Synthesis, Characterization and Antimicrobial activity of *Leptadaenia reticulata* Silver Nanoparticles

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Abstract

Recently green synthesis of silver nanoparticles (SNPs) from plants is an extreme rising filed in nanotechnology. We have reported a green method for synthesis of Silver Nano Particles from aqueous plant leaves extract of *Leptadaenia reticulata*, an endemic medicinal plant of South-Eastern Ghats. These green-synthesized nanoparticles ar made public by color modification pattern, and thus the broad peak obtained at 421 nm with UV–Vis surface plasmon resonance studies check that that the synthesized.DLS analysis, it was located that Ag nanoparticles size changed into in the range of 80-120nm. Zeta capability famous the energetically very unstable. The particles undergo aggregation to stabilize themselves. Antimicrobial studies of the synthesized Silver nanoparticles on clinically separated Microorganisms were showed sensitivity against the biosynthesized nanoparticles. It indicates that Plant leaf extract of *Leptadaenia reticulata* is suitable for synthesizing stable silver nanoparticles which act as excellent antimicrobial agents.

Keywords:*Leptadaenia reticulat*a- Green synthesis- Silver nanoparticles -UV–Vis - FT-IR - SEM - DLS - Zeta potential analyser- Antimicrobial activity.

Inroduction:

Nanotechnology is a discipline of contemporary studies managing synthesis, design, and manipulation is an vital particle structure from about 1-100 nm variety in a single measurement. Remarkable growth in this up-and-coming technology has opened novel fundamental and rapidly gaining importance in a number of field [Colvin, V et al.,1994; Wang, Y et al.,1991]. The materials were synthesized and devices are fabricated by using the Nanotechnology method. Incorporation of nanoscale building blocks into purposeful assemblies and any into multifunctional devices may be achieved through a “bottom-up approach”. Research at the synthesis of nanosized material is of brilliant interest due to their precise houses like optoelectronic, magnetic, and mechanical, which differs from bulk [Atul, R.et al.,2010].

Nanoparticles is generally classified into 2, namely, organic nanoparticles that embody carbon nanoparticles (fullerenes) and semiconductor nanoparticles (like pigment and metallic element oxide). There is a growing interestingness in inorganic nanoparticles i.e. of metallic element nanoparticles (gold and silver) as they supply superior material properties with practical skillfulness.Because of their size decisions and endowments over available compound imaging drug specialists and prescriptions, inorganic particles are analyzed as likely apparatuses for clinical imaging moreover concerning treating illnesses. Inorganic nonmaterial have been broadly utilized for cell conveyance due to their adaptable capacities like significant accessibility, rich ability, legitimate similarity, and ability of focused medication conveyance and controlled arrival of medication [Xu, Z et al.,2006].

Silver nanoparticles square measure of interest attributable to the distinctive properties (e.g., size and form relying optical, electrical, and magnetic properties) which might be incorporated into antimicrobial applications, biosensor materials, composite fibers, refrigerant superconducting materials, cosmetic merchandise, and electronic parts. Various physical and chemical strategies have been used for synthesize and establishing silver nanoparticles [Klaus, T, et al.,1999; Senapati, S., et al.,2005]. The most fashionable chemical approaches, as well as chemical reduction employing a sort of organic and inorganic reducing agents, chemistry techniques, chemical science reduction, and lysis square measure wide used for the synthesis of silver nanoparticlesMethods for nanoparticle synthesis Physical processes, the absence of solvent infection inside the organized thin films and the similarity of nanoparticle spatial association are the advantage of bodily processes in evaluating with chemical methods.. [Magnusson, M. et al.,1999].It turn out to be incontestable that silver nanoparticles might be synthesized through a small ceramic heater with a local heating source [Jung, JO., et al.,2006; Mafune, F et al.,2001; Sylvestre, et al.,2004; Kim S et al., 2005].

