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Antibacterial Activity and Characterization of Silver Nanoparticles Using Ficus Tomentosa Leaf Extract

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Abstract

In the present study deals synthesis of Silver nanoparticles using Ficus Tomentosa leaf extract. The AgNPs were characterized using the following methods. Bio-synthesized Silver nanoparticles were primarily confirmed by change in colour from brown to dark brown. UV Visible Spectroscopy analyzed the Absorbance of synthesized FT-AgNPs. FT-IR was used to find out the specific functional groups present in the FT-AgNPs. The XRD pattern of Ficus Tomentosa Silver nanoparticles was found in the form of face centered cubic (FCC). Field Emission Scanning Electron Microscopy characterized the synthesized FT-AgNPs were shape and size was confirmed and the Energy Dispersive X-ray analysis spectrum confirmed the elemental compound peaks. The synthesized FT-Ag nanoparticles were analyzed to know the average size 170 nm was carried out by Dynamic Light Scattering. The two gram positive and one gram negative bacterial strains such as Staphylococcus epidermis, Bacillus subtilis and Klebsiella pneumonia were experienced to analyze the antibacterial activity of the Silver nanoparticles.

Keywords: Ficus Tomentosa, XRD, FT-AgNPs, FESEM, EDX, FTIR, UV, DLS and Antibacterial activity.

1. INTRODUCTION

Nanomaterials are foundations of nanoscience and nanotechnology. Nanostructure science and development is a wide and interdisciplinary zone of inventive work activity that has been getting perilously worldwide in the earlier year's [1-3]. It has the potential for reforming the way materials and things are made and the range and nature of functionalities that can be gotten. It is presently having a basic business influence, which will augment later on. Nanoscale materials are described as a ton of substances where in any occasion one estimation is not by and large around 100 nanometers [4-5].

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Nanomaterials are of interest considering the way that at this scale exceptional optical, appealing, electrical, and various properties create. These prominent properties have the potential for extraordinary impacts in equipment, prescription, and various fields [6]There are various engineered, physical, and natural green association strategies to set up the nanoparticle shape and size for the recently referenced needed applications.The momentum work is an undertaking to coordinate Silver compound precipitation methodology using plant eliminate in watery course of action. As of late, research on composite materials made the chance of abusing size-subordinate effects opens accordingly the way toward the improvement of new utilitarian materials and advanced contraptions [7-9]. Nano-particles regularly suggested as particles with a size up to 100 nm. Nano-particles show absolutely new or improved assets reliant on express ascribes, for instance, size; allocation and morphology, at whatever point differentiated and greater particles of the mass material they are made of Nano-particles present a higher shallow to volume extent with reducing size of nano-particles [10-12]. In this examination, we screened Ficus Tomentosaplant leaf remove and contemplated their mix of silver nano-particles by checking the describe of UV-obvious spectroscopy, optical release of FT-IR, structure choose of XRD, morphology imaging of FESEM, presence of segments in EDX, size spread of DLS and their natural depict of antibacterial activities. The antibacterial activites of the examples were concentrated on Staphylococcus epidermidis (MTCC 737), Bacillus subtilis (MTCC 2451) and Klebsiella pneumonia (MTCC 3384) [13-16].

2. PLANT AND MATERIALS

2.1 Materials

Silver Nitrate (Analytical evaluation) was purchased from Sigma-Aldrich Chemical Pvt. Ltd. All the watery arrangements were readied utilizing twofold refined de-ionized water. 8 mM (0.1358 g) of silver nitrate was weakening in 800 ml water underneath continued blending for 1 hour to get AgNO3 fluid arrangement.

2.2 Collection of Plant Material

The given below figure 1 shows the Ficus Tomentosa that leaves were gathered from maiyanur village kallakurichi district in Tamil Nadu state. The gathered leaf

was firmly gathered with Polythene pack and afterward moves to the research center. At that point it was washed a few times under running water and afterward washed with refined water twice to eliminate dust particles and held under room temperature for about fourteen days in dark

Figure 1 Ficus Tomentosa

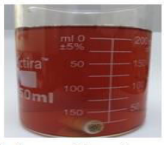
Condition and it was making into powder utilizing blender [17-19].

