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Phytochemical Mediated Synthesis of Silver Nanoparticles using *Musa paradisiaca* Peduncle Latex and Its Photocatalytic and Antimicrobial Activity

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ABSTRACT

In the present work, green synthesis of silver nanoparticles (AgNPs) using a natural phytochemical agent has been described. The aqueous latex from *Musa paradisiaca* peduncle has been utilized as reducing as well as stabilizing agent. The formation of AgNPs was optimized by varying latex and $AgNO_3$ concentrations and finally reaction time. Ultraviolet-visible spectroscopic analysis showed the surface plasmon resonance peak between 350 and 450 nm confirms the formation of silver nanoparticles. X-ray powder diffraction analysis revealed the crystalline nature of AgNPs, Fourier transform infrared spectroscopy analysis revealed that AgNPs were stabilized by polyphenols and other aromatics present in the *Musa Paradisiaca* peduncle latex, while X-ray energy dispersive spectroscopy confirms the metallic nature. The field emission scanning electron microscopy and high resolution transmission electron microscopy showed the spherical shape of the particles and size distribution of AgNPs measured by dynamic light scattering which are in the range of 40 to 50 nm. The synthesized AgNPs showed photocatalytic activity on the degradation / removal of the methylene blue dye and the antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebseilla* bacterial species.

1. Introduction

Nowadays, metallic nanoparticles (MNPs) received wide interest as a result of their strong optical properties [1] in the areas of energy, catalysis, medicine and water treatment, etc., [2]. Currently, various chemical and physical processes are used to prepare MNPs, which enable nanoparticles to be obtained with the special characteristics. However, these synthesis methodologies are often expensive, laborious and harmful to the environment. Therefore, there is a necessity for alternative strategies such as the production of profitable nanoparticles that are both safe and environmentally friendly. In recent years, many studies have been shown that plants, algae, yeasts, fungi and bacteria can transform inorganic metal ions into MNPs [3-5].

Among various MNPs, silver nanoparticles (AgNPs) became the main focus of intensive research because of their huge range of applications in areas like antimicrobials, catalysis, optics and biomaterial production. Properties of AgNPs depend on their morphology, size and distribution. Numerous approaches have been used for the synthesis of MNPs using plant extracts [6]. These approaches have several benefits over chemical, physical and microorganism methods because these require no hazardous chemicals, high energy and also the elaborate culturing method [7]. In Asian countries, phytochemical compounds from plants have been used as drugs in ancient time. As an example, it's better-known that tea leaf extract contains powerful antioxidants like polyphenols, which is a regular drink offers necessary health benefits [8].

Table 1 shows some of the recently reported literature on the synthesis of AgNPs using plant extracts such as Setaria italic husk, Grewiaflaviscences, *Saccharomyces cerevisiae*, Citrus lemon, *Parkia speciosa* Hassk, *Acalypha hispida*, *Descurainia sophia*, *P. nigrum* (Black pepper) leaves, Belgian endive, *Cassia auriculata* and flower extract, Nelumbonucifera, Alpiniakatsumadai seeds extract, Tamarind fruit extract etc., [9-21].

The literature survey showed that naturally obtainable agricultural wastes were not studied for the preparation of MNPs. A classic example of a natural material so abundantly available is the *Musa paradisiaca* latex which is obtained by cutting the peduncle after the ripening of bananas.

Musa paradisiaca popularly called banana is a perennial herb like tree, a major crop in several semitropical and tropical regions around the world. Different plant parts of *Musa* are used topically or orally as remedy in folk medicine and roots, pseudostem, leaves, peel and fruits of *Musa* plants have shown antimicrobial activity, antioxidant and anti ulcerogenic activity and also shows anti diabetic and hypoglycemic activity [22, 23].