In chemical methods unique lowering agents consisting of sodium citrate, tollens reagent, ascorbate, sodium borohydride ($NaBH_4$) polyol technique, N, , elemental hydrogen, N-dimethylformamide (DMF), and poly (ethylene glycol)- block copolymers are used for discount of silver ions (Ag^+) in binary compound or non-aqueous solutions. The said reducing agents cut back silver ions (Ag^+) and cause the formation of aluminiferous silver (Ag^0), which is followed by agglomeration into oligomeric clusters. These clusters ultimately lead to the formation of metal colloidal silver particles [Wiley, B. et al.,2005; Oliveira, M et al.,2005]. Biological approaches in recent years, the event of economical inexperienced chemistry strategies using natural reducing, capping, and helpful agents to arrange silver nanoparticles with desired morphology and size have become a major focus of researchers. Biological techniques can be used to synthesize silver nanoparticles without using any harsh, poisonous and luxurious chemical materials [Ahmad, A et al.,2003].

The bioreduction of metal ions by combos of biomolecules found within the extracts of sure organisms (e.g., enzymes/proteins, amino acids, polysaccharides, and vitamins) is environmentally benign, nonetheless with chemicals advanced.Many studies have said the a success synthesis of silver nanoparticles the use of organisms (microorganisms and biological systems) [Korbekandi, H. et al.,2009; Iravani, S. et al.,2011]. There are some microorganisms involved in synthesis of silver nanoparticles such as bacteria (Pseudomonas stutzeri AG259 strain) [Haefeli, C., et al.,1984; Vaidyanathan R., et al.,2010] fungi (F. oxysporum)[Ahmad A. et al.,2003]. The major advantage of victimisation plant extracts for silver nanoparticles synthesis that they're simply out there, safe, and nontoxic in most cases, have a broad variety of metabolites that can aid within the reduction of silver ions and square measure faster than microbes within the synthesis. The primary phytochemicals involved square degree terpenoids, flavones, ketones, aldehydes, amides, and institution acids. Water-soluble phytochemicals which might be responsible for the immediately reduction of the ions. The phytochemicals are involved directly in the reduction of the ions and the formation of silver nanoparticles [Prasad, K., et al.,2009].

Materials and methods

Synthesis of silver nanoparticles

The whole plant of *Leptadaenia reticulata* was collected from in the summer season from the localities of Ariyalur and Perambalur districts agroecosystems. The plant substances have been coarsely powdered. Finely ground 25 g of powdered plant material was kept in a beaker for 24 hrs with water. After 24hrs content was filtered, and marc was discarded. One part of the filtrate volume was reduced in a vacuum oven, then the aqueous extract was dried in Lyophilizer. Another part of the extract was used for the reduction of silver ions (Ag+) to silver nanoparticles (Ago). For the synthesis of silver nanoparticles, silver nitrate ready at the concentration of 10-3 M with pre-sterilized Milli Q water was used.

A amount of 1.5ml of every extract was mixed with thirty cc of 10-3 M of caustic for the synthesis of silver nanoparticles. Silver nitrate became taken in similar quantities of 1.5 ml each with out including plant extracts to fundamental respective controls.The saline bottles were tightly lined with aluminium foil so as to avoid exposure reduction of Silver ions, incubated at room temperature under darkish condition and observations had been recorded at 15 min, 30 min, 1 and 2hrs.

Characterization of Silver Nanoparticles

UV-Vis Analysis

The optical property of AgNPs was determined by a UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35, Germany).After the addition of $AgNO_3$ to the plant extract, the spectra had been taken in distinct time durations as much as 24hrs between 350 nm to 500 nm.Then the spectrum turned into taken after 24hrs of $AgNO_3$ addition.

FTIR analysis:

The chemical composition of the synthesized silver nanoparticles was studied by using the FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75^{o}C and the dried powders were characterised in the range 4000–400 cm^{-1} using the KBr pellet method.