2.3 Preparation of Plant Extract

The powder of Ficus Tomentosa leaf was weighed 5g and disintegrated in 80ml of double distilled water and warmed to 50˚C with nonstop mixing time for 30 minutes. The blends were cooled to get temperature, separated with whatmann No.1 channel paper to get the concentrate (100%). The Leaf extract was gathered and put away in test bottle [20].

2.4 Synthesis of Silver nanoparticles utilizing Ficus Tomentosa leaf extract

A 200 ml of the fluid arrangement was readied which comprises of 2mM Silver nitrate is (AgNPs) and this arrangement is utilized for the blend of silver nanoparticles. To accomplish a productive amalgamation, 20 ml of Ficus Tomentosaleaf separate was included into 200 ml fluid arrangement of silver nitrate with consistent blending on an attractive stirrer for 1-hour and under kept the room temperature 24 hour in dim condition. The shading change of the arrangement was checked. At that point the examples were at consistent 8000 rpm, the completely dense arrangement was centrifuged for around 10 minutes. The total breakdown of the metal particles was gotten. The incorporated silver nanoparticles were gathered and put away in test bottle.



Before color change

After color changed

Silver Plant Extract Silver nanoparticles

Fig 2 Color change of plant extract after the addition of $AgNO_3$

The earthy colored shade of arranged nanoparticles from dull demonstrates 100% transformation of silver nanoparticles as appeared in the figure 2 [21-22].

3. EXPERIMENTAL CHARACTERIZATION

3.1Characterizations

The formation of silver nanoparticles proved by UV-Visible spectroscopy using UV-VISIBLE SPECTROPHOTOMETER LAMBDA 365 PERKIN ELMER showing ranges between 190 nm to 1100 nm. FTIR analysis was accomplished for the reduction of silver ions with the spectral range of 400-4000 cm-1using FTIR SPECTRUM RX I PERKIN ELMER SPECTROMETER instrument. The silver nanoparticles having crystalline structure was determined by the XRD examples of chose examples exist recorded use (XPERT-PRO) diffract meter. Anode material Cu, K-alpha frequency 1.54060 Å, was utilized to record the example at 25°C of estimation temperature. To examine the morphology of silver nanoparticles by employing Field Emission Scanning Electron Microscopy by using the sample exist recorded use (make and model) FESEM : CARL ZEISS (USA), SIGMA WITH GEMINI COLUMN, Resolution 1.5 nm, In focal point Detector, SE2 Detector, BSD Detector. with an Energy Dispersive X-ray Spectroscopy the basic examination (Point check, Area Scan, Line Scan and Elemental Mapping) of the sample affirmed by EDX (make and model) EDAX MAKE : BRUKER (GERMAN) , MODEL :Nano XFlash Detector. Dynamic Light Scattering analysis were used to determine the size of the silver particles by the help of instrument by (DLS) molecule size analyzer (make and model) MICROMERITICS Nanoplus.

3.2 Antibacterial activity

3.2.1 Collection of test organisms:

To examine the antibacterial activity of Euphorbia Heteropylla silver nanoparticles, two gram positive bacterial strains Staphylococcus epidermidis (MTCC 737) and Bacillus subtilis (MTCC 2451). One gram negative bacterial strains Klebsiella pneumonia (MTCC 3384) were prepared as test organisms. All the strains were procured from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India. Bacterial strains were cultivated at 37ºC and maintained on nutrient agar (Difco, USA) slant at for 4ºC.

