

Green Synthesis and Characterization of Silver Nanoparticles using Banana and Papaya Leaves Extracts and their Applications

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Abstract – Nanoparticles display improved characteristics due to their size, distribution and are widely used in numerous scientific fields. Among metallic nanoparticles, silver nanoparticles (AgNPs) are very important especially due to their physiochemical and antimicrobial properties which help in therapies. The current study assessed the green synthesis and characterization of AgNPs using banana and papaya leaves extracts and their applications in antibacterial, antioxidant activity and as eco-friendly dyes degraders. In the present study, phytochemical analysis was performed for the screening of bioactive chemical constituents of both plants leaves extracts. The synthesized AgNPs were confirmed by the color changed from colorless to colloidal brown. The AgNPs were characterized by FTIR analysis. Synthesized particles were further investigated for their antibacterial activity by agar well diffusion assay, antioxidant activity using Butyl Hydroxytoluene (BHT) as standard and dye degradation ability. AgNP_s synthesized from banana leaf extract have shown antibacterial activity against all the tested organisms with high inhibitory action than that of nanoparticles AgNP_s from papaya plant extract. Highest antioxidant activity was exhibited by AgNP_s obtained using Papaya leaf extract compared to AgNPs obtained using banana leaf extract. In dye degradation assay, the both green synthesized AgNPs were able to degrade Methylene Blue, Safranin and Acridine Orange dyes and were unable to degrade of Methyl Orange, Methyl Red and Ethidium Bromide dyes. In conclusion, the green synthesized AgNPs from both the plants have shown antibacterial and antioxidant activities and were able to degrade dyes and thus can be used in processes of pollution remediation.

Keywords – Silver Nanoparticles, Phytochemicals, FTIR, Antioxidant Activity, Dye Degradation.

I. INTRODUCTION

In recent times the noble metal nanoparticles are subject of focus in research due to their unique optical, electronic, mechanical, magnetic and chemical properties. The synthesis of nanoparticles and applications are gaining intense importance in biomedicine, the smaller size of nanoparticles (1–100 nm), high surface area and reactivity provide them the ability for therapeutic purpose in different dosage forms and dosing routes (Jyoti and Singh, 2016). Physical and chemical methods are more popular for nanoparticles synthesis but the uses of toxic compounds limit their application and are economically not feasible. Microbial enzymes or the plant phytochemicals with anti-oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. Silver nanoparticles (AgNPs) are produced by the reduction of silver ions to neutral silver atoms. This is done by the reduction of silver ions using a reducing agent (Imran *et al.*, 2015). Papaya and banana are the medicinal plants which are used the synthesis.

Nanotechnology has been extended to the wastewater treatments in the recent years. Due to high surface area, AgNPs exhibits an enhanced reactivity (Kumar *et al.*, 2016). In the race of AgNPs preparation, plant mediated green synthesis of AgNPs is considered a widely acceptable technology as it is environmental friendly, rapid, cost effective, and a single-step method for biosynthesis. The current study assessed the green synthesis and characterization of silver nanoparticles using banana and papaya leaves extracts and their applications in anti-

-bacterial, antioxidant activity and in eco-friendly dyes degradation.

II. METHODOLOGY

The laboratory work was carried out from 25th July 2019 to 29th February 2020 at the Microbiology Department of Dolat-Usha Institute of Applied Sciences and Dhiru-Sarla Institute of Management & Commerce, Gujarat, India.

Sample Collection

The leaves of banana and papaya plants were collected from local areas of Valsad District. The fresh leaves were collected in polyethylene zipper bags and washed twice with tap water which was followed by washing with distilled water. Fresh weights of washed leaves were determined.

Extraction Method

Banana and Papaya leaves extracts were prepared by 10 g of fresh leaves, taken in separate beakers. Leaves were thoroughly washed twice with tap water followed by distilled water and cut into small pieces. The chopped leaves were boiled in 100ml of distilled water for 20 minutes, cooled and filtered by whatman No.1 filter paper. The obtained leaves extracts which appeared light green in color were stored 4°C after covering the beaker with aluminum foil for further use (Murugan et al., 2017).

Synthesis of Silver Nanoparticles

Stock solution was prepared by dissolving 1mM aqueous solution of AgNO₃ and used for the synthesis of silver nanoparticles (AgNPs). 10 ml of each leaf aqueous extracts (banana and papaya leaf extract) was added into 90 ml of AgNO₃ solution in separate 100 ml Erlenmeyer flasks and kept at room temperature for 24 h. All the reactions were performed in dark to avoid photo activation of AgNO₃ at room temperature. Complete reduction of AgNO₃ to Ag⁺ ions was confirmed by the color change from colorless to colloidal brown and was stored at 4°C for further use (Murugan et al., 2017).