SEM Analysis:

The morphological features of synthesized silver nanoparticles from *L. reticulate* plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs of the addition of AgNO3, the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of atomic number 78 was coated to form the samples semiconductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

DLS & Zeta-Potential Analysis

Dynamic mild scattering (DLS) which is primarily based on the laser diffraction technique with multiple scattering strategies turned into employed to observe the common particle length of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultra-sonication. Then the solution was filtered and centrifuged for 15 min. at 250C with 5000 rpm and the supernatant was collected. The supernatant was diluted four to five times and so the particle distribution in the liquid was studied in an exceedingly computer-controlled particle size analyzer (ZETA sizer Nano series, Malvern instrument Nano Zs).

Testing of antimicrobial activity

Microbial strains were tested for antimicrobial sensitivity using the good diffusion method. This technique become used to assess in vitro antibacterial and antifungal interest of take a look at samples towards certain human pathogenic microorganisms on Muller Hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the standardized bacterial suspension on the surface of agar plate for even growth. The 2.5, 5 and 10 μL of test solutions (prepared with 100% of DMSO) was poured in each well (6 mm diameter), separately. One separate well was used for the control study by taking 100% of DMSO (without test sample).

The plates were incubated at 37±1°C for 24–48 h (for bacteria) and twenty ±1°C for 48-72 h (for fungus). After incubation, the quarter of inhibition changed into measured with ruler/HiAntibiotic ZoneScale-C. The assays were performed in triplicate and the average values are presented. Ampicillin (A); Erythromycin(E); Kantrex (K); penicillin (M); antibacterial drug (Na); Trimethoprien (Tr); bactericide (T); Gendamicin (G). (for bacteria) and Ketoconazole (K) = fungus; Chloramphenicol (Ch) (for fungus) was used as a positive control, and DMSO (100%) used as a negative control.

Result & Discussion:

UV-Vis Spectrophotometer Analysis

The reduction of silver ions into silver nanoparticles during vulnerability to *L. reticulate* plant extracts changed into discovered as a result of the colour deviate. The colour alternate is due to the Surface Plasmon Resonance (SPR) phenomenon. The metallic nanoparticles have unfastened electrons, which give the SPR absorption band, due to the combined vibration of electrons of metallic nanoparticles in resonance with lightwave. The sharp bands of silver nanoparticles have been observed around 421 nm inside the case of L. *reticulate*. From one of a kind styles of literature, it was determined that the silver nanoparticles display SPR height at around 420 nm. From our research, we discovered the SPR top for *L. reticulate* at 421 nm. So we showed that *L. reticulate* leaf extract has extra capacity to lessen Ag ions into Ag nanoparticles, which lead us for similarly research on the synthesis of silver nanoparticles from *L. reticulate* leaf extracts. The intensity of the absorption height increases with an growing time period.

This feature colour variation is because of the excitation of the SPR inside the metal nanoparticles. The discount of the metal ions takes place fairly rapidly; extra than 90% of discount of Ag+ ions is whole inside 2 Hrs. After addition of the metal ions to the plant extract. The steel particles have been decided to be stable in answer even 4 weeks whilst their synthesis. By stability, we have a propensity to mean that there was no substantial version inside the optical properties of the nanoparticles solutions with time. On the behalf of UV-vis statistics it was cleared that reduces steel ions. So the further characterizations had been performed with *L. reticulate* [Figure.1]. The UV-Vis absorption spectroscopy is one of the important strategies followed to examine size and form of the nanoparticles in the aqueous suspensions Huang et al. suggested formation of silver nanoparticles when steady aqueous $AgNo_3$ at 50 ml, 1 mM with 0.1 g bio-mass produced silver nanoparticles as indicated via sharp absorbance at around 440 nm in *Leptadaenia reticulate.*

FTIR analysis:

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles [Figure: 2] wherein some pronounced absorbance was recorded in the region between 4000 and 400 cm^{-1}. They include 3435 (secondary amine, free, N-H asymmetric stretching), 2076 (Diazo, RCH=N=N Stretching), 1638 (Nitrate, O-NO2 Stretching asymm), 1371 (Alkanes, CH3 symmetric bending, R-CH3), and 695 cm-1 (C-S, R-C-CH3 stretching for sulfur compounds). Therefore, the synthesized nanoparticles were enclosed by proteins and metabolites like terpenoids having purposeful teams. From the analysis of FTIR studies, we confirmed that the carbonyl groups from the amino acid residues and proteins have the stronger ability to bind metal indicating that the proteins may presumably from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules may presumably perform twin functions of the formation and stabilization of silver nanoparticles within the liquid medium. Carbonyl teams tested that flavanones or terpenoids absorbed on the surface of metal nanoparticles. Flavanones or terpenoids may possibly be adsorbate on the surface of metal nanoparticles, probably by interaction through carbonyl teams or π-electrons within the absence of different robust ligating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and the formation of the corresponding metal nanoparticles. It is additionally attainable that the terpenoids play a task within the reduction of metal ions by a reaction of organic compound teams within the molecules to radical acids. These issues can be addressed once the various fractions of the plant extract are separated, identified and individually assayed for reduction of the metal ions. This rather elaborate study is currently underway (Figure 2).

SEM Analysis

SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes several nano meters i.e. between 1-100 nm. The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles (

DLS analysis

The Figure 4 shows the particle size distribution (PSD) of synthesized silver nanoparticles, it was found that Ag nanoparticles size were in the range of 80-120nm. However, beyond 100 nm range the percentage of nanoparticles present is very less. The highest fraction of AgNPs present in the solution was of 73nm is very appropriate since it gives lowest average size of nanoparticles.

Zeta Potential Analysis

The Figure 5 shows the zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticles and is related to the electrophoretic mobility and stability of the suspension of nanoparticles of the nanosilver. The overall absorbance of Zeta Potential revealed the energetically very unstable. Therefore, the particles undergo agglomeration/aggregation to stabilize themselves. So there were some potential charges on the surface of the nanoparticles which makes them stable. These charge potential we got from this analysis. Zeta potential (Surface potential) has direct relation with the stability of a form/structure as mentioned below (Table 1; Figure 5).

Antimicrobial activity of nanoparticles

Silver nanoparticles were tested in triplicates for antimicrobial activity. The values were recorded and averaged (Tables 2). L. reticulate has tested and recorded the results for the gram-positive (Plate 1), gram-negative (Plate 2) bacteria and fungi (Plate 3). The gram-positive were highly sensitive than gram-negative bacteria. Selected microorganisms were showed significant sensitivityagainst the biosynthesized nanoparticles. The antimicrobial activity of the test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) well diffusion test. The results of the antimicrobial activities are summarized in Table 2.

The 3 tested concentrations like two.5, five and ten µL /well turn out the zone of inhibition on MHA and PDA plates for bacterium and fungi, severally.

In the present study, higher (10 µL/well) concentration of the sample got greater sensitivity than (2.5 and 5 µL/well) lower concentration in all the tested microorganisms. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples, in bacteria, the test sample was most effective against B2, B3, B4, B5, and B9. While the moderate effect was noticed from B1, B6, B7, and B8. In fungi, which was effective against F4 and F6 whereas the average effect was observed in F1, F2, F3, and F5. All the microbial strains depict higher sensitivity to the higher concentration (10 µL) for the test samples. There is no antimicrobial activity in a solution devoid of sample used as a vehicle control (100% DMSO), reflecting that antimicrobial activity was directly related to the sample. Overall good antimicrobial activity was observed.