3.2.2 Screening of Antibacterial Activities (disc diffusion method):

Antibacterial activity of Euphorbia Heteropylla silver nanoparticles (disc diffusion method) was determined using the disc diffusion method. The petridishes (diameter 60 mm) was prepared with Muller Hinton Agar and inoculated with test organisms. Sterile disc of six millimeter width were impregnated with 10 µl of various samples

respectively. Prepared discs were placed onto the top layer of the agar plates and left for 30 minute at room temperature for compound diffusion. Positive control was prepared using the 10 µl of Amoxicillin as standard antibiotic disc. The dishes were incubated for 24 h at 37ºC and the zone of inhibition was recorded in millimeters and the experiment was repeated twice.

4. RESULTS AND DISCUSSION

4.1 UV-Visible Spectroscopy

UV-Visible spectroscopy is a staunch non-destructive technique to explore the optical properties of silver nanoparticles. The UV-Visible absorption spectrum of the colloidal $AgNO_3$ nanoparticles has been carried out using UV-Visible spectrometer in the wavelength range 200nm to 1100nm at room temperature shown in figure 3(a).

The absorption peak is observed at wavelength 433 nm which clearly indicates the synthesized AgNPs and 396 nm presents the bio-colloidal Ficus Tomentosa AgNPs. During the synthesis, the color of suspension has changed from brown to dark brown which shows the formation of nano sized silver metals and their given table 1 shows the peak values [24].

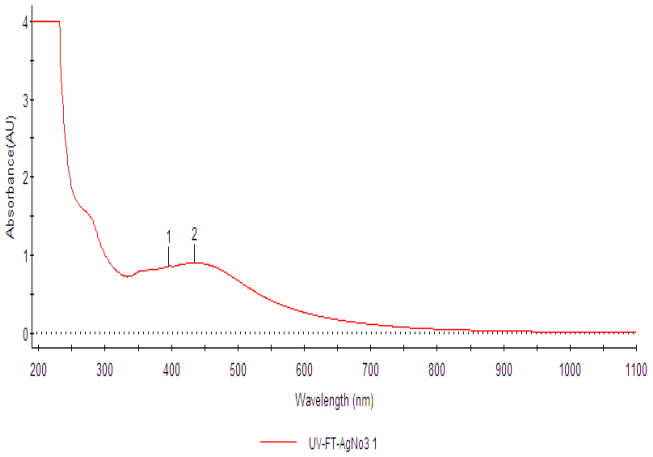


Fig 3 UV-Visible absorbance spectrum of Ag NPs using Ficus Tomentosa

Table 1: UV-Visible absorbance spectrum of FT-AgNPs

S.No	Peak (nm) Wavelength	Peak (AU) Absorbance
1	396.30	0.8624
2	433.50	0.9006

4.2 Fourier Transform Infrared Spectroscopy Analysis

The given below figure 4 shows the FT-IR spectrum of AgNPs of Ficus Tomentosa in the region 400-4000 cm^{-1}. The vibrations frequencies of the various chemical bonds in the nanoparticles can be assigned from the spectrum in terms of band position are given table 2. The sample can be identified by the assignments of stretching and bending modes of vibration frequencies [25].

The FT-IR spectrum showed the twelve major bands at 3412 cm^{-1}, 2923 cm^{-1}, 2426 cm^{-1}, 2093 cm^{-1}, 1611 cm^{-1}, 1516 cm^{-1},1384 cm^{-1}, 1270 cm^{-1}, 1072 cm^{-1}, 795 cm^{-1}, 777 cm^{-1}, and 575 cm^{-1}. The spectrum indicates a strong broad band at 3412 cm^{-1} is attributed to O – H stretching vibration of alcohols compound class. While the broad peak at 2923 cm^{-1} belongs to O – H unit of –COOH group of carboxylic acid. While the weak peak 2426 cm^{-1} specifies the S-H group of Thiol compound. A peak at 2093 cm^{-1} represent the stretching vibration N=C=S group of Isothiocyanate compound. A small peak at 1611 cm^{-1} specifies the C = C stretching vibration of α,β unsaturated ketone compound and 1516 cm^{-1} wave number is N – O group of stretching vibration of nitro compound and the peak at 1384 cm^{-1} C - H group of bending vibration of alkene compound. The peaks appeared at 1270 cm^{-1} and 1072 cm^{-1} are C – O group of stretching vibration compounds respectively alkyl aryl ether and vinyl ether. The wave number 806 cm^{-1} and 777 cm^{-1} are bending vibrations of C=C , C-H group of alkene and 1,2,3 - trisubstitued compound. The wave numbers 619 cm^{-1} and 558 cm^{-1} are related to C – Br stretching vibration and C – I stretching vibration group of halo compound strong intensity. In these below given table 3, which have described at wavelength, vibration, compound class, appearance and which functional group presented are analyzed.

