



GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MEDICINAL PLANTS AND THEIR CHARACTERIZATION

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ABSTRACT:

Green synthesis of nanoparticle is a novel way to synthesis nanoparticles by using biological sources. It is gaining attention due to its cost effective, ecofriendly and large scale production possibilities. In this present study six plants *Hibiscus rosa sinensis*, *Moringa oleifera*, *Azadirachta indica*, *musa balbisiana*, *ocimum tenuiflorum*, and *Punica granatum* were taken to investigate their potential for synthesizing silver nanoparticle. The silver nanoparticles synthesized were confirmed by their change of color to dark brown due to the phenomenon of surface plasmon resonance. The characterization studied was done by UV-VIS spectroscopy. All the six plants synthesized silver nanoparticles show good antimicrobial activity against clinically important pathogens *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The studies describing the biosynthesis of silver nanoparticles followed by the investigation of antimicrobial and scavenging activities may be useful for green nanotechnology research opening a new area in this field.

Keywords:- Silver Nanoparticles, medicinal plants, antimicrobial activity.

INTRODUCTION :

Nanotechnology can be termed as the fabrication, characterization, exploration and application of nanosized [1-100 nm] materials for the development of science. It deals with the study of extremely minute structure and the prefix “nano” is a Greek word which means “dwarf or miniature”. The concept of nanobiotechnology emerged on 9th century. For the first time in 1959, Richard Feynman gave a talk on the concept of nanotechnology and described about molecular machines built with atomic precision where he discuss about nanoparticles and entitled that “there plenty of space at the bottom” [1].

“Nano-technology” mainly consists of the processing of separation consolidation, and deformation of materials by one atom or by one molecule [2]. In the 1980s the basic idea of this definition was explored in much more depth by Dr. K. Eric Drexler, who promoted the technological significance of nano scale

phenomenon and devises through speeches and the books Engines of creation: the coming era of nanotechnology (1986) and Nanosystems: molecular machinery, Manufacturing and Computation (and so the term required its current sense[3].

Nanotechnology and Nanoscience got started in the early 1980s with two major developments: the birth of cluster science and the invention of the scanning tunneling microscope (STM). In another development, the synthesis and the properties of semiconductor nanocrystals were studied; this led to fast increasing number of metal and metal oxide nanoparticles and quantum dots.

In 2000, the United States National Nanotechnology initiative was founded to coordinate federal nanotechnology research and development and is evaluated by the president’s Council of Advisors on science and technology. The nanoparticles (NPs) received a particular attention for their positive impact in

improving many sectors of economy ,including consumer products, pharma- ceutics, cosmetics, transportation, energy and agriculture etc. and are being increasingly produced for a wide range of new applications within industry are emerging rapidly[4].

In the recent days silver nanoparticles have been synthesized from the naturally occurring sources and their products like neem (azardica indica), various leaf broth, Hibiscus rosa sinensis, Moringo oliefera, Cucurbita maxima, etc,. With respect to the microbes, the silver nanoparticles get attached to the cell wall, there by disturbing the permeability of cell wall and cellular respiration. Besides the potency of the antibacterial effects corresponds to the size of the nanoparticles. The smaller particles have higher antibacterial activities due to the equivalent silver mass content. with respect to the clinical applications of nanoparticles, microorganisms including diatoms, fungi, bacteria and yeast producing inorganic materials through biological synthesis either intra or extracellular made nanoparticles more biocompatible[5].

Plants are known to harbor a big range of metabolites that are most likely to be responsible in the green synthesis of metal nanoparticles. Biotechnological approaches, exclusively plant tissue cultures provide a better platform in producing specific phytoconstituents at a rate similar or superior to that of intact plants [6]. Explants are cultured under appropriate physiological conditions and the desired product is extracted from the cultured cells/tissue by means of plant tissue culture. However, the biosynthetic capacity of cultured plant tissue can be enhanced by regulating environment factors, which are effective for the high production of NPs [7].

Recent developments in plant tissue culture techniques in fabrication of NPs have shown promising results to improve the productivity

many folds [8]. As plants potentially eliminate the environmental issues by making the NPs more biocompatible, it is necessary to increase the efficiency of the locally available and unexplored plants resources for the green synthesis of AgNPs and clarify the possible mechanism involved in synthesis, is still infancy. The silver nanoparticles have wide spread antimicrobial resistance [9].

The principle of preparation of silver nanoparticles by using plant extract and microorganism is a bioreduction process ;the silver metal ions are reduced by the extracellular reductase enzymes produced by the microorganisms to silver metal in nanometer range.

