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Preparation of silver nanoparticles using leaf and fruit extracts of *Morinda coreia* Buck., Ham. –A green approach

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Abstract

Nanotechnology has been a recent developed field of science. Several methods were employed to synthesize different types of nanoparticles. In the present study, we demonstrate the synthesis of silver nanomaterials from leaf and fruit aqueous extracts of *Morinda coreia* Buck., Ham. using the biological method. The silver nanoparticles formation was confirmed by the colour change of the mixture and further confirmed by spectral analysis. The UV-Visible spectrum of colloidal solutions have absorbance peaks at 421.6- 434.4 nm regions with fruit extract and 431.2-442.4 nm regions observed with the leaf extract measured at various time intervals. These nanoparticles were further used to test the antimicrobial activity against five human pathogenic bacteria.

Keywords: Silver nanoparticles, *Morinda coreia* Buck., Ham., Characterization, Antimicrobial activity.

Introduction

Nano-biotechnology deals with the biotic and abiotic materials in the size varies from 1nm to 100 nm.[1] Nanoparticles have new properties and these are depending on the specific characteristics such as size, morphology and distribution.[2] The silver nanoparticles have various important applications. Historically, silver has been known to have disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganism at low concentrations without any side effects.[3]

Nanomaterials and nanodevices contain potential to reduce pollutions such as water and air pollutions and can be used for more efficient alternate energy production.[4] The use of plant parts like stem, leaf, flower, fruit, seed, bark, etc. for the synthesis of nanoparticles is a quite novel method leading to truly green chemistry compare to other methods like chemical and physical method. This is cost effective, environment friendly and easily scaled up process for large scale synthesis.[5, 6] Now days researchers are using bacteria, fungi for the synthesis of nanoparticles but use of a leaf extract reduce the cost as well as we do not require any special culture preparation and isolation techniques.

Silver nanoparticles synthesized using aqueous extracts of different plant species were tested for their antimicrobial activities by several researchers and found these highly positive.[7-10]

Morinda coreia Buck., Ham. (belongs to Rubiaceae family) is an important medicinal plant. The plant parts contain valuable secondary metabolites such as, anthraquinone, vitamin A and C, flavonoids phenolics compounds, etc. This plant is used by the traditional medical practitioners in all kinds of systems of medicine.[10]

In the present study, the aqueous solutions of silver nitrate, which represent silver ions, were converted into silver nanoparticles with the help of fruit and leaf extracts of *M. coreia* and their screening of antibacterial assessment was performed.

Materials and Methods

The plant material was collected from the coastal area of Pondicherry, India. Fresh, green and mature fruits and leaves were harvested and thoroughly washed with distilled water. The fruit and leaves were finely cut into small pieces. The plant fruit pulp and leaf broth solutions were prepared by using 5 gm of washed and cut fruit pulp and leaves in a 250 ml Erlenmeyer flask with 50 ml of sterile distilled water and boiling the mixture for 5 minutes. The aqueous extracts were collected in separate conical flasks by standard filtration method and stored at 4ºC.

1mM aqueous solution of Silver nitrate (Himedia, Mumbai) was prepared for synthesis of silver nanoparticles. For the synthesis of SNPs, three boiling tubes were taken, one containing 10 ml of 1mM $AgNO_3$ solution as control, the second containing 9 ml of 1mM Silver nitrate solution and 1 ml of plant fruit pulp extracts, third one test tube 9 ml of 1mM Silver nitrate solution and 1 ml of plant leaf extract as test solution. These were incubated at room temperature for 1-2 hours. The color change of the leaf extracts from pale yellow to dark brown was checked periodically up to 48 hours. They were centrifuged at 5000 rpm for 15 minutes in order to obtain the pellet which was used for further study. Supernatants are discarded and the pellets are dissolved in deionised water. The silver nanoparticules were confirmed by color changes and qualitatively characterized by UV-Visible spectrophotometer.

The reduction of metallic Ag^+ ions was monitored by measuring the UV-Vis spectrum after about 12 hours of reaction. A small aliquot was drawn from the reaction mixture and a spectrum was taken at a wavelength from 300 nm to 700 nm on UV-Vis spectrophotometer (Systronics Double beam spectrophotometer 2202).