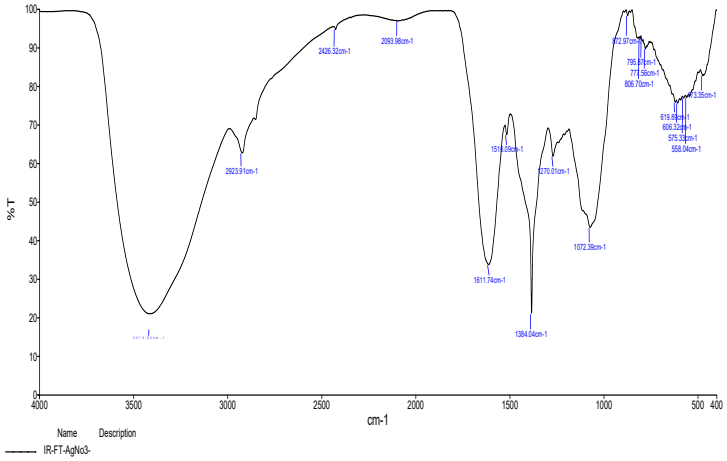


Figure 4 FT-IR Spectrum of AgNPs Capping Ficus Tomentosa leaf extract

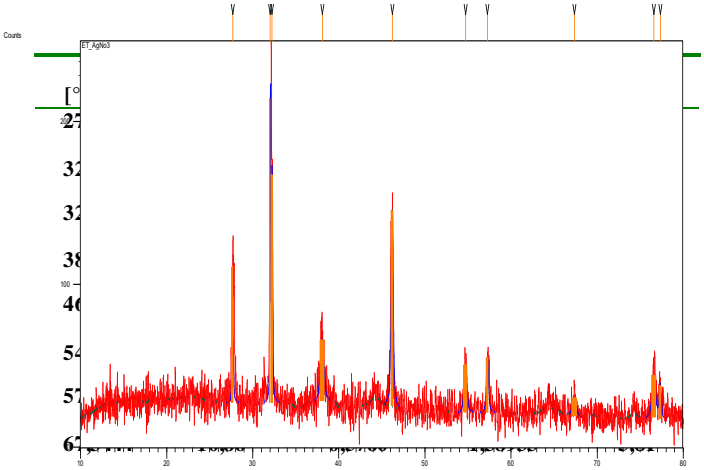
Table 2: FT-IR Spectrum Values of FT-AgNPs

Wave number peaks (cm^{-1})	Functional group	Type of vibration	Compound Class	Appearance
3412	O – H	Stretching	Alcohols	Strong, broad
2923	O – H	Stretching	Carboxylic acid	Strong, broad
2426	S – H	Stretching	Thiol	Weak
2093	N = C = S	Stretching	Isothiocyanate	Strong
1611	C = C	Stretching	α,β unsaturated ketone	Strong
1516	N – O	Stretching	Nitro compound	Strong
1384	C – H	Bending	Alkane	Medium
1270	C – O	Stretching	Alkyl aryl ether	Strong
1072	C – O	Stretching	Vinyl ether	Strong
806	C = C	Bending	Alkene	Medium
795	C = C	Bending	Alkene	Medium
777	C – H	Bending	1,2,3-trisubstituted	Strong

