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GREEN SYNTHESIS OF GOLD NANOPARTICLES FROM BANANA PITH EXTRACT AND ITS EVALUATION OF ANTIBACTERIAL ACTIVITY AND CATALYTIC REDUCTION OF MALACHITE GREEN DYE

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ABSTRACT

In this paper, we report the synthesis of gold nanoparticles (BPAuNPs) using banana pith extract. Biosynthesized BPAuNPs were screened for phytochemicals coated over them and further characterized by UV-visible spectroscopy, FTIR, XRD and particle size analyser. UV-Visible spectrscopic analysis confirmed the production of BPAuNPs at 530 to 560 nm, where the colour change in the solution from light yellow to deep purple indicated the formation of BPAuNPs. FT-IR analysis confirmed the capping of BPAuNPs with organic residues like, proteins, amino acids and polyphenols present in the extract, which led to stabilization of BPAuNPs. Negative zeta values indicated the stability of BPAuNPs. XRD results prooved the crystalline nature of BPAuNPs. The synthesized BPAuNPs were tested for their antibacterial activity against both Gram positive (*Bacillus subtilis*) and negative (*E.coli*, *Pseudomonas aeruginosa*) bacteria with known antibiotic as control. BPAuNPs showed significant bacteriostatic effect. The BPAuNPs were found to have a positive catalytic activity of reduction of malachite green dye, which was confirmed by the time dependent reduction in absorbance maxima, it can be ascribed to the electron relay response.

Keywords: Banana pith extract, gold nanoparticles (BPAuNPs), biosynthesis, antibacterial activity, catalytic reduction

INTRODUCTION

Synthesis of nanoparticles (NPs) using biological materials has gained importance in recent years because it is cost effective, environmental friendly, easy scale up ability for large scale synthesis and further, there is no need to use high pressure, energy, temperature and toxic chemicals **(Jayseelan *et al* ., 2013).** Researchers have attempted synthesis of nanoparticles of different size and shape using variety of biological materials **(Saifuddin *et al*., 2009).** Biosynthesis of NPs using plant extracts are more stable and the rate of synthesis is faster, in addition, it would be advantageous over other biological processes because it eliminates maintenance of cell cultures and can be suitably scaled up for large scale synthesis **(Shankar *et al*., 2004).** Biosynthesis of AuNPs using aqueous extract of *Abelmoschus esculentus***(Saifuddin *et al*., 2009)** seed, *Aloe vera* **(Chandran *et al*., 2006) ,** tamarind **(Ankamwar *et al*., 2005) ,** *Cinnamomum camphora* **(Huang *et al*., 2007)**, lemon grass **(Shankar *et al*., 2005),** pear fruit extract **(Ghodake *et al*.,2010),** glucan of edible mushroom **(Sen *et al*., 2013)**, *Geobacillus stearothermophi*lus **(Girilal *et al*., 2013)** and *Punica Granatum* **(Ganeshkumar *et al*., 2013)** have been reported.
It is a known fact that AuNPs display antimicrobial activity **(Arulkumar *et al*, 2010; Mubarak *et al*, 2011).** The binding strength of the nanoparticles with the bacterial membrane depends on the surface area of interaction and high percentage of surface will have higher interaction with microbial membrane leading to opsonization of bacterial membrane hence antimicrobial effect **(Krishnaraj *et al*., 2010).** The exact mechanism behind the antimicrobial effect of metal nanoparticles is still not clearly understood. There are reports in the literature suggesting that the electrostatic attraction of negatively charged bacterial cell membrane and positively charged nanoparticles is crucial for the antibacterial activity of the nanoparticles **(Edison *et al*., 2012).** Nanoparticles are known to accumulate on the bacterial cell membrane causing significantly increased permeability leading to leakage of cytoplasmic contents and cell death by Donnan effect **(Valodkar *et al*., 2011).**
Nanoparticles synthesized using biological materials can be used for bioremediation of environmental pollutants like textile dyes **(Arunachalam *et al*, 2012; Xin *et al*, 2016)**.Iron nanoparticles synthesized using Guanyin tea extract have been used for degradation of bromothymol blue **(Xin *et al*., 2016)**. Silver nanoparticles synthesized using *Coccinea grandis* leaf extract for coomassie brilliant blue G-250 dye degradation (**Arunachalam *et al*., 2012)**. Components of biological material cap around the nanoparticles, thereby stabilize the nanoparticles.
In this paper, we report the synthesis of gold nanoparticles (BPAuNPs) using banana pith extract as banana stem is available throught the year and is usually discarded as a waste material **(Li *et al*, 2010; Nguyen** et al, **2017).** Biosynthesized BPAuNPs were characterized by UV-visible spectroscopy, FTIR, XRD and Particle size analyser. The interaction of the proteins, carbohydrates and other constituents (*p*-Hydroxybenzoic and gallic acids) present in the cell free extract **(Li *et al*., 2010; Nguyen *et al*, 2017)** with the surface of the synthesized AuNPs were confirmed by Fourier transform infrared (FT-IR) spectroscopy. The AuNPs were tested for their antibacterial activity against both Gram positive (*Bacillus subtilis*) and Gram negative (*E.coli*, *Pseudomonas aeruginosa*) bacteria. The synthesized BPAuNPs were evaluated for catalytic activity by Malachite green dye reduction test.

