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GREEN SYNTHESIS CHARACTERIZATION BIOLOGICAL ACTIVITIES CATALYTIC ACTIVITY AND ANTI- OXIDANT ACTIVITY OF SILVER NANOPARTICLES USING LAGERSTROEMIA LEAF EXTRACT

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Abstract
Nano-technological development has groomed the creativity which led to the innovative ideas that have produced innovative methods and innovations. Nanotechnology has unlocked different methods such as physical, chemical, biological and green methods to synthesize the nanoparticles. Though each method has its own significance and necessity at its own time yet green method is preferred over these methods because of its cost-effectiveness and eco-friendliness. We have used green method i.e. lagerstroemia plant leaves to synthesize the silver nanoparticles and subjected them to characterization using different techniques such as Ultraviolet-visible spectrophotometry, Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Fourier Transformer Infrared (FT-IR), X-Ray Diffraction (XRD). All these techniques are used to study the morphological parameters. The so-prepared silver nanoparticles were further studied for various activities such as anti-diabetic activity, anti-oxidant activity, catalytic activity, antimicrobial activity.

Keywords: Lagerstroemia Leaf Extract, Silver nitrate, Uv-visible spectrophotometer, α-amylase, 2,2,Diphenyl picryl-1 hydroxyl, Nitrophenol, sodium borohydride.

Introduction
Eco-friendly processes (Green) in chemistry and chemical technologies are gaining popularity day by day as they have the potential to end the problems which the world is facing concerning the environment[1]. Their significant role is not only been noticed

in the fields of high sensitive biomolecular detection, catalysis, biosensors and medicine but have also been accepted to possess challenging inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities[2,3]. Large number of methods are there which are used for synthesize of silver nanoparticles like ion sputtering, chemical reduction, sol gel, etc[4,5,6,7]. Unluckily many synthetic approaches of the nanoparticle either use harmful chemicals or high energy/temperature, which are rather difficult and involve wasteful purifications[8]. It is worth to mention that whichever method is followed always tend to the chemical contaminations either during their synthetic procedures or in later applications with associated limitations. Yet; no one can refuse their ever growing applications in day-to-day life. Their eminent role in science and technology compels us to find the alternative synthesis approach that is not only cost effective but should also be eco-friendly. Such method is called green method which stands at atop of all methods available. By this method nanoparticles are prepared using naturally occurring reagents such as sugars, biodegradable polymers (chitosan, etc.) and plant extracts as reducing, stabilising and capping agents are considered attractive for nanotechnology[9,10,11]. Greener synthesis of nanoparticles favoured over other methods as they are simple, one step, cost-effective, environment friendly and relatively reproducible and often results in more stable materials[12]. In recent times plant extracts of large number of plants are used to produce nanoparticles some of which are Ziziphora tenuior[13], Abutilon indicum[14], Solanum tricobatum[15], Ocimum tenuiflorum[16], Spirogyra varians[17], olive[18], leaf extract of Acalypha indica with high antibacterial activities[19] and of Sesuvium portulacastrum[20] also reported in literature. In the work we have used Lagerstroemia plant to synthesize the silver nanoparticles.

Material and Methods

Materials required:- Leaves of Lagerstreomia plant, glass beaker, filter paper, test tubes, magnetic stirrer,

Chemicals required:- Silver nitrate, Diphenyl picryl hydroxyl (Dpph), α-amylase, sodium borohydrate, nitrophenol, congo red, methylene blue, methyl orange

Methodology

Preparation of leaf extract:- 10gm of leaf powder was taken in a glass beaker and 100ml of water was added. The mixture was boiled for 30 minutes at 70˚c and

then filtered. The filtrate was kept in a beaker with a label of lagerstroemia leaf extract and then kept at 4˚c in refrigerator.

Synthesis of Silver nanoparticles:- Mulberry leaves extract (5ml) was taken in 250ml beaker and kept on magnetic stirrer which was adjusted at 700rmp and 70˚c. 50ml of 0.001M $AgNO_3$ were added dropwise by burett to the beaker containing leaf extract. The mixture was kept at same rmp value and temperature until the colour change was observed i.e. light yellow to greyish brown.

