



ISSN: 0976-3031

Available Online at <http://www.recentscientific.com>

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 10, pp. 21098-21106, October, 2017

**International Journal of
Recent Scientific
Research**

DOI: 10.24327/IJRSR

Research Article

ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLE USING BANANA (*MUSA ACUMINATE COLLA*) PEEL, ITS PHYTOCHEMICAL, ANTIMICROBIAL AND ANTICANCER ACTIVITY

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DOI: <http://dx.doi.org/10.24327/ijrsr.2017.0810.1014>

ARTICLE INFO

Article History:

Received 15th July, 2017
Received in revised form 25th
August, 2017
Accepted 28th September, 2017
Published online 28th October, 2017

Key Words:

BPE; antioxidant activity; silver nanoparticle; characterization; nano conjugation; cytotoxicity study

ABSTRACT

Green synthesis of nanoparticle is gaining more attention of research towards eco-friendly biosynthesis of NPs. In this study, possible role of banana peel extract (BPE) in reducing silver nitrate into silver NPs is highlighted. The biosynthesical NPs were characterized using UV, FTIR, SEM, and XRD. The data revealed that the silver nanoparticle was synthesized after 24-48 hrs of incubation. The UV – VIS surface plasmon resonance peak at 380nm confirmed the Ag⁺ and Ag⁰ ions. FTIR spectra implicate the absorption band at 987.25cm⁻¹ to 3867.39 cm⁻¹ in the formation of SNPs. SEM analysis showed 20-50 nm NPs were within the average and mostly spherical in shape. XRD analysis showed 3 peaks at 250, 370, 510, Confirmed the size of the NPs (82nm). The Phytochemical and antioxidant study was confirmed before and after synthesis of Nps. The synthesized NPs was combined with Azithromycin and the drug release study was carried out. 10mg of drug combination gave good drug release study (78%). The antibacterial activity was observed against all pathogens tried which proved the better candidate for pathogens. The BPE NPs showed the 51.75 % of cell death against HeLa Cell Line using MTT assay. The drug coated BPE NPs showed maximum zone of inhibition against *E.coli*. This recent research suggests the potential application of BPE AgNPs as novel alternative agent in medical application.

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INTRODUCTION

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology (Natarajan *et al.*, 2010). Nanoparticles research is inevitable today not because of only application and also by the way of synthesis (Gopinath *et al.*, 2012). Nanoparticles have unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nano device fabrication and in medicine (Jain *et al.*, 2008).

The unique properties of nanoparticles, especially in the size range 1–100 nm, has made nanotechnology the most interesting area of research. The size, shape, and morphology are the crucial parameters deciding the property of nanoparticles (Nilesh and Raman, 2016) Hence, many studies were conducted in controlling the size and shape of nanoparticles during the synthesis leading to the conclusion of the method of synthesis playing a major role. Though there are many methods of synthesis including chemical and physical methods, the green synthesis of nanoparticles has gained prominence in recent years (Ahmed *et al.*, 2016). Many nano materials have been developed such as copper, zinc, titanium magnesium, gold, alginate and silver (Das *et al.*, 2014). However, silver

nanoparticles (AgNPs) outperformed their counterparts in the ability to work as antimicrobial against bacteria, viruses, as well as other eukaryotic microorganisms.

Among different metal nanoparticles, silver (Ag) nanoparticles have been used enormously due to their potential anti-bacterial (Mohanta *et al.*, 2016) anti-fungal and anti-proliferative activity (Nayak *et al.*, 2015). Silver nanoparticles are important materials that have been studied extensively, such nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nano device fabrication and in medicine (Nair and Laurencin, 2007). Silver nanoparticles (Ag-NPs) are now widely studied nanoparticles due to their antimicrobial, anticancer and antiviral activities and their use in consumer products including electronics, paint, clothing, food and medical devices. The diverse applications of silver nanoparticles are due to their characteristic properties of magnetic and optical polarization, catalysis, electrical conductivity, DNA sequencing and surface-enhanced Raman scattering (Pratibha, 2015).

Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer

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and heart disease (Kanazawa, and Sakakibara, 2000). Bananas are one of the most popular fruits on the world and it well be known that fruits contain various antioxidants compounds such as gallicocatechin (Someya et al., 2002) and dopamine (Kanazawa and Sakakibara, 2000). Since the banana fruits are widely available, they have been used as food without apparent toxic effect. The peel could be a potential source of antioxidant and antimicrobial activities.

Bananas are consumed all over the world, after consumption of the pulp, the peels are generally discarded (Bankar et al., 2010), banana a tropical plant may protect itself from the oxidative stress caused by strong sunshine and high temperature by producing large amounts of antioxidant (Lii et al., 1982).