MATERIAL AND METHODS

The BPAuNPs are synthesized using banana pith extract by the reduction of $HAuCl_4$. The details of the steps in the biosynthesis of BPAuNPs are given below.

Preparation of Pith extract

Banana pith was washed in deionized water, chopped into small pieces and used for the preparation of 10% (w/v) extract by homogenizing the chopped banan pith in deionized water. The extract was kept in the water bath for half an hour at 80ºC. Whatman No. 1 filter paper was used to filter the cell free extract and the filtrate was then used for BPAuNPs ynthesis by using $HAuCl_4$.

Phytochemical screening of extract

Various phytochemicals (Carbohydrates, proteins, alkaloids, flavanoids, tannins, phenols, saponins and resins) present in the aqueous extract of banana pith were screened as per standard protocols **(Kokate *et al*., 2008).**

Synthesis of gold nanoparticles

As reported in the literature **(Krishnaraj *et al*, 2010; Voladkar *et al*, 2011; Aromal *et al*, 2012** and **2013; Sahu *et al*,2013)** biosynthesis of gold nanoparticles

was undertaken by using 1mM aqueous $HAuCl_4$ prepared in deionized water. 25ml of 10% (w/v) banana pith extract in deionized water was added to 225ml of 1mM $HAuCl_4$ and mixed thoroughly. The resultant mixture was kept in a shaker at 120 rpm at room temperature. Color change in the resulting solution was measured at regular intervals by measuring absorbance at 360 to 700 nm using UV visible spectrophotometer. The reaction mixture was centrifuged at 10000 rpm for 30 mins and the product obtained was washed thrice with deionized distilled water and finally with ethanol, and stored at - 4ºC for further analysis.

Characterization of BPAuNPs

UV- visible spectroscopic Characterization of BPAuNPs

Colour change in the solution takes place during the biosynthesis of nanoparticles, which can be accounted for surface plasmon resonance **(Ramteke *et al*., 2013)** measured at regular intervals by noting the absorbance at 360- 700 nm using UV-visible spectrophotometer (UV1- Thermo electronic corporation, Merck).

FT-IR spectroscopic studies

The functional groups present in the phytoconstituents of banana pith extract and their involvement as capping agents in the biosynthesized BPAuNPs was determined by recording the IR on Bruker Alpha. Biosynthesized nanoparticles are known to be capped with proteins, amino acids, and polyphenols, which can be determined by FT-IR **(Raghunandan *et al*, 2010; Edison *et al*, 2012; Ramteke *et al*, 2013).**

XRD analysis

X-ray diffraction profile was obtained using X-ray diffractometer (Rigaku Miniflex 600, Rigaku Co., Tokyo, Japan) using cu-kα X-ray diffraction patterns of the tested material was recorded over the 2θ range of variable degrees at a scan rate of 4°/min.