Anti-diabetic (α-amylase Assay)

The inhibition of α-amylase was carried out by the method[21] with slight modifications. 20 µl of alpha amylase (.5mg/ml) were taken in test tubes. Different concentrations i.e. 15µl, 20µl, 30µl of test samples (plant extract, copper nanoparticles) and 10µl of 0.02m phosphate buffer (ph 6.9) were added to test tubes and the mixture is incubated for 10minutes. 1ml of 1%starch solution was added to the mixture and again incubated for 20minutes. Finally 400µl of DNS reagent were added to stop the reaction and then the reaction mixture was boiled for 5minutes. Control was prepared wherein amylase is not added. Absorbance was measured at 540nm.

Antimicrobial (Agar Well Diffusion)

The antibacterial assay was performed by agar well diffusion method on Escherichia coli (E.Coli), Staphylococcus, Bacillus cereus which were obtained from Food Microbiology, Defence Food Laboratory Mysore. The bacterial culture medium used, was nutrient agar medium which consists of 2.0 g beef extract, 17.5 g casein hydrolysate, 1.5 g starch and 17.0 g agar, dissolved in 1 litre of distilled water and pH adjusted to neutral at 25 °C. The dissolved medium was autoclaved at 15 lbs pressure at 121 °C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30ml/plate) while still molten. After 30 minutes, the cultured medium was inoculated with the test organisms Petriplates containing 20 ml Muller Hinton medium were seeded with 24hrs culture of bacterial strains. Wells were cut and 100 µl of the metal nanoparticles were added along with the standard antibacterial agent containing disc were placed onto an agar plate. Metal salt solution was used as a standard antibacterial agent. The plates were then incubated at 37 °C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well. The inhibition cleared zone around the sample decides the efficiency of the antibacterial agent to inhibit the growth of bacteria.

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Catalytic activity

1ml of 0.2M freshly prepared sodium boro-hydride was taken in a cuvette and 1.9ml of 0.2Mm of dye was added to the cuvette containing sodium boro-hydride. Cuvette was shaked and placed in the uv-visible spectrophotometer to record the absorbance. The cuvette was removed and 0.1ml of test sample was added and shaken vigorously and kept in Uv-visible spectrophotometer and absorbance was recorded[22].

Antioxidant (Dpph Assay)

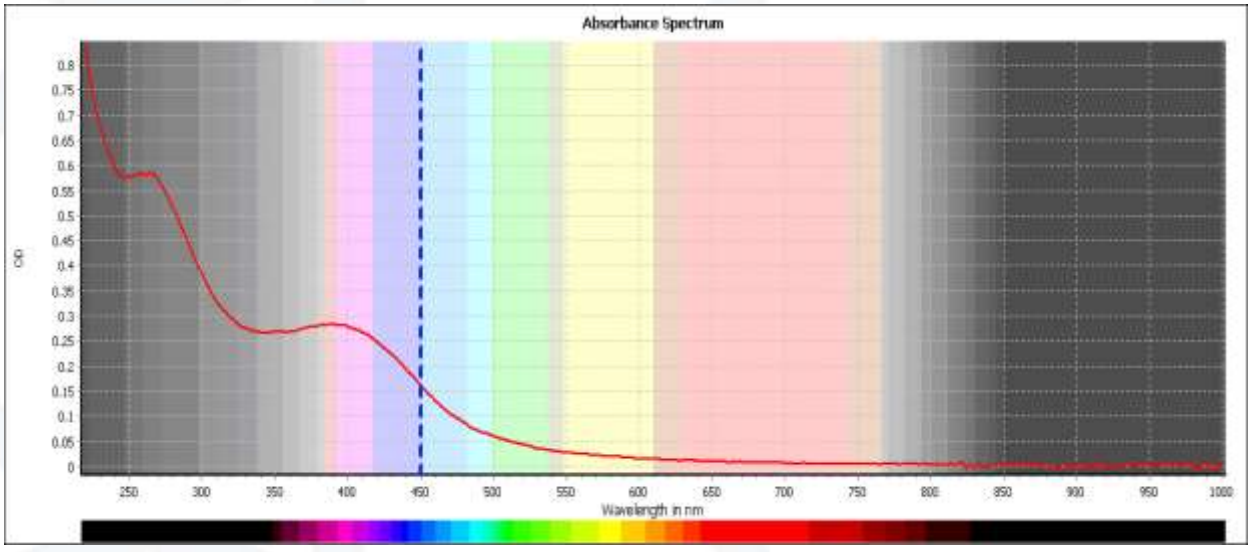
Standard procedure described by[23], was followed with a little modification. A stock solution of dpph (7mg in 10ml of methanol) was prepared. To 1ml of stock solution 10ml of methanol is added which was labelled as working solution. Different concentrations of test samples (plant extract, copper nanoparticles) were taken in test tubes. To each sample 800µl of methanol and 400µl of dpph was added. Dpph solution without test sample was used as control. All the samples were incubated for half an hour in dark and then absorbance was measured at 517nm.

