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GREEN CELLULAR DELIVERY OF SILVER NANOPARTICLE FROM *MIRABILIS JALAPA* FLOWER EXTRACT AND ITS ANTIPATHOGENIC ACTIVITY

[1]C R Rathi, [2] S N Suresh,

Department of Biotechnology

Sree Narayana Guru College of Arts and Science, Coimbatore, Tamil Nadu

ABSTRACT

Bio friendly green modest synthesis of nanoparticles are the present research in the extremity of nanotechnology. This study has been undertaken to explore the determinants of silver nanoparticles from 1 mM $AgNO_3$ solution through profuse concentration of aqueous flower extract of *Mirabilis jalapa* reducing besides immobilizing agent. The attribute of silver nanoparticles was studied by using UV-VIS spectroscopy SEM and XRD. The XRD spectrum of the silver nanoparticles established the presence of elemental silver signal. Green synthesized silver nanoparticle manifests the zone of inhibition against isolated human pathogenic (*Streptococcus species*, *Bacillus* species, *Staphylococcus species*, *Klebsiella species* and *E. coli*) bacteria. The analytical chassis contains the flower pigment betalain the natural food dye resources can efficiently use in the production of silver nanoparticle and it could be utilized in various fields in therapeutical and nanotechnology.

Index Terms **- Nanoparticles, *Mirabilis jalapa*, Antibacterial activity, UV-VIS spectroscopy, SEM, XRD.**

I. INTRODUCTION

Mirabilis jalapa (four O' Clock plant) belongs to the family *Nyctaginacea*e. Mirabilis jalapa bloom all the summer long. They have antifungal, antimicrobial, antiviral, antispasmodic and antibacterial properties (Dimayuga, 1998: Yang, 2001). Mirabilis jalapa flower contains betalain pigments are classified in to red(crimson) betacyanin's and yellow betaxanthins (Strack et al., 2003). Antibacterial properties of silver are documented since 1000 B.C., when silver vessels were used to preserve water (Richard *et al.*, 2002; Castellano *et al.*, 2007). Environmental-friendly antimicrobial nano paint can be developed (Kumar *et al.*, 2008). Silver has been known to possess strong antimicrobial properties both in its metallic and nanoparticle forms hence; it has found variety of application in different fields. The Fe_3O_4 attached Ag nanoparticles can be used for the treatment of water and easily removed using magnetic field to avoid contamination of the environment (Gong *et al.*, 2007). The silver nanoparticles are reported to show better wound healing capacity, better cosmetic appearance and scar less healing when tested using an animal model (Tian *et al.*, 2006). Although few silver-containing compounds are approved by the FDA for direct food contact, silver-incorporated food packaging is quite widespread in Japan (Appendini and Hotchkiss, 2002). Silica gel micro-spheres mixed with silica thio-sulfate are used for long lasting antibacterial activity (Gupta and Silver, 1998) and treatment of burns and various infections (Feng *et al.*, 2000). Silver has been known to possess strong antimicrobial properties both in its metallic and nanoparticle forms hence; it has found variety of application in different fields. The Fe_3O_4 attached Ag nanoparticles can be used for the treatment of water and easily removed using magnetic field to avoid contamination of the environment (Gong *et al.*, 2007). Silver nanoparticles can be used for water filtration (Jain and Pradeep, 2005). Use of plant sources offers several advantages such as cost-effectiveness, eco-friendliness and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods (Sun and Xia, 2002).