Particle size distribution and zeta potential

Zetasizer nano instrument (Malvern) was used for data acquisition and for estimating the particle size distribution as well as zeta potential distribution of a colloidal solution of BPAuNPs.

Antibacterial activity of BPAuNPs

The BPAuNPs were tested for their antibacterial activity against both Gram positive (*Bacillus subtilis*) and Gram negative (*E.coli*, *Pseudomonas aeruginosa*) bacteria. BPAuNPs at a concentration of 10 (mg/ml) were prepared in DMSO (14M) and 50µl was loaded in the well to study antimicrobial activity using agar diffusion method. 1% bacterial nutrient agar plates were prepared and four zones were marked from outside with a permanent marker. Spread plate culture of each bacterium was prepared by taking 100µl of fresh culture of the bacterium. In the middle of each zone marked on the plate, 5 mm well was punched using agar punch. In each well, 50µl of BPAuNPs prepared in DMSO were added. DMSO, Ciprofloxacin and 1mM aqueous $HAuCl_4$ were used as control. Light zones around the well were considered as zone of inhibition, which was measured in mm from the periphery of the well to the periphery of the zone. All Experiments were conducted in triplicates.

Malachite green dye degradation by biosynthesized BPAuNPs

Malachite green was purchased from Loba chemicals. Catalytic activity of synthesized BPAuNPs was assessed by carrying out two reactions in a quartz cuvette of 3.5 ml capacity and absorbance was measured using UV-Vis spectrophotometer. A mixture of 1ml of Malachite green (1×10^{-4} M) along with 0.2ml of aqueous pith extract and 1.8ml of water was prepared in the first reaction, which was then monitored after 30 mins as control. In the second reaction, a mixture of 1ml of Malachite green with 0.2ml of aqueous pith extract and 1.8ml of synthesized BPAuNPs was prepared, and this reaction was monitored continuously at different time intervals till maximum degradation was achieved as reported by **Edison *et al*., 2012**. The total reaction volume of the mixture was made up to 3ml and the absorption maxima (λ max) values were checked in comparison with that of pure Malachite green and percentage degradation was calculated using the formula as given below (**Edison *et al*., 2012)**

$$\%\ Decolorization = \frac{Initial\ absorbance - Observed\ absorbance}{Initial\ absorbance} x100$$

RESULTS AND DISCUSSION

Phytochemical screening of Banana pith extract

The phytochemical tests conducted for the banana pith extract showed the presence of carbohydrates, proteins and secondary metabolites like alkaloids. These phytochemicals cap around the nanoparticles and stabilize the nanoparticles by preventing agglomeration of nanoparticles for better antimicrobial properties **(Donald *et al*, 2015; Das *et al*, 2016)** and better catalytic activity **(Aromal *et al*., 2012).**

Table 1 Phytochemical screening of Banana Pith aqueous extract

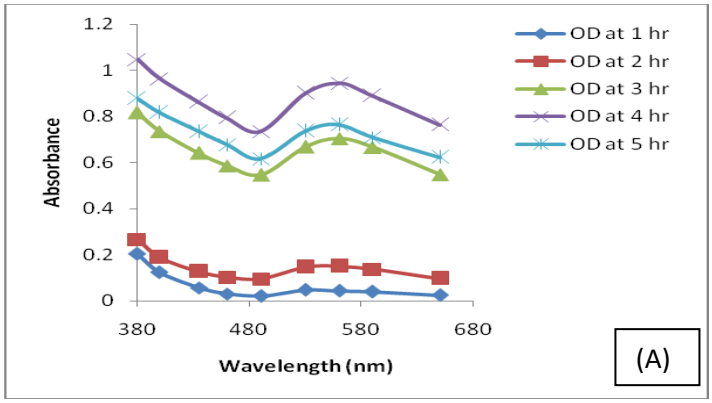
Parameter		*Banana Pith* Extract
Carbohydrates	Molish test	+
	Fehlings test	+
Proteins	Biuret test	+
	Xanthoprotic test	+
Alkaloids	Hagers test	+
	Wagers test	+
Flavonoids	Ferric chloride test	-
	Lead acetate test	-
Tannins		-
Phenols		-
Saponins		-
Resins		-