619	C – Br	Stretching	Halo compound	Strong
606	C – Br	Stretching	Halo compound	Strong
575	C – Br	Stretching	Halo compound	Strong
558	C – Br	Stretching	Halo compound	Strong

4.3 X-ray Diffraction Spectrometer Analyses

In this study, the X-ray diffraction pattern of the prepared Silver nanoparticles is recorded using the XPERT-PRO diffractometer system. Cu K_α line with wavelength of 1.5406 Å is generated with a setting of 30 milli amperes and 40 kV with the electrode. The diffracting angle is scanned from 10.00 degrees to 80.00 degrees with a step size of 0.02 degrees. The whole process takes place at a temperature of 25°C. The XRD profile of AgNPs of Ficus Tomentosa prepared at room temperature is shown in Fig.5. From the figure the diffraction peak indicated at $2\theta=27.67^0$, 32.03^0, 38.06^0, 46.20^0, and 76.57^0 corresponding indices to (110), (111), (200), (220) and (311) planes of the metallic silver nanoparticles with a face-centered cubic (FCC) crystal structure. From the figure obtained spectrum the corresponding values of 2θ and their intensities for the peaks are noted the Joint Committee on Powder Diffraction Standards (**JCPDS**) files to identify the crystal structure and small particles size of the as-synthesized silver nanopartices. The figure 5 show the peak at 32.03 pertaining to (111) diffraction peak specify the presence of the pure silver and the Ficus Tomentosa capped layer on the silver nanoparticles prevents silver particles from oxidizing. No diffraction peaks pertaining to other impurities are detected [26]. In addition, the background noise is attributed to the Ficus Tomentosa extract adsorbed the silver nanoparticles .This can be explained that, because of the surface adsorption of Ficus Tomentosa extract, the silver nanoparticles maintain good oxidation resistance. The (111) specify the resultant diffraction peak indicate that the products consisted of pure aspects. The given below table 3 shows the XRD peak values for synthesized FT leaf extract of silver nanoparticles.



76,5784	25,84	0,4800	1,24316	13,82
77,3350	20,58	0,2880	1,23287	11,01

Fig 5 XRD analyses of AgNPs Capping Ficus Tomentosa leaf extract

Table 3: XRD Peak Values of AgNPs capping Ficus Tomentosa leaf extract

4.4 Field Emission Scanning Electron Microscopy Analysis

The surface morphology of AgNPs using Ficus Tomentosa was investigating that FESEM. The micrograph observations showed synthesize nanoparticles was not in direct contact even within the aggregates, indicating stabilization of the AgNPs. The synthesized nanoparticles were found to be predominantly spherical, round, rod, square, rectangle and even shapes. The morphological shapes and size of the aggregations were described in terms of the fractal dimensions and box-counting method [27]. The result indicates the reduction process being held in the surface by representative FESEM micrograph. The Figure 6.(a), (b), (c), (d),(e) &(f) shows in morphology with sizes ranging from 20 µm, 2 µm, 1µm, 200 nm, 150 nm and 100 nm under the various microscopic analyses.

4.5 Energy Dispersive X-ray Spectrometer Analyses

The composition of the Silver nanoparticles is calculated using (EDX) elemental of point scan, area scan, line scan and elemental mapping. The elemental composition of green synthesis prepared silver nanoparticles was confirmed by EDS. The Figure 7 reveals that the results of EDX analysis. In this result determined the elemental peaks 0 Kev to 10 Kev and their strong elemental peak at around 1.6 kev to 3.4 kev is found which is in congruence