The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burns and open wound. Silver ions (Ag^+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells (Prema, 2011). It has also been proposed that the antibacterial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage (Danilczuk *et al.*, 2006; Kim *et al.*, 2007). The silver nanoparticle containing poly vinyl nano-fibers also show efficient antibacterial property as wound dressing (Jun *et al.*, 2007). Silver zeolite is used in food preservation, disinfection and decontamination of products (Matsuura *et al.*, 1997; Nikawa *et al.*, 1997). It was also observed that when treated with Ag^+, *E. coli*, a gram-negative bacterium, sustained more structural damages than the gram-positive *Staphylococcus aureus* (Feng *et al.*, 2000). Silver impregnated medical devices like surgical masks and implantable devices show significant antimicrobial efficacy (Furno *et al.*, 2004). Feng *et al.*, (2000) conducted a study to observe the effects of silver ions on gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*). Use of plant sources offers several advantages such as cost-

effectiveness, eco-friendliness and the elimination of high pressure, energy, temperature, and toxic chemicals necessary in the traditional synthesis methods (Sun and Xia, 2002).

Cell membrane detachment from the cell wall, cell wall damage, and electron dense granules outside and, in some instances, inside the cell. It was proposed that condensation of DNA occurred as a protective measure in order to protect the genetic information of the cell (Feng *et al.*, 2000). Silver zeolite is used in food preservation, disinfection and decontamination of products (Matsuura *et al.*, 1997; Nikawa *et al.*, 1997). Silver sulfadiazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids (Fox and Modak, 1974). Toxicity from silver is observed in the form of argyria, only when there is a large open wound and large amount of silver ions are used for dressing. There are no regular reports of silver allergy (Leaper, 2006). Studies shows that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria (Morones *et al.*, 2005). cell membrane detachment from the cell wall, cell wall damage, and electron dense granules outside and, in some instances, inside the cell. It was proposed that condensation of DNA occurred as a protective measure in order to protect the genetic information of the cell (Feng *et al.*, 2000). Silver sulfadazine depicts better healing of burn wounds due to its slow and steady reaction with serum and other body fluids (Fox and Modak, 1974). The main objectives of this study were to (1) Synthesize the silver nanoparticles using aqueous flower extract of Mirabilis jalapa, (2) characterization of silver nanoparticles by using UV-Vis spectroscopy, SEM-XRD (3) analyze antimicrobial properties against human pathogenic bacteria.

II. MATERIALS AND METHODS

i. SAMPLE COLLECTION

Mirabilis jalapa flower extract were collected from Thodupuzha, Idukki district of Kerala state, India.

The fresh flowers were collected and washed with distilled water. The plant materials were thoroughly washed with distilled water and fresh weight were determined. The samples are then oven dried at 50°C for 24 h. The dried samples were powdered using a waring blender and stored in air-tight bottles until further analysis.

ii. *Extraction Method*

Mirabilis jalapa flower extract was prepared with 10 g of fresh flower taken in a beaker. It was thoroughly washed with tap water and then with distilled water for at least 2 times and cut into small pieces. The chopped flowers were boiled in 50ml of distilled water for 5 minutes. The

flower pigment extract was then cooled and filtered. The filtered flower pigment samples collected and stored in air tight bottles at 4°C until further analysis.

iii. *Synthesis of Sliver Nanoparticles*

Stock solution was prepared by dissolving 1mM sliver nitrate ($AgNO_3$; Merck, Chennai, India) and volume made up to 250 ml with distilled water. 5ml of Mirabilis jalapa flower extract was added to 100 ml of 1mM $AgNO_3$ solution and allowed to react at room temperature.

iv. *Test Microorganisms*

The common human pathogenic organisms used (*Staphylococcus, Bacillus, Streptococcus, Salmonella, Klebsiella* and *Escherichia coli*). The test organisms were obtained from microbial stock cultures, District hospital Thodupuzha, Idukki, Kerala.

v. *Characterization of Silver Nanoparticles*

UV –Vis Spectroscopy the periodic scans of the optical absorbance between 380 and 500nm with a UV- Vis spectrophotometer (Model 118, Systronics, Mumbai, India) at a resolution of 1 nm were performed to investigate the reduction rate of silver ions by *Mirabilis flower* pigment extract. The reaction mixture was diluted 20 times and used for UV-Vis spectrophotometry. Deionised water was used to adjust the baseline.