Characterization of Synthesized Silver Nanoparticles

Determination of Phytochemicals

The chemical tests were performed on aqueous extracts to identify the various phytochemicals using standard procedures as described by Bisht et al., (2016). Both the plant extracts were accessed for phytochemical constituents namely Alkaloids, Saponins, Phenolic compounds, Tannins, Terpenoids, Flavonoids, Carbohydrate and Steroids.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was used to detect the presence of possible functional groups of biomolecules present in AgNPs acted as reducing and capping agent. For the analysis, 200 mL residual solution of silver nanoparticles was centrifuged at 10,000 rpm for 30 min and the precipitate was resuspended in 10 mL ethanol and then in sterile distilled water. The centrifugation and resuspension processes were repeated for 3–4 times. The purified suspension was dried in an oven to obtain the powder and analysed by FTIR, Thermo Nicolet AVATAR 330 (Nicolet, USA).

Determination of Antibacterial Activity

The synthesized silver nanoparticles from banana and papaya were assessed for their antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antibacterial was done using agar well diffusion method. The microbial culture was swabbed on nutrient agar plates. Plates containing media were divided into three equal parts; sterile cork borer was used to make a well on plates. Wells were loaded with 40µl AgNPs, 40µl of 1mM AgNO₃ and 40µl Leaf extract. The plates were incubated at 37°C for 24h to 72h. Inhibition zones were measured for each test organisms (Bisht et al., 2016).

Determination of Antioxidant Activity

In determination of antioxidant activity of synthesized AgNPs, purified butylated hydroxy-toluene (BHT) was used which served as standard for determining antioxidant power. 0.2 M phosphate buffer was added in to AgNPs along with 1% K₃ [Fe (CN) 6] and incubated at 50°C for 20 minutes. After incubation, 10% tri-chloro acetic acid was added and mixture was centrifuged for 10mins at 2000rpm. 1ml of supernatant with 1ml of deionised water and 250µl of 0.1% FeCl₃ was mixed and absorbance was recorded at 700nm (Nakkala et al., 2014).

Determination of Dye Degradation by Synthesized AgNPs

The catalytic study of dye degradation was performed without involvement of any chemical reducing agent. The degradation study was carried out by adding 1ml silver colloid into 10ml of dye solution and mixed. A control was also maintained without addition of silver nanoparticles.

Then 3ml of each dye solution was used to evaluate the dye degradation activity. The effect of volume variation of silver colloid on the degradation, different dye has also been investigated through performing the same experiments by adding 2 ml and 3 ml silver colloid. Absorbance measured at 660nm at different time interval (1h, 12h, 24h, and 48h). The dye degradation was indicated by color change and decrease in absorbance value (Jyoti and Singh, 2016). Percentage of dye degradation was estimated by the following formula:

$$\% \text{ Decolourization} = 100 \times (C_0 - C) / C_0 \quad (1)$$

Where, C₀ is the initial concentration of dye solution and C is the concentration of dye solution after degradation.

III. RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles from Banana and Papaya Plants Extracts

In the present study, AgNPs were synthesized from banana and papaya leaves extracts where leaves extracts were added in a AgNO₃ solutions and the color of the solutions were changed from faint yellowish brown to reddish brown and finally to colloidal brown indicating AgNPs formation (Figure 1 and 2).



Fig. 1. Synthesis of AgNPs using Banana leaf extract.



Fig. 2. Synthesis of AgNPs using Papaya leaf extract.

Characterization of Synthesized AgNPs

Phytochemical Analysis of Banana And Papaya Leaves Extracts

In the present study, the phytochemical constituents in both the plants were investigated and summarized in Table 1.

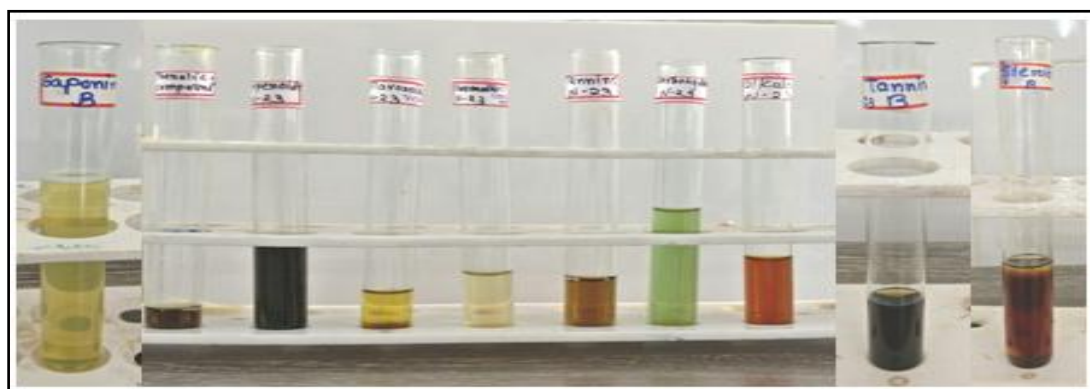


Fig. 3. Phytochemicals found in Banana Leaf Extract.