Antibacterial activities of plant extract-mediated silver nanoparticles were assessed using standard well-diffusion method. The test bacteria (human pathogenic bacteria) such as Bacillus subtilis, Escherichia coli, Klebsiella pheumoniae, Pseudomonas aeroginosa and Shigella flexneri were included in this study to assess the susceptibility pattern of the nanoparticles. Nutrient Agar (NA) was prepared for cultivation of the bacteria. 100µl of fresh overnight grown cultures of the bacteria were spread on Nutrient Agar containing Petri plates. With a sterile borer 1 mm holes were punched in the medium. 100 µl of the solution containing nanoparticles was inoculated in this hole and the plates were incubated at 37ºC for 24-48 hours of observing zone of inhibition.

Results and Discussion

The green synthesis of silver nanoparticles using fruit and leaf extracts of *M. coreia* was carried out in the present study. The color was changed in the cell free fruit and leaf extracts when challenged with 1mM $AgNO_3$ from pale yellow to brown (Fig. 1C) and dark brown (Fig. 1F) within 48 and 24 hours respectively. These attained the maximum intensity after 12 hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticle. Fruit pulp extract when subjected to 1mM $AgNO_3$ it did attain slow color changes with time duration as compared to leaf extract. The reaction was completed within 48 and 24 hours respectively. Control (without silver ions) showed no change in color of the cell filtrates when incubated under the same conditions (Fig. 1A, B, D and E).

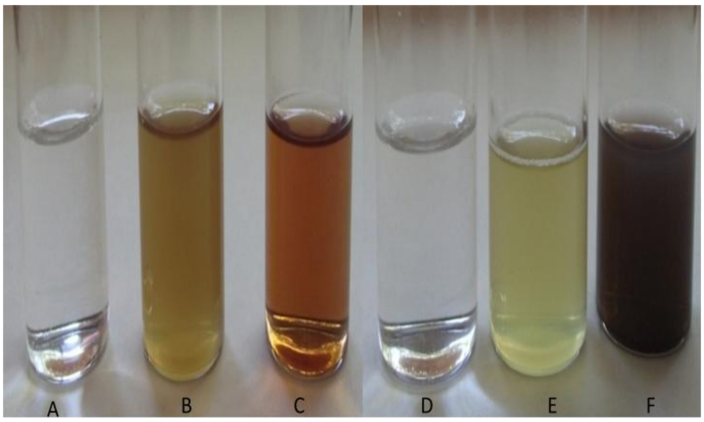

Figure 1: Change in color of the cell filtrates

Extracts from plants may act as reducing and capping agents in silver nanoparticles synthesis. The reduction of Ag+ ions by combinations of biomolecules found in these extracts (e.g. enzymes/proteins, amino acids, polysaccharides, vitamins, etc.) is environmentally benign, yet chemically complex.[11] The extract of lower plants (algae, fungi, etc.) was also used to synthesize SNPs at room temperature. Proteins in the extract provide a dual function of Ag^+ reduction and shape control in the nanoparticle synthesis. The carboxyl groups in aspartic and/or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins were suggested to be responsible for the Ag^+ ion reduction.[12]

The silver nanoparticles exhibit dark brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles.[13] The appearances of brown and dark brown colour in the reaction vessels suggest the formation of silver nanoparticles.[14]

SNPs synthesis had been confirmed by measuring the UV-Visible spectroscopy of the reaction media. The UV-Vis spectrum of colloidal solutions of AgNPs synthesized from *Morinda coreia* fruit pulp and leaf extracts have absorbance peaks at 421.6 – 434.4 nm regions measured at various hours (20, 24, 44, 48 and 50 hrs for fruit pulp, Fig. 2) and 431.2 – 442.4 nm regions observed at 20, 21, 22, 23 and 24 hrs for leaf (Fig. 3), which are identical to the characteristics UV-visible spectrum of metallic silver. The reaction mixture of fruit extract took 48 hrs to complete the reaction and gave the stable reading in spectral analysis. The weak absorption peaks at shorter wave lengths was due to the presence of several organic compounds which were known to interact with silver ions.