Table 1 Represented recent publications of bio-synthesis of AgNPs

Plant	Size (nm)	Shape	Reference
Nelumbo nucifera seed extract	10-15	Quasi-Spherical	[18]
P. nigrum	20-50	Spherical	[15]
Alpinia katsumadai seed extract	12.6	Quasi-Spherical	[19]
Tamarind fruit extract	10	Cubic & Spherical	[20]
Belgian endive	19-64	Quasi-Spherical	[16]
Cassia auriculata flower extract	10-35	Spherical &Triangle	[17]
Cassia javanica. L	435	Spherical	[12]
Carissa spinarum	20-50	Cubic	[13]
Descurainia sophia	35	Spherical	[14]
Setaria italica husk	30	Spherical	[8]
Grewia flaviscences	60	Spherical	[9]
Saccharomyces cerevisiae	10	Spherical	[10]
Citrus limon	10-30	Spherical	[11]

In this work, we synthesized AgNPs using latex of *Musa paradisiaca* peduncle as a reducing agent and the effective factors for its synthesis process by varying the metal ion and latex concentrations and time. AgNPs were characterized by Fourier transform infra-red spectroscopy (FTIR), X-ray diffractometer (XRD), particle size analyzer, field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). Antimicrobial and photocatalytic efficiency of the synthesized AgNPs are also studied.

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2. Experimental Methods

2.1 Materials

Silver nitrate ($AgNO_3$) was purchased from SD Fine Chemicals, Mumbai, India. Metheylene blue (MB) powder was purchased from Merck, Mumbai. The raw white latex was collected by cutting the green peduncle after separating fruits of the *Musa paradisiaca* plants near the place of Pulivendula, Kadapa (Dist.), Andhra Pradesh, India. White slurry latex was stored at 4 °C for further experiments. All solutions were prepared using double distilled water.

2.2 Preparation of Latex Extract

In a typical procedure, 1.75 mL of crude *Musa paradisiaca* peduncle latex was diluted in 49.25 mL of distilled water to make it 2.5 wt% solution and boiled at 80 °C for 15 mins. Using whatman No.1 filter paper the latex was filtered and stored at 4 °C for later use.

2.3 Qualitative and Quantitative Estimation of Phytochemicals

The qualitative and quantitative analysis of *Musa paradisiaca* peduncle latex was performed by the following methods of Arunachalam et.al. [24, 25] to determine the presence of phytochemical compound such as triterpenes, alkaloids, flavonoids, phenolics, saponins, and tannins [26-28]. The quantitative analysis of the total phenolic content was measured using the method of *Ciesla and Mariita et.al* [29, 30] whereas flavonoids were estimated using colorimetric method of aluminum chloride. Gallic acid has been used as a standard for the analysis of total antioxidant capability.

2.4 Synthesis of AgNPs using Musa paradisiaca Peduncle Latex

AgNPs were prepared by adding 2.5 mL of latex to 47.5 mL of 1 mM $AgNO_3$ solution and the reaction mixture was kept under dark conditions at room temperature in a laboratory environment. The reducing process of Ag^+ ions to AgNPs was monitored by measuring UV-Vis spectra at regular time intervals. The effect of $AgNO_3$ concentration (1-5 mM) was studied keeping the latex concentration constant (2.5%) and the effect of the latex concentration was varied (2.5%, 5%, 7.5% and 10%) maintaining the concentration of $AgNO_3$ constant at 1 mM. Once the reduction was complete, the AgNPs were isolated at 8000 rpm for 20 minutes in Remi (C24 plus modal) centrifuge and then washed with double distil water three times under centrifugation. Finally, the silver nanoparticles were dried and stored.

2.5 Characterization of Synthesized AgNPs

The formation of AgNPs was monitored using UV-Vis spectrophotometer (Lab India, Model 3092, Mumbai). The X-ray diffraction technique (XRD) is used to analyze the crystalline nature of synthesized Ag particles. The XRD pattern of dried AgNPs was recorded on Rigaku mini 600 using Cu Kα radiation X-ray diffractometer. Dried AgNPs were grinded with KBr to make pellet and Fourier transforms infra-red spectroscopy (FTIR) spectra was recorded using PerkinElmer spectrophotometer. Field emission scanning electron microscopy (FESEM) with energy dispersive X-ray analysis (EDAX) was carried out on SUPRATM with co-relatively microscope SEM machine. Dried powdered sample was placed on the SEM grid and the images were taken for size and morphology and EDAX spectra were also taken along with SEM images to find out the chemical composition. TEM sample was prepared by placing a droplet of aqueous dispersion on 200 mesh copper grids coated with carbon and dried at ambient conditions for 10-12 hours. TEM images were collected using a JEOL 3010 at 200 kv microscopy. The average size and distribution was measured using Zeta Sizer model Nano-S90 (Malvern U.K) using nanoparticles dispersion.