Fig. 4. Phytochemicals found in Papaya Leaf Extract.

Table 1. Results of Phytochemical Analysis.

Phytochemicals	Observation	Banana leaf extract	Papaya leaf extract
Saponins	Presence of foam	+	+
Phenolic compound	Color changes to dark green or bluish black.	+	+
Steroids	Green or red color precipitates	+	+
Tannins	Formation of green precipitates.	+	+
Terpenoids	reddish brown precipitates	+	+

Phytochemicals	Observation	Banana leaf extract	Papaya leaf extract
Flavonoids	Yellow precipitates, which become colorless on addition of diluted HCL	+	+
Alkaloids	Appearance of brown precipitates	+	+
Carbohydrate	Appearance of green, yellow, orange or red color or precipitates	+	+

All the tested phytochemicals, namely Saponins, Phenolic-compounds, Tannins, Flavonoids, Carbohydrate, Steroids, Alkaloids and Terpenoids, were found present in banana and papaya plants leaves extracts (Table 1). In 2012, Amin et al., from their studies, have reported that plant crude extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids and also states that the compounds are mainly responsible for the reduction of ionic into metallic nanoparticles formation. Similar work was also carried out by Bisht et al. in 2016 where they have reported alkaloids present in papaya leaves and served as an important element in defense against plant pathogens. Similar findings were also reported in our studies.

FTIR Analysis

FTIR spectroscopy was employed to study the possible bio molecules and functional groups involved in reduction, capping, efficient stabilization and characterization of synthesized AgNPs from Banana and Papaya leaf extracts shown in Figure 5 (A) and (B).

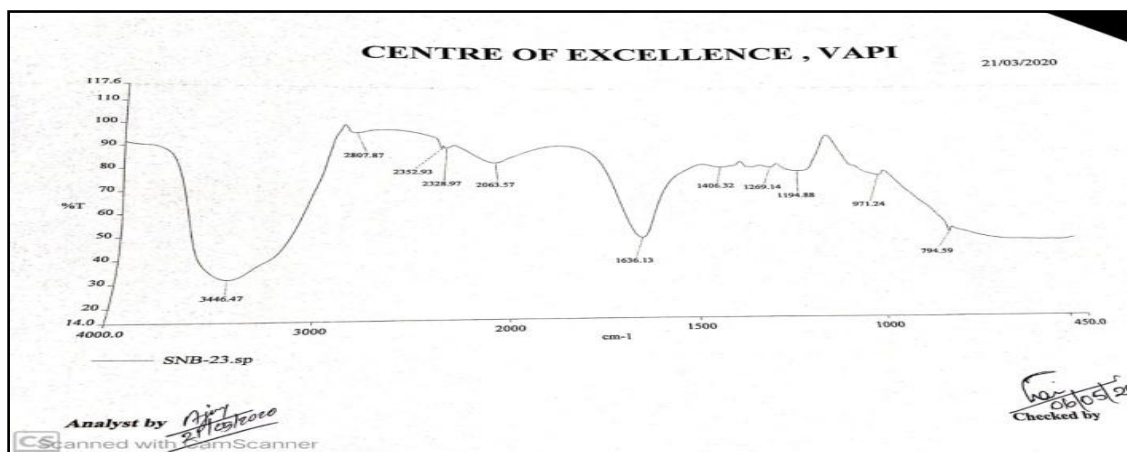


Fig. 5(A). Graph of FTIR analysis of AgNPs synthesized from Banana leaf extract.

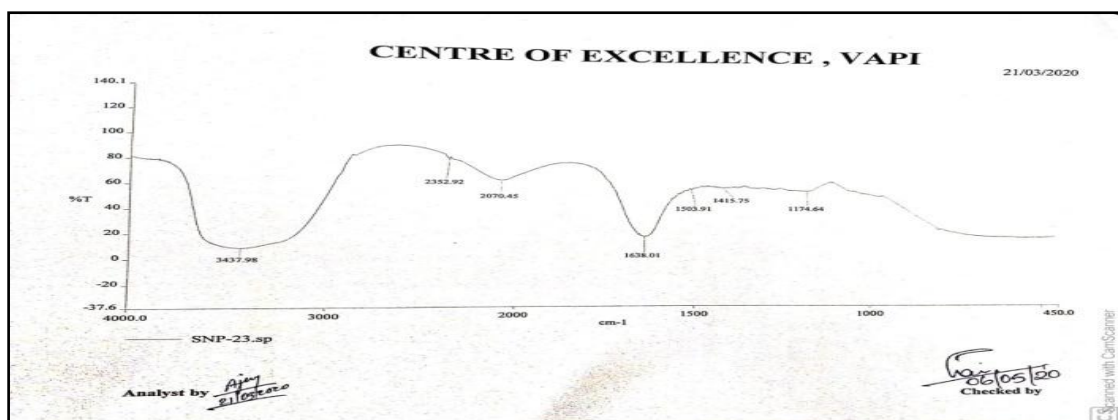


Fig. 5(B). Graph of FTIR analysis of AgNPs synthesized from Papaya leaf extract.

