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Plant mediated synthesis of silver nanoparticles using five different Lantana leaf extract and assessing their antioxidant, antibacterial and photocatalytic properties.

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Abstract

Silver nanoparticles (AgNPs) are used in a wide range of applications due to their antimicrobial, antioxidant, and photocatalytic activity. Cost-effective and eco-friendly methods for AgNP synthesis was thoroughly researched with available technologies. In this research varieties of Lantana leaves were used to synthesise AgNPs and determine antioxidant, antibacterial, and photocatalytic activity. The AgNPs was optimized at room temperature as overnight incubation. To characterize AgNPs UV-Vis spectroscopy and SEM were used. Phytochemicals such as tannin, saponins, alkaloids, terpenoids, carbohydrates, quinones, anthocyanin and coumarin were analyzed. The antioxidant capacity of AgNPs was measured and compared using Total flavonoid content (TFC), Total phenolic content (TPC) and Total antioxidant capacity (TAC). The water-soluble characterization of flavonoids in leaves were able to spectacularly reduce AgNPs. Their free radical scavenging activity was measured using DPPH and IC_{50}. The significant photocatalytic activity was demonstrated by K5NP through the degradation of Erichrome Black-T with sodium borohydride. *Escherichia coli* and *Staphylococcus aureus* were used to determine antimicrobial activity, where AgNPs showed significant effects. All the results were statistically analyzed using Microsoft Excel 2016 and one-way ANOVA statistical analysis was performed using IBM SPSS Statics 23 software. In this study green synthesis of AgNPs was studied significantly to provide valuable information to many fields for the betterment of the environment.

Keywords: Nanoparticles, Lantana leaves, Nanotechnology, phytochemicals, EBT

Introduction

Nanotechnology is one of the most active substantial research areas in modern science where it provides an understanding of the fundamental properties of objects. It is a study of manipulating materials at their atomic level by a combination of chemical, biological, and engineering approaches, and the synthesis of nanoparticles.[1] There is a rising commercial for nanoparticles because of their wide applications such as chemistry, energy, electronics, and medicine. There are two main types of nanoparticles as organic and inorganic nanoparticle.[2] Metal nanoparticles are the most widely studied nanoparticles due to their wide range of applications and easier to synthesize. In nanotechnology, different types of metals are being used but silver is the most preferred metal due to its properties such as chemical stability, catalytic activity, good conductivity, nontoxic, and antimicrobial activity. Silver has the ability to inhibit bacterial growth by damaging bacterial cell walls and disrupts cell metabolism.[3]

Two general approaches for the synthesis of nanoparticles are the top-down approach and the bottom-up approach. The top-down approach involves the formation of nanosized structures by breaking down the bulk materials. The bottom-up

approach involves building up material from the bottom: atom-by-atom, molecule-by-molecule, or cluster-by-cluster. Physical, chemical, and biological methods are used to synthesis nanoparticles.[4] However physical and chemical methods involve the usage of harmful chemicals which leads to a requirement of ecological and nontoxic nanoparticles. The biosynthesis method uses environmentally affable materials like bacteria, plant extract, and enzymes which appeared facile, cohesive for different biomedical application and better alternative for complex chemical synthetic methods. Compared to microorganism, the leaf extract is eco-friendly and compatible.[5]

Biological synthesis eliminates the usage of high pressure, energy, temperature, toxic chemicals and release environment-friendly products and byproducts. However, plant-mediated AgNps are non-hazardous and can be easily synthesized compared to microorganism mediated AgNPs. Plant extract can be obtained in various way since water-based extraction is ecofriendly compared to other chemical methods. Biological synthesis combines metal salts and plant extract to obtain nanoparticles. Nanoparticles synthesized using medicinal plants shows more benefits and AgNP enhance the antibacterial activity. Natural functional groups like amine, hydroxyl, carboxyl, and carboxylic groups are non-toxic reducing agents which facilitate the formation of metal ion complexes and reduces metal ions by oxidization. Plant extract mediated nanoparticle synthesis is influenced by factors such as time of reaction, pH and temperature.[6,7]

Lantana is a genus under the Verbenaceae family which is mostly known as a medicinal and ornamental herb. It is mostly found in tropical, subtropical countries and temperature regions.[8] *Lantana camara, Lantana involucrate, Lantana montevidensis, Lantana horrida and Lantana trifola* are the species that are studied under this project. Phytochemicals are natural bioactive compounds found in parts of plants and play important roles in the stabilization and reduction of nanoparticles. The leaves extract exhibit antimicrobial, antitumor and insecticidal activities and contain verbascoside, which possesses antimicrobial, immunosuppressive, and antitumor actives.[9]

According to Kalita *et al*. (2011), lantana leaves are rich in various phytochemical constituents such as triterpenes, steroids, glycosides, flavonoids, and essential oils.[10] It has various medicinal properties and used in the traditional medicinal system to cure various diseases like skin disease as dermatitis, cuts, itching, scabies, leprosy, rheumatisms, and chickenpox. Lantanoside, linaroside and camarinic acid are isolated from lantana which is investigated as potential nematicides.[11]

The function of an antioxidant is to trap free radical species, intercepting radical chained reactions and repairing oxidative damage. Free radicals are uncharged molecule having unpaired valency electron which is highly reactive and short lived. Free radicals and reactive oxygen species (ROS) are generated by normal metabolic actions. Oxidative stress is caused due to the imbalance of ROS and antioxidants which are an important risk factor in numerous pathogeneses of chronic diseases.[12]

Oxidative damage mediated by ROS to macromolecules incriminates within the pathogenicity of diseases like cancer, ageing, autoimmune diseases, rheumatoid arthritis, and cardiovascular disease.[13] There is increasing interest in natural antioxidants due to their wide-ranging activity compared to synthetic antioxidants. Antioxidant compounds such as polyphenols, phenolic acids and flavonoids scavenge free radicals inhibits the oxidative mechanisms that lead to degenerative disease. Since ancient times medicinal plants are considered to possess good antioxidant.[14,15] Organic dye is one of the major pollutants widely used in textile, medicine, and many other industries. Carcinogenic or toxic dye and their N-

substituted aromatic biotransformation products can be harmful to humans and the environment. The presence of aromatic rings and nitrogen bonds makes azo dyes non-degradable.[16]

The water bodies are accumulated with these dyes which cause eutrophication, toxic effects on aquatic life form by hindering the infiltration of sunlight and reduction of reoxygenation capacity. It also causes harmful effects on humans like allergic, some dyes are carcinogenic and tumor formation in the kidney, bladder, and liver. In wastewater treatment, the dominance of photocatalytic degradation by nanoparticles is due to its advantages over traditional methods, such as rapid oxidation, the absence of polycyclic materials and the oxidation of contaminants.[17] Recent studies show metal nanoparticles degrade dyes as an efficient photocatalyst at ambient temperature with visible light illumination. Biosynthesis nano catalysts are now commonly used for the effective removal of dye pollutants.[18,19]

The present study aims to synthesis AgNPs using five different types of Lantana leaves, characterization of AgNPs and assess their antioxidant, antibacterial and photocatalytic activity. Antioxidant activity will be assessed using TFC, TPC, TAC, DPPH and IC_{50} assays. Erichrome black-T will be used in determining photocatalytic activity. *Staphylococcus aureus* and *Escherichia coli* strains will be used to assess the antimicrobial activity of AgNPs. This research data is expected to be used in relevant future studies for the advancement of green mediated nanoparticle synthesis and betterment of prevailing environmental issues.