vi. *Antibacterial Assay*

Nutrient agar and Muller Hinton agar plates were made according to standard microbiological protocol. Filter paper discs of approximately 6 mm diameter were soaked with 10µl, 20µl, 30µl, 40µl, 50µl respectively of the flower pigment extract, $AgNO_3$ and silver nanoparticle separately and allowed to dry at room temperature for 15 minutes. Muller Hinton Agar plates were prepared and the test microorganisms were inoculated by the spread plate method. Prepared discs were placed in the previously prepared agar plates. Each plate of every test organisms contained discs impregnated with Ag nanoparticle, flower extract, silver nitrate solution and an antibiotic disc. The discs were pressed down to ensure complete contact with the agar surface and distributed evenly so that they were not closer than 24 mm from each other, to Center. The agar plates were then incubated at 37ºC. After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of inhibition were measured, including the diameter of the disc where the antibiotic was used as control (NCCLS, 1997).

Figure 1: a) *Mirabilis jalapa* plant with flower b) *Mirabilis jalapa* fresh flower extract c) Mixture of *Mirabilis jalapa* flower extract and silver nitrate over 2 hours incubation.

III. RESULTS AND DISCUSSION

i. Synthesis of Sliver Nanoparticles

After the addition of *Mirabilis jalapa* flower extract to $AgNO_3$ solution a visible color change from transparent to dark brown was observed which indicates the formation of silver nanoparticle. This occurred due to the reduction of silver ions present in the solution due to terpenoids present in *Mirabilis jalapa* flower extract. After 90 minutes there was no change in the intensity of color developed, which indicates the completion of reduction reaction. The reduced silver particles are in the range of nano size.

ii. Characterization of Sliver Nanoparticles

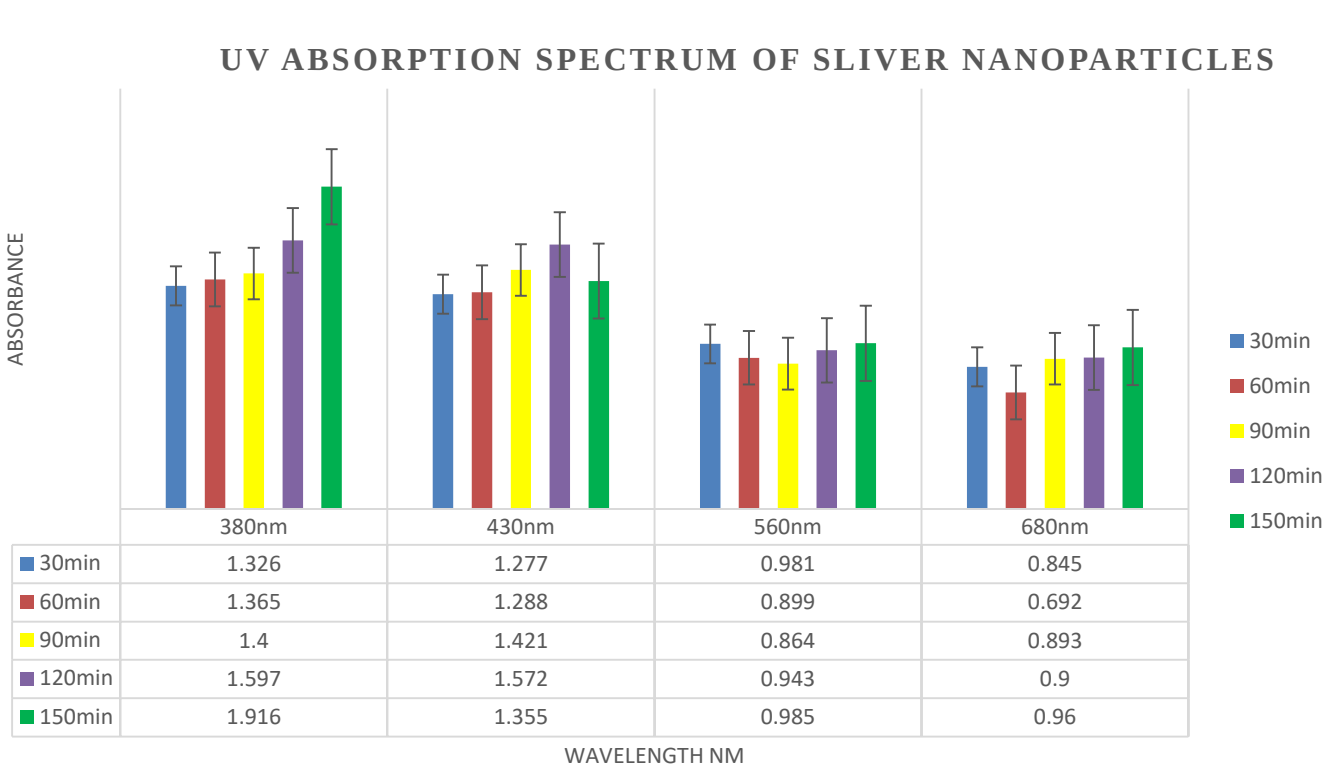
UV Spectrometry the UV absorption spectrum of silver nanoparticles from *Mirabilis jalapa* flower extract of different concentrations was obtained as given in Figure 2.