The IR spectrum, of synthesized AgNPs from banana, shows peak at 3446.47, 2807.87, 2352.93, 2328.97, 2063.57, 1636.13, 1406.32, 1269.14, 1194.88, 971.24 and 794.59 cm^{-1} that indicates the presence of functional groups -N-H, -C-H, $\text{-C}\equiv\text{N}$, $\text{-C}\equiv\text{N}$, $\text{-C}\equiv\text{C}$, $\text{-C}=\text{C}$, -C-O-H , -C-O , -C-O , $=\text{C-H}$ and $=\text{C-H}$ respectively which confirms the AgNPs synthesis. The IR spectrum, of synthesized AgNPs from papaya, shows peak at 3437.98, 2352.92, 2070.45, 1638.01, 1503.91, 1415.75, 1174.64 cm^{-1} that indicates the presence of functional groups -N-H, $\text{-C}\equiv\text{N}$, $\text{-C}\equiv\text{C}$, $\text{-C}=\text{C}$, $\text{-C}=\text{C}$, -C-O-H and -C-O respectively which confirms the AgNPs synthesis.

Antimicrobial Activity

Antibacterial Activity of Synthesized AgNPs using Banana and Papaya Leaves Extracts

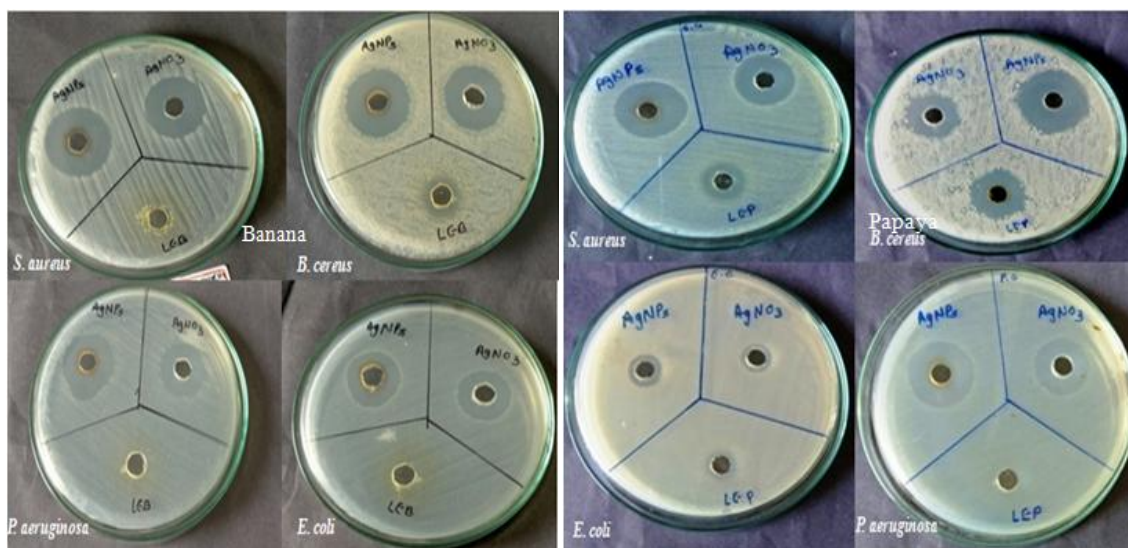


Fig. 6(A). Antibacterial activity of AgNPs from banana and papaya leaves extracts.