Banana peels are major agricultural wastes which have been used as medicine, animal feeds, blacking of leathers, soap making, fillers in rubber and so on (Arawande and Komolafe 2010). Banana peel form about 18- 33% of the whole fruit and are considered as a waste product. They are the good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health (Larrauri et al., 1999, Wolfe et al., 2003). Banana peel is also rich in dietary fibre, proteins, essential amino acids, polyunsaturated fatty acids and potassium (Emaga et al., 2007).

At present, these peels were not being used for any other purposes and are mostly dumped as solid waste at large expense. The present study reveals the phytochemical constituents and antioxidant activity of banana peel extract, synthesizing of banana peel extract (BPE) with AgNO₃ in different concentration, its characterization by UV – VIS Spectrophotometer, FTIR, SEM and XRD. The synthesized nanoparticle were used as antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, nano conjugation with azithromycin, its efficacy study, drug releasing capacity, anti textile activity against multidrug resistant pathogens and anticancer activity against HeLa cell line.

MATERIALS AND METHODS

Collection of Samples and materials used

Banana (*Musa acuminata Colla*) fruits were purchased from local market (Mavelikara, Kerala, India) at yellow stage without any ethylene treatment and stored at 37°C for 24hr before being extracted. Silver nitrate (Molecular weight 169.87) was purchased from Hi Media, Mumbai. HeLa cells were purchased from Pune, India.

Preparation of Banana Peel Extracts (BPE) and identification of bioactive compounds

Preparation of Banana Peel Extracts (BPE)

Banana peels were cut into small pieces, and air dried under the shade of sunlight for 2-3 days, 10g of banana peels were taken and crushed with 100ml of distilled water, after mixing, this were heated in a water bath at 40°C for 1 hour and the extract thus formed was filtered through a What man No. 1 filter paper to remove insoluble fractions and macromolecules. The resultant filtrate was stored at 4°C for further study.

Phyto chemical assay

The BPE samples were tested with the presence or absence of the phytohormones (alkaloids, terpenoids, phenol, sugar, saponins, flavanoids, quinines, protein, and steroids) it determined by the colour change of solution for the presence or absence of the phytohormones in the BPE sample, according to the procedure of Monisha et al., (2017).

Total phenol and total antioxidant

Total phenol content of the BPE was determined by the protocol which is followed from Nisha et al., (2016).

The antioxidant activity was evaluated by the phospho molybdenum method, the phosphomolybdate reagent solution was prepared by adding 1 ml of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate to 20 ml of distilled water and the volume was made up to 50 ml by adding distilled water. The extract of BPE was added to each test tube individually containing 1ml of BPE and synthesized BPE and 1 ml of molybdate reagent solution. These tubes were incubated at 95 °C for 90 min. After incubation these tubes were normalized to room temperature for 20–30 min and the absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as positive reference standard mg/g of total antioxidant was calculated.

Synthesis of BPE with AgNO₃

1mM Silver nitrate solutions were prepared by using a standard formula. Solution of 1mM Ag ions was prepared by dissolving 0.017 g of AgNO₃ in 100 ml of deionized water. The effect of the silver was determined by different AgNO₃ concentration. In this study 3 different type of synthesizing were done i.e. 1) 5ml of banana peel extract and 2.5 ml of 1mM AgNO₃ solution (Half volume) 2) 5ml of banana peel extract and 5ml of 1mM AgNO₃ solution (equal volume), 3) 5ml of banana peel extract and 10ml of 1mM AgNO₃ solution (double volume). Banana peel extract (BPE) were used as a control (AgNO₃ free sample). The prepared all samples were incubated at dark room temperature for 24-48 hrs.

Characterization of silver nanoparticles

Visual Identification and UV–Visible analysis

The preliminary detection of AgNPs was carried out by visual observation of the color changes of the reaction solutions. These changes were attributed to the excitation of surface plasmon resonance (SPR) in the metal nanoparticles. Typically, UV–visible absorption is used to investigate SPR. The UV-vis spectroscopy measurements (300nm-600nm) were recorded on a UV visible ELICO SL 159 spectrophotometer.

FTIR

To determine the functional groups of banana peel extract and predict their role in the synthesis of silver nanoparticles, Fourier Transform Infrared spectroscopy analysis was performed. A Perkin–Elmer Frontier FT-IR spectrometer with Universal Single bounce Diamond ATR attachment was used to analyse samples over a range starting at 400cm⁻¹ up to a maximum of 4000 cm⁻¹ in steps of 4 cm⁻¹. FTIR analysis For FTIR measurements, the air-dried powder samples were

grinded with KBr pellets and analyzed in the diffuse reflectance mode operating at a resolution of 4 cm⁻¹.

