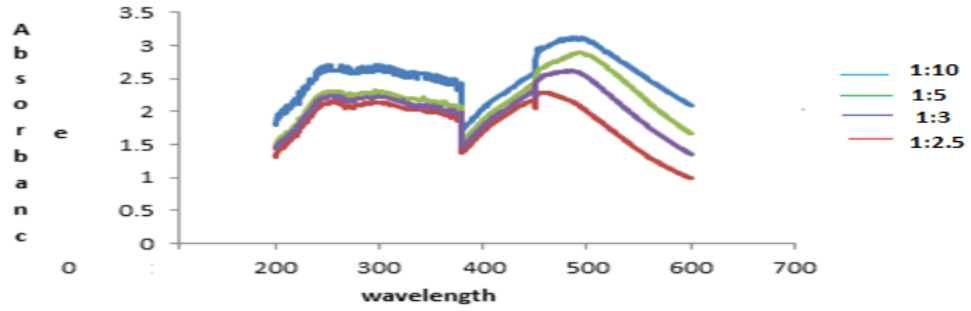

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Fig 3: UV -Visible Spectra of synthesized nanoparticles at different concentration of silver nitrate

3.2.1.2 Analysis of UV-Visible spectra of synthesized silver nanoparticles by varying the time interval of reduction of silver nitrate by root extract of S.lappa

The formation of Silver nanoparticles were monitored at time intervals of 1hr, 2 hr, 3hr, 4 hr, 5hr using Uv- visible spectrophotometer as indicated in Fig.4 at different concentrations.Form Fig 3, it is evidentthat the formation silver Nanoparticle started at very first hours of synthesis and increases with increase in time. At fifth hour the intensity of peak increases indicating that more number of nanoparticles was formed. At twenty fourth hour, no much changes in SPR bands was observed thus formation of Silver nanoparticles were due to completely reduction Ag^{+} ion to Ag^{0} at fifth hours. Root extract of *S.lappa* contains many phytoconstituents such as Sesquiterpenoid, alkaloids, etc., which might have helped in reduction of silver ions to silver. These formed nanoparticles were stabilized due to capping by suitable capping agents. Compounds such as amine, carbonyl, and hydroxyl functional groups are present in root extract of *S. lappa*. They act as capping agents and there by helpful in stabilizing the Silver nanoparticles. The same result holds true for other prepared Silver nanoparticles by using root extract of *S.lappa* as reducing and capping agent.