2.6 Antimicrobial Activity

Antimicrobial efficiency of synthesized AgNPs was examined against *Pseudomonas aeruginosa*, *Staphylococcus aureus, Klebseilla* and *Escherichia coli* microbial species. This was carried out with 24 h active cultures of the selected strains of bacteria by disk diffusion method [9]. The four tested organisms are swiped over the agar medium with help of sterilized spreader, and the silver nanoparticles containing disks were kept over the medium using sterile forceps. AgNPs disks were prepared separately at different concentrations (10, 20, and 30 μL) using 1 mg/mL aqueous dispersion and 1% streptomycin solution (antibiotic) was used as control .The plates were incubated at 35 °C for 24 h, finally the zone of inhibition zone (ZoI) around the well was measured.

2.7 Photocatalytic Activity of Synthesized AgNPs

Photocatalytic activity of synthesized AgNPs were studied for the degradation of methylene blue (MB) dye prepared in an aqueous medium. The photocatalytic degradation reaction was carried out under sunlight with continuous agitation. In a typical experiment, 15 mg of AgNPs catalyst was loaded in 50 mL of 20 μg/mL aqueous MB solution. The average ambient temperature during the experiment was found to be around 30 °C at our university. The intensity of sunlight is around 80,000 flux. At regular time intervals, 2 mL of suspension were pipetted and centrifuged at 5,000 rpm for 10 min to eliminate the catalyst and the absorbance was measured using UV-Vis spectrophotometer.

3. Results and Discussion

The preliminary quantitative and qualitative analysis of aqueous latex confirms the presence of phenolics, flavonoids, alkaloids, tannins, terpenoids and sterols as shown in Table 2. Phenolic, terpenoids and flavonoids compounds shows a broad range of biological activity, such as antioxidants, inhibition of lipid per-oxidation etc. The activity of the compounds depends mainly on their molecular structure and position of functional groups, such as the hydroxyl.

Table 2 Estimated quantity of phytochemical compounds present in *Musa paradisiaca* latex extract

Phytochemical Compounds	μg/g
Phenolics	102.59 ± 0.6
Terpenoids	39.48 ± 1
Alkaloids	0.045 ± 0.0002
Tannins	0.035 ± 0.0001
Flavonoids	26.55 ± 0.3
ABTS	85.76 ± 0.25

The size, shape, concentration, agglomeration state and refractive index near the surface of MNPs are responsible for the observed optical properties, hence, UV-Vis spectroscopy becomes an important tool to identify, characterize and study the MNPs [31]. After the addition of latex to the $AgNO_3$ solution, a visible color change was observed [9, 10] and the intensity of the color increased with reaction time, the latex and silver nitrate concentrations. Finally, a dark brown transparent dispersion was formed (Fig. 1) which suggested the formation of AgNPs. The presence of phytochemical compounds such as phenols, alkaloids, terpenoids, tannins, flavonoids and antioxidants in latex could be involved in reducing Ag^+ ions and stabilization of formed AgNPs during the reaction [32].

Fig. 1 Visual observation of change in color of the solution during the reduction reaction

The green synthesis of AgNPs has been studied using 1 mM aqueous solution of $AgNO_3$ with different concentrations of latex and the results are shown in Fig. 2. The characteristic absorption band of surface plasmonic resonance (SPR) at 360-450 nm was observed. The absorbance of this band increases with increasing the concentration of the latex to 5% and further increase in concentration decreases the absorbance. The increase in the concentration of the latex obviously increases the percentage of AgNPs formation because of availability of more reducing agents. However, the observed decreases in the absorbance of the characteristic band greater than 5% concentration of the latex could be due to the formation of larger aggregates.

