



BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES FROM MANGO PEELS REVEALING THEIR ANTIBACTERIAL EFFECT ON BACTERIA *ESCHERICHIA COLI*.

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ABSTRACT

Nanoscience is developing very rapidly in all fields of science and so in the field of pharmacy, where it is actually very demanding, as the global problem of multi drug resistance is arising all over. Therefore to overcome this issue nano science is proving beneficial, by the production of nanoparticles from various organic and non organic materials. Here we have synthesized silver nanoparticles from the peels of raw mango. After keeping the material for 24 hours at stationary condition change in colour was observed this indicated the production of silver nanoparticles. Further characterization using UV spectroscopy, XRD, FTIR, SEM and TEM of the solution was done which confirmed the synthesis of silver nanoparticles. Later antibacterial assay was followed to determine the antibacterial potential of the solution against the bacteria *E. coli*. Maximum zone of inhibition of 21mm is observed in the concentration of 3mm. thus this proves that silver nanoparticles (SNPs) synthesized by the raw mango peels is potent against the bacteria *E. coli*.

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INTRODUCTION

Nanotechnology is the approaching field of science, it has proved beneficial in various sectors of physics, chemistry and biology and in environmental science as well. Human life is greatly benefited from the new discoveries which nanotechnology is exploring each day (Dahl *et al.*, 2007). Discovery of metal nanoparticles is one of biggest gift to mankind as these metal nanoparticles is of great importance in different ways, every metal nanoparticles have their own specific property and thus are useful in their own specific ways, silver nanoparticles is the one which is of utmost utility. (Jose *et al.*, 2005). There are several techniques of synthesizing silver nanoparticles from different sources (Naik *et al.*, 2002). although it was found that physically and chemically synthesized nanoparticles were very distinct in nature and are of good purity level, but chemicals and other physical means have their own set of caution and are even hazardous to the environment and cost of synthesizing is also considerably high. (Song *et al.*, 2008, Gole *et al.*, 2004 and Meltzer *et al.*, 2001) today everyone is concerned about the environmental condition and getting conscious about the facts of improving the environmental conditions, this concept gave rise to the idea of organic synthesis of silver nanoparticles.

Therefore synthesis of silver nanoparticles is done in the most organic and eco-friendly method (Zhang *et al.*, 2011, Hsiao *et al.*, 2006 and Egorovas *et al.*, 2000). Silver nanoparticles were synthesized from the waste of mango fruit peel and then their antibacterial property is analyzed on bacteria *Escherichia coli*. Various techniques were used to characterize the true property of silver nanoparticles UV visible spectroscopy, XRD, FTIR, SEM with EDS and TEM was done, which confirms the presence of silver nanoparticles in the solution which is prepared from the peels of mango and silver nitrate in a specific concentration. The synthesized solution of silver nanoparticle showed good zone of inhibition against the bacteria *Escherichia coli*. Thus the nanoparticles synthesized were proved efficient against water isolated *Escherichia coli*.

MATERIALS AND METHOD

Materials

Silver nitrate was purchased of 99.95% from Merck India. Mango peels of raw mangoes were collected from the local market during the mid summer season. Powder preparation of the peels was done by collecting the peels of mango, washing them with distilled water and then drying the peels under shade for upto 15 days. The needed glassware were also washed and sterilized. Double distilled water is used in the preparation of all kinds of solution used in the synthesis of silver nanoparticles. Bacteria on which antibacterial analysis was carried out was isolated from contaminated source of water and identified from the NABL pathology and microbiology Laboratory of Agra.