MATERIAL AND METHOD :

Six Indian medicinal plants *M. balbisiana*, *A. indica* ,*O. tenuiflorum* , *M. oleifera* ,*P. granatum* , *Hibiscus rosa sinensis* were collected from the locality of Nagpur on the basis of cost effectiveness, ease of availability and medicinal property. Fresh and healthy leaves were collected locally and rinsed thoroughly with tap water followed with distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature. About 10 gm of these finely incised leaves of each plant type were weighed separately and transferred into 250 ml Erlenmeyer beakers containing 100 ml distilled water and boiled for about 20 min. The extract were then filtered through Whatmann no. 1 filter paper to remove particulate matter and to get clear solution which were then refrigerator (4°C) in 250 ml Erlenmeyer flasks for further experiments.

Preparation of 1mM AgNO₃ solution

Accurate concentration of 1 mM AgNO₃ was prepared by dissolving 0.017 gm of AgNO₃ in 100 ml of distil water..20 and stored in amber colored bottle in cool and dry place.

Synthesis of silver nanoparticles

Aqueous solution (1mM) of silver nitrate (AgNO_3) was prepared in 250 ml Erlenmeyer flasks. To 50 ml of 1 Mm silver nitrate (AgNO_3) solution, 5 ml of extract was added drop wise for reduction of Ag^+ ions for each type of plant extract. The composite mixture was then kept on dark condition to avoid photo activation for 24 hrs at room temperature. In the mean time, color change of the mixture from faint light yellow to reddish brown to colloidal brown was monitored periodically.

UV-spectra analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-VIS spectrum of the spectrophotometer at a resolution difference of 20 nm (from 400 to 600 nm) in 2 ml quartz cuvette with 1 cm path length.

Antimicrobial activity

The comparative antibacterial activities of the plant extract and of the Ag NPs synthesized from the respective extract were effectively accessed against gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and gram negative (*E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*).

Well diffusion method was followed for testing each type of plant leaf extract and their respective Ag NPs containing solution. The plates containing nutrient agar media were prepared by swabbing them with the 24 hr old microbial cultures prepared in nutrient broth. Plates containing media as well as culture divided into four equal parts and wells were prepared with the help of sterile borer. The extract were placed in well in the following order. 10 μl of distilled water as a negative control, 10 μl plant leaves extract, 10 μl 1 mm silver nitrate and 10 μl of synthesized silver nanoparticles. [10]

RESULT AND DISCUSSION

According to literature studies silver nanoparticle solution has dark brown or dark reddish in color. In *H. sinensis* before addition of

AgNO_3 its color was grey but after its treatment with AgNO_3 its color changes to dark brown which indicated the formation of AgNPs. Likewise all the other plants extract (*H. sinensis*, *O. tenuiflorum*, *A. Indica*, *M. oleifera*, *M. balbisiana*, *punica granatum*) color changed to dark brown after treatment with AgNO_3 . This color change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles [11].

The UV- vis spectra recorded after time interval of 24 hrs from the initiation of the reaction. Absorption spectra of Ag NPs formed in the reaction media has absorption maxima in the range of 435-465 nm due to surface Plasmon resonance of Ag NPs. The UV spectra recorded, implied that most rapid bioreduction was achieved *Moringo oliefera* extract followed by other plant extract. These were denoted by the broadening of the peak which indicated the formation of polydispersed large nanoparticles due to slow reduction rate. [12]

Determination of antimicrobial activity

In these study antibacterial activity of AgNPs were investigated by growing *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumonie* colonies on nutrient agar plates supplemented with AgNPs. A control plate was separately maintained for above organisms. Results obtained are shown in fig.4-5. The inhibition zones obtained indicates maximum antibacterial activity of the prepared test sample. Results obtained in previous studies [13] also support the antibacterial potential of AgNPs. The zone of bacterial inhibition by AgNPs prepared from *Moringo oliefera* leaf extract show maximum inhibition for *B. subtilis*, and *K. pneumonie*, which may be concluded from the fact that these particles had the smallest diameter than those prepared by other five plant extract. Also, in comparison to AgNO_3 and AgNPs, there is no such prominent antimicrobial activity in case of

the plant extract when used in crude form. No zone of inhibition was obtained in case of control.

CONCLUSION :

In this study silver nanoparticles were successfully obtained from the bioreduction of silver nitrate using *H. sinensis*, *M. balbisiana*, *O.tenuiflorum*, *P.granatum*, *M. oleifera*, *A. indica*. The UV absorption peak at 435-635 nm clearly indicates the synthesis of AgNPs. The AgNPs showed good antimicrobial activity against *K. pneumonia*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and *E. coli* that were resistant to majority of antibiotics.

