Journal of Scientific and Innovative Research 2016; 5(3): 83-86
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2016; 25(3): 83-86

Received: 25-12-2015
Accepted: 21-06-2016

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Green synthesis, characterization and antimicrobial activity of silver nanoparticles using *Morinda pubscens* J.E. Smith root extract

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Abstract

Nanotechnology deals with the synthesis of nanoparticles with controlled size, shape and dispersity of materials at the nanometer scale and their potential use for human well being. This leads to focus on "Green Synthesis" of nanoparticles which seems to be an easy, efficient and eco- friendly approach. In this study, the green synthesis of silver nanoparticles was carried out using root extract of *Morinda pubescens* as reducing agent. It was found that aqueous silver ions can be reduced by aqueous root extract of *Morinda pubescens* to generate extremely stable silver nanoparticles in water. The silver nanoparticles (AgNPs) formation was confirmed by the colour change of the mixture and further confirmed by spectral analysis. UV-Visible spectrum of the aqueous medium containing silver nanoparticles showed a peak around 416.5 nm. FT-IR analysis confirmed reduction of Ag^+ ions to Ag^0 ions in synthesized silver nanoparticles. Further, the produced silver nanoparticles showed bactericidal effect against *Staphylococcus aureus, Escherichia coli* and *Aspergillus niger*. From this study concluded that the root extract of *Morinda pubescens* reduces Ag^+ to Ag^0 and enhances synthesis of silver nanoparticles with antimicrobial activity.

Keywords: Nanoparticles, *Morinda pubescens*, UV-Visible, FT-IR, Antibacterial activity.

INTRODUCTION

Nanotechnology deals with the synthesis of nanoparticles with controlled size, shape and dispersity of materials at the nanometer scale length and their potential use for human well being. Metallic silver and silver nanoparticles were recently applied as antimicrobial agents in various products such as cosmetics, animal feed, coating of catheters, wound dressing, and purification with a minimal risk of toxicity in humans. Biological methods of nanoparticle synthesis using microorganism, enzyme and plant or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticle synthesis can be advantageous over other biological processes as it eliminates the elaborate process of maintaining cell cultures. *Morinda pubescens* J.E. Smith commonly known as Aal or Indian Mulbery is a species of flowering plant in the family Rubiaceae, native to Southern Asia**.** Traditionally the leaf juice was given orally to children before food for easy digestion. The charred leaves made into a decoction with mustard were a favourite domestic remedy for infantile diarrhoea. The expressed juice of leaves was externally applied to gout to relieve pain. The leaves are administered internally as a tonic and febrifuge. The roots were styptic, constipating, anti- inflammatory, alexeteric and tonic, and were useful in haemorrhages, dysentery, inflammations, boils and general debility**.** *Morinda pubescens* its medicinal potential has yet to be studied scientifically, and, therefore, this present study was initiated the green synthesis of silver nanoparticles was carried out using root extract of *Morinda pubescens* as reducing agent. The antibacterial activity of silver nanoparticles has been tested against various pathogens.

MATERIALS AND METHODS

Materials

**Morinda pubescens* aqueous extract
*1 mM silver nitrate solution

Methods

For synthesis of silver nanoparticles, 10 ml of aqueous extract of *M. pubescens* was added to 90 ml 1 mM solution of silver nitrate in 250 ml conical flask and kept at room temperature for 2 hrs. The primary detection of synthesized silvernanoparticles (AgNPs) was carried out in the reaction mixture by observing the colour change of the medium. The AgNPs solution thus obtained was purified by repeated centrifugation at 5,000 rpm for 20 minutes. The supernatant as discarded and the pellet were dissolved in double distilled water. AgNPs confirmed by colour change.

Characterization

The produced nanoparticles were subjected to UV-Vis spectroscopy analysis, Fourier Transform Infrared radiation (FT-IR) spectroscopy.

UV-Vis spectroscopy

The samples were diluted with 2 ml of doubles distilled water and subsequently measured by the UV-Vis spectroscopy. The UV-Vis spectroscopy analysis of silver nanoparticles produced was carried out as a function of bioreduction time at room temperature on Jasco Spectrophotometer 530.

FT-IR Spectroscopy

To remove biomass residue or compound that is not capping ligand of the nanoparticles, the residue solution of 100 ml after reaction was centrifuged at 5,000 rpm for 10 minutes. The supernatant was again centrifuged at 10,000 rpm for 60 minutes and the pellet was obtained. Then the pellet of AgNPs was redispersed into 1 ml deionized water. The purified suspension was dried to obtain dried powder. Finally, the dried AgNPs were analysed by FT-IR analysis.

1: Antimicrobial assay of Green synthesized silver nanaoparticles

The antimicrobial activity of green synthesized AgNPs was performed by agar- well diffusion method.

Test Micro-organisms

1. *Escherichia coli (gram –ve)*
2. *Staphylococcus aureus (gram+ve)*
3. *Candida albicans (fungus)*

Standard: Gentamycin (100µg/ml)

Medium used: Nutrient agar medium

Preparation of Extract

For microbiological studies silver nanoparticles of different concentration were prepared. For this the synthesized nanoparticles were dissolved in water so that a concentration of 50 µg/ ml, 100µg/ml was obtained. Control was also used for each solution with the solvent alone.

The antibacterial and antifungal activity of the various isolated compounds was studied in the following manner.