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Preparation of peel extracts solution

Raw mangoes were purchased, washed and peeled. Peels of the mangoes were washed and dried properly under shade for about 15 – 20 days, then after powder preparation of the dried peels was done using domestic grinder. Powder was stored in air tight container for future use. Peel extract solution was prepared by adding 4grams of mango peel powder into 100ml of distilled water then this solution was kept in water bath at 100 degree for about 30 minutes. Before keeping the glassware in water bath, it was covered with aluminum foil. When the solution gets boiled it was double filtered using whatmann filter paper No.1. Now the solution was centrifuged at 10000 rpm for 15 minutes to remove residual material if any and again filtered using filter paper No.1. This solution was stored in air tight container to avoid contamination and kept in the refrigerator for future use.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, three different concentration of silver nitrate was prepared 1mM 2mM and 3mM. Solution of silver nitrate is prepared by dissolving silver nitrate salt in these three concentrations in 50ml distilled water in each. 5 ml mango peel solution was added to the silver nitrate solutions of three different concentrations and these solutions are then left for 24 hours at room temperature for the synthesis of silver nanoparticles. No change in color is observed in the first hour then slowly the solution starts changing color from light orange to deep wine and finally to dark grey colour. The dark grey color appears in the time interval of 24 hours which indicates the possibility of formation of silver nanoparticles. Best activity of synthesis was observed in the concentration of 3mM therefore for further characterization 3mM solution was selected. (Yang and Li 2016)



A



B

Fig. (1) Color change in the silver nanoparticles solution (A) Change in color in the first hour
(B) Change in color in 24 hours

Disc diffusion method (Espinel-Ingroff et al., 2007)

Antibacterial analysis of biogenically synthesized silver nanoparticles was done using Kirby Bauer method commonly known as disc diffusion method on petri plates having nutrient agar. Sterile disc of 10mm diameter is purchased from Hi

media labs reference no. DD036-1VL. The zones obtained around the discs and were measured and interpreted using meter ruler.

Characterization of silver nanoparticles

The synthesized silver nanoparticles from mango peel extract solution were characterized by techniques such as UV visible spectroscopy, Fourier transform infrared (FT-IR), X ray diffraction (XRD), Scanning electron microscope (SEM) with EDS and Transmission Electron Microscope (TEM).

Antibacterial analysis

Antibacterial analysis was carried out against bacteria *E.coli*. Disc diffusion method was used for the analysis 10mm disc were purchased from himedia labs reference no. DD036-1VL. Then these discs are diffused in synthesized silver nanoparticles solution. Inhibition zone was observed in the discs containing synthesized silver nanoparticles solution. The zone obtained was measured using mm scale. Antibiotic sensitivity was also observed along as the standard for the experiment.

RESULTS

UV-Spectroscopy of synthesized Silver Nanoparticles

Analysis of synthesized silver nanoparticles from mango peels extract is carried out using double beam ultraviolet spectrophotometer 2201 which reveals the absorption band of the AgNPs. SPR peak at 445nm was observed in the synthesized silver nanoparticles solution, which indicated the synthesis of silver nanoparticles as the SPR band was in the range of silver nanoparticles.

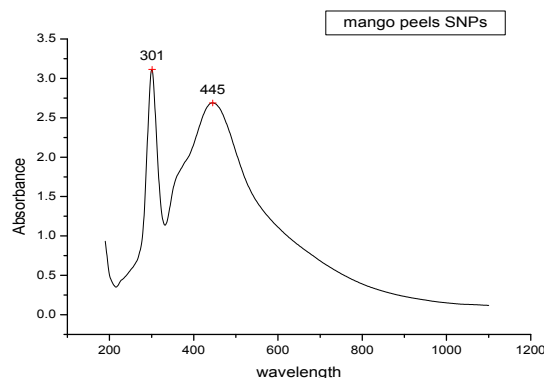


Fig 2 UV spectroscopy graph of Mango peels SNPs.

X-Ray diffraction

X ray diffraction confirms the crystalline property of silver nanoparticles. Peaks of the silver nanoparticles were observed at a specific degree. Data was analyzed for 2θ range of 30 to 80 degrees with a step of 0.02 degree. Peak at 38.00 degrees was observed which confirms the presence of silver nanoparticles and its crystalline form with reference to JCPDS file No. 030921. This peak was indexed as 111. Therefore the particles are crystalline Face Centered Cubic FCC. Average particle size was calculated 26.2nm using Scherrer equation.