2. Methodology

The five different lantana plants (*Lantana camara, Lantana horrida, Lantana involucrate, Lantana trifola* and *Lantana montevidensis*) were collected from Rajagirya area. The leaves were washed, and shade dried. The leaves were labelled according to Table 1. Dried leaves were crushed using mortar and pestle. 2 g of each powdered sample was added into 50 ml distilled water (d.H_2O) separately and heated in a dry oven at 95°C for 20 min. Then it was let to cool and filtered into 50 ml falcon using Whatman filter paper No. 1. The falcons were labelled and stored at 4°C for further use.

Table 1. Labelling of samples.

Label	Sample
K1	*Lantana camara*
K2	*Lantana horrida*
K3	*Lantana involucrate*
K4	*Lantana trifola*
K5	*Lantana montevidensis*

2.1. Phytochemicals testing. Water extracts have been used to test for the presence of phytochemicals (Table 2).[20]

Table 2. Procedure for phytochemicals.

Phytochemicals	Procedure
Tannin	0.5 ml of leaf extract and few drops of 10% $FeCl_3$ were added into the test tube.
Saponins	0.5 ml of leaf extract and 1.5 ml of d.H_2O were added into a test tube and shaken vigorously.
Alkaloids	0.5ml of leaf extract and 0.5ml of Wagner's reagent were added into a test tube
Terpenoids	0.5ml of leaf extract, 1 ml of chloroform and 3 drops of conc. H_2SO_4 were added into the test tube.
Carbohydrates	0.5ml of leaf extract, 0.5 ml of conc.H_2SO_4 and few drops of Molisch reagent were added into the test tube.
Quinones	0.5ml of leaf extract and 0.5ml of conc. HCl were added into a test tube.
Anthocyanin	0.5ml of leaf extract, 0.5ml of NH_3 and few drops of conc. HCl was added to the test tube.
Coumarin	0.5 ml of leaf extract and 0.75ml 10% NaOH were added into a test tube.

2.2. Synthesis of silver nanoparticle (AgNP) using five different lantana species leaf extract. To 1 ml of each leaf extract, 9 ml of 1mM $AgNO_3$ was added into labelled test tubes. Then it was covered with a foil and stored overnight in a dark place at room temperature (RT). Colour change was observed. Samples were shaken and mixed well. The absorbance was measured at the wavelength of 300-540 nm. Optimization was carried out at 90°C for 30 min and 60 min using 1 ml of leaf extract and 9 ml of prepared AgNO3. Then the absorbance was measured at the wavelength of 300-540 nm.

2.3. Scanning Electron Microscopy (SEM) analysis. 2 ml of K5 AgNP was added into Eppendorf and centrifuged at 10 RPM for 2 min. The supernatant was discarded, and it was repeated till the pellet is observed. Then the pellet was dried at 40°C in the dry oven for 3 hours. SEM analysis was carried out at the Sri Lankan Institute of Nanotechnology (SLINTEC), Homagama, using Hitachi SU6600 SEM.

2.4. Dilution of samples. 1 ml of each leaf extract and AgNPs was added with 14 ml with d.H_2O to have 1:15 dilution. Diluted samples were stored at 4°C for further use.

2.5. Antioxidant assays. Diluted water extracts and AgNPs were used for the following assays.

2.5.1. Analysis of Total flavonoid content (TFC). 1.5 ml of sample was added into a test tube and 0.2 ml of 10% $AlCl_3$ and 0.2 ml of 1M potassium acetate was added. Then it was let to incubate for 20 min at room temperature (RT) and absorbance was measured in triplicates at 420 nm using a spectrophotometer with d.H_2O was used as a blank. TFC was expressed in equivalents of Quercetin in µg QE/100g.[21]

2.5.2. Analysis of total phenolic content (TPC). To 0.5 ml of dilute sample, 2.5 ml of 10% Folin-Ciocalto reagent and 2 ml of 7.5% sodium carbonate were added into each tube. Then it was let to incubate at RT for 30 min. The absorbance was measured in triplicates at 765 nm using a spectrophotometer with d.H_2O was used as a blank. TPC was expressed in equivalents of Gallic acid in mg GAE/100g.[21] (John et al., 2014).

2.5.3. Analysis of total antioxidant content (TAC). To 1.5 ml of dilute sample, 0.5ml reagent solution (mixture of 28mM Sodium phosphate, 0.6M H_2SO_4, 4mM Ammonium molybdate in 1:1:1 ratio) was added into tube. It was let to incubate at 95°C for 90 min. The absorbance was measured in triplicates at 695 nm using a spectrophotometer with d.H_2O was used as a blank. TAC was expressed in equivalents of Ascorbic acid in mg AAE/100g.[21]

2.5.4. Analysis of 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity. To 1 ml of 0.004% DPPH, 10 µl of the sample was added. Then it was let to incubate in a dark place for 30 min at RT. Absorbance was measured in triplicates at 517 nm using UV-Vis spectrophotometry with methanol as a blank. TAC was expressed in equivalents of Ascorbic acid in mg AAE/100g.[22]

2.5.5. Analysis of medium Inhibition concentration (IC_{50}). To 1 ml of 0.004% DPPH, 1ml of samples and d.H_2O were added in series of concentration (Table 3). Then it was incubated in a dark place for 30 min at RT. Absorbance was measured in triplicates at 517 nm using UV-Vis spectrophotometry with methanol as a blank.[22] DPPH scavenging activity was expressed in percentage inhibition. It was calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_{CONTROL} - A_{SAMPLE}) / A_{CONTROL}] \times 100$$

Where $A_{CONTROL}$ is the initial absorbance of DPPH reagent, and A_{SAMPLE} is the absorbance of the sample.

Table 3. Concentration of the sample.