Fig :1 UV-VIS Spectral analysis Ag nanoparticle

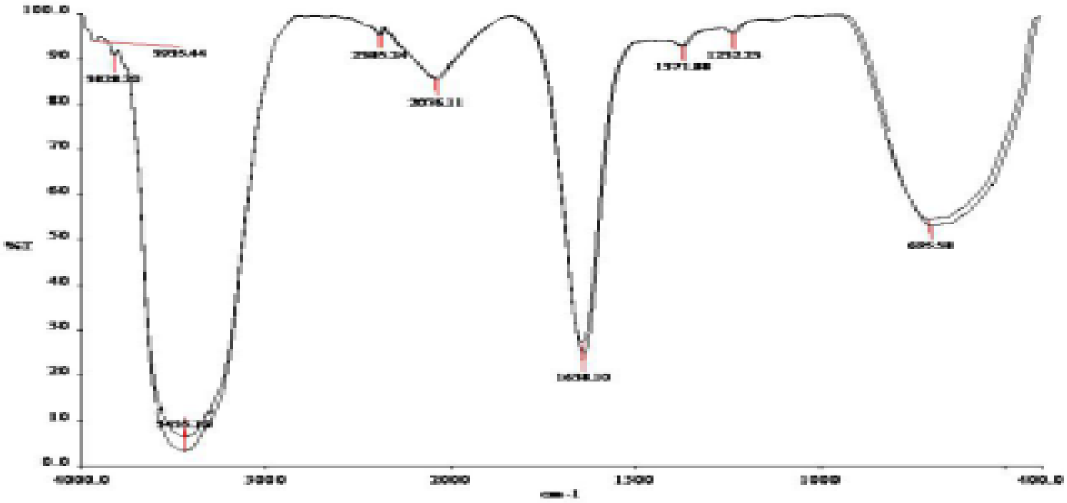

Fig. 2. FTIR analysis of vibration modes and function groups of *L. reticulate*

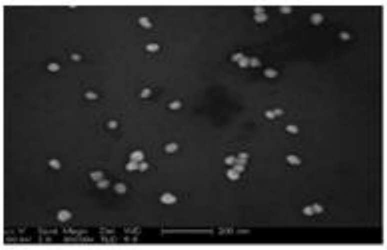

Fig.3: SEM-Microscopic view of *L. reticulate* reduced silver nano particles

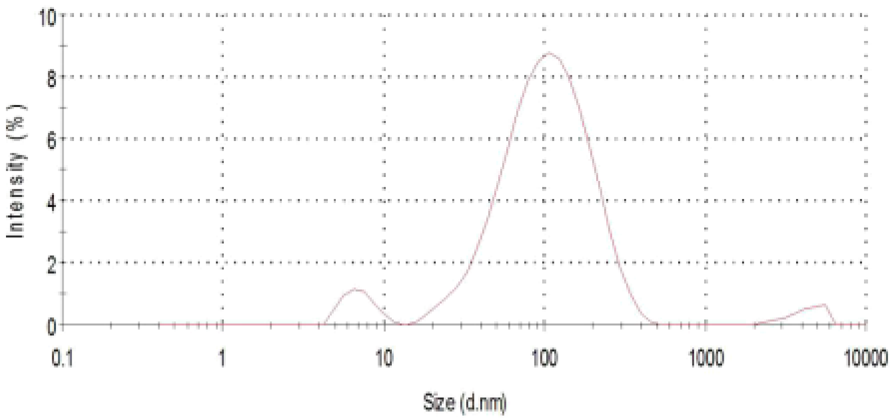

Figure 4. Dynamic Light Scattering ofParticle Size Analyzer of Nanoparticles

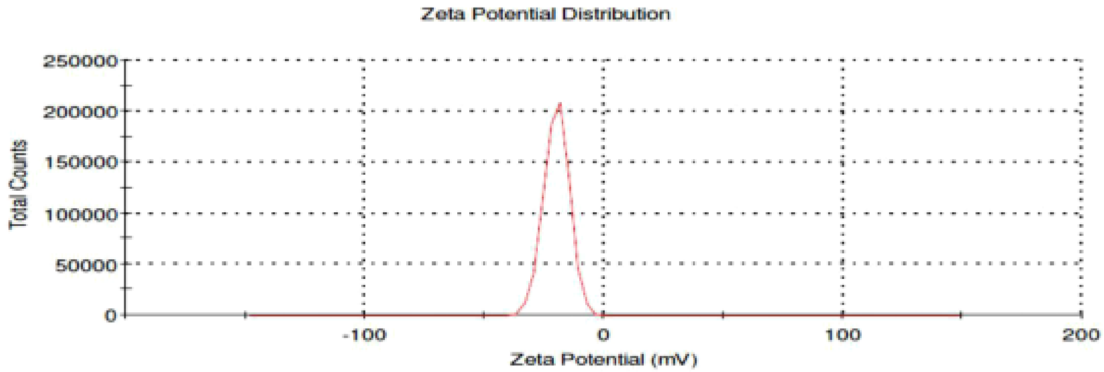

Fig.5. Zeta Potential Measurement of Ag Nanoparticles

L. *reticulata* (Silver Nano particles); Z-Average (d.nm): 73.84

		Mean(mV)	Area (%)	Width (mV)
Zeta potential (mV):	Peak 1	-19.7	100	5.29
Zeta Deviation (mV):	Peak 2	0.00	0.0	0.00
Conductivity (mS/cm):	Peak 3	0.00	0.0	0.00

Zeta Potential (mV): from ±10 to ±30 = Incipient instability

Zeta potential [mV]	Stability behavior of the colloid
from 0 to ±5	Rapid coagulation or flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability

from ±40 to ±60	Good stability
more than ±61	Excellent stability

Table 1. A table showing the stability of the NPs according to the potential charge

Organisms	Silver Nanoparticles (mg/ml)			Standard
	2.5	5	10	
Gram-positive				
Bacillus cereus (B1)	9	13	16	33 (T)