By studying the different characteristic data we could conclude that AgNPs synthesized from *H. sinensis* were less stable than the other five plant extracts which could be due to the absence of capping and stabilizing materials as petals mainly contains pigments for attracting different insects for pollination. Present study results denoted by *Moringa oleifera* and *A. indica* extract SNPs to be a better reducing agent and showed excellent antimicrobial activity against tested MDR strains. *Pseudomonas aeruginosa* exudates SNPs can extracellularly biosynthesize thermodynamically stable desired size and shape of silver nanoparticle by optimizing pH and temperature. The bacterial exudates SNPs were showed good antimicrobial activity against all pathogens but it is most sensitive against *P. aeruginosa*.

Based on the result presented in this work green synthesize AgNPs can be recommended as a good alternative for the control of microorganisms with less risk of toxicity. Further studies should investigate the combination of AgNPs and antibiotics against various MDR strains for the development of new materials and substances for medical application.

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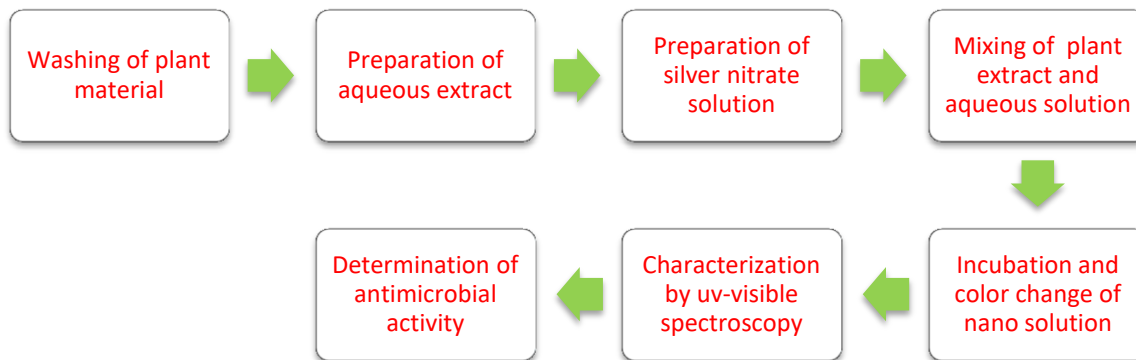
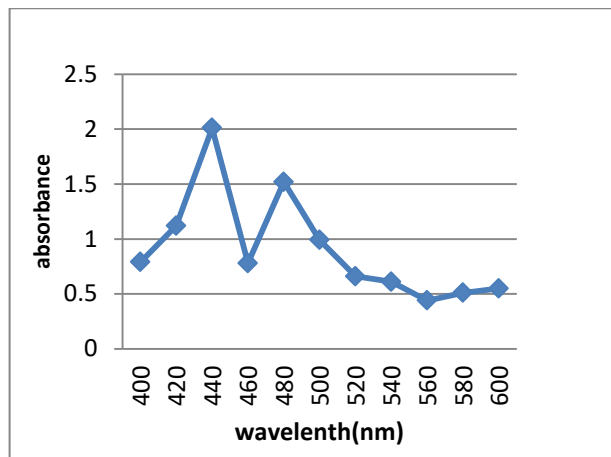


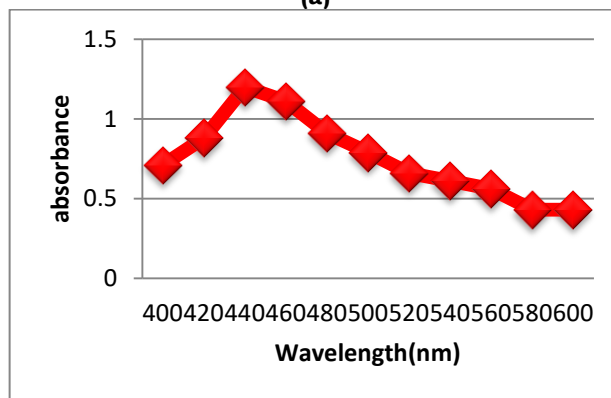
Fig 1: schematic representation of various steps involved in the execution of experiment in the present study.