Concentration	Sample	d.H_2O
100	10 µl	0
80	8 µl	2 µl
60	6 µl	4 µl
40	4 µl	6 µl
20	2 µl	8 µl

*2.6. Analysis of antibacterial property***.** 13.3 g of Muller-Hinton agar was dissolved in 350 ml d.H_2O and 9.8 g of nutrient agar was dissolved in 350 ml d.H_2O. It was autoclaved and poured into Petri plates. *E. coli* and *S. aureus* were inoculated from the mother culture. The plates were labelled and streaked with *E. coli* and *S. aureus* respectively. 1 ml of leaf extract and AgNP was dried in the watch glass at 90°C for 5 min. Three wells were made in the plate using clean pipette tips for sample (2 replicant) and negative control (saline). Gentamycin was used as a positive control. The procedure was done in a fume hood. All the plates were incubated at 37°C overnight. The inhibition zone was measured by using a ruler in cm.[8]

2.7. Analysis of photocatalytic activity. To 50 ml of 2 mM Erichrome black-T (EBT) and 0.188 ml of 10 ppm, K5 AgNPs was added, and the absorbance was measured from 340-780 nm for 210 min using a spectrophotometer. The same procedure was carried out for 100 ppm and 500 ppm K5 AgNPs. To 50 ml of 2 mM EBT, 0.188ml of 10 ppm K5 AgNPs sample and 20µl of NaBH4 was added and the absorbance was measured from 340-780 nm for 210 min. The same procedure was done for 100ppm K5 AgNPs.[23]

2.8 Statistical analysis. Using Microsoft Excel 2016 One-way ANOVA statistical analysis was performed and using IBM SPSS Statistics 23 software correlation graphs were generated. $p<0.05$ was used as the significance cut-off for the analysis.

3. Results

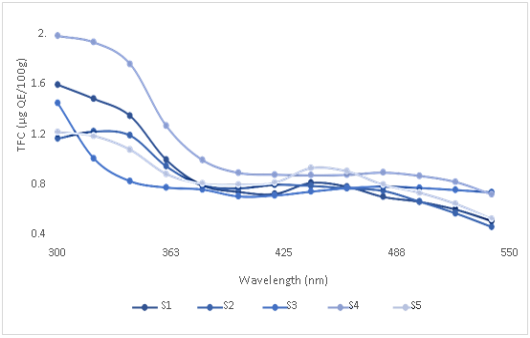

Figure 2. Spectrometric analysis of AgNP at RT.

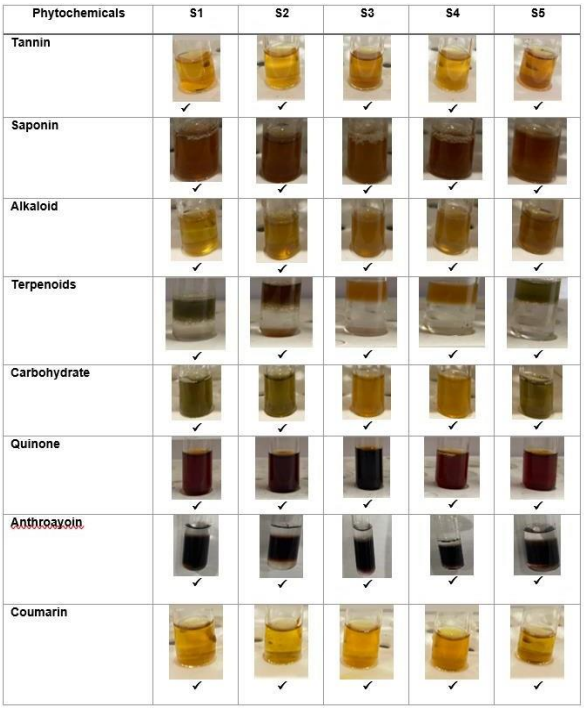

Figure 3. Results of phytochemicals

Table 5. Spectrophotometry analysis of AgNP for optimization

Sample	30min (90C)	60min (90C)
K1	Absent	Absent
K2	Absent	Absent
K3	Absent	Absent
K4	Absent	Present
K5	Absent	Absent

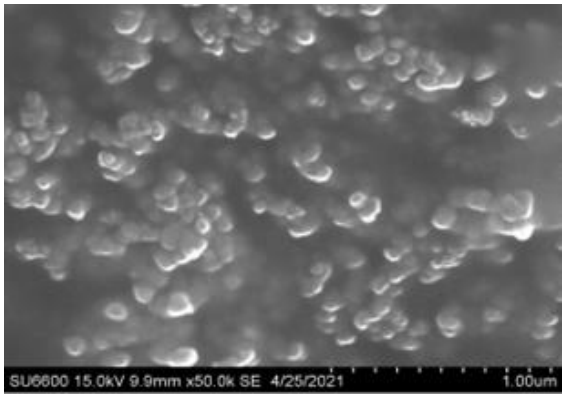

Figure 4. SEM imaging at 15.0kV 9.9mm x50.0k

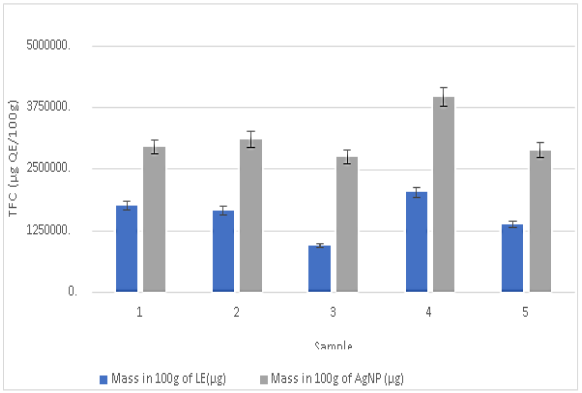

Figure 5. TFC of leaf extract and AgNPs.