(+ : Positive test , - : Negative test)

Characterization of synthesized nanoparticles

Characterization using UV-Visible spectrophotometer

Colour change of the solution from light yellow to deep purple indicated the formation of gold nanoparticles at 530 to 560 nm **(Krishnaraj *et al*., 2010; Voladkar *et al*, 2011; Aromal *et al*., 2012** and **2013; Sahu *et al*., 2013; Ramteke *et al*., 2013)**, which was confirmed by UV-Visible spectrophotometer. The intensity of the peak steadily increased up to 4hrs and then started decreasing, which is directly proportional to the density of nanoparticles in solution as shown in the figure 1.



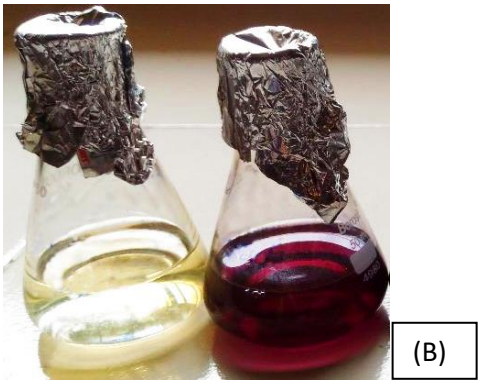


Figure 1(A) Visual observation of UV-Visible spectra of biosynthesized BPAuNPs (B) Conical flasks confirming BPAuNPs Synthesis

Characterization using FT-IR

The IR absorption spectra seen in the aqueous extract was also seen in phytocapped BPAuNPs. Capping of nanoparticles with organic residues like, proteins and amino acids present in the extract lead to the stabilization of nanoparticles and protect the BPAuNPs from aggregation which was confirmed by FT-IR **(Raghunandan *et al.*, 2010; Edison *et al.*, 2012; Ramteke *et al.*, 2013)**.

Figure 2 and figure 3 shows the FT-IR measurements of banana pith extract and AuNps synthesized from pith extract. BPAuNPs potentially possess rocking vibration of CH_3 (648 cm^{-1}), C –OH stretching of secondary alcohols (1049 cm^{-1}) **(Annamalai *et al.*, 2013),** C=O stretching vibration of carbonyl and carboxylic group of hydroxy benozoic acid and gallic acid (1632 cm^{-1}), C=H stretching vibration of aldehydic amine group(2927 cm^{-1}), O-H stretching vibration of hydroxyl functional groups of polyphenols and alcohols and –NH stretching vibrations of amide (II) or amine (3437 cm^{-1}) functional groups on the surface **(Emmanuel *et al.*, 2014).** The most common rocking vibration found between the extract and the BPAuNPs was found to be C=H stretching vibration of aldehydic amine group(2927 cm^{-1}). The Drastic reduction in peak was seen at (1049 cm^{-1}) and (1632 cm^{-1}) C –OH stretching of secondary alcohols and C=O stretching vibration of carbonyl and carboxylic group of hydroxy benozoic acid and gallic acid peaks seen at 1308 cm^{-1} and 1382 cm^{-1} in the extract, which completely vanished in the BPAuNPs indicating the involvement of these groups in the catalytic reduction of $HAuCl_4$ to BPAuNPs **(Annamalai *et al.*, 2013).**