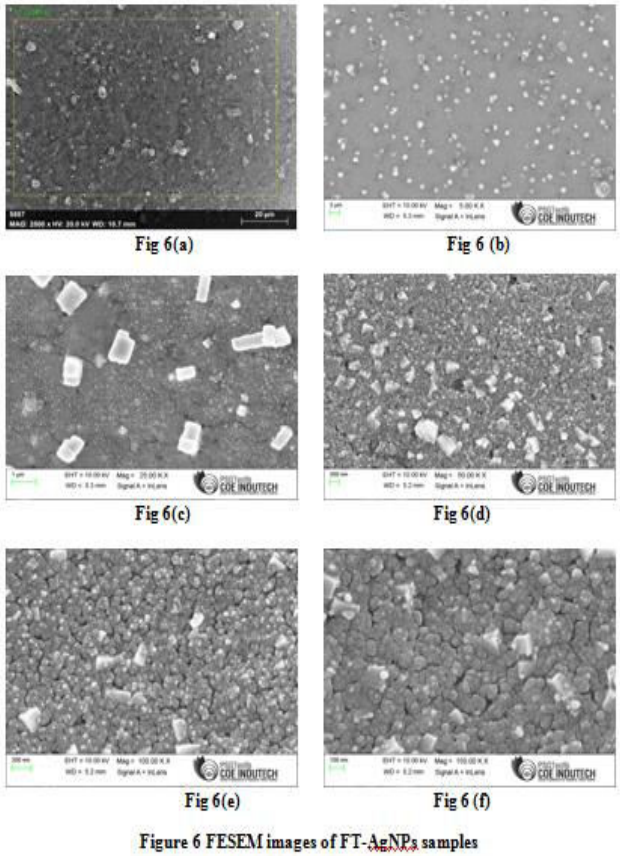


Fig 6(a) Fig 6 (b)

Fig 6(c) Fig 6(d)

Fig 6(e) Fig 6 (f)

Figure 6 FESEM images of FT-AgNPs samples

with the major emission peaks specified for metallic silver, indicating the formation of silver nano particles.

This result is consistent with the literature values. Along with this, small peak of oxygen and carbon were also observed because of the capping of silver nanoparticles with bio-molecules of Ficus Tomentosa leaf extract. Apart from these peaks, no other peak can be formation of pure silver nanoparticles.

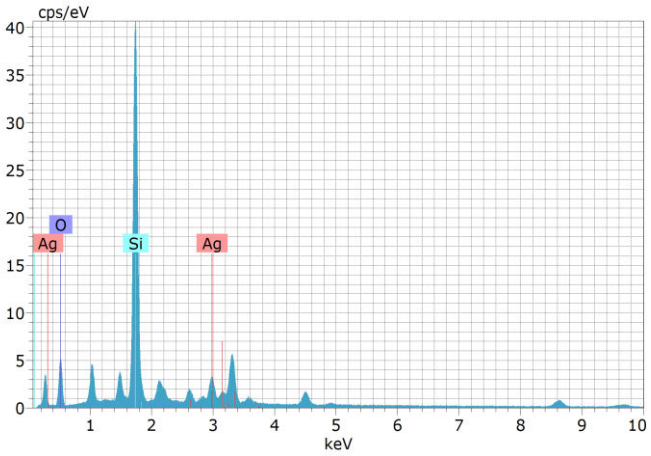


Figure 7 EDX Spectrum of Silver nanoparticles capping Ficus Tomentosa leaf extract

4.6 Dynamic Light Scattering Analysis

Dynamic Light Scattering measurements were done to determine the size of the silver nanoparticles formed is shown in the below figure 8. The particle size distribution peaks determined at various sizes of the particles ranging from 34.20nm to 372.50 nm and had an average particle size of 170.5 nm of synthesized Ficus Tomentosa silver nanoparticles as shown in the Figure 8 [28].