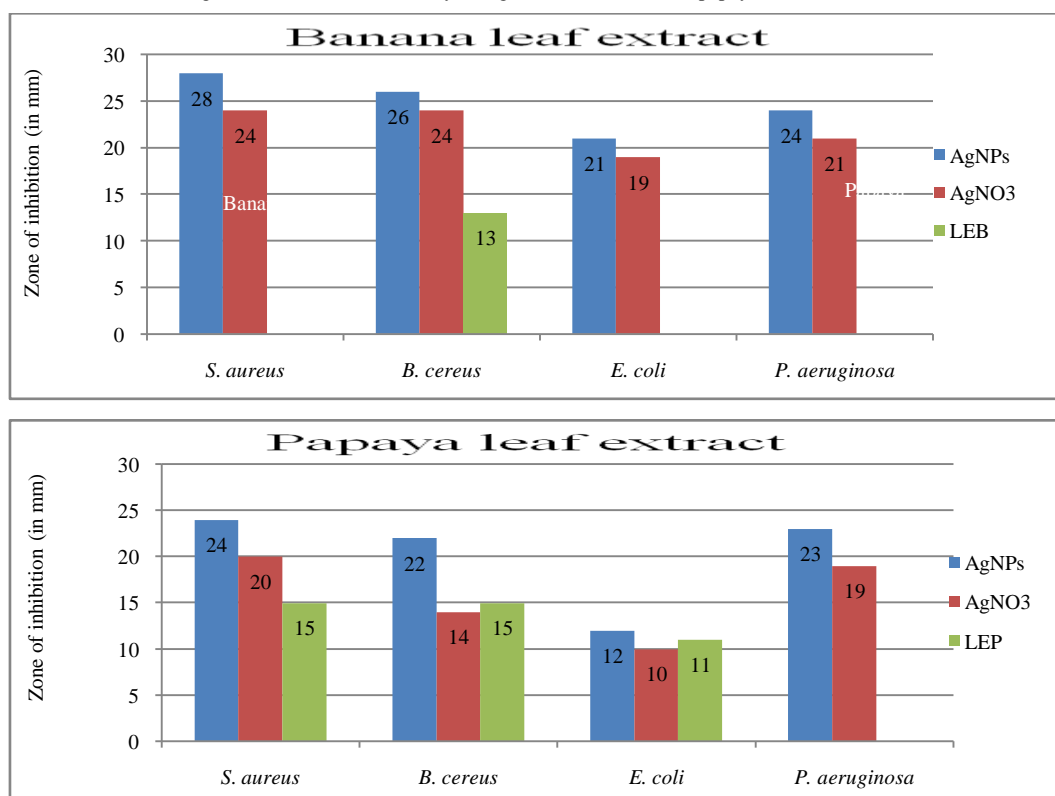


Fig. 6(B). Graph of antibacterial activity of AgNPs.

The results of anti-bacterial activity are presented in Figure 6. The anti-bacterial activity of the synthesized AgNPs obtained higher than that of plant extracts and control. Based on the inhibitory zone produced, synthesized AgNPs have shown good antibacterial activity against all the tested organisms. The highest antimicrobial activity of synthesized AgNPs from both the plants was found against *Staphylococcus aureus* and the lowest was found against *E. coli*.

In the present study, AgNPs synthesized from banana leaf extract have shown antibacterial activity against all the tested organisms with high inhibitory action than that of silver nanoparticles synthesized from Papaya plant extract. From all the four tested organisms, *S. aureus* was the highly sensitive towards both synthesized AgNPs (Figure 7).

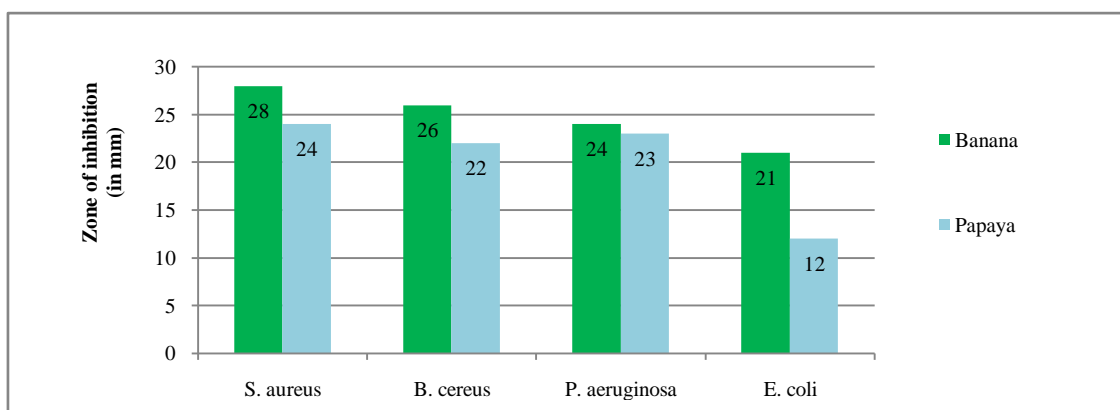


Fig. 7. Comparative study of antibacterial activity of synthesized AgNPs.

Similar study was also performed by Bisht et al. in 2016. In their study, have also performed antibacterial activity of synthesized AgNPs and found the zone of inhibition with AgNPs was 21mm for *Staphylococcus aureus*, 23mm for *Bacillus cereus*, 22mm for *Pseudomonas aeruginosa* and 11mm for *Escherichia coli*.