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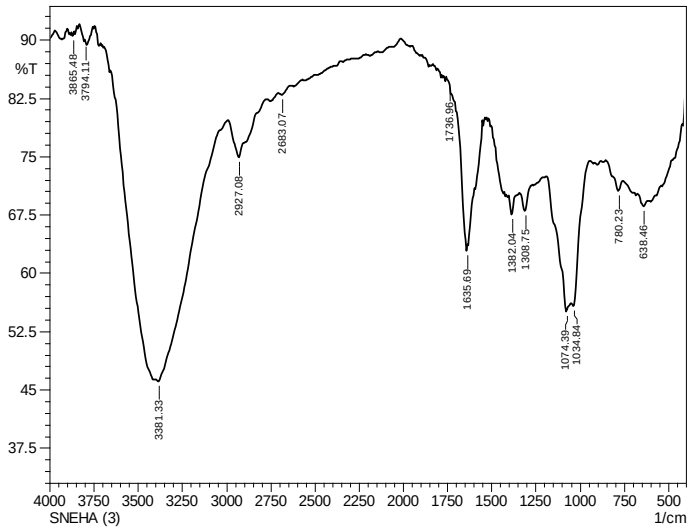

Figure 2 FT-IR of *banana pith* extract

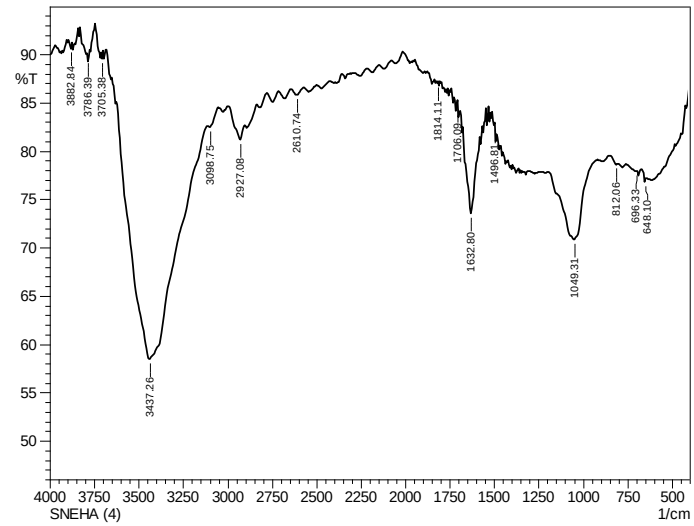

Figure 3 FT-IR of Au - nanoparticles produced from *banana pith* extract

Earlier studies of IR spectra of biologically synthesized nanoparticles have shown the presence of biomolecules on their surface **(Arulkumar *et al.*, 2010; Mubarak *et al.*, 2011; Edison *et al.*, 2012)**. From the present study, it is also evident that the AuNPs synthesized are capped with organic residues like proteins and amino acids. It is well known fact that amines of the proteins facilitate the binding to Au nanoparticles and therefore, stabilization of AuNPs through surface bound proteins occurs **(Arulkumar *et al.*,2010).**

Charecterization using XRD

Figure 4 shows the XRD patterns for BPAuNPs, which exhibited four prominent Bragg reflections at around 37.68, 43.90, 64.11, and 77.17°, which correspond to (1,1,1), (2,0,0), (2,2,0), and (3,1,1) reflection planes of face-centered cubic structure of BPAuNPs, **(JCPDS file no 01- 1174, Poojary *et al.*, 2016)**. Further, Scherrer equation was used to calculate the average crystal sizes of BPAuNPs and were found to be 17.5 nm and the crystal size was in the range of 17-20nm. The observed XRD pattern, thus, confirmed that the BPAuNPs formed by the reduction of $HAuCl_4$ − ions by phytochemicals of *Banana pith* extract are crystalline in nature. Apart from normal peaks, some additional and unidentified peaks were also noticed at the vicinity of the characteristic peaks of BPAuNPs. These peaks may be due to some bioorganic compounds or proteins and carbohydrates found in the extract **(Kalishwarlal *et al.*, 2010).**