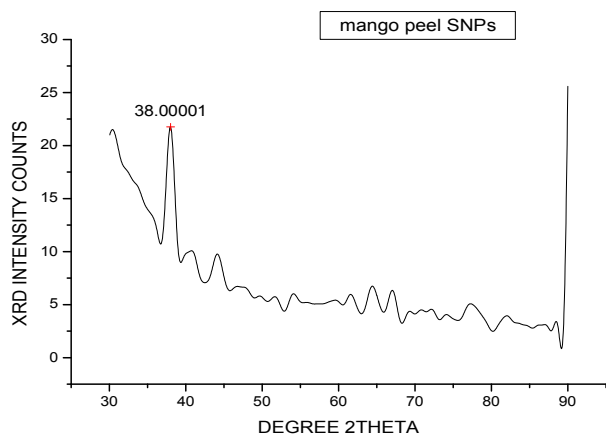


Fig 3 XRD Graph of Mango peels SNPs

Fourier Transform Infrared Radiation (FT-IR)

FT-IR is the technique which analyzes the surface functional groups involved in the reduction of silver nitrate to silver nanoparticles. Silver nanoparticles of Mango peel extract were subjected to FT-IR analysis in the range of 350 to 4500 cm^{-1} . The bands were observed at 1640.5 and 3327.6. 3327 peak indicates hydroxyl group of phenol or alcohol whereas peak at 1640 resembles $\text{C}=\text{C}$ group.

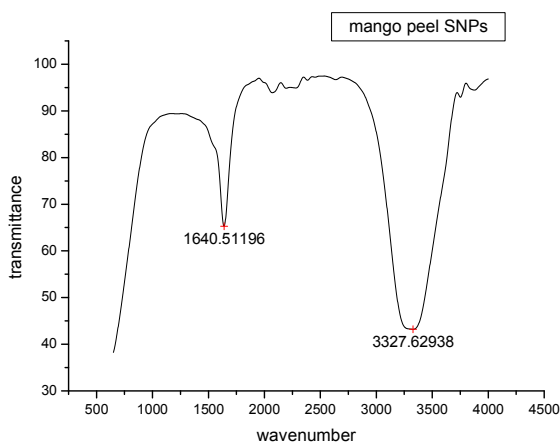


Fig 4 FT-IR Graph of Mango peels SNPs

Scanning electron microscopy with Energy Dispersive Spectroscopy (EDS)

SEM is the technique which reveals the morphology of the silver nanoparticles synthesized from mango peels extract it was observed that the silver nanoparticles are crystalline FCC with the symmetry in cubic, particles are oval in shape and are dispersed, agglomeration is observed in some places.

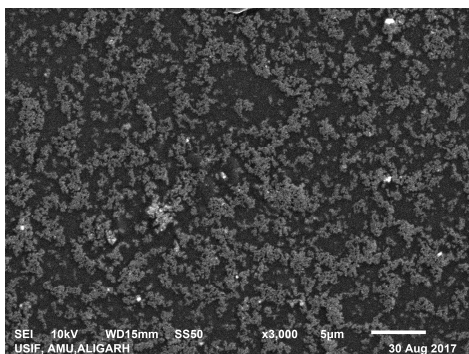


Fig 5 Image of Scanning electron Microscope of Mango peel synthesized SNPs.

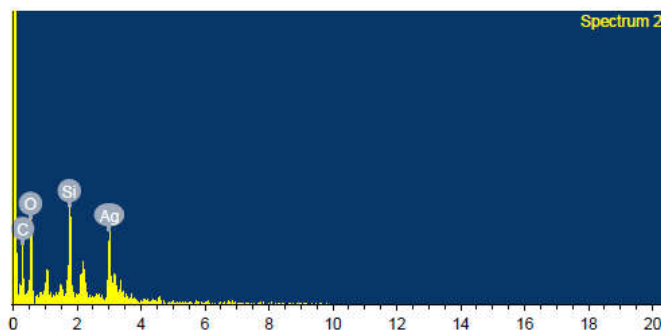


Fig 6 EDS report of SNPs

Energy Dispersive Spectroscopy revealed the silver present in the synthesized sample of silver nanoparticles