iii. Anti-Bacterial Assay

For *Mirabilis jalapa* flower the zone of inhibition was found to be 14-26 mm for *Klebsiella species*, 15-28 mm for *Bacillus species*, 14-211mm for *E. coli*, 13-27 mm for *Staphylococcus species*, 15-25 mm for *Salmonella species*, and 12-27 mm for *Streptococcus species*. The study done by Gavane *et al.*, (2012) the zone of inhibition found was 14-26 mm for *Klebsiella species*. Silver ions and silver salts are used as antimicrobial agents (Russel *et al.*, 1994). However, the high concentrations of silver salts restrict the use of them in present day medicine. Use of metal nanoparticles decreases the concentration of silver and other metal salts. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio which allows them to interact closely with microbial membranes and is not merely due to release of metal ions in solution or in culture plates (Morones *et al.*, 2005). The mode of action of both silver nanoparticles and silver ions were reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentration than that of the ions (Morones *et al.*, 2005). According to Lok (2006), the attachment of both silver ions and nanoparticles to the cell membrane caused acclimatization of envelope protein precursors causing dissipation of the protein motive force.

iv. SEM-XRD Analysis

SEM-XRD analysis proved the effective formation of silver nanoparticles in *Mirabilis jalapa* flower pigment extract. SEM-XRD analysis was carried out in instrument JSM 6390 with acceleration voltage 20 kV. SEM reveals information about the sample including external morphology, chemical composition and crystalline structure and orientation of materials making up the sample (Yeo SY and Jeong SH (2003). SEM provides detailed high-resolution images of the sample by rastering a focused electron beam across the surface and detecting secondary or back scattered electron signal. The XRD imaging of the silver nanoparticles was performed to confirm the presence of elemental metal signal and provides quantitative compositional information (Oberdorster G, Oberdorster E and Oberdorster J (2005).



	380nm	430nm	560nm	680nm
30min	1.326	1.277	0.981	0.845
60min	1.365	1.288	0.899	0.692
90min	1.4	1.421	0.864	0.893
120min	1.597	1.572	0.943	0.9
150min	1.916	1.355	0.985	0.96

Figure 2: ***Mirabilis jalapa*** **flower extract UV absorption spectrum of sliver nanoparticles**