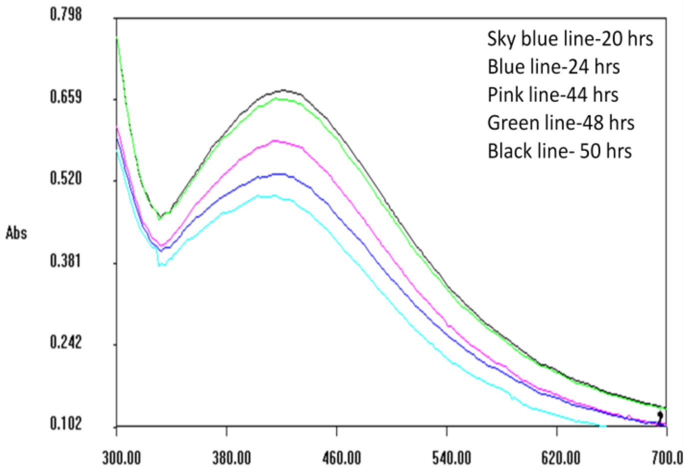

Figure 2: UV-Vis spectrum of colloidal solutions of AgNPs synthesized from *Morinda coreia* fruit pulp extracts

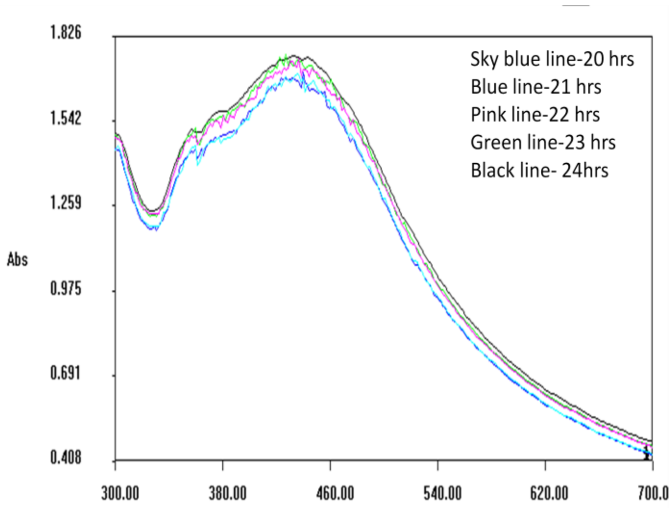

Figure 3: UV-Vis spectrum of colloidal solutions of AgNPs synthesized from *Morinda coreia* leaf extracts

Silver nanoparticles synthesized by biologically method using medicinal plants were found to be highly toxic against different pathogenic bacteria. Silver nanoparticles are very effective against micro-organisms because of their enormously high surface area. Antibacterial activity of SNPs was studied through Well-Diffused method in present investigation. Five human pathogenic bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pheumoniae*, *Pseudomonas aeroginosa* and *Shigella flexneri* were used for the screening of antibacterial activity of synthesized silver nanoparticles from extracts. The maximum zone of inhibition was found out with the *Escherichia coli*, and *Klebsiella pheumoniae*. Moderate zone of inhibition was noticed in *Bacillus subtilis* and *Shigella flexneri* but *Pseudomonas aeroginosa* was not affected by silver nanoparticles (Fig. 4).

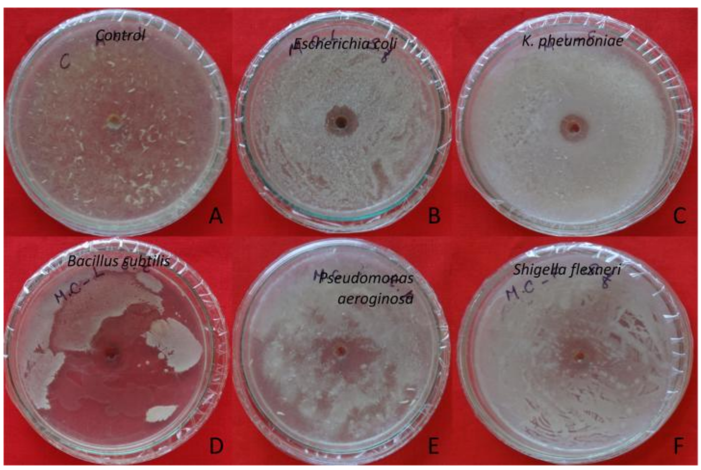

Figure 4: Zone of inhibition by silver nanoparticles

Several studies proposed that SNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. Smaller SNPs having the large surface area available for interaction would give more bactericidal effect than the larger SNPs.[15] It is also possible that SNPs not only interact with the surface of the membrane, but can also penetrate inside the bacteria.[16]

Conclusion

The present study is a first attempt for green and eco-friendly biosynthesis of silver nanoparticles with the aid of aqueous extracts of various parts of *Morinda coreia*. This technique of biosynthesis of nanomaterials using plant extract is a cost-effective and an easy approach to produce stable silver nanoparticles in bulk. These particles could be further used in the field of medicine and animal health care industry.

Acknowledgement

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Conflict of Interest

We declare that we have no conflict of interest.

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