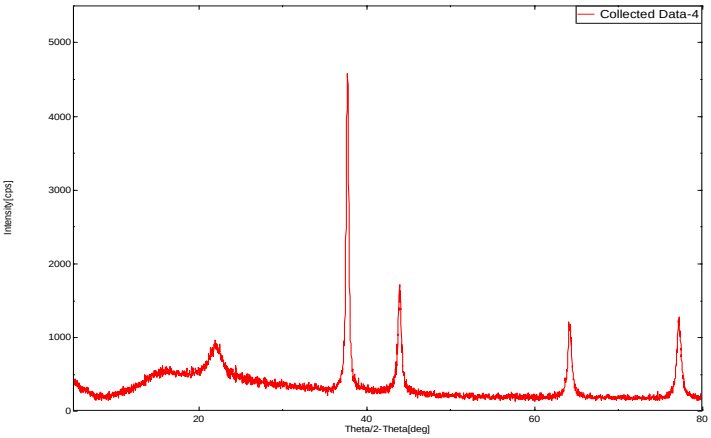

Figure 4 XRD analysis of biosynthesized BPAuNPs

Particle Size distribution and zeta potential

Figure 5A shows the particle size distribution of BPAuNPs in the range of 470 nm, which are comparable to gold nanoparticles synthesized using lemon grass extract, which had average size of 200-500nm **(Sen *et al.*, 2013)** and those synthesized using pear fruit extract had average size of 200-500nm (**Girilal *et al.*, 2013**). BPAuNPs had a polydispersity index (PDI) of 0.704 which is low and Zeta potential of – 6.07 (Fig. 5B). This indicates the capping agents existing on the surface of BPAuNPs mainly comprise of negatively charged groups, which may be responsible for the moderate stability of nanoparticles.

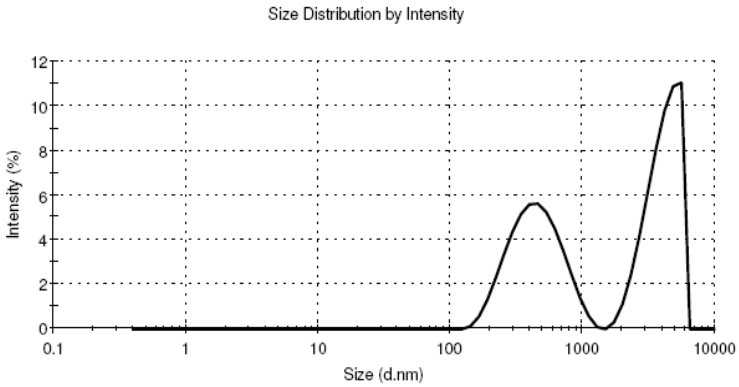

Figure 5A Size distribution BPAuNPs

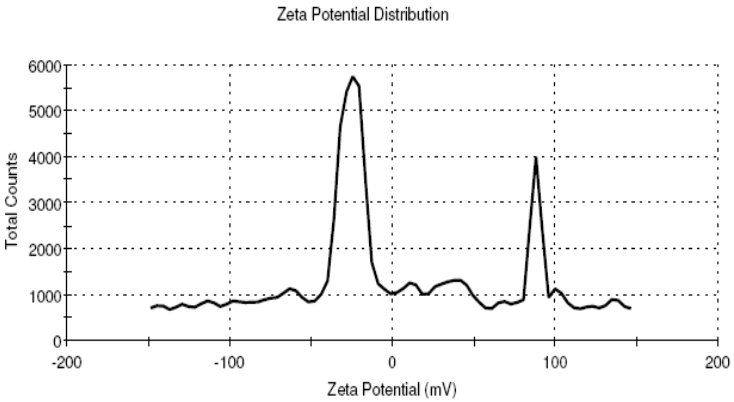

Figure 5B Zetasizer and Zeta potential distribution of biosynthesized BPAuNPs

Antibacterial activity of BPAuNPs

Biologically synthesized BPAuNPs displayed antimicrobial activity towards the Gram-negative *E.coli and Pseudomonas aeruginosa* and against Gram-positive *Bacillus subtilis* bacteria as shown in figure 6. The light zones around the wells were considered as the zone of inhibition which are comparable with literature reports where gold nanoparticles synthesized using *extreme bacteria Deinococcus radiodurans* showed inhibition zone of 9mm against *E.coli* **(Li *et al.*, 2016).** Gold nanoparticles synthesized using *Abutilon indicum* extract showed inhibition zone of 7.5mm against *Bacillus spp*. **(Chandrika *et al.*, 2018).** Antibacterial activity of BPAuNPs against Gram-positive *Bacillus subtilis* and Gram-negative *E.coli* was significantly higher than that of Gram-negative *Pseudomonas aeruginosa* bacteria and controls. From this observation, it can be said that the two different species of bacteria of same category (Gram negative) respond differently to nanoparticles synthesized from the same source.