Antioxidant Activity

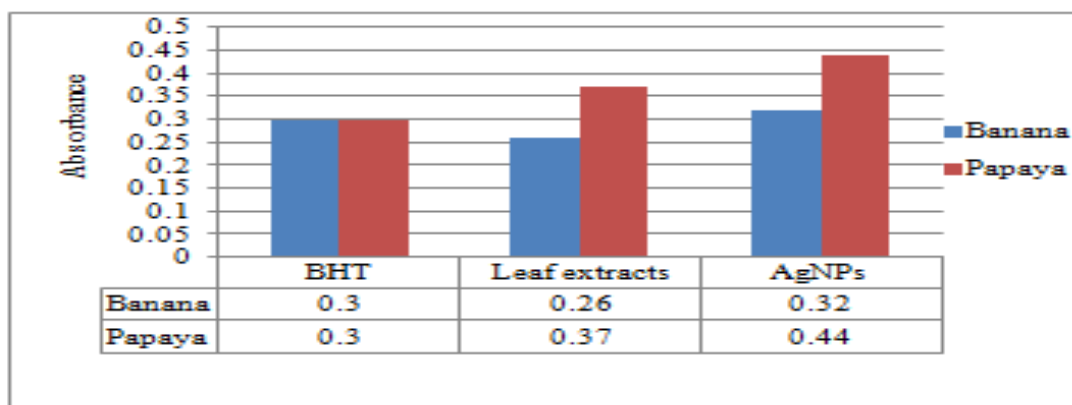


Fig. 8. Graph of activity and color observed with leaf extracts and AgNPs in reducing power assay.

In this assay, purified Butylated hydroxy-toluene (BHT) was synthetic antioxidant used as a standard. Highest antioxidant activity was exhibited by silver nanoparticles obtained using Papaya leaf extract compared to AgNPs obtained using banana leaf extract (Figure 8).

In 2016, Bhakya et al. have observed that that an increased absorbance can be correlated with the reducing ability of compound. In previous studies, similar findings were also reported by Nakkala et al., (2014) who

observed that the total reducing power of AgNPs and leaf extracts were determined by the standard ferric ion reducing assay where the Fe^{3+} ion gets reduced to Fe^{2+} by antioxidants. The present study indicated that the synthesized AgNPs have higher reducing potency showing higher antioxidant activities than that of leaves extracts (Figure 8).

Dye Degradation By Synthesized AgNPs

The release of dye effluents from textile industry is a major source of water pollution. This is one of pollutant of waste water and has potential threat to the environment; there-fore, treatment of that dye is highly desirable (Jyoti and Singh, 2016). Kumar et al., (2013) have reported that the AgNPs are highly efficient photo catalyst for degrading organic compounds and dyes under ambient temperature with visible light illumination.

In the present study, green synthesized AgNPs were tested for their dye degradation ability wherein six dyes were selected namely Methylene Blue, Safranin, Acridine orange, Methyl orange, Methyl red, and Ethidium bromide dyes. Out of six dyes, both synthesized AgNPs were able to degrade Methylene Blue, Safranin and Acridine orange and unable to degrade rest three dyes. The extent of dye's degradations using synthesized AgNPs were monitored by visual observation and through absorbance value taken measured by colorimeter. The main absorption decreased gradually with the exposure time indicating the degradation of dye.

Degradation of Acridine Orange Dye using Green Synthesized AgNPs

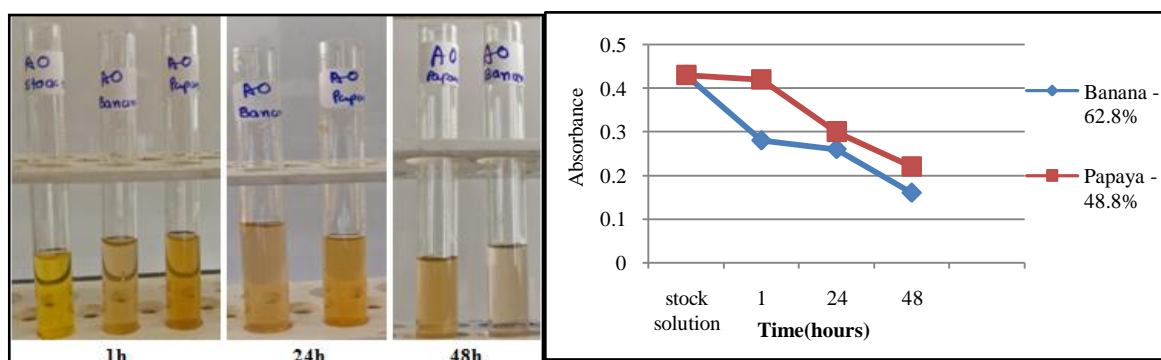


Fig. 9. Visual observation of color change and Graph indicates the degradation of Acridine Orange dye at different time intervals.