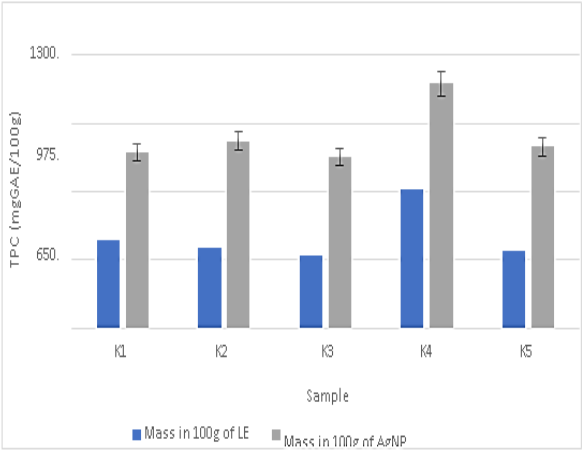

Figure 6. TPC of leaf extract and AgNP

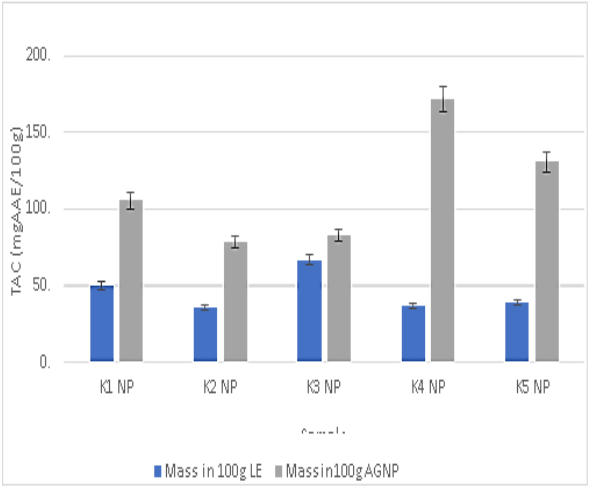

Figure 7. TAC of leaf extract and AgNPS.

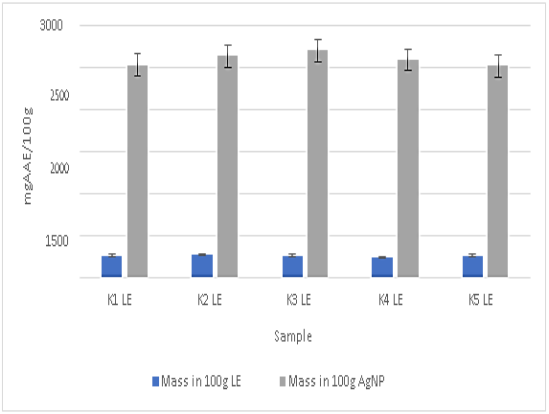

Figure 8. DPPH inhibition activity of leaf extract and AgNP.

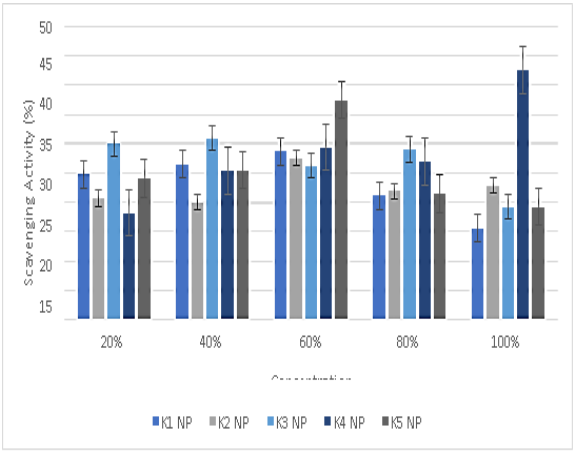

Figure 9. Inhibition concentration of the leaf extract.

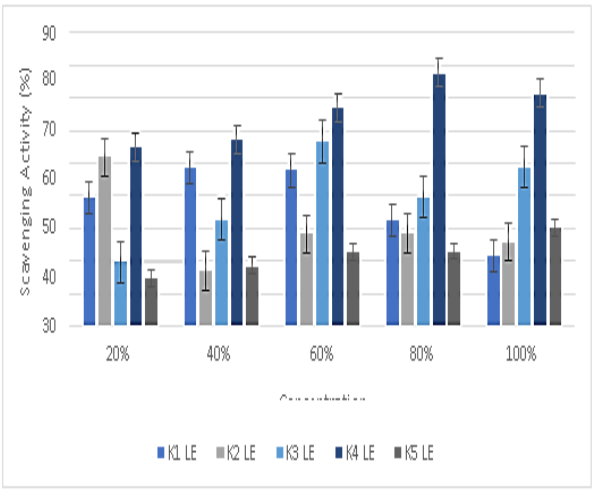

Figure 10. Inhibition concertation of the synthesized AgNP.

According to Figure 9 and 10, the highest IC50 was observed in K4LE and K4NP.

Table 6. IC50 of the samples expressed in the specific activity (%).

Sample	Leaf extract	AgNP
K1	66.231	24.543
K2	52.847	22.313
K3	36.279	28.265
K4	62.843	25.982
K5	20.146	13.154

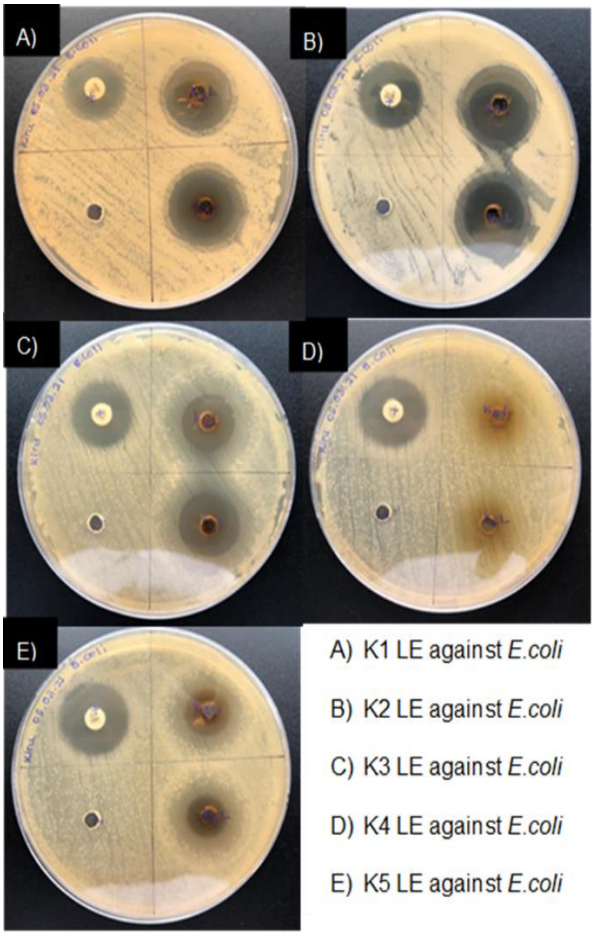

Figure 11. Antibacterial activity of leaf extract against *E. coli*.