Results and Discussion

Characterization

Ultraviolet visible spectrophotometry

The UV-vis absorption spectra of the green synthesized silver nanoparticles was taken by Uv –visible spectrophotometre (NANO STAR SPECTRA BMG Lab tech). An absorption peak was observed at 410nm which testifies the formation of silver nanoparticles. The wavelength was set between 300nm and 600nm and absorption band was obtained at 410nm which is characteristic silver SPR (surface plasmon resonance) band[24].



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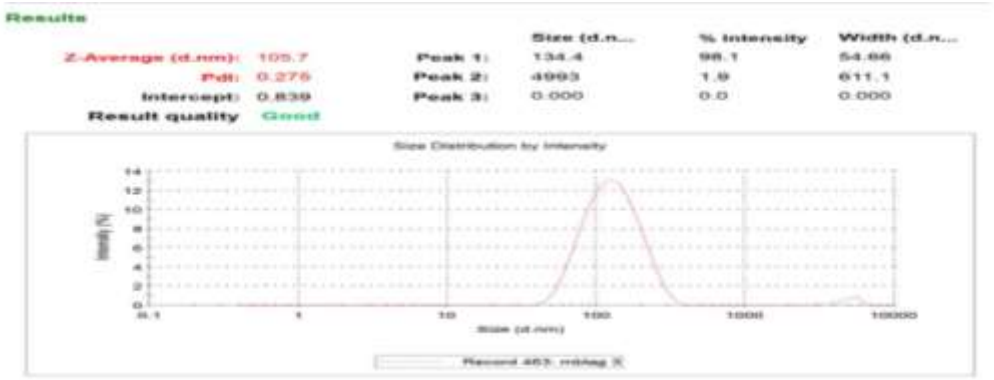
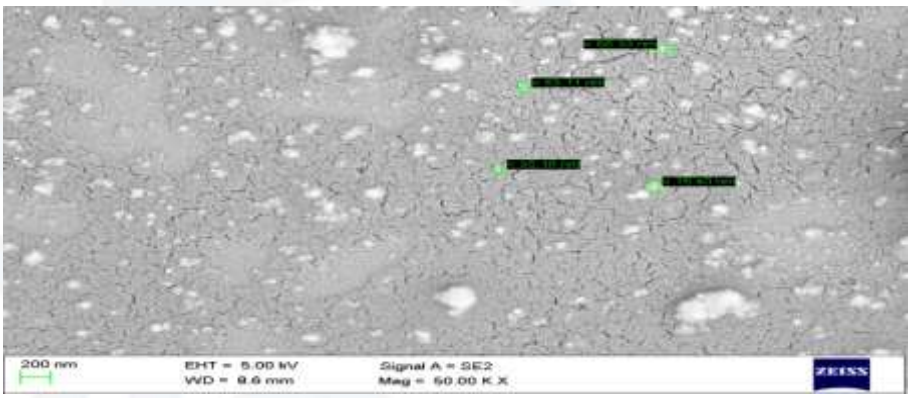
Dynamic light scattering (DLS)

Dynamic light scattering (DLS) was used to determine the particle size distribution and average particle size of all metal NPs at a scattering angle of 90˚. The average particle size of silver nanoparticles was found 105nm. Poly-distribution index was found 0.275.