A concentration variation study of silver nitrate (1-5 nM) using 2.5% of latex as a constant was studied and results are presented in Fig. 3. As the concentrations silver ions increases the intensity of the SPR band between 360-450 nm also increases suggest that more and more quantity of silver particles formed [32]. Fig. 4 shows the UV–vis spectra of AgNPs

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synthesized at various time durations. The characteristic SPR band between 360-450 nm of AgNPs appeared within 10 min of reaction time and the intensity of the band increases with increase in time.

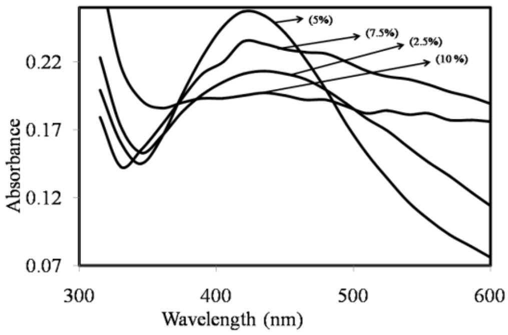

Fig. 2 UV–Vis spectra of silver nanoparticle dispersions with varied concentration (2.5%, 5%, 7.5% and 10%) of latex

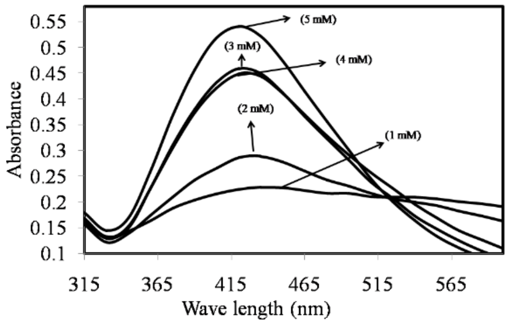

Fig. 3 UV–Vis spectra of AgNPs dispersions with varied concentration (1, 2, 3, 4 and 5 mM) of $AgNO_3$ solutions

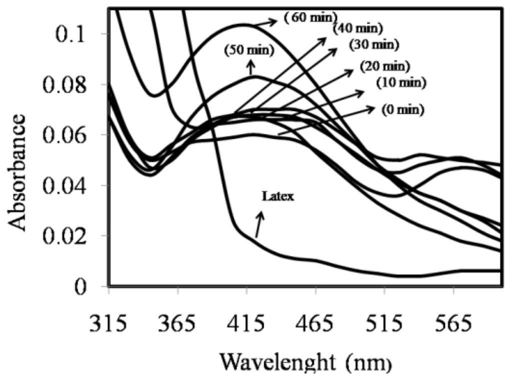

Fig. 4 UV–Vis spectra of AgNPs formation at different time intervals with 2 mM of $AgNO_3$ and 2.5% of latex

From all the above variations studied, it was decided to use 2 mM $AgNO_3$ and 2.5 % latex concentration and 50 min of reaction as standard for all further studies [33].

XRD pattern of AgNPs displayed in Fig. 5 showed the Bragg's reflections with 2θ values 38.15°, 44.31°, 64.48° and 77.42° lattice planes in the diffractogram. These values could be indexed to (111), (200), (220) and (311) planes of the face centered cubic (FCC) structure, which confirms the crystallinity. The data obtained were compared with the database of the joint committee on powder diffraction standards (JCPDS file No. 04-0783) which is in good agreement with the standard values [31, 32].

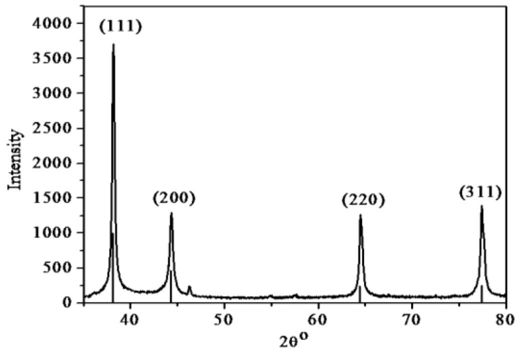

Fig. 5 XRD pattern of synthesized AgNPs by using *Musa paradisiaca* peduncle latex