SEM analysis

The silver nanoparticles synthesized from the banana peel extract were characterized by a Scanning Electron Microscope. Usually Scanning Electron Microscope is used to characterize the internal properties like exact size, shape, dimensions of nanoparticles. The synthesized BPE were made into a powdered form then the filtrate embedded with silver nanoparticle was dried under vacuum and subjected to SEM studies. Thin films of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 mins for emitting characteristic X-rays.

XRD

The crystalline nature of AgNPs was further confirmed by X-ray diffraction (XRD) analysis. X-ray diffraction is used to characterize crystallographic structure, grain size, and preferred orientation in polycrystalline or powder solid samples. Sample preparation consisted of depositing two to three drops of colloid onto a glass slide via a clean glass pipette fitted with a rubber bulb. The droplets were dispersed over the slide and then dried under vacuum for a period of 4 h. Spectroscopy was performed on each sample in turn at room temperature. XRD operated at 35 kV and 28 mA in flat plane geometry mode with each scan taking 2 seconds. The respective diffraction patterns were collected over a 2θ range of 20° to 90°.

Anti bacterial activity by well diffusion method

The antibacterial activity of biosynthesized BPE was tested against three bacterial strains; *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. To determine the antibacterial activity of Ag-NPs from banana peel extract (BPE), Mueller Hinton Agar medium were prepared and sterilized, sterile media were poured aseptically into sterile petri plates and the plates were allowed to solidify at room temperature in sterile condition. After solidification and drying, the plates were seeded with microorganisms (*Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*) by evenly onto the surface of the medium with a sterile cotton swab and wells (5 mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 50μl of the each synthesized silver nanoparticles samples (half, equal, double amount of AgNO₃ with BPE, and control – without AgNO₃) in respective wells. Then the plates were incubated at 37 °C for 24 hours. The next day, zones of inhibition was measured with a measuring scale.

Nano conjugation with drug (Azithromycin) and its efficacy study

Nano conjugation

The optimum concentration of BPE was selected (equal volume of sample and AgNO₃), and this was used for drug coating with banana peel extract. Different concentrations of drug (5mg, 10mg, and drug free sample used as control) were used for drug coating. Pellets of the synthesized BPE were taken after centrifugation and washed with phosphate buffer (twice). After that, the weighed 5mg, 10mg of drugs were added to the 10mg

of dried pellets. The phosphate buffer solutions were used to dissolve the drugs and pellets. The OD value measurements (254nm) were recorded on a UV visible ELICO SL 159 spectrophotometer. After that it incubated at 37 °C for 24 hours and again OD measurements (254nm) were recorded.

Efficacy test for the conjugated drug

Mueller Hinton Agar medium plates were seeded with microorganism (*Escherichia coli*) by evenly onto the surface of the medium with a sterile cotton swab and wells (5mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 50μl of the each synthesized silver nanoparticles solution (5mg, 10mg, drug and control - BPE) in respective wells. Then the plates were incubated at 37 °C for 24 hours. The next day, zones of inhibition were measured with a measuring scale.

Drug releasing study

The drug coated nanoparticle BPE was added to 500μl phosphate buffer and incubated for 30 minutes, 10cm pretreated dialysis bag was taken and activated by rinsing in double distilled water. After that it washed with phosphate buffer and the one end of the dialysis bag was tightly tied and the drug coated sample recovered was taken inside the bag. The other end of the dialysis bag was tightly tied to prevent the leakage. After that, dialysis bag was suspended in a beaker containing phosphate buffer. The time dependent release study at 0-24 was performed and the release quantified spectro photometrically.

Anti Textile activity

The medical sterile gauge cloths were pre-coated with synthesized BPE, drug coated BPE, AgNO₃ and BPE as a control incubated at 37 °C for 24 hours and after drying, placed on the pre swabbed with multidrug resistant pathogens *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* on Mueller Hinton agar plates. The plates were further incubated at 37 °C for observation. The zone of inhibition was measured and tabulated.

In Vitro Cytotoxicity Study

Cytotoxicity study of the synthesized BPE against HeLa cell line in 3 different concentrations (5, 10, 15μl) was did with MTT assay methods. The HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with sodium bicarbonate, glucose and 10% fetal bovineserum (FBS) and Penicillin (10mg/ml). The cultured DMEM medium was prepared in a T-flask and kept it in 5% CO₂ incubator and incubated for 24-72 hrs at 80-90% humidity. The cell maturation was confirmed by checking bilayer formation and checking the cells by inverted microscope. After maturation this were transferred to, 96 well plate, samples in the different concentration of 5,10,15μl along with 100μl of the cell lines were taken, DMSO was used as a blank, cell lines were used as a control without the addition of any other solution, after adding this were incubated in the same condition for 24hrs. After incubation cells were washed and lysed with DMSO and trypsin solution. After washing 10 μlof MTT dye was added in each well and allowed to incubate for 24 hours in 5% CO₂ incubator. After 24 hours, readings were taken by using ELISA reader at 570nm.