Element	Weight%	Atomic%
C K	23.93	36.31
O K	48.66	55.43
Si K	7.56	4.91
Ag L	19.84	3.35
Totals	100.00	

Transmission electron microscope

In the analysis of TEM it was observed that the particles size is ranging between 10.27 nm to 27.62nm. Agglomeration is observed in the images obtained in TEM.

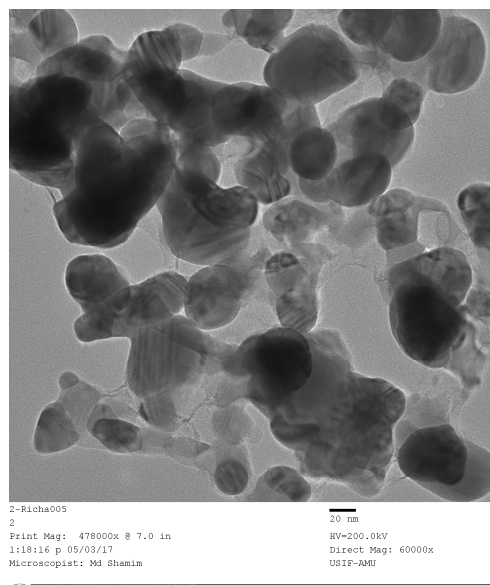


Fig 7 Image of Mango Peels SNPs showing agglomeration.

Antibacterial Analysis

Antibacterial analysis is done using synthesized silver nanoparticles in from mango peel extract against bacteria *E.coli* using disc diffusion method. Effective inhibitory zone appeared which are measured 21mm using meter ruler. Zone of inhibition obtained using synthesized silver nanoparticles are equivalent to the zone of inhibition obtained using antibiotic Gentamycin. Zone of inhibition revealed the antibacterial characters of synthesized silver nanoparticles from peels of mango, therefore this indicate the good possibility of mango peels nanoparticles as the target future drug against bacteria and may be used as a substitute of antibiotic to reduce the global concern of multi drug resistance.

Table 1 Zone of Inhibition Results of SNPs

Bacteria	Control peel extract	Silver nanoparticles	Antibiotic	Control AgNO ₃ Soln.
<i>Escherichia coli</i> ₁	0	21mm	19mm	0
Replicate plate ₁	0	20.5mm	19mm	0
Replicate plate ₂	0	21mm	18.5mm	0

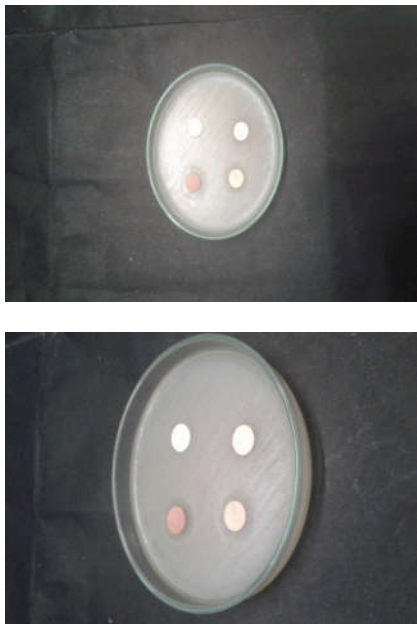


Fig 8 Images showing the zone of inhibition from silver nanoparticles synthesized biologically from mango peels in comparison with antibiotic gentamycin against *E.coli*.

CONCLUSION

Biogenic synthesis of silver nanoparticles from mango peel extract is an efficient method of synthesizing silver nanoparticles as it is less time consuming and very cost effective method, along with the utilization of the waste. The synthesized nanoparticles were effective against bacteria *E.coli* and thus can be an alternative to antibiotics in future.

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