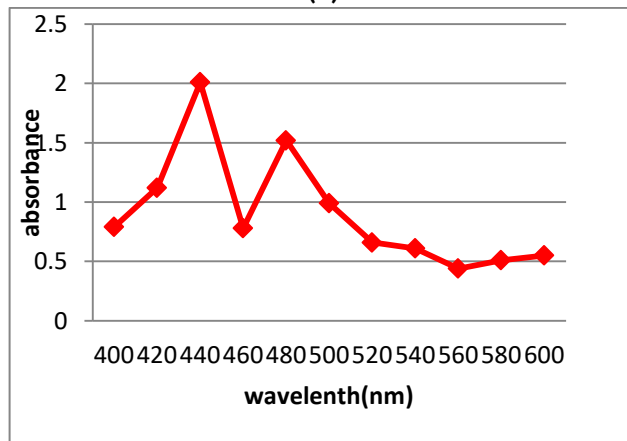
Fig 2:- Color change of Moringa oleifera plant extract before and after addition of AgNO3



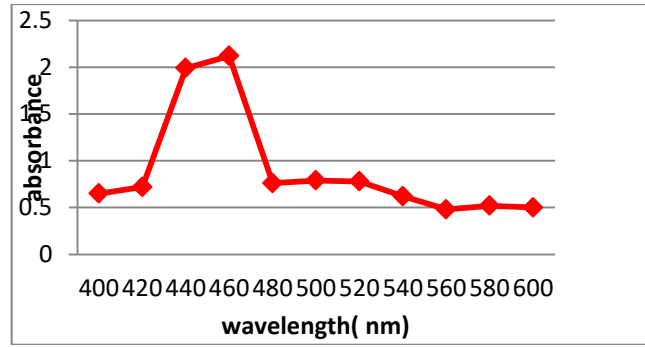
(a)



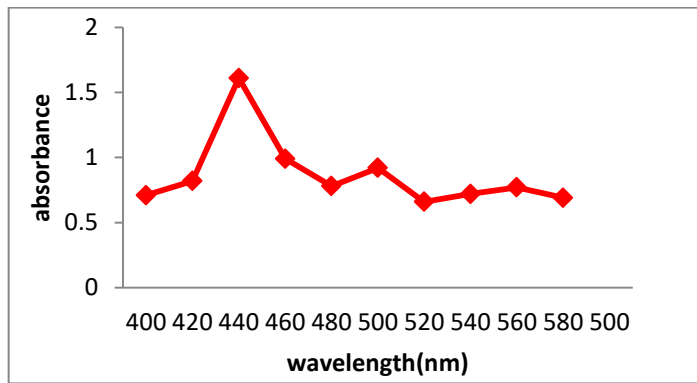
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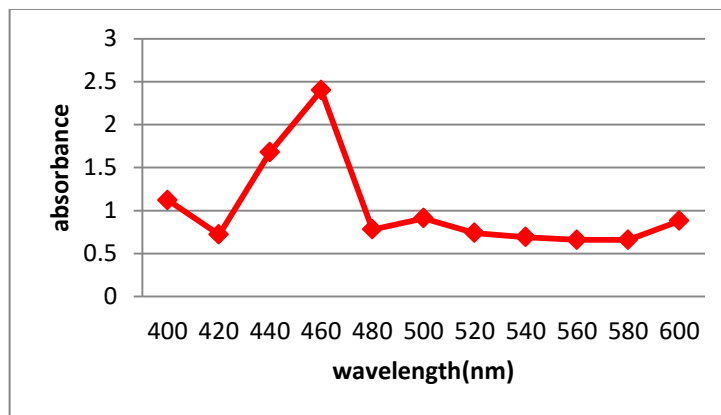
(c)



(d)



(e)



(f)

Fig 3: UV spectra analysis of SNPs synthesized from (a)Moringo oliefera (b)Azardichata indica (c)Hibiscus rosa sinensis (d)Ocimum tenuiflorum (e)Punica granatum (f)Musa balbisiana



Fig 4:- Antimicrobial effect of synthesized AgNP_s on 1) Moringa oliefera (K.Pneumoniae) 2) A. Indica (E. coli)

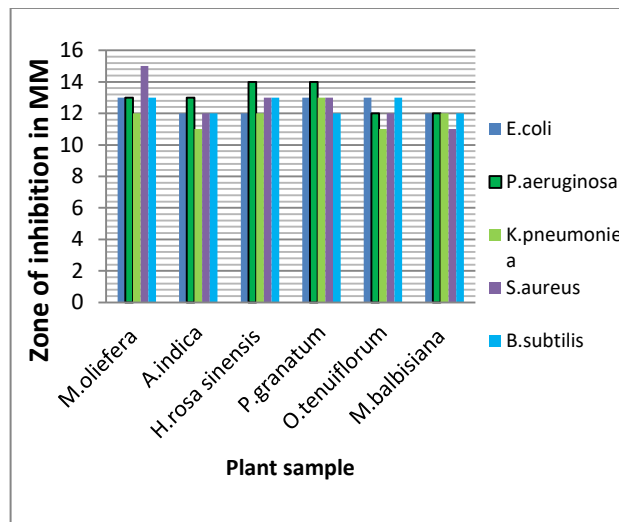


Fig 5:- Graphical representation of the size of the zone of inhibition for the five tested bacterial culture against SNPs synthesized from different plant extract