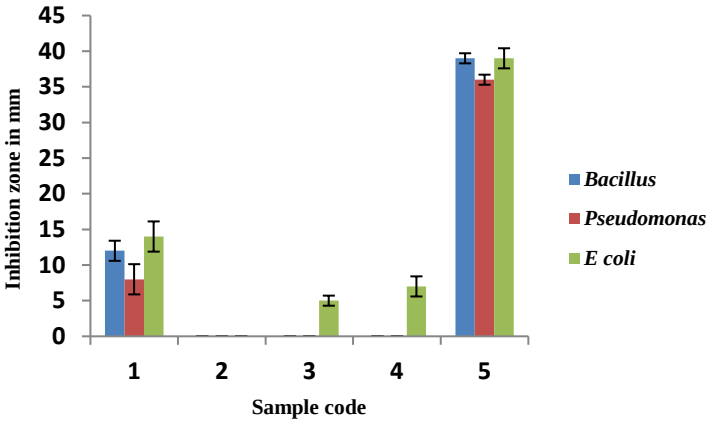

Figure 6. Results of Antibacterial activity . 1= BPAuNPs, 2= Banana pith extract , 3=$HAuCl_4$, 4= DMSO , 5= Ciprofloxacin .

Malachite green Dye degradation study

Figure 7 shows Malachite green dye degradation by BPAuNPs. When compared to literature reports where 89% of malachite green dye is degraded using *Trichoderma hpyocrea lixii* under optimal condition of 30ºC, pH of 5.8 , yeast extract of 5.81 mg/l and incubation period of 10 days **(Saravanakumar *et al.*, 2014)** , the present study using BPAuNPs acheives 96% malachite green dye degradation in 5 days. Hence the present study does not require laborious maintenance of cultures and can be easily scaled up for large scale applications **(Shankar *et al.*, 2004).**

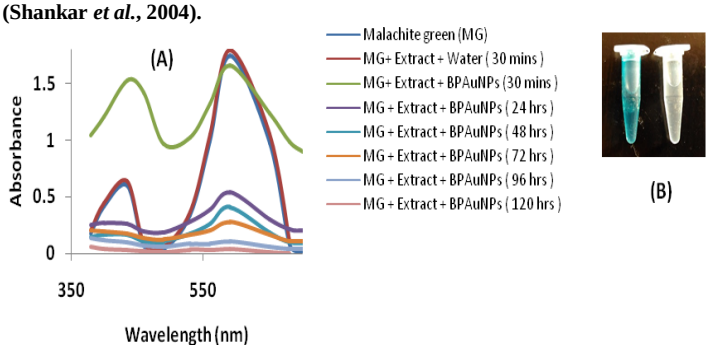

Figure 7 (A) UV- Visible absorption spectra of Malachite green dye degradation by BPAuNPs (B) Eppendorf tube on left side containing Malachite green dye and on right hand side confirming biodegradation of Malachite green dye

CONCLUSION

By employing economical and ecofriendly biological approach for the synthesis of gold nanoparticles (BPAuNPs) using banana pith extract, where biosynthesized BPAuNPs were found to capped with proteins and other organics present in the banana pith. The surface plasmon resonance peaks observed for BPAuNPs were comparable to the literature reports. Negative zeta values indicated the stability of nanoparticles. XRD results confirmed the crystallinity of nanoparticles. The synthesized BPAuNPs showed antibacterial activity against both Gram positive and Gram negative bacteria. BPAuNPs showed significant bacteriostatic effect hence can be used in biomedical applications as an antimicrobial agent. BpAuNPs were also capable of degrading Malachite green dye effectively, hence can be used for bioremediation of textile industry effluent containing Malachite green dye.

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