The green synthesis of AgNPs has reduced the pollution load in the environment to a greater extent by avoiding the use of hazardous chemicals. Degradation of Acridine orange dye, using green synthesized AgNPs, was visualized by a color change yellowish orange to light orange or near to colorless and decrease in absorbance value represented in Figure 9. The complete degradation was achieved within 48 h and was detected by the color change of reaction mixture. Absorbance was measured and was found to be decreased. 62.8 And 48.8 percent degradations were obtained after 48h of exposure time with AgNPs synthesized from Banana and Papaya leaf extracts respectively.

Kumar et al., (2016) reported that AgNPs are highly efficient photo catalyst for degrading organic compounds and dyes under ambient temperature with visible light illumination. The catalytic degradation of the AO occurs through N-de-methylation pathway through multistep process involving the attack of $\bullet OH$ radical which is a most powerful oxidant. In their study, researchers found that 3 ml silver colloid was sufficient for the complete degradation of AO without using any reducing agent within 120 min.

Degradation of Methylene Blue Dye using Green Synthesized AgNPs

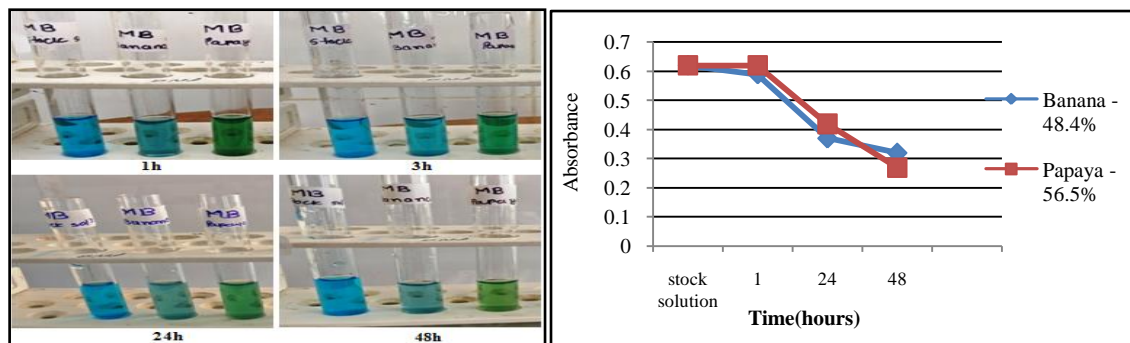


Fig. 10. Visual observation of color change and graph indicates the degradation of Methylene Blue dye at different time intervals.

Methylene Blue is a heterocyclic azo dye which is commonly released through the effluents of textile industries. It depletes oxygen from the surface of water bodies which in turn affects aquatic flora and fauna. In addition to being an environmental hazard, it is also known to cause toxicity in humans (Indana et al., 2016).

In the current study, degradation of Methylene Blue dye, using green synthesized AgNPs, was visualized by a color change from blue to light blue or near to colorless and decrease in absorbance value represented in Figure 10. The complete degradation was achieved within 48 h and was detected by the color change of reaction mixture. Absorbance was measured and was found to be decreased. 48.4 And 56.5 percent degradations were obtained after 48h of exposure time with AgNPs synthesized from Banana and Papaya leaf extracts respectively.

In 2012, Edison et al. have carried out catalytic activity of synthesized Ag NPs in the reduction of Methylene blue and was studied by UV-visible spectrophotometer. In their study, the synthesized AgNPs was found to have a good catalytic activity on the reduction of Methylene blue dye with AgNPs synthesized from *T. chebula* and was confirmed by decrease in absorbance of maximum values of Methylene blue with respect to time using UV-visible spectrophotometer.

Degradation of Safranin Dye using Green Synthesized AgNPs

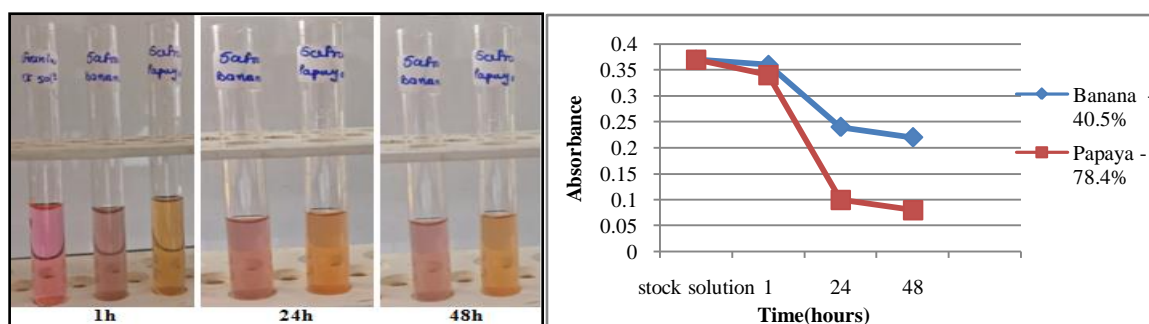


Fig. 11. Visual observation of color change and Graph indicates the degradation of Safranin dye at different time intervals