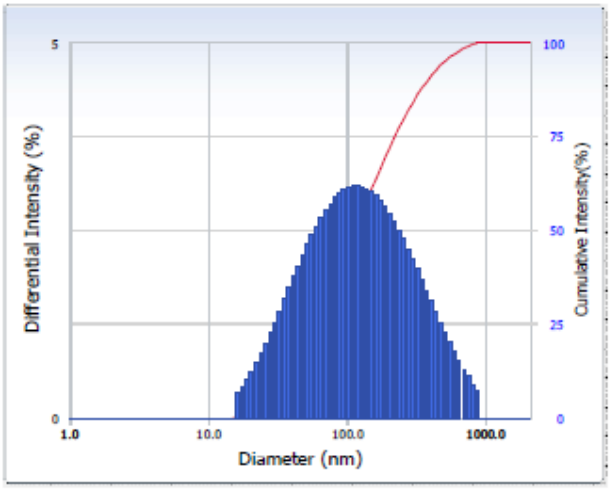


Figure 8 DLS analyses of Ficus Tomentosa-AgNPs

4.7 Antibacterial Activities of FT-AgNPs

The given below figure 9 shows the antibacterial activity of Silver nanoparticles capping Ficus Tomentosa by using disc diffusion method, the results of the antibacterial activity of different samples was tested against pathogens are shown in Table.4. The inhibitory activity against positive strains Staphylococcus epidermidis (1mm) and Bacillus subtilis (2 mm) were shown by sample D whereas at sample D, exhibited the antibacterial activity in all the four bacteria. The leaf extract and synthesized nanoparticles manifested better inhibitory actions against pathogens [29].

Table 4: Antibacterial activity of Ficus Tomentosa silver nanoparticles

Samples	Concentrations (µl/ml)	Organisms/Zone of inhibition (mm)		
		Staphylococcus epidermidis	Bacillus subtilis	Klebsiella Pneumoniae
A (silver nitrate)	10	0	0	0
B (Amoxicillin)	10	8	8	8
C (Plant extract)	10	2	1	0
D (Nanoparticles)	10	4	2	0

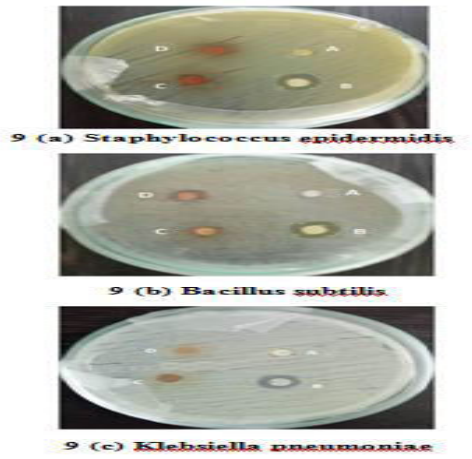


Figure 9 Antibacterial Activities

5. CONCLUSION

In this bio-synthesis study environmentally not harmful in effect. Ficus Tomentosa leaf extract can be used as an effective capping as well as reducing agent for the synthesis of silver nanoparticles. This green physical approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be economic viability, etc. applications of such eco-friendly nanoparticles used to anti-biotic, rupture treatment, fungal disease, abscesses, and body pain. The silver nanoparticles synthesized by Ficus Tomentosa leaf extract were characterized by UV-Visible spectra has confirmed the reduction of silver ions 433 nm. The presence of functional groups was confirmed by Fourier Transform Infrared Spectroscopy. XRD analysis confirms the crystalline face centered cubic structure [30-34]. The shape and size of the nanoparticles were strongly produced 1µm and 200-100 nm to confirm by Field emission scanning electron microscopy and Energy-Dispersive X-ray spectroscopy has confirmed the presence of strong elemental peak at around 1.6 KeV and 3.4 KeV. DLS study showed various sizes of the particles ranging from 34.20 nm to 372.50 nm and had an average particle size of 170.5 nm [35]. As synthesized silver nanoparticles capping Ficus Tomentosa showed excellent antibacterial activity against pathogenic Two Gram-positive bacterial strains Staphylococcus epidermidis and Bacillus subtilis (MTCC 2451) and One gram negative bacterial strains Klebsiella pneumonia (MTCC 3384) [36].

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