Cell viability was calculated by using formula;

% relative cell viability = (test OD/control OD) × 100

RESULTS AND DISCUSSION

Identification of bioactive compounds

Phytochemical assay

The presence or absence of phytohormones in the banana peel extracts were determined by phytochemical assay. It was identified by the colour change of the BPE. The results obtained by the qualitative screening of phytochemicals constituents of BPE is given in Fig 1. Five phytochemicals were found to be present in the banana peel extract. A significant amount of alkaloids, terpenoids, saponins, sugar and proteins is present and phenol, flavanoids, quinines, steroids were not present in the banana peel extract.

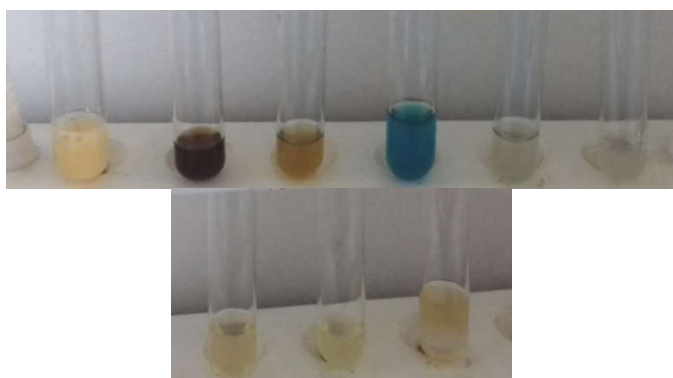


Figure 1 Phytochemical assay

This study results were correlates the results of Yugal, (2017) the phytochemical study of biosynthesized silver Nanoparticles from *protium serratum* revealed the existence of flavanoids, tannins, phenolic, sugars and triterpenoids where as glycoside, steroids and sterols were found absent.

Wolfe *et al.*, (2003) reported that, banana peels are the good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health. Emaga *et al.*, (2007) also recorded; banana peel is also rich in dietary fibers, proteins, essential amino acids, polyunsaturated fatty acids and potassium.

Total Phenol and Total Antioxidant activity

Total Phenol and Total Antioxidant activity were determined by using quantitative method. Total phenol was measured at 765nm and the total antioxidant was measured at 695nm using spectrophotometer, the results obtained are shown in the Table 1. The presence of phenol and total antioxidant activity showing that which might have possess super anti-oxidative capabilities and considered strong free radical scavengers.

Table 1 Total Phenol and Total Antioxidant activity

Sample	Total phenol (mg/g)	Total antioxidant (mg/g)
BPE	83mg/g	172mg/g
BPE NPs	66mg/g	78mg/g

Abdel-Aziz *et al.*, (2014) reported, antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *chenopodium murale* leaf extract of the antioxidant activities were highly correlated with total phenolic levels. However, the

result showed that the anti-oxidant activities of reproductive parts surpassed the anti-oxidant activities of the vegetative organs, including the pods that have the highest total phenolic and flavanoid contents.

Characterization of silver nanocomposites

Visual Identification and UV-Visible analysis of Synthesized BPE

Nanocomposites were synthesized from BPE in various concentrations when comparatively with different variations were shown in fig 2.

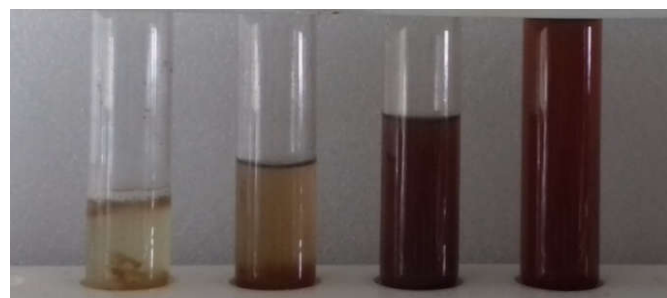


Figure 2 synthesised sample in different concentration BPE (without AgNO₃), sample with half vol. of AgNO₃, sample with equal vol. of AgNO₃ and sample with double vol. of AgNO₃

This study revealed that, the composites turned reddish dark brown in colour, after the incubation of 48hrs it indicates that the nanoparticles were formed. This was confirmed by characterization study. Alvakonda (2016), reported, natural synthesis of silver nanoparticles by banana peel extract shows formation of silver nanoparticles was predicted by the color change from yellowish brown to reddish brown. Kokila *et al.*, (2015) also reported, biosynthesis of silver nanoparticles from *Cavendish* banana peel extract shows the reduction of silver ions takes place within 30 min at room temperature and the color change of the solution brownish-orange color was observed after incubation, this indicating the formation of silver nanoparticles.