The FTIR spectra of AgNPs synthesized using latex were presented in Fig. 6. The band at 3430.91 cm^{-1} represents to the O-H groups and also the H-bonded alcohols and phenols of latex. The band at 2933.36 cm^{-1} represents to C-H vibrations indicates the presence of alkanes. The band at 2417.14 $cm^{-1}$and 1613.28 cm^{-1} represents and primary amines groups present within the latex. The band at 1383.16 cm^{-1} indicates C-H rock alkenes and at 1105.72 cm^{-1} to that of C-O stretching carboxylic acids, esters, alcohols, and ethers. This analysis provides evidence for the presence of metabolites and proteins such as terpenoids with functional groups of ketones, alcohols, aldehydes, phenols, alkaloids, tannins, flavonoids and carboxylic acids, which act as reducing agents and help to increase stability of the synthesis AgNPs [31, 32].

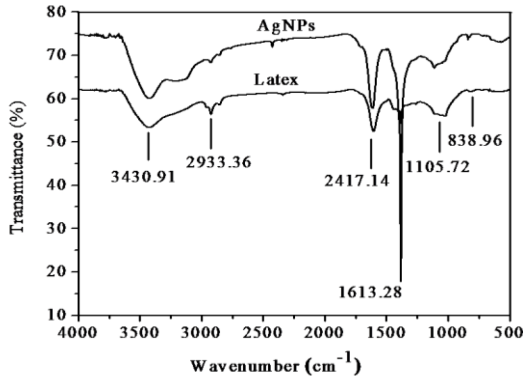

Fig. 6. FTIR spectra of synthesized AgNPs by using *Musa paradisiaca* peduncle latex

Scanning electron microscopy (SEM) will provide information of the morphology and size of the nanoparticles. FESEM micrograph presented in Fig. 7 suggests that the AgNPs were well dispersed without much aggregation and had a spherical shape. The average particle size is around ~ 40 nm. EDAX spectra shows (Fig. 8) strong signal for silver at the energy level of 3 keV and also some of the weak signals, which might due to the present of C, K, O, Ca, Mg, Na and Si elements.

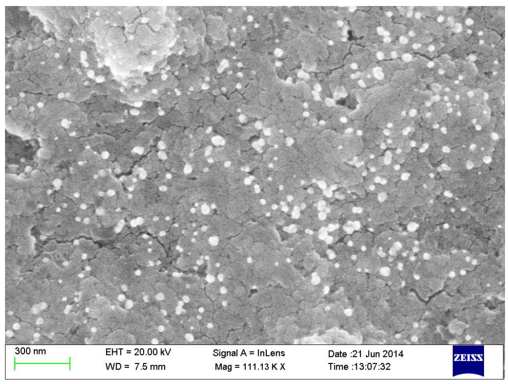

Fig. 7 FESEM image of synthesized AgNPs by using *Musa paradisiaca* peduncle latex

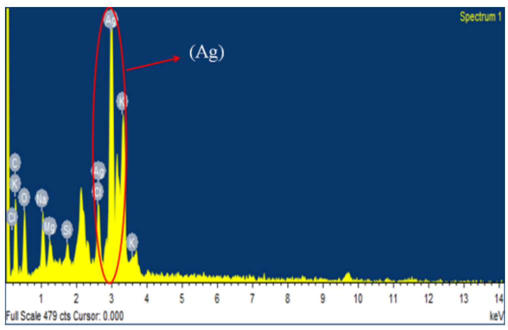

Fig. 8 EDAX spectrum of AgNPs synthesized by using *Musa paradisiaca* peduncle latex

TEM image of the AgNPs displayed in Fig. 9 reveals that the prepared AgNPs are dispersed and spherical in shape with some agglomeration at some places. Average size of the AgNPs was around ~40 nm. A selective area electron diffraction pattern depicted in Fig. 9 confirms the crystallinity of AgNPs. Particle size and distribution of AgNPs were also measured using particle size analyzer and the results are displayed in Fig. 10. The histogram showed that most of the particles are within 35 nm, however, the size of the particles are ranged between 30-50 nm.