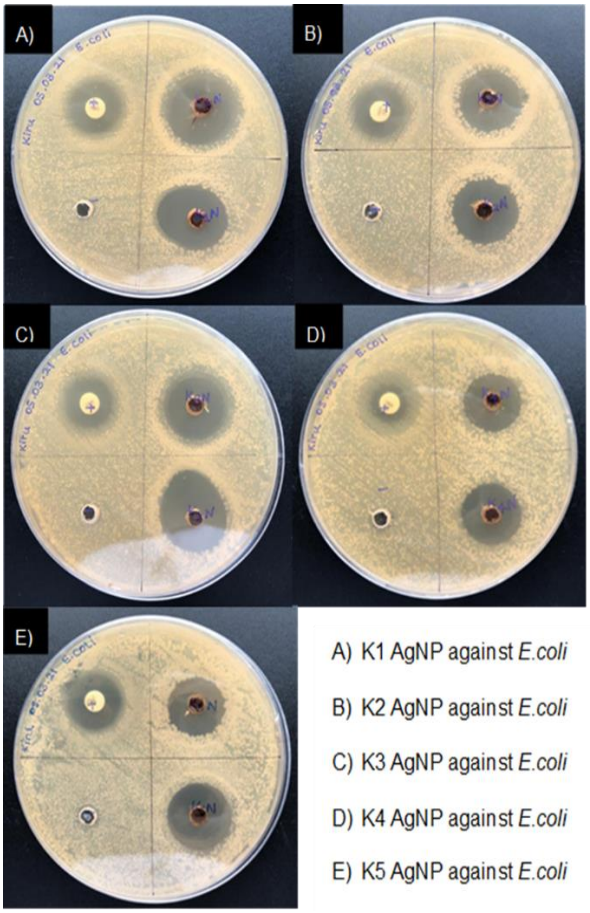

Figure 12. Antibacterial activity of AgNP against *E.coli.*

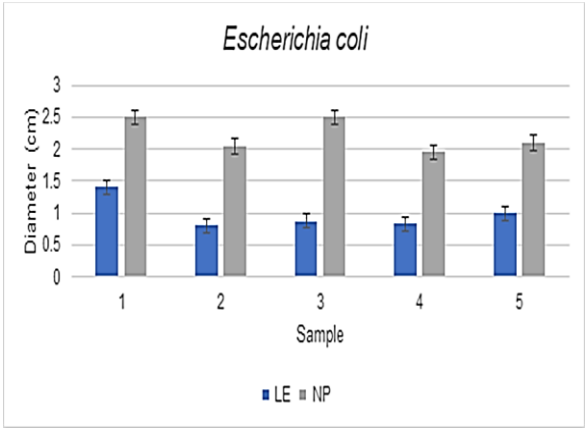

Figure 13. Antimicrobial activity against *E.coli* using leaf extract and AgNPs.

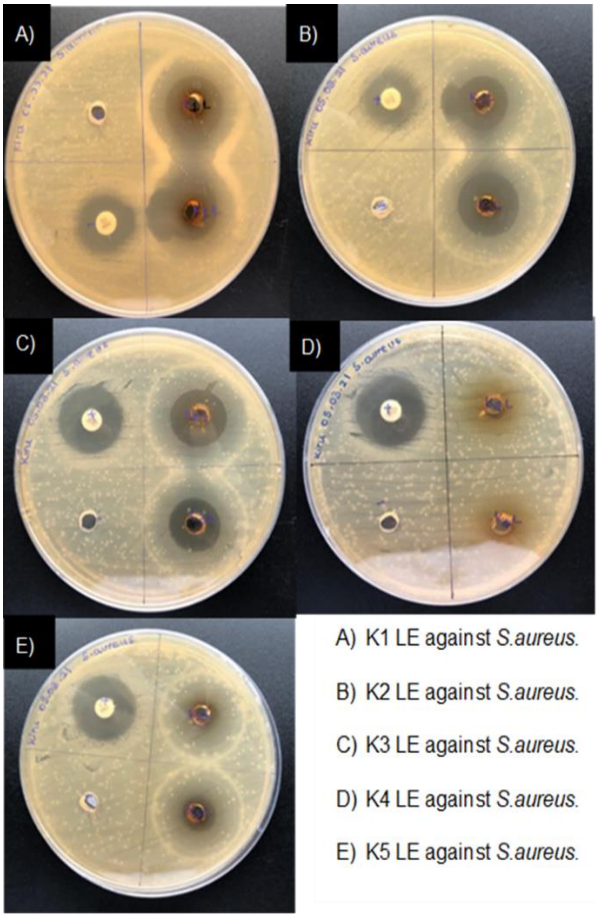

Figure 14. Antibacterial activity of Leaf extract against *S. aureus.*

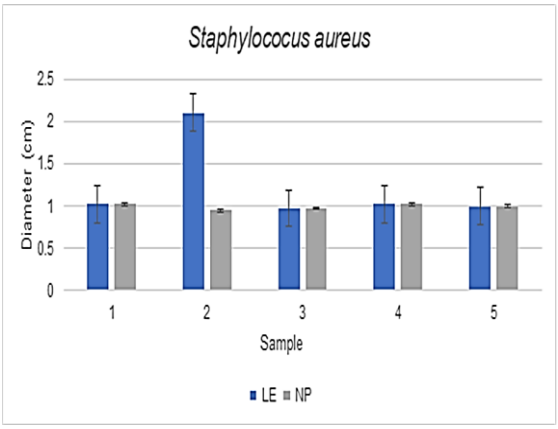

Figure 15. Antimicrobial activity against *S. aureus* using leaf extract and AgNPs

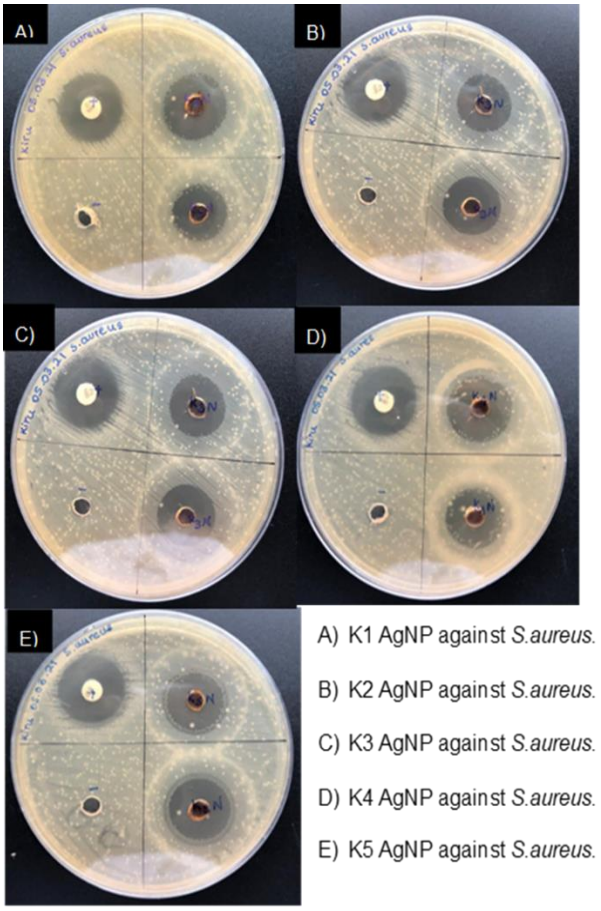

Figure 16. Antibacterial activity of AgNPs against *S. aureus*.