In UV-Visible study it has been found that the optimum concentration for the synthesized AgNPs is equal vol. of sample and AgNO₃ extract. Plasmon peak an observed at nearly 385 nm in size. Plasmon peak were observed as 360, 380, 385nm respectively for the synthesized sample. It was clearly indicate the presence of silver nanocomposites. There is small increase in the intensity of SPR band from 2.5 to 5ml. However, when the concentration is increased further, there is a decrease in the intensity of SPR band.

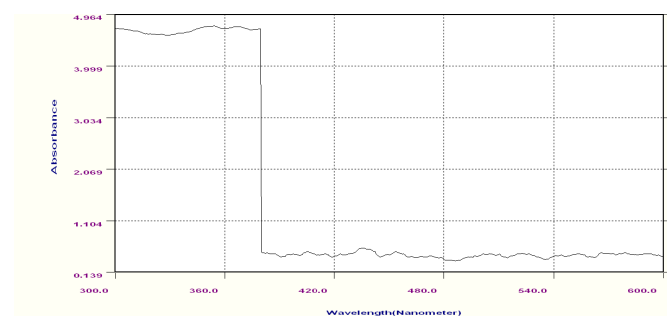


Fig 3 BPE with half vol. of AgNO₃

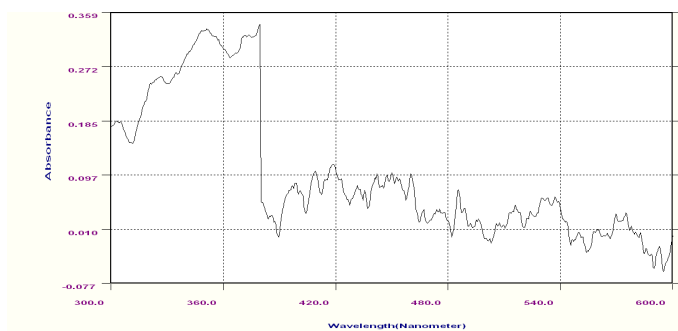


Fig 4 BPE with equal vol. of AgNO₃

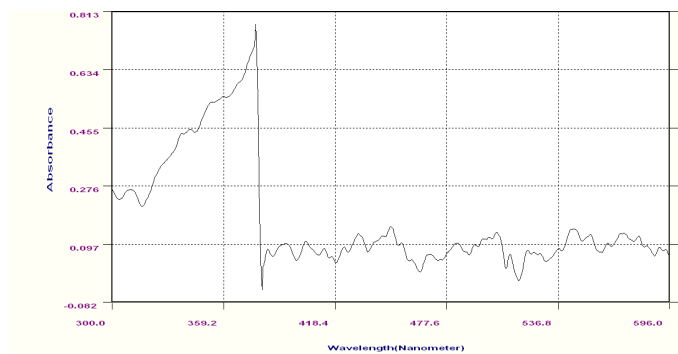


Fig 5 BPE with double vol. of AgNO₃

Around 385nm is showing in double vol. is due to the formation of more AgNPs because of high initial concentration of Ag⁺ ions. The regular decrease in SPR band intensity from curve (360nm) supports the formation of large sized AgNPs. The AgNPs prepared from 5ml concentration of Ag⁺ are used for other characterizations.

Nanda *et al.*, (2015), the maximum absorbance of Ag NPs solution and the strong absorbance peak shown at 420 nm confirming the presence of Ag NPs. Elgorban *et al.*, (2015), finding could be attributed to changes in electron density at the surface of silver due to collective excitation of electron.

Basavaraj (2015), reported, the fruit juice synthesized by silver nanoparticles were characterized by UV-Vis spectroscopy. The absorption spectra of silver nanoparticle solution showed a surface plasmon absorption band with a maximum of 420-430 nm.

Fadel and Al-Mashhedy, (2017) reported their study shows the UV absorption spectra of the synthesized AgNPs using an extract of *R. sativus* roots. The reduction of AgNO₃ into NPs was showing an absorbance sharp peak at around 430 nm with high absorbance which is very specific of silver nanoparticles.

FTIR

FTIR analysis of sample giving the peaks at 987.25, 1644.40, 2130.86, 3459.12, 3813.25, 3867.39 FTIR measurements were carried out to identify the major functional groups of the BPE AgNPs and their involvement in the synthesis and stabilization of silver nanoparticles, and the compounds were presented is butyramide, acetaldoxime- 95%, phorone-93%, nylon, nonanoic acid -98%, lanthanum sulfate nonahydrate 99.999%, gold label, benzamide-99%, 1-6-heptadien-4-ol-97%, 3 nonanone-99%, D(-)-fructose-98%.