In the current study, visualization of color changed from pink to light pink or near to colorless confirms the Safranin dye degradation. The complete degradation was achieved within 48 h and was detected by the color change of reaction mixture. Absorbance was measured and was found to be decreased. 40.5 and 78.4 percent degradations were obtained after 48h of exposure time with AgNPs synthesized from Banana and Papaya leaf extracts respectively (Figure 11).

In 2016, Jyoti and Singh have reported that AgNPs are successfully used as a photocatalysts because of high surface to volume ratio, non toxic, cost effective and novel way of treatment of several dye pollutants. In their

studies, degradation of Safranin O was monitored by UV-visible spectrophotometer. The results revealed that the rate of degradation of Safranin was visualized by a decrease in peak intensity within 24 h of incubation time.

Degradation of Ethidium Bromide, Methyl Orange and Methyl Red Dyes using Green Synthesized AgNPs

In the present study, both the synthesized AgNPs were unable to degrade these dyes. The color of dyes were not changed with time and absorbance value measured was found to increased instead of decreasing that indicated that the synthesized AgNPs were unable in degradation of Ethidium Bromide, Methyl Orange and Methyl Red dyes tested.

Table 2. Comparative Study of Dye Degradation by Both Synthesized AgNPs.

AgNP, from two plant extracts	DYES					
	Acridine orange	Ethidium bromide	Methyl orange	Methyl red	Methylene blue	Safranine
Banana	62.8%	No Degradation			48.4%	40.5%
Papaya	48.8%				56.5%	78.4%

In the present study, the green synthesized AgNPs were able to degrade Methylene Blue, Safranin and Acridine Orange dyes. AgNPs synthesized from Banana leaf extract showed the higher degradation efficiency for Acridine orange than the AgNPs synthesized from Papaya leaf extract. AgNPs synthesized from Papaya leaf extract showed the higher degradation efficiency for Methylene blue and Safranin than the AgNPs synthesized from Banana plant leaf extract. On the contrary, the synthesized AgNPs were unable to degrade of Methyl Orange, Methyl Red and Ethidium Bromide dyes (Table 2). In 2019, López-Miranda et al. have reported that AgNPs have catalytic properties and they can be employed in the photo degradation of several compounds. The efficiency rate of dye degradation depends on the nanoparticles concentration. From their studies, they have reported the best results when highest concentration was employed.

IV. CONCLUSION

In the present study, banana and papaya leaves extract mediated AgNPs were synthesized successfully and the procedure was found to have fast and less expensive. The phytochemical constituents in both the plants extracts were investigated where all the tested phytochemicals, namely Saponins, Phenolic-compounds, Tannins, Flavonoids, Carbohydrate, Steroids, Alkaloids and Terpenoids, were found present in both the extracts. The both synthesized AgNPs were characterized by FTIR analysis where IR spectrum of AgNPs from banana have shown peaks that indicated the presence of functional groups -N-H, -C-H, -C \equiv N, -C \equiv N, -C \equiv C, -C=C, -C-O-H, -C-O, -C-O, =C-H and =C-H while IR spectrum of AgNPs from papaya have shown peaks that indicated the presence of functional groups -N-H, -C \equiv N, -C \equiv C, -C=C, -C=C, -C-O-H and -C-O which confirmed that the AgNPs have synthesized. In antibacterial activity, AgNPs synthesized from banana leaf extract have shown antibacterial activity against all the tested organisms with high inhibitory action than that of nanoparticles AgNPs from Papaya plant extract. Moreover, all four tested organisms, *S. aureus* was the highly sensitive towards both synthesized AgNPs. Highest antioxidant activity was exhibited by AgNPs obtained using

Papaya leaf extract compared to AgNPs obtained using banana leaf extract. In dye degradation assay, the both green synthesized AgNPs were able to degrade Methylene Blue, Safranin and Acridine Orange dyes and were unable to degrade of Methyl Orange, Methyl Red and Ethidium Bromide dyes. In summary, the green synthesized AgNPs are found to be safe to discharge in environment and can be utilized in processes of pollution remediation and agricultural research to obtain better health of crop plants. Moreover, green synthesis method offers efficient, economic and eco-friendly approaches to synthesize AgNPs without sophisticated instruments.

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