Scanning electron microscopy

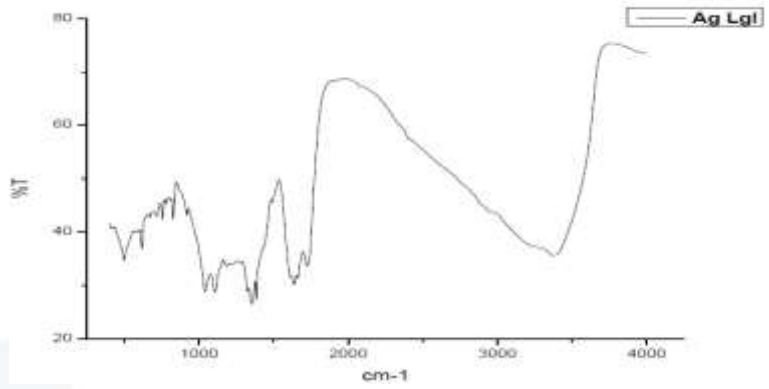
The SEM study provides the information about morphology of nanoparticles. Figure below shows the SEM image of green synthesized AgNPs in which nanoparticles of different size i.e. 68nm, 63nm, 35nm, 76nm etc. are seen clearly. The morphology of the silver nanoparticles made using lagerstroemia leaves were spherical in shape. Aggregations or impurities were also observed. Particles could be seen well dispersed. These bigger particles are either waste plant material, or bigger particles formed due to agglomeration.



Fourier transformer-infrared

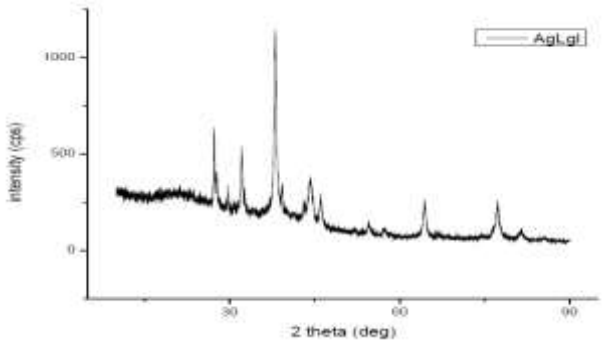
FTIR spectroscopic analysis was used to determine the functional groups present in the synthesized silver nanoparticles from lagerstroemia leaf extract. FT-IR spectrum of dry powder of synthesized AgNPs is shown in (Figure). The IR-spectrum of the AgNPs showed absorption bands at 1038, 1380, 1635 and 3430 cm−1 . The absorption

bands at 1038 cm^{-1} correspond to C-N stretching vibrations of the amine. The absorption bands at 1635 cm^{-1} correspond to amide 1 band of proteins due to carbonyl stretch in proteins and absorption bands at 3430 cm^{-1} are due to the O-H stretching in alcoholic compounds. The sharp band at 1380 cm^{-1} is due to C-H stretching vibrations of aromatic and aliphatic amines. The two bands observed at 1379 cm^{-1} and 824 cm^{-1} can be assigned to the C N stretching vibrations of aromatic and aliphatic amines, respectively. The existence of diverse types of functional groups that contribute to the synthesis of AgNPs was investigated using various modes of vibration[25,26].



X-ray diffraction

The formation of AgNPs nanoparticles was detected by X-ray diffraction (XRD). The crystallinity, phase structure and purity of the silver nanoparticles nanoparticles was determined by its typical powder XRD diffraction patterns. All the diffraction peaks corresponds to the lattice planes of (110), (111), (200) and (211) in between 2θ values: 38.17°, 47.29°, 66.42° and 78.71° is in good agreement with the Agnps which can be indexed on the basis of JCPDS card no. 65-2309. Sharp peaks in diffraction pattern show the crystalline nature of the particles.