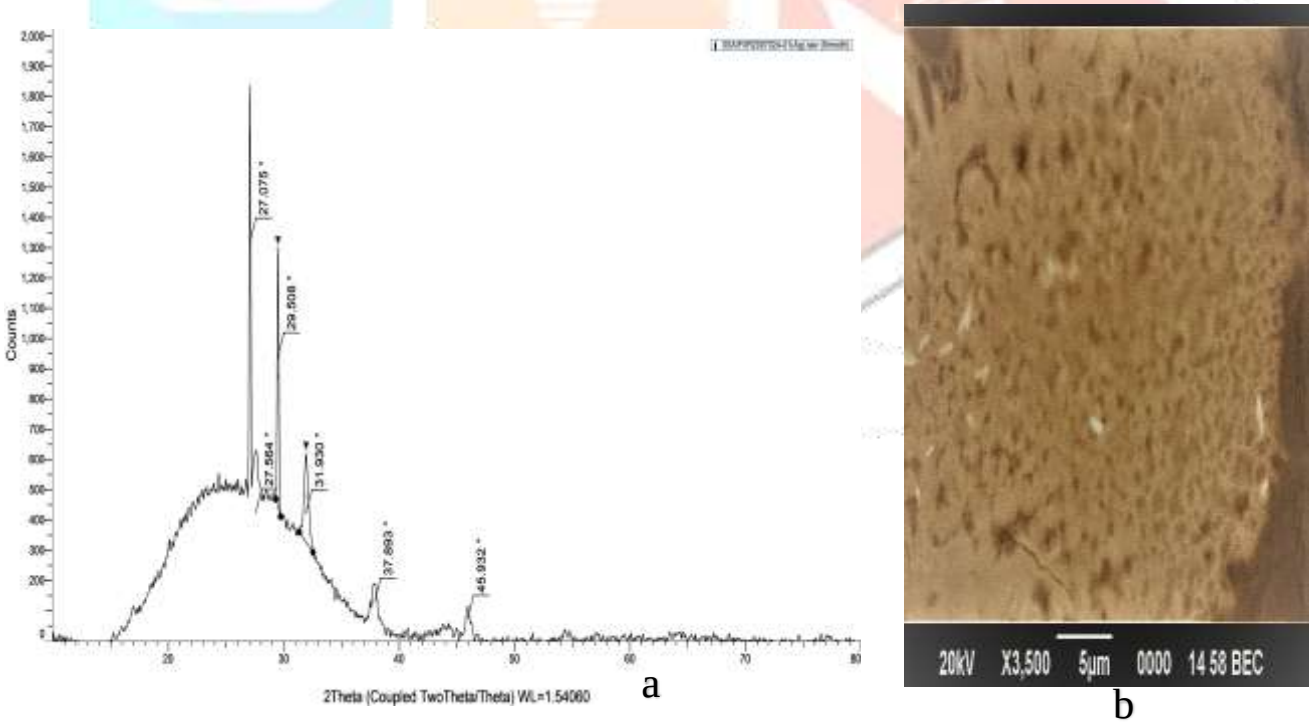


Figure 3: ***Mirabilis jalapa*** **flower extract silver nanoparticles a) X-Ray Diffractogram Ag (Coupled Two Theta/Theta); b) SEM microgram of silver nanoparticles.**

Table 1. Zone of inhibition against various bacteria (*Escherichia coli, Salmonella typhi, Staphylococcus species, Klebsiella species, Bacillus species,)* using silver nanoparticles produced by *Mirabilis jalapa* red pigment 1/100 dilution.

Microorganism	control	Sample	Measure zone of inhibition in mm				
			10 µl	20 µl	30 µl	40 µl	50 µl
Escherichia coli	12	10	14	16	19	20	23
Salmonella typhi	13	11	15	17	20	22	25
Staphylococcus aureus	14	12	13	15	17	21	27
Klebsiella species	11	10	14	16	19	20	26
Bacillus species	14	13	15	18	21	24	28
Streptococcus species	13	12	12	14	17	23	27