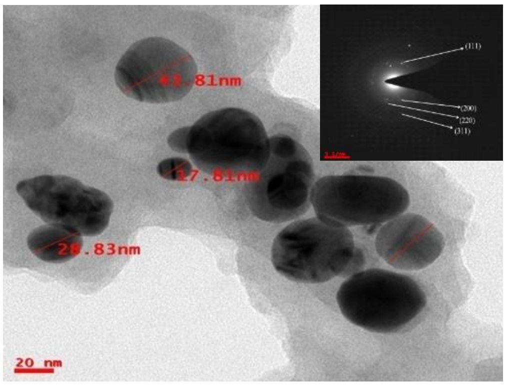

Fig. 9 HRTEM Image of synthesized AgNPs (inset: SAED pattern) by using *Musa paradisiaca* peduncle latex

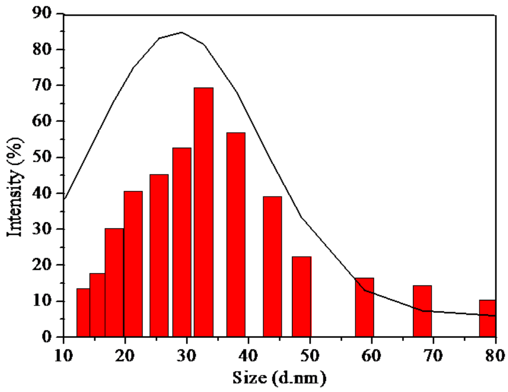

Fig. 10 Histogram of particle size distribution of synthesized AgNPs by using *Musa paradisiaca* peduncle latex

It is very difficult to find out exact mechanism behind the formation of nanoparticles by using natural phytochemical compounds because they contain diverse molecular structures, and need more detailed studies. In recent years some research groups have suggested possible mechanisms, while synthesizing gold and silver nanoparticles from various plants extracts such as geranium, neem, lemon grass and chickpea seeds etc. The proposed mechanism suggested by Mittel et al. [5] demonstrated that organic compounds such as alkaloids, flavonoids, phenolic compounds, triterpenes, saponins and tannins are responsible for the reduction and stabilization of MNPs. In this work, *Musa paradisiaca* peduncle latex also contained good amounts of these compounds, which can be effectively utilized for biosynthesis of AgNPs [26, 28, 34].

The phytochemical compounds such as, flavonoids, phenolics, triterpenes, saponins, and tannins etc., contains a high quantity of hydroxyl groups. The latex was added to Ag^+ solution, the Ag^+ reacts with the phytochemical complex and forms an intermediate and later converted to Ag^0 particles followed by coalescence, cluster formation and growth of aggregation,. The phenolic groups present within latex are oxidized and converted to the quinone form. Difference in electrochemical potentials of Ag^+ and the phytochemicals is the driving force for the reaction. The AgNPs formation might be stabilized due to the presence of lone pair of electron and the π electrons of the quinone structures [31].

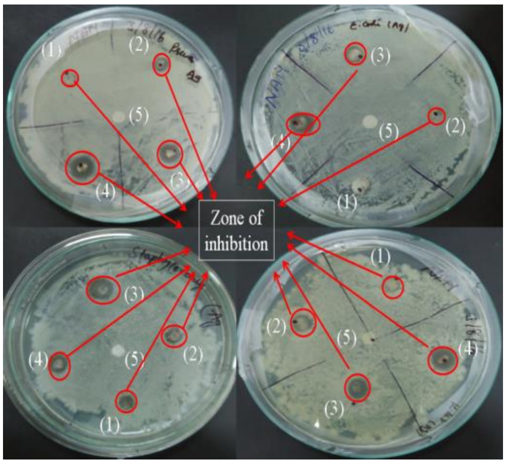

Fig. 11 Antimicrobial activity of synthesized AgNPs by using *Musa paradisiaca* peduncle latex against gram negative and gram positive bacterial culture

Antimicrobial activity of synthesized AgNPs against *Klebsiella, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli* Sp, was studied and zone of inhibition (ZoI) results were presented in Table 3 and the images were displayed in Fig. 11. Five sterilized disks were placed in each plate. Disk nos. 1, 2 and 3 is filled with 10, 20 and 30 µL of 1 mg/mL aqueous dispersion of AgNPs. The disk no. 5 is filled with pure latex and disk 4 is filled with 30 µL of 1% *streptomycin* (antibiotic) solution as standard. The results suggested that increase in concentration of AgNPs increases the inhibition towards the growth of bacteria. Compared to well no 3 and 4 which were filled with same quantity of silver nanoparticles and *streptomycin,* the antibiotic showed better ZoI values. No inhibition was observed in disk 5 which is filled with pure latex.