Bacillus subtilis (B2)	9	18	23	26 (A)
Staphylococcus aureus (B3)	12	16	23	45 (M)
Staphylococcus epidermidis (B4)	9	16	23	29 (T)
Gram -negative				
Aeromonas hydrophila (B5)	11	16	23	20 (Tr)
Escherichia coli (B6)	16	18	19	30 (K)
Klebsiella pneumoniae (B7)	10	11	11	30 (K)
Proteus mirabilis (B8)	12	19	20	25 (E)
Proteus vulgaris (B9)	11	19	23	30 (T)
Pseudomonas aeruginosa (B10)	12	17	23	20 (K)

Salmonella paratyphi(A) (B11)	9	12	15	30 (G)
Salmonella typhi (B12)	10	17	20	20 (Na)
Fungi				
Aspergillus fumigatus (F1)	13	16	17	(30) K
Aspergillus niger (F2)	12	14	17	(12) K
Candida albicans (F3)	13	17	21	(24) K
Microsporum canis (F4)	14	15	23	(32) Ch
Microsporum gypseum (F5)	16	18	21	(30) Ch
Trichophyton rubrum (F6)	17	19	25	(32) K

S= Standard; - = No activity; Measurements are given in mm; Ampicillin (A); Erythromycin(E); Kanamycin (K); Methicillin (M); Nalidixic acid (Na); Trimethoprien (Tr); Tetracycline (T); Gendamicin (G); Ketoconazole(K) = fungus; Chloromphenicol (Ch).

Table 2. Antimicrobial activity of Silver nanopartilces

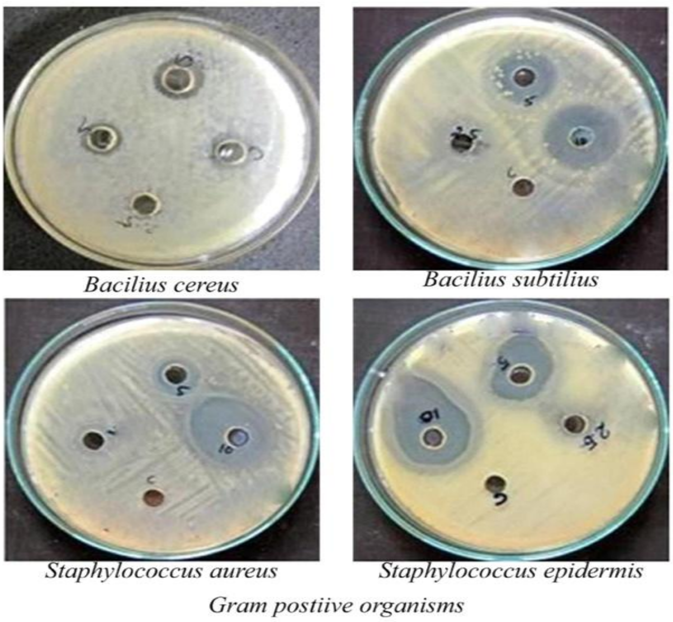

Plate 1. Antibacterial activity of biologically synthesized silver nanoparticles against Gram positive organisms