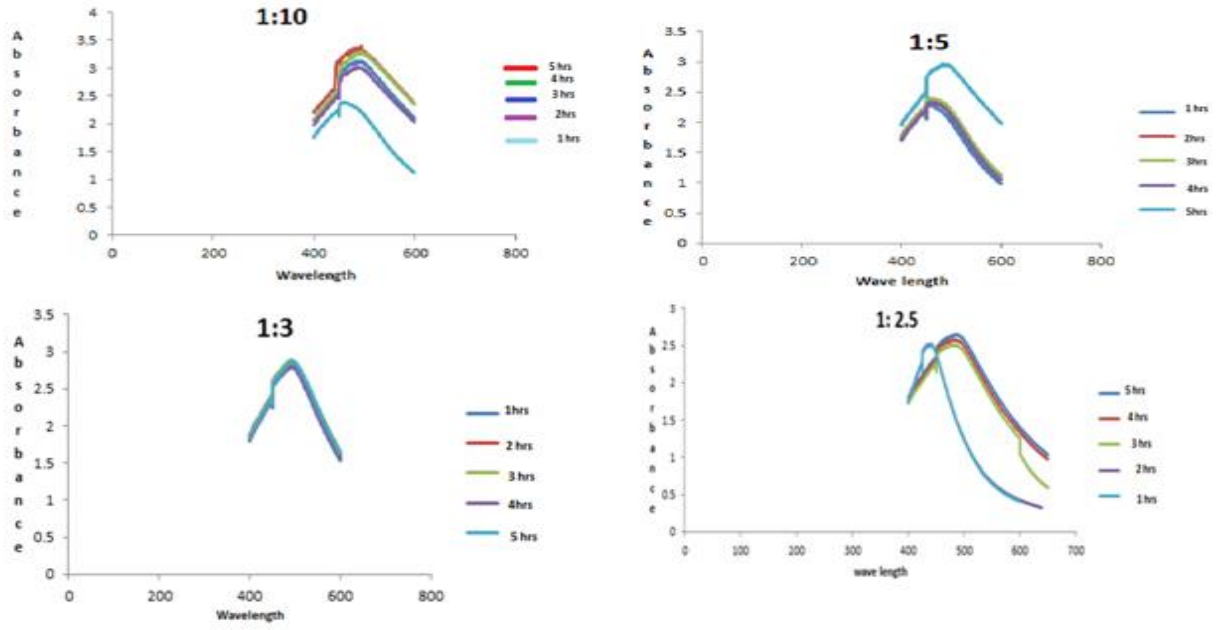

Fig 4: UV-Visible Spectrophotometer of Silver nanoparticles prepared by 1:10, 1:5, 1:3 and 1:25 concentration of root extract of *S.lappa* and $AgNO_3$ respectively.

3.2.2. Scanning electron microscope (SEM): To understand the morphological shape and size of synthesized silver nanoparticle Scanning Electron microscopy (SEM) studies were carried out. SEM image of synthesized silver nanoparticles at various magnifications such as 500 nm, 2 and 10 μm exhibited uniformly distributed cube shaped silver. Though agglomeration of silver

nanoparticles has been noticed at some places but no direct fusion had been observed. This might be due to the presence of different functional groups acting as capping agents in the root extract of *S. lappa*(Fig.5). Obtained results greatly agree with earlier reports in which green synthesis of silver nanoparticles using plant entities have shown the cube shaped particles.

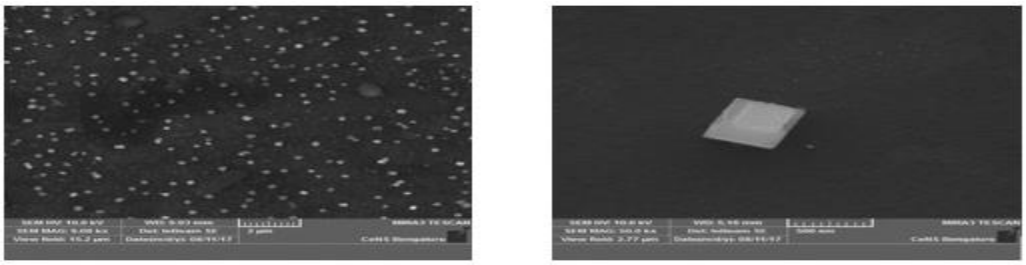

Fig 5: SEM micrograph size and shape of synthesized silver nanoparticles by using root extract of *S.lappa*

3.2.3. Energy Dispersive X-Ray (EDX):

Elemental analysis was carried out using energy diffraction X-ray. EDX analysis, displayed that the elements present were silver, chlorine, oxygen, nitrogen respectively (Fig.6 and Table 1). A sharp peak at 3.Kev was observed which is well agreed accordingly to the report[20].

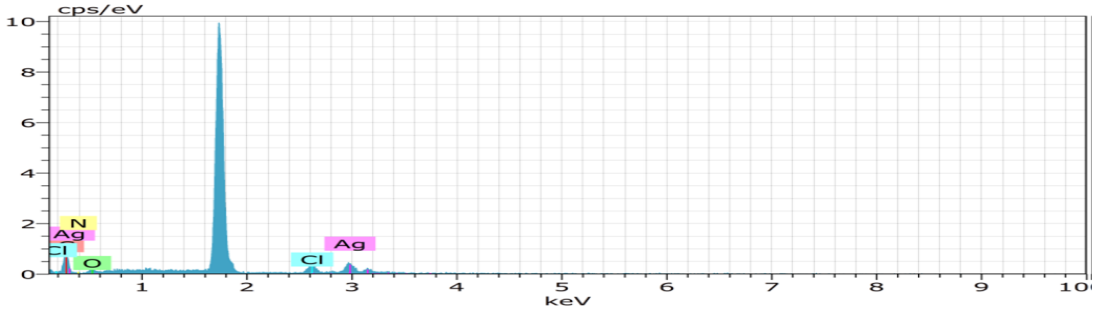

Fig 6: Energy Dispersive X-ray analysis spectrum shoes the silver metal of synthesized Silver nanoparticles

Table 1: Energy Dispersive X-ray analysis of composition from synthesized Silver nanoparticles from root extract of *S.lappa*

Elements	Weight %	Atomic %
Silver K	51.49	12.99
Chlorine K	11.68	8.97
Oxygen K	8.58	14.55
Nitrogen L	1.71	3.32