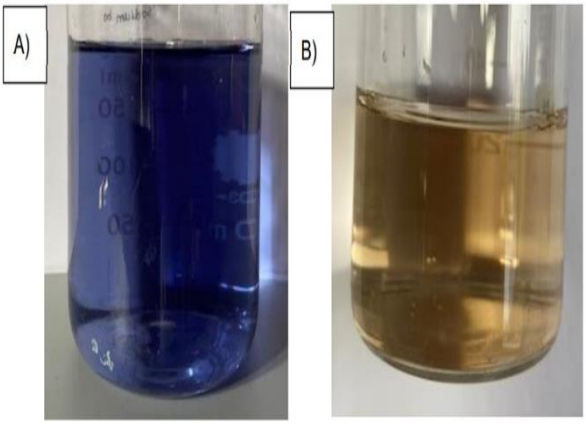

Figure 17. Photocatalytic degradation of EBT by K5NP. A) Before the addition of K5NP. B) After the addition of K5NP at 210min.

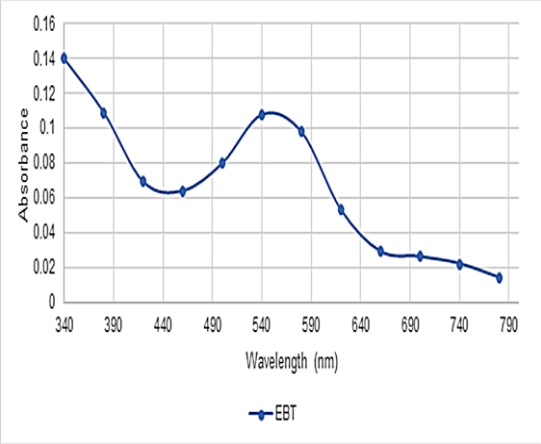

Figure 18. EBT dye absorbance.

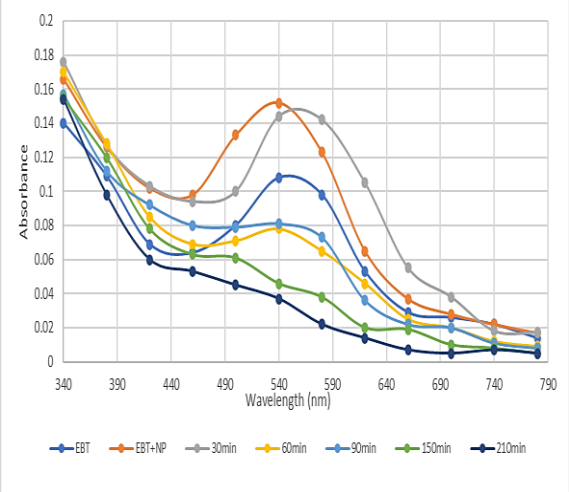

Figure 19. Photocatalytic activity at 10ppm of K5 AgNP

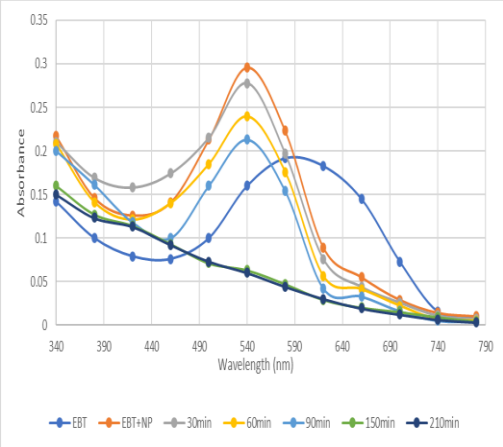

Figure 20. Photocatalytic activity at 10ppm of K5 AgNP with sodium borohydride.

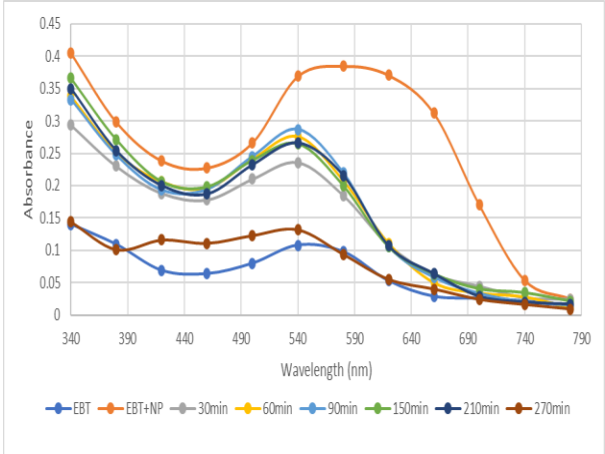

Figure 21. Photocatalytic activity 100ppm of K5 AgNP.

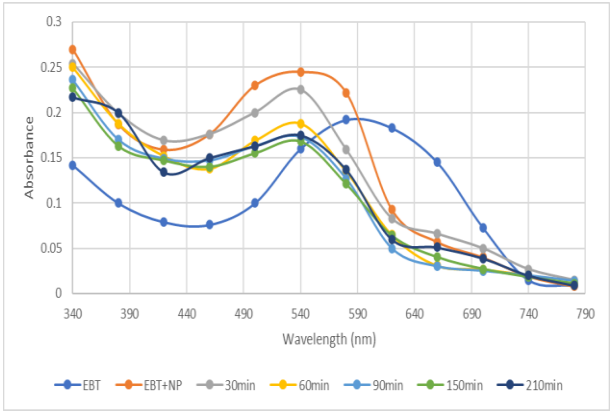

Figure 22. Photocatalytic activity 100ppm of K5 AgNP with sodium borohydride.

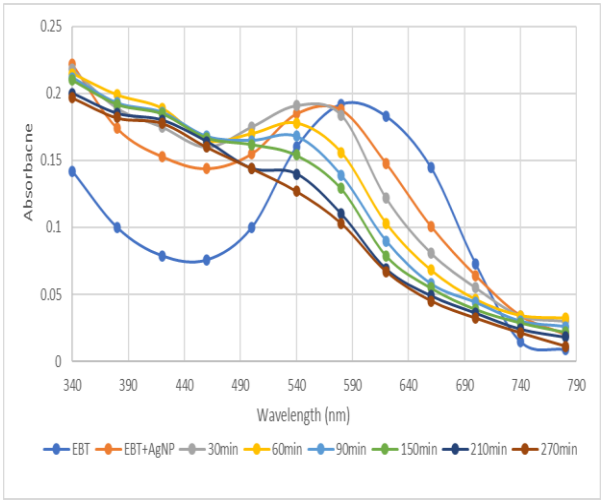

Figure 23. Photocatalytic activity at 500ppm of K5 AgNP.