Anti-diabetic Activity

Green synthesized silver nanoparticles were found to show strong antidiabetic activity while plant leaf extract showed moderate inhibition. The study confirmed that silver nanoparticles, plant extract possesses a maximum and minimum inhibition of 87% , 70% at 60µl respectively. It has also been observed that with the increase in the concentration of AgNPs the activity of αamylase was more efficiently inhibited. Similar results were obtained using silver nanoparticles prepared from Halymenia poryphyroides that showed significant in-vitro antidiabetes efficiency in a dose-dependent manner with an increase in percentage alpha-amylase inhibitory activity[27]. The Lagerstroemia leaf extract made silver nanoparticles reduced the amylase level by hydrolysing complex carbohydrates into lower carbohydrates to increase the utilisation of glucose. Likewise results were also recorded[28,29,30]. Our results are in agreement with the previous studies[31,]. According to various in vivo studies, for diabetes care, alpha-amylase inhibition is supposed to be one of the most operative methods[32,33].

Concentration (µl)	Control %	Lagerstroemia leaf %	Silver nanoparticles %
20	0	56.18	77
40	0	63.14	81
60	0	68.01	87

Antimicrobial Activity

Antimicrobial properties of silver nanoparticles have made them to be used vastly in the medicine, health industry, wound dressing, textile coatings, food storage, dye reduction, antiseptic creams and a number of environmental applications[34]. Since earlier times, native silver and its compounds have been serving as antimicrobial agents; and was used to protect water in form of silver coins/silver vessels[35,36]. We have investigated Lagerstroemia specio extract mediated silver nanoparticles as possible antibacterial agents. The plant extract and synthesized silver nanoparticles were studied for their antimicrobial activities towards bacteria culture of E.coli, Staphylococcus aureus and Bacillus cereus. Both plant extract and silver nanoparticles show zone of inhibition. Zone of inhibition witnesses that silver nanoparticles possess high antibacterial activity as compared to plant extract. The results of antibacterial activities of standard, plant extract, silver nanoparticles obtained from the agar well diffusion method are given. The silver nanoparticles showed efficient antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms[37].

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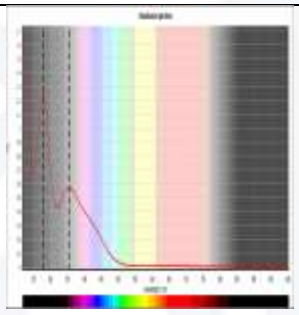
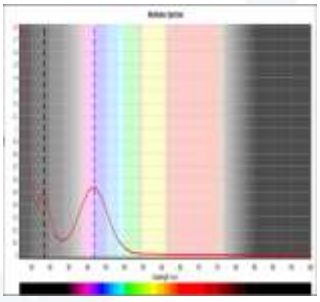
Samples	Zone of inhibition by well diffusion assay		
	E.coli ATCC 10536	Staphylococcus ATCC 11632	Bacillus cereus ATCC 14579
Silver standard	16mm	18mm	15mm
Lagerstroemia Leaf extract	12mm	13mm	11mm
Silver Nanoparticles	22mm	24mm	20mm