Carbon K	26.56	60.17
Total	100	100

3.3. DPPH assay

DPPH (1, 1-diphenyl-2-picrylhydrazyl) method is a well-recognized and more stable free radical assay and it is based on the reduction of accepting electron or hydrogen from the donors. Here in the present work the synthesized Silver nanoparticles from the root extract of *S. lappa*is the donor for DPPH. DPPH which showed a deep purple band at 517nm in UV –visible spectra, disappeared upon addition of silver nano particles and color of sample turned to pale yellow within 30 minutes of incubation in dark place. The various functional groups such as amines, hydroxyl groups help in donating the electron to the free radical generated in the reaction thereby stopping the chain reaction. Scavenging activity of synthesized silver nano particles is shown in Table2.Ascorbic acid was used as control and no change in color was seen for the control.

Table 2: Scavenging activity Calculation

Concentration µ/ml	Absorbance	Scavenging	IC50
25	0.241	3.984	
50	0.139	13.9	70.8
75	0.286	44.62	
100	0.009	96.4	
Blank	0.0251		

3.4 Antimicrobial Study

The results invitro antibacterial studies of prepared silver nanoparticles using *S. lappa*root extract showed exhibited good antibacterial activity against *Escherichia Coli* with high degree of zone of inhibition. However zone of inhibition was less in *Bacillus Cereus* (Table 3 and Figure7).High bacterial activity of the prepared silver nano particle is due to the release of silver ions from silver nano particles[20]-[21].

Table 3. *In vitro* antibacterial activity of prepared silver nanoparticles from root extract of *S. lappa*against *Escherichia coli* and *Bacillus Cereus*

Bacterial Species	Zone of inhibition (mm) average ± standard deviation (SD)

	Different concentration of AgNP				Chloramphenicol
	25%	50%	75%	100%	
Escherichia .Coli	1.5 ±0.05	1.7 ±0.4	2.6 ±0.6	2.2 ±0.4	2.8 ±0.3
Bacillus Cereus	1.0 ±0.08	1.6 ±0.06	2.1 ±0.06	1.8 ±0.03	2.2 ±0.02

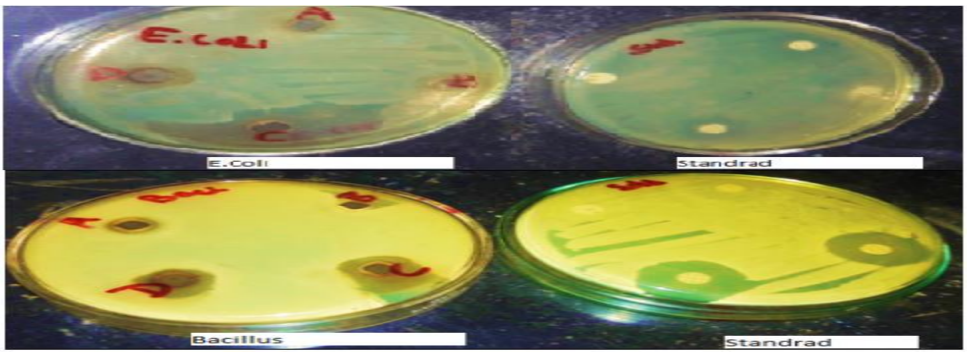

Fig.7. Zone of inhibition of antibacterial activity of prepared silver nanoparticles from root extract of *S. lappa*against *Escherichia Coli* (i), and *Bacillus Cereus* (ii) with the standard

4. CONCLUSION

Green synthesis of Silver nanoparticles was carried out successfully by using the root extract of *S. lappa*. The bioactive compound present in the *Root extract of S. lappa*like flavonoids, alkaloids, terpenoids are responsible for the reduction of Ag^{+} to Ag^{0}. Further these functional groups stabilize the nanoparticles by acting as capping agents which further confirmed by FTIR studies. EDX reports revealed that synthesized silver nano particles are crystalline.Synthesized nanoparticles showed remarkable and promising antioxidant activity (IC_{50}= 70.8%) in DPPH assay than comparative to ascorbic acid. Silver nanoparticles of root extract of *S. lappa*also emerged as a promising antibacterial nanomaterial. Thus biosynthesized silver nano particles found to be multifunctional with good antibacterial and antioxidant activities and can be used to develop new drugs for the biomedical purposes.

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