4. Discussion

Nowadays the development of NP has become an attribute of the development of Richard Feynman laid down the concept of nanotechnology. The green synthesis of nanoparticle is a promising method to replace more complex physiochemical synthesis since it's free from toxic chemicals and hazardous byproducts.[2] Lantana species was hypothesized to be an efficient way to synthesize and stabilize NPs. AgNPs are the significantly used nanomaterial due to their physical, chemical, and biological characteristics and their superiority stem mainly from the size, shape, composition, crystallinity, and structure of AgNPs. In this study, leaf extracts were extracted using distilled water due to their ecofriendly nature. Phytochemical tests which were carried out on the Lantana sample gave positive results for tannin, saponin, alkaloid, terpenoids, carbohydrate, quinone, anthocyanin and coumarin (Figure 3). The presence of these reducing agents in the plants acts as natural capping agents for the stabilization of AgNPs.[24] However, Kalita *et al.* 2011, review of literature done with other solvent indicate the absence of these compounds. This difference might be due to the different polarity of the solvents.[10]

In this study, five lantana species leaf extract formed AgNPs successfully which was confirmed by primary indication, colour change of leaf extract to reddish after overnight incubation at RT with $AgNO_3$ solution. The colour change is due to the reduction of Ag+ ions to form Ag(O). Subsequently conducted spectroscopic studies for AgNPs confirmed this finding due to the unique optical properties of AgNPs making them strongly interact with a specific wavelength of light. AgNP showed an absorption peak in the visible region between 420-480nm, corresponding to surface plasma resonance (SPR) of K5NP shows elevated absorbance compared to other samples (Figure 2). Depending on the particle size, dielectric medium and chemical surrounding the absorption of AgNPs varies.[25]

The effect of temperature and time on the formation of AgNPs was analyzed using UV-Vis spectroscopy by heating AgNPs at 90°C for 30min and 60min. It was observed that there was no appearance of the peak which led to the conclusion of the absence of AgNPs except K4 at 60min (90°C) (Table 5). Due to prolonged exposure at 90°C, K4 sample was able to produce AgNP by stabilizing it with the phytochemicals. The AgNPs physiochemical properties are important for their bio-distribution, behaviour, safety, and efficacy.[26] Therefore, it has been characterized using scanning electron microscopy (SEM) in order to evaluate the functional aspects of the synthesized particles. SEM imaging results show surface morphology, agglomeration of nanoparticles and size was around 40 nm with spherical shape (Figure 4).[27,28.]

The SPR absorption band is formed due to the collective oscillation of the free electrons of AgNPs in resonance with a light wave. The conduction band and valence band of AgNPs lie very close to each other in which electrons move freely. The energy difference between the top of the valence band and the bottom of the conduction band in insulators (>4eV) and semiconductors (<3eV) is referred as the band gap.[29] According to the results, all the synthesized AgNPs are semiconductor (Table 7).

$$\text{Band gap energy } (E) = h \times C/\lambda$$

where h (Planck's constant) = 6.629 x10-34Js, C (Speed of light) = 3 x 108 m/s, and λ (Cutoff wavelength) which ranges from 420-460nm of the samples.

where h (Planck's constant) = 6.629 x10-34Js, C (Speed of light) = 3 x 108 m/s, and λ (Cutoff wavelength) which ranges from 420-460nm of the samples.

The classification of the samples is shown in Table 2 calculated using band gap energy.[30]

Table 7. Classification of conductance in synthesized AgNPs.

Sample	Band gap energy	Conductance
S1	2.82	Semiconductor
S2	2.95	Semiconductor
S3	2.58	Semiconductor
S4	2.58	Semiconductor
S5	2.82	Semiconductor

By using the aluminum chloride colourimetric method, TFC (QE) was estimated. Al^{+3} reacts with OH groups of the flavonoids establishing a stable flavonoid-Al^{+3} complex with a yellow colour where the intensity is proportional to the concentration of flavonoids.[31] The TFC of AgNPs was observed higher than leaf extract with AgNP of K4 being the highest among AgNPs followed by K2NP≈K1NP≈K5NP>K3NP and leaf extract followed by K4LE>K1LE≈K2LE≈ K5LE>K3LE where K4LE shows higher TFC among leaf extract (figure 5). The ONE- way ANOVA shows F > Fcrit (F-30.63463, Fcrit- 5.317655) indicating there is statistical significance between the groups. TFC in Lantana leaves study conducted by El-Sayed *et al*. in 2016 showed lesser value which might be due to the geographical difference, plant season and the extraction method.[32]

Using colourimetric assay is used to determine TPC (GAE) using Folin- Ciocalteu reagent which is a mixture of phosphomolybdate and phosphotungstate. The Folin-Ciocalteu reaction is a redox reaction where phenolic groups transfer an electron to phosphomolybdic and phosphotungstic acid compounds, in an alkaline medium. Sodium carbonate is the alkali that extents an optimum pH. The reducing acids change colour from light yellow to blue colour (reduced state) depending on the number of reacting phenolic groups.[33] The TPC of AgNPs was observed to be higher than leaf extract with AgNP K4NP was highest and other AgNPs have shown similar TPC (Figure 6). K4LE showed higher TPC among leaf extract followed by K1LE≈K2LE≈K5LE>K3LE. The ONE- way ANOVA shows F>Fcrit (F-30.66322, Fcrit-5.317655) indicating there is statistical significance between the groups. A similar study conducted by Kumar, Sandhir and Ojha in 2014 showed similar TPC in Lantana leaves.[13]

The TAC (AAE) of leaf extract and AgNPs were depicted and showed AgNPs have higher TAC compared to leaf extract. TAC in leaf extract is mainly constituents of the redox potential of Phyto constitutes, which satiating singlet and triplet oxygen and nullifying the free radicals. Therefore, the higher antioxidant activity of AgNPs might be due to preferential absorption of the antioxidant material from the extract onto the surface of the nanoparticles.[11] The overall highest TAC was observed to be higher than in K4NP among followed by K5NP>K1NP> K3NP≈K2NP. K3LE showed higher TAC among leaf extract followed by K1LE>K2LE≈K4LE≈K5LE (Figure 7). The ONE- way ANOVA shows F>Fcrit (F-14.12283, Fcrit-5.317655) indicating there is statistical significance between the groups. The recent study conducted by Kim and Lee in 2020 showed similar TAC results in Lantana leaves.[34]