Catalytic activity

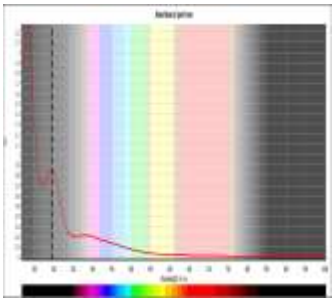
The green synthesized silver nanoparticles were studied for their catalytic activity towards nitrophenols (ortho nitrophenol and paranitrophenol) and organic dyes such as congo red, methyl orange, methylene blue etc. The Uv-visible spectrum of all compounds showed absorption peaks at 317, 355nm, 500nm, 600nm, 450nm. Upon addition of sodium borohydrate to nitrophenols and organic dyes Uv-visible spectrum was again taken which showed the absorption peaks at 400nm, 420nm 550nm, 650nm, 460nm. The shift in absorption bands is due to the partial reduction of nitrophenols and the organic dyes into intermediates. The absorption bands remained unchanged until the addition of silver nanoparticles. As soon as silver nanoparticles were added to solution the absorption peaks disappeared and new peaks were seen at 292nm, 290nm,250nm,255nm , 248nm which indicated that silver nanoparticles served as catalysts. Silver nanoparticles reduced the energy barrier of reaction between sodium borohydrate and nitrophenoles, organic dyes which otherwise couldn't have been possible.



Orthonitrophenol uv-visible spectrum



OrthO-nitrophenol with $NABH_4$ uv-visible spectrum



Mixture of Ortho-nitrophenol, NABH4, Agnp uv-visible spectrum

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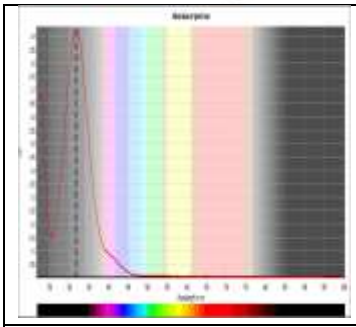
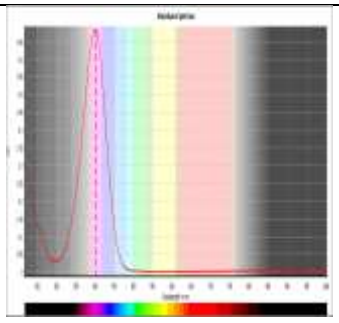
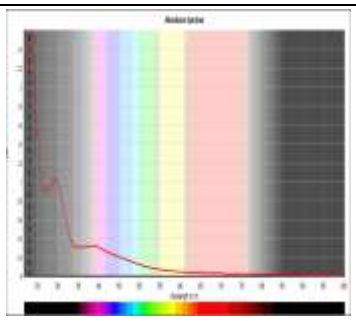
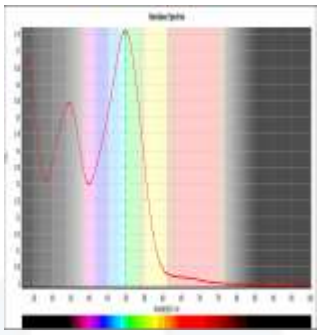
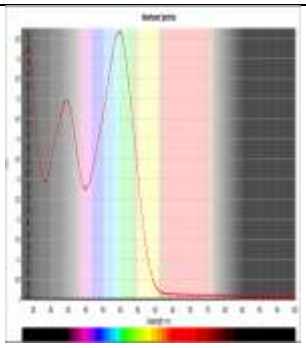
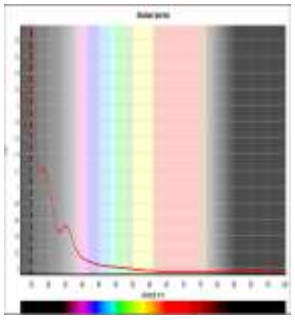
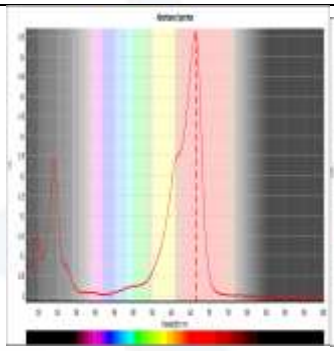
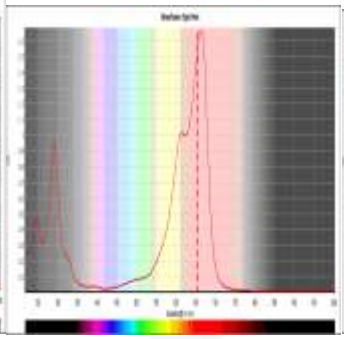
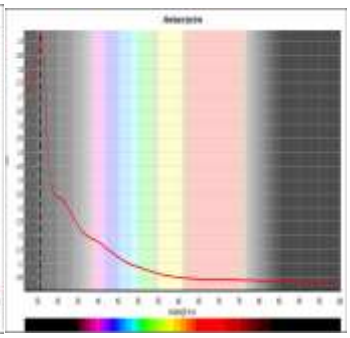
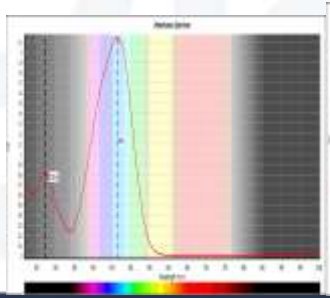
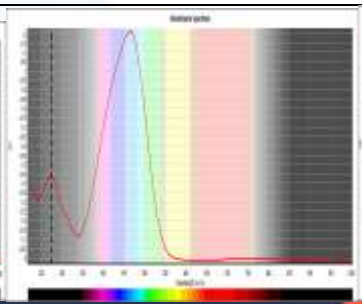
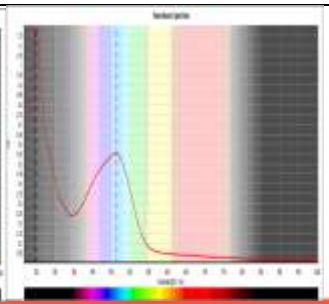
		