Table 3 Results of antimicrobial activity of synthesized AgNPs

S. No	Tested Pathogens	Zone of Inhibition (mm)				
		10 µL	20 µL	30 µL	Streptomycin	Latex
1	*E.coli*	0	7	9	14	0
2	*Staphylococcus aureus*	7	9	12	20	0
3	*Pseudomonas aeruginosa*	0	8	10	13	0
4	*Klebsiella*	7	9	10	15	0

Many researchers were studied photocatalytic activity in the area of dye removal/degradation using AgNPs and their composites [35-37]. Compared to irradiation and other techniques, solar light irradiation technique was found to be faster in the decolorization of MB in the presence of AgNPs as a catalyst [38]. In this work, photo-catalytic activity for degradation of MB using synthesized AgNPs by solar irradiation technique was studied and the degradation results in terms of absorbance of dye solution were presented in Fig. 12.

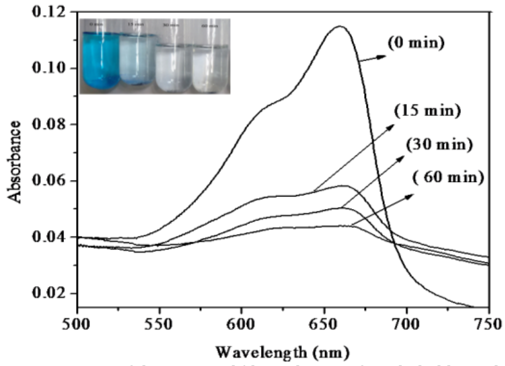

Fig. 12 UV – Vis spectra of dye removel/degradation of methyle blue solution using synthesized AgNPs by *Musa paradisiaca* peduncle latex (inset: Decolorization image of MB)

The characteristic absorbance of methylene blue solution was found to be 664 nm. Removal/degradation of MB was visualized by decrease in peak intensity within 1 hr under solar irradiation. Initially, the adsorption of AgNPs in MB solution is low and further increased with increase in time. This confirms that AgNPs acted as an electron transfer mediator between MB due to the electron relay effect [33]. The dye removal/degradation(%) was calculated using the equation,

$$\text{Dye degradation\%} = \left[\left(C_0 - C_t\right)/C_0\right] \times 100$$

where, C_0 is the initial concentration of the MB solution and C_t is the concentration of the dye solution at time *t* min of exposure to solar irradiation, and the results are shown in Fig. 13.

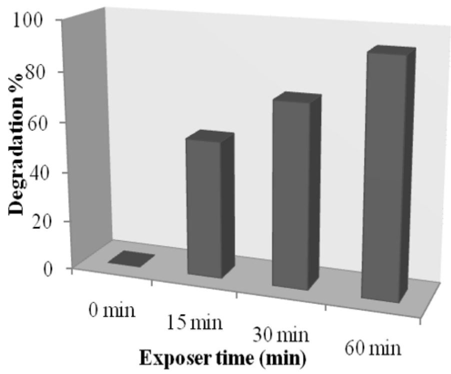

Fig. 13 Percent removal/degradation of MB dye at different exposure time using synthesized AgNPs by *Musa paradisiaca* peduncle latex

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4. Conclusion

Easy, rapid and well dispersed AgNPs were effectively synthesized by using *Musa paradisiaca* peduncle latex at room temperature. The effects of latex and $AgNO_3$ concentrations and also the time on formation of AgNPs have also been studied and optimum concentration of $AgNO_3$ and latex were found to be 2 mM and 2.5 %, respectively in 50 min of reaction time. These AgNPs were well characterized and found to be spherical and dispersed well with average size of ~40 nm. These spherical shaped AgNPs exhibited good photo-catalytic activity under solar light irradiation for the degradation of MB dye and also showed good antimicrobial activity towards *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* and *E. coli* bacterial species.

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