Pearson's correlation coefficient was applied to evaluate the relationship between the antioxidant assays, including TFC, TPC and TAC. The correlation between TFC versus TAC was higher than the rest of the correlation (Figure 24). Flavonoid content is more responsible for the antioxidant property compared to phenolic content. A similar finding was recorded by Aryal and his coworkers in 2019.[35] The phenolic and flavonoid compounds have been reported to have high potent antioxidants because they possess the ability to neutralize free radicals. This possibly suggests that Lantana leaf extracts have higher antioxidant activity.[35]

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a stable compound that is reduced by accepting hydrogen/electrons. Antioxidants have the ability to donate an

electron to DPPH free radical leading to colour change from purple to yellow. The reducing activity of Lantana species leaf extract and AgNPs was quantified using spectrophotometer. DPPH scavenging activity of all the leaf extract was similar as well as the AgNP (Figure 8). Due to the encapsulation of bioactive molecules on the spherical surface of AgNPs through the electrostatic attraction between negatively charged bioactive compounds and neutral or positively charged NPs the antioxidant efficacy is higher in AgNPs compared to leaf extract. The AgNPs showed higher DPPH scavenging activity compared to leaf extract with AgNP being highest in K3NP.

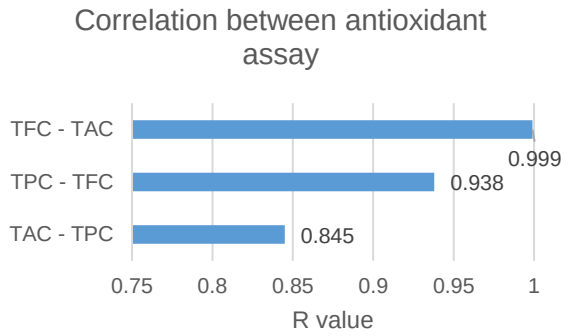

Figure 24. Correlation between antioxidant assays.

The ONE- way ANOVA generated for leaf extract vs AgNPs shows F>Fcrit value (F-4645.267, Fcrit-5.317655) indicating there is a significant difference between leaf extract and AgNPs.[36]

The IC_{50} value was intended to determine the concentration of the sample required to inhibit 50% of radicals. The lower the IC_{50} the higher the antioxidant activity of the sample (Figure 9 and 10). IC_{50} of the samples shows that leaf extract has a higher value compared to AgNPs followed by K1LE>K4LE>K2LE>K3LE>K5LE (Table 6). The highest IC_{50} was observed in K5NP followed by K3NP>K4NP>K1NP>K2NP. Leaf extract shows higher specific activity (%) than AgNPs. A similar study in 2013 by Khan and his coworker showed higher results, due to the geographical difference and different extraction procedure.[37]

AgNPs seems to be the potential antibacterial agent among the several promising nanomaterials due to their large surface to volume ratios and crystallographic surface structure. AgNPs have various mechanisms to inhibit the growth and proliferation of gram-positive and gram-negative bacteria. AgNPs damages the bacterial cell membrane, disrupts protein synthesis and the formation of ROS which lead to bacterial death. The leaf extract and synthesized AgNPs were able to inhibit the growth of *E.coli* and *S. aureus* to an extent. Compared to leaf extract, AgNPs showed a better inhibition zone. Samples showed higher inhibition in *E.coli* compared to *S. aureus* (Figures 11-16)

The ONE- way ANOVA generated for leaf extract vs AgNPs shows F>Fcrit value (F-59.45288, Fcrit-5.317655) indicating there is a significant difference between leaf extract and AgNPs. The recent study conducted by Mansoori and his co-worker in 2020 shows similar results. AgNPs indicate stronger antibacterial capabilities due to the antibacterial property of leaf extract enhanced with Ag ions.[38]

The catalytic activity of AgNPs was assessed by using EBT dye. It's a known fact that AgNPs shows greater catalytic activity in the reduction and removal of dye. Mallick *et al.* studied the catalytic activity of AgNPs synthesized using Lantana leaves on the reduction of phenosaffarin dye and Ajitha *et al.* studied the reduction of methylene blue by green aqueous extract of Lantana mediated AgNPs.[6,39]

In this study, green synthesized Lantana AgNPs is reported for EBT reduction. EBT was recorded to have maximum absorbance at 530 nm. In addition to sample at various concentration, EBT was reduced within a period of time but a remarkable decrease in absorbance

peak was observed at 10 ppm. Higher concentration 500 ppm was not able to fully degrade EBT in 270 min. At 10ppm K5NP was able to degrade EBT within 210 min but with the help of sodium borohydride, it degraded at 150 min (Figure 17-23).

At 100ppm K5NP degrade EBT within 270min however the addition of sodium borohydride didn't enhance the degradation at 100ppm. On the other hand, there was a blue shift observed at 540 nm, due to the decrease in particle size and accumulation of byproducts. Sodium borohydride ($NaBH_4$) is a reducing agent which acts as a catalyst in the degradation of dye. Since BH_4- ions are nucleophilic (donor) nanoparticles accept the electron and transfer to electrophilic nature dye (acceptor).[40] In 2021 a similar study conducted by Surendra and his coworker showed similar results.[41]

The rate constant was analyzed for further confirmation. According to Table 8, a comparison of each rate constant reveals that 10 ppm with sodium borohydride shows a better rate constant. This reveals that the lower concentration is the best concentration to degrade the EBT dye.[42]

Table 8. Rate constant of AgNPs degrading EBT at different concentration.

Concentration	Rate constant
10 ppm	0.2986
10 ppm + $NaBH_4$	0.3587
100 pm	0.103
100 ppm + $NaBH_4$	0.0755
500 ppm	0.0677

Conclusion

The present study demonstrates that Lantana leaves contain most of the phytochemicals which Phyto stabilized the Ag ions for the formation of AgNPs. All the variety of Lantana leaves were able to synthesize AgNPs. The water-soluble characterization of flavonoids in leaves were responsible for the spectacular reduction process of AgNPs. SEM analysis revealed the spherical shape of the AgNP. AgNP contains higher TAC compared to leaf extract. As well as AgNPs shows higher antibacterial activity compared to LE.

They have higher antibacterial activity against *E.coli* compared to *S. aureus*. Photocatalytic degradation of EBT by K5NP at lower concentration with the catalyst shows greater activity within a short period of time. It shows natural renewable and eco-friendly reducing agent used for the synthesis of AgNPs exhibits excellent photocatalytic activity and can be used in dye effluent treatment. Leaf mediated synthesis of AgNPs by using Lantana shows more compatible, eco-friendly, low cost, and less time-consuming approach.

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