Paranitrophenol Uv-visible spectrum	Paranitrophenol with $NABH_4$ Uv-visible spectrum	Mixture of para-nitrophenol, NABH4, Agnp uv-visible spectrum

		
Congo red Uv-visible spectrum	Congo red with $NABH_4$ Uv-visible spectrum	Mixture of , Congo red ,NABH4, Agnp uv-visible spectrum

		
Methylene blue Uv-visible spectrum	Methylene blue with $NABH_4$ Uv-visible spectrum	Mixture of Methylene blue ,NABH4, Agnp uv-visible spectrum

Methylorange Uv-visible spectrum	Methyl-orange with $NABH_4$ Uv-visible spectrum	Mixture of Methyl-orange,NABH4, Agnp uv-visible spectrum

Antioxidant Activity

Freshly prepared silver nanoparticles displayed highest antioxidant activity when compared with plant extract from which they were prepared. The scavenging activity of silver nanoparticles and plant extract were found 85% and 65% respectively. The reaction was carried out in dark under room temperature. The results obtained are almost similar with the findings of Kharat and Mendhulkar[38], who showed that green-synthesized AgNPs using the aqueous leaf extract of E. scaber had strong scavenge DPPH free radicals (85.90%), but it was observed that AgNPs prepared by using aqueous garlic, green tea, and turmeric extracts showed efficient antioxidant activity compared to the ascorbic acid[39]. AgNPs synthesized by a green method using 4-N-methyl benzoic acid, which is a phenolic derivative isolated from Memecylon umbellatum Burm F., had the potential scavenge DPPH radical effect (81.57%)[40].

Concentration (µl)	**Control %**	**Lagerstroemia leaf %**	**Silver nanoparticles %**
20	**0**	**53.80**	**75**
40	**0**	**61.95**	**80**
60	**0**	**70.10**	**85**

Conclusion

It is need of hour to have such a method of synthesis which is environmental friendly and cost- effective. One such method is green synthetic approach which involves the use of plant parts and other vegetable waste materials. By this method we have synthesized silver nanoparticles using lagerstroemia leaf extract. Instruments like Uv-visible spectrophotometry, dynamic light scattering , scanning electron microscopy, Fourier transformer-infrared, X-ray diffraction were used to confirm the formation of silver nanoparticles and other morphological parameters i.e. shape ,size and structure. The synthesized silver nanoparticles were found to possess significant antimicrobial, catalytic antidiabetic, antioxidant activity.

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