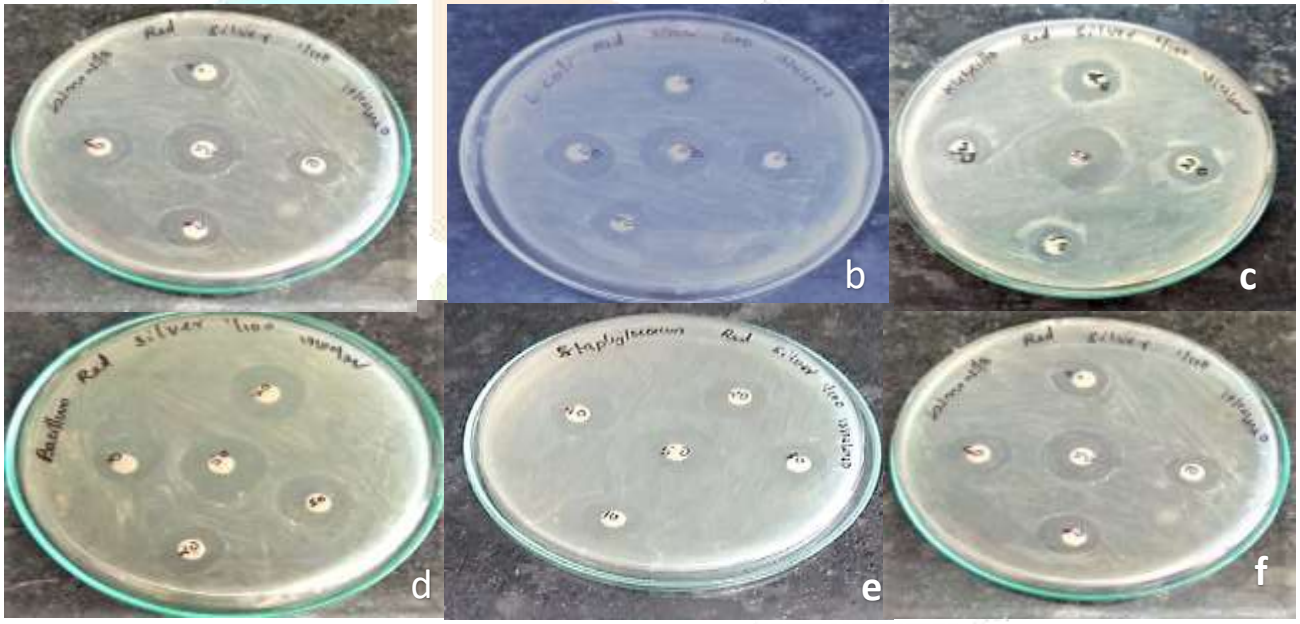


Figure 4: Antimicrobial activity of *Mirabilis jalapa* flower extract green synthesized silver nanoparticles against a) Streptococcus species b) Escherichia coliform c) Klebsiella species d) Bacillus species e) Staphylococcus species f) Salmonella species at the concentration of 10µl, 20µl, 30µl, 40µl, 50µl respectively.

IV. CONCLUSION

The *Mirabilis jalapa* flower extract was draw up from fresh flower by boiling it 5 minutes. The prevail extract was of reddish in colour. Freshly prepared flower extract was appended to 1mM silver nitrate solution and the reaction takes place at room temperature which resulted in the synthesis of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-VIS spectrometry, SEM and XDX measurements. The UV-VIS spectra of silver nanoparticles formed in the reaction media has absorbance peak at 380 nm. It has been indicated that *Mirabilis jalapa* extract is competent of fabricating silver nanoparticles that shows good firmness in solution. The synthesized silver nanoparticles were distinguished by UV-VIS spectrum, SEM and XDX measurements. This green synthesis method is substitute to chemical method, since it is contemptible, non-polluting and green-friendly. The results showed that *Mirabilis jalapa* flower plays a salient role in the reduction and stabilization of silver to silver nanoparticles. Further, these synthesized silver nanoparticles from *Mirabilis jalapa* shows antibacterial activity on human pathogenic bacteria.

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