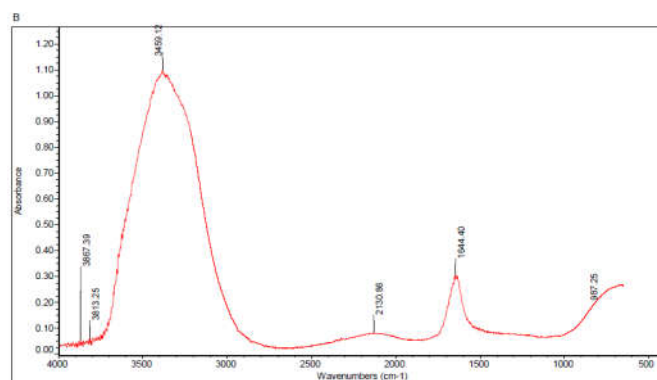


Fig 6 shows the IR spectrum obtained from the biosynthesized BPE

Graph represents the FT-IR spectra indicating bands at 987.25, 1644.40, 2130.86, 3459.12, 3813.25, 3867.39 which are respectively assigned to the stretching vibrations of O-H of carboxylic acids, C-H of aromatic, N-H of amines, C-N of aliphatic amines, C-Cl of alkyl halides, and C=C of alkynes. This result corresponds to the data reported by Shakeel (2016), a broad band between 3454 cm⁻¹ is due to the N-H stretching vibration of group NH₂ and OH the overlapping of the stretching vibration of attributed for the extract molecules. The band at 1636 cm⁻¹ corresponds to amide C=O stretching and a peak at 2083 cm⁻¹ can be assigned to alkynes group present in phyto constituents of extract.

Kokila, (2015) reported, the peak located at 1,641 cm⁻¹ could be assigned to the C = O stretching in carboxyl or C = N bending in the amide group. A shift in this peak (from 1,641 to 1,643 cm⁻¹) indicated the possible involvement of carboxyl or amino groups of the CBPE powder in nanoparticle synthesis. The peak at 771 and 760 cm⁻¹ corresponds to C-H stretching of aromatic compounds. Shanmuga *et al.*, (2015) described, the functional groups reported were OAH stretching, H-bonded alcohols and phenols, Carbonyl stretching, NAH bond 1_ amines corresponding to CAN stretching of the aromatic amino group and CAO stretching alcohols, ethers, and others. The result obtained not only indicates the involvement of sample in the reduction of AgNO₃ but also proves to work as a capping agent for AgNPs.

SEM

The scanning electron micrograph reveals the morphology of silver nanoparticles, it was observed that they were approximately round in shape with smooth surface Fig 7.

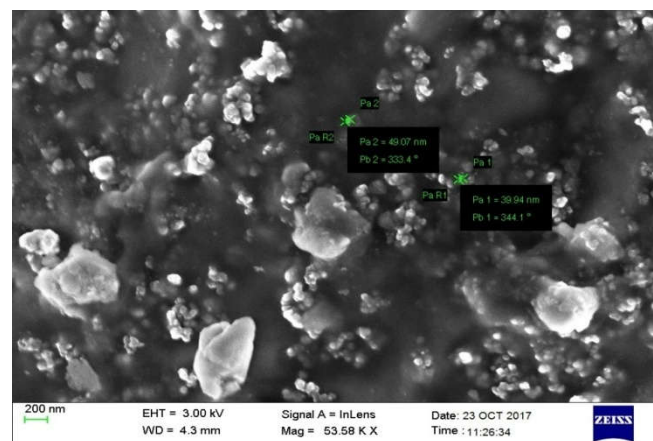


Figure 7 Scanning electron microscopy image of biosynthesized BPE AgNPs

The shape of the particles has correlated with SPR band at 200 nm for silver nanoparticles. This also revealed that the powder form particles are slightly agglomerated. The insert picture also shows agglomerated with spherical shape in 20–50 nm range at 53.58 KX magnification scale.

Ahmed (2016), investigated about the green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms-also he found that the shape of the synthesized silver nanoparticles from BPE is spherical in shape and the size is 20-34nm.

XRD

The crystalline nature of AgNPs was further confirmed by X-ray diffraction (XRD) analysis. X-ray diffraction is used to characterize crystallographic structure, grain size, and preferred orientation in polycrystalline or powder solid samples.

The X-ray Diffraction pattern of synthesized AgNPs is presented in graph 5, Silver nanoparticles synthesized from Banana peel extract showed Bragg Reflection peaks at 210, 280, 350,410 and 500 in the 2θ range between 100–800 which can be indexed to the (100), (200), (250) and (400) planes of face centered cubic (fcc) crystal, respectively. Mohamed et al., (2017) find the characterization and anti-*Aspergillus flavus* impact of nanoparticles synthesized by *Penicillium citrinum* illustrates the XRD of biosynthesized Ag NPs. The AgNPs are clearly polycrystalline and no crystallographic impurities spurious diffraction was observed. All the reflections correspond to pure silver metal with face centered cubic symmetry.