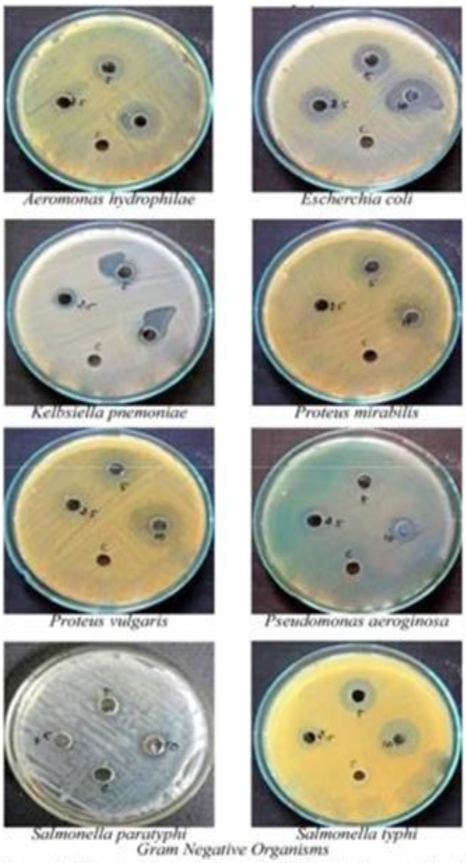

Plate 2. Antibacterial activity of biologically synthesized silver nanoparticles against Gram negative organisms

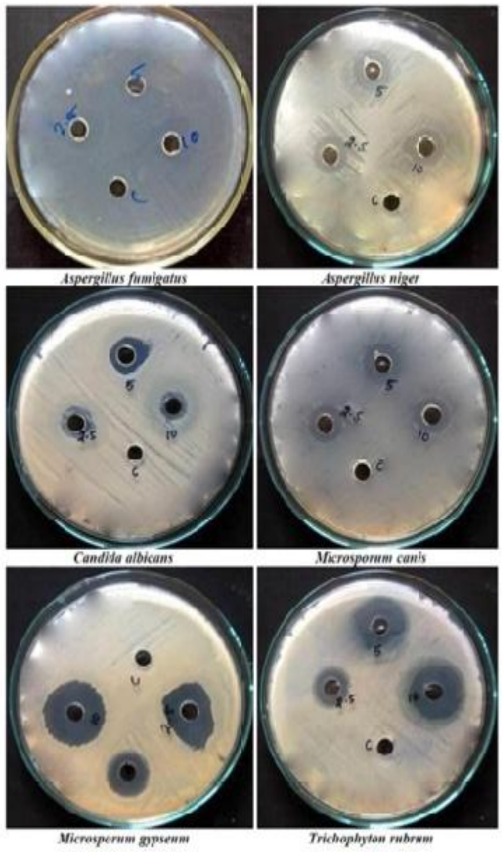

Plate 3. Antifungal activity of biologically synthesized Silver nanoparticles against the fungal organisms

Conclusion

The rapid biological synthesis of silver nanoparticles using L. reticulate leaves extract provides an environmentally friendly, simple and efficient route for the synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical and sheet-shaped and the estimated sizes were 160-180 nm. The size was bigger as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization victimization UV-vis photometer, SEM, DLS, Zeta Analyzer, XRD, and FTIR techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had a sheet-shaped appearance whereas the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different concentrations were also different which depend on the reduction of metal ions. From the data of DLS it was found that the 30:1 ratio solution had sharp nanoparticles of around 5 nm and some have around 180 nm and they had the potential of around 15.5 mV. Antimicrobial studies on some human pathogens, show excellent results in the test sample and also give good results. From the technological purpose of reading, these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications furthermore as massive-scale industrial production.

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