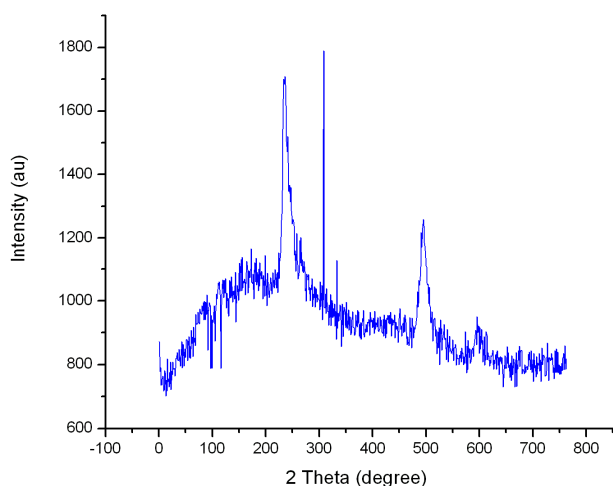


Fig 8 The XRD graph of BPE silver nanoparticles

Antibacterial activity of silver Nanoparticles

Antibacterial activity were performed against *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* on Mueller Hinton agar plates treated with different concentrations of Ag nanocomposites (different amount of AgNO₃ & BPE as a control).



Figure 9 Anti bacterial activity by well diffusion method

Bacillus subtilis showing larger zones of inhibition, compared to *Escherichia coli*, and *Staphylococcus aureus*. Zone of inhibition diameter values were obtained for the synthesized nanoparticles tested against *Escherichia coli*, *Bacillus subtilis*, (Gram negative bacteria) and *Staphylococcus aureus* (Gram positive bacteria). The results are presented as average values in Table 2.

Table 2 Anti bacterial activity

Samples used	Zone of inhibition and organism used		
	<i>E.coli</i>	<i>B.subtilis</i>	<i>S.aureus</i>
BPE with half vol. of AgNO ₃	2mm	2mm	1mm
BPE with equal vol. of AgNO ₃	2mm	16mm	2mm
BPE with double vol. of AgNO ₃	2mm	14mm	1mm
AgNO ₃ alone	2mm	4mm	1mm
BPE alone	Nil	1	Nil

Patra and Baek, (2017) find that there are several probable prospective mechanisms are exists for the decisive antibacterial activity of AgNPs which comprise the enzyme degradation, inactivation of major cellular proteins and impairment of genetic materials.

This study results compared with the study of Haytham, (2015), green synthesis and characterization of silver nanoparticles using banana peel extract in gram negative bacteria (*E. coli* and *P. aeruginosa*) showed larger zones of inhibition, compared with the gram positive bacteria (*B. subtilis* and *S. aureus*). Alvakonda (2016) reported, the natural synthesized silver nanoparticles by banana peel extract showed excellent antimicrobial activity against clinically isolated Multidrug-resistant human pathogens such as Gram-positive bacteria *Staphylococcus aureus*, and Gram-negative bacteria *E.coli*. Haytham, (2015) also reported, silver nanoparticles showed efficient antimicrobial property compared to other due to their extremely large surface area providing better contact with cell wall of microorganisms.

Nano conjugation of drug (Azithromycin) and its efficacy

The optimum concentration of BPE was selected (equal vol.), and this was used for drug conjugation. Different concentrations of drug (5mg, 10mg) were used for drug

coating. The efficacy study done on Mueller Hinton Agar plates, which is seeded with microorganism (*Escherichia coli*), with a sterile cotton swab and wells (5mm diameter) were cut out from the agar plates using a sterile stainless steel bore and filled with 50µl of the each synthesized silver nanoparticles solution (5mg, 10mg, drug and control - BPE) in respective wells. This study concluded that 10mg concentration of Ag nanocomposites shows higher activity fig 10.

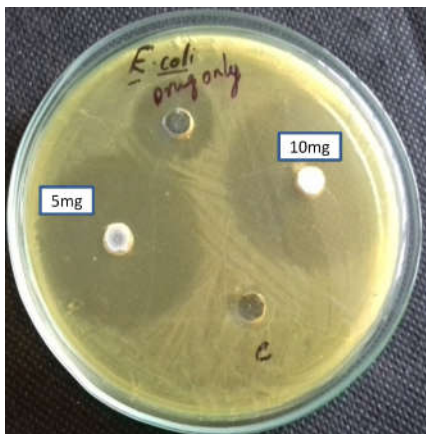


Figure 10 Efficacy study

Table 3 efficacy study

Efficacy test for Azithromycin with BPE sample	Zone of inhibition
5mg	12mm
10mg	13mm
Drug alone	5mm
Control	1mm

Emmanuel *et al.*, (2015) reported, combination of AgNPs with drugs (Azithromycin and Clarithromycin) is highly effective against dental caries and periodontal disease causing microorganisms compared to AgNPs and drugs alone.

Drug releasing study

The drug release study of the nanoparticles can be occur in two types ie; sudden release and sustained release. Sudden release of drug in the body can quickly reach an effective therapeutic concentration and sustained release can make the drug in the body to stay at the effective therapeutic concentration range. After incubation of 0-7 hrs showing 76% drug release for the nanoparticle and without nanoparticle which is released 91%, and after that the nanoparticle had the effect of prolonging the drug release Sun *et al.*, (2017). This results correlate the results of present study, which is also showing better release in 0-9 hrs (78%) and then this were decreasing gradually (nanoparticle with drug).

Anti Textile activity

In the present study three multidrug resistant pathogens *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used for the antitextile activity. *Pseudomonas aeruginosa* nanocomposites coated gauge cloth had more antimicrobial activity compared with *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The zones of inhibition are reported in Table 4 and Fig11.

Table 4 anti textile activity against multidrug resistant pathogens

Sample used	Organism used		
	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>
Banana extract	15mm	1mm	1mm
Drug alone	10mm	10mm	18mm
AgNo3	5mm	2mm	7mm
Equal vol. of BPE and AgNO ₃	12mm	3mm	3mm

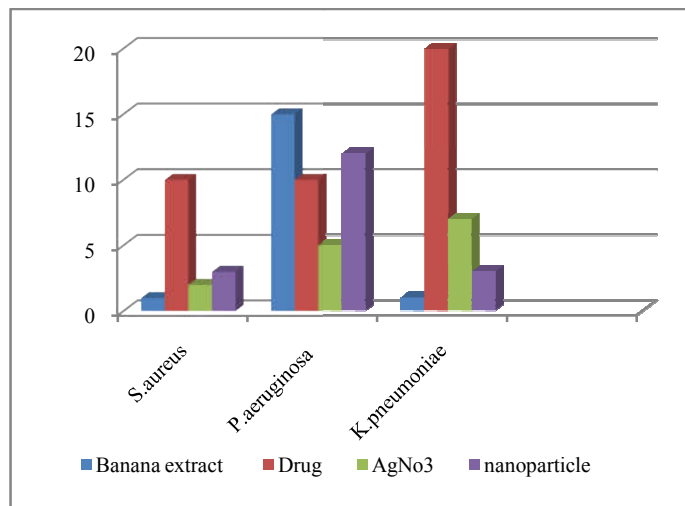


Fig 11 Anti-textile activity against multidrug resistant pathogens

Amar *et al.*, (2012) also reported synthesized silver coating on the fabrics showed the anti microbial activity against ESBL-*Pseudomonas* and this fabric can be implemented as an application of inhibiting wound infections.

In Vitro Cytotoxicity Study

In vitro cytotoxicity test were done by the synthesized BPE were against HeLa cell line in 3 different concentrations (5, 10, 15µl) and determined by MTT assay. And the percentage of the cell viability was calculated, the values for 5µl is observed as 49.5%, 10µl value shown 48.25%, and 15µl cell viability is 52.32%. The percentage of the cell death 49.5%, 51.75%, 47.68% respectively. The data from this experiment suggest that higher concentrations between 10 and 15µl had significantly more impact on cell viability than 5µl. At 24 hours of treatment, AgNPs were found to be significantly toxic to the cells at concentrations of 5µl and higher.

This results were correlates with the results of an earlier study (Sowemimo *et al.*, 2009) where *Sapium* leaves showed the highest cytotoxic activity against HeLa cell line.

Yugal (2017), find the activity against fibroblast cells and observed very low level activity against L-929 cell line at lower concentrations. The percentage of cell viability of normal fibroblast cells is declined with an increase in concentration of AgNPs. The percentage of cell viability of normal fibroblast cells is declined with an increase in concentration of AgNPs.

Preetha *et al.*, (2013) studied, the cytotoxic activity of AgNPs synthesized by using *cannonball* leaf extract was determined by MTT assay, the minimum inhibitory concentration (IC₅₀) of AgNPs on MCF-7 cells was obtained at 20µL/mL at 24 hours.

Exposure to increasing concentration of AgNPs shows dose-dependent cytotoxicity on MCF-7 cells.

Acknowledgement

The authors thank Centre for Bioscience and Nanoscience Research, Eachanari, Coimbatore, Tamilnadu, India, for providing necessary laboratory facilities to carry out this work.

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How to cite this article:

Susan K Thomas *et al.* 2017, Eco-Friendly Synthesis of Silver nanoparticle Using Banana (*Musa Acuminate Colla*) Peel, Its Phytochemical, Antimicrobial And Anticancer Activity. *Int J Recent Sci Res.* 8(10), pp. 21098-21106.
DOI: <http://dx.doi.org/10.24327/ijrsr.2017.0810.1014>