Method

Preparation of medium

Boiled the solid contents in 1000 ml of distilled water using a conical flask. The pH of the medium should be adjusted to 7.4 sterilized by autoclaving at 121°C for 15 minutes. Cooled to 50-55°C. When the media was Luke warm, the organisms were inoculated separately in separate nutrient agar media and poured aseptically into the sterile petridishes and allowed to solidify.

Well diffusion method

The prepared culture plate was inoculated with different selected strains of bacteria using streak plate method. Wells are made on agar surface with 6 mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37^0C $\pm 2^0$C for 24 hrs for bacterial activity. The plates were observed for the zone clearance around the wells. A blank well containing vehicle without plant extract was used as negative control and the reference drug Gentamycin (100µg/ml) was used as positive control. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Media: Nutrient agar media having the following composition was used.

Table 1: Composition of nutrient agar

Constituents	**gm/L**
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Agar	15
Distilled Water up to	1000 ml

2: Antifungal Activity

Antifungal activity of plant extract was tested against *Candida albicans*

Standard drug used : Clotrimazole (100µg/ml)

Media used : Sabouraud's dextrose agar medium.

The composition of the medium was as follows:

Table 2: Composition of Sabouraud's dextrose agar medium (SDA)

Constituents	**gm/L**
Mycolical peptone	300
Dextrose	20
Agar	20
Distilled Water upto	1000 ml
pH at 25^0C	5.6 ± 0.2

Inoculum Preparation

3-5 colonies of standard strain *Candida albicans* was suspended in 2 ml of sterile normal saline and vortexed. The turbidity of the homogenous

suspension was adjusted. The sterile swab was dipped in suspension and swabbed on dried plates of Sabouraud's dextrose agar medium.

Method

Disc Diffusion method

6 mm sterile filter paper discs were purchased and sterilized. These discs were saturated with different drug concentration, placed and inoculated on SDA plates. These plates were incubated at 37°C. Zone of inhibition was noted around the disc at 24 and 48 hrs.

RESULTS AND DISCUSSION

Aqueous root extract of *M. pubescens* was used for the synthesis of silver nanoparticles. For synthesizing 1mM silver nitrate solution was used. The primary detection of the synthesized silver nanoparticles (AgNPs) was carried out in the reaction mixture by observing the colour change of the medium. The colour of the medium was changed into dark brown after the addition of silver nitrate. The AgNPs solution thus obtained was purified by repeated centrifugation at 5,000 rpm for 20 minutes. Then the pellet obtained is dissolved in double distilled water and then it was characterized by UV-Vis spectroscopy and FT-IR spectroscopy.

A) Pure AgNO3 solution **B)** Pure M. pubescens root aqueous extract **C)** Colour changes after adding root extract with $AgNO_3$ solution

Figure 1: Preparation of silver nanoparticles

UV-Vis absorbance spectroscopy

The produced AgNPs used for analysis were diluted with 2 ml of double distilled water and subsequently measured at UV-Vis spectroscopy. The results of the UV-Vis absorption showed increasing colour intensity with increased time intervals and this might be due to the production of the silver nanoparticles and the formation of the dark brown colour might be due to the excitation of the surface Plasmon vibration of the synthesized AgNPs. The maximum absorption of silver colloids was observed at 416.5 nm. The intensity of absorbtion band increase with increasing time period of aqueous component and consequent colour changes were observed from colourless to reddish brown. This characteristic colour variation is due to the excitation of the surface plasmon resonance in the metal nanoparticles.

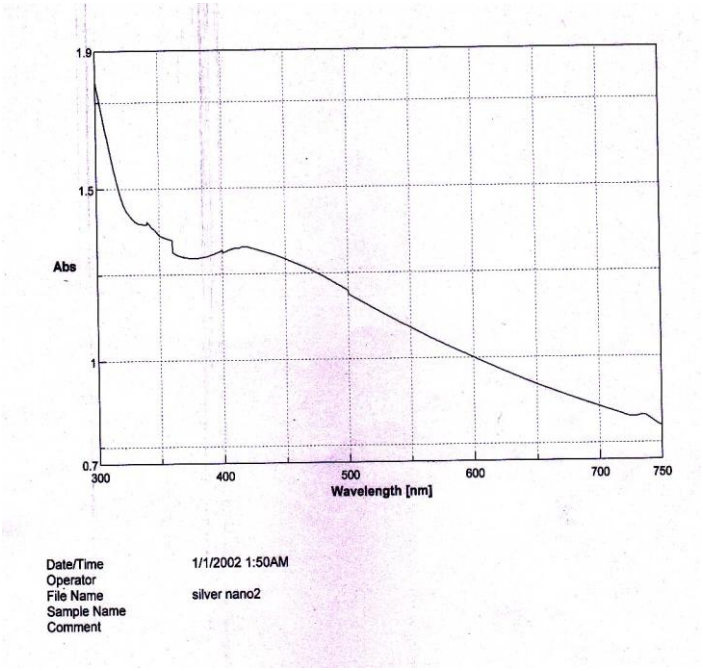

Figure 2: UV- Vis absorbances of silver nanoparticles

FT- IR analysis

Peak observed at position 3600- 3200 cm^{-1} may be due to the presence of phenolic OH group. Peak for CH_2 streching observed at 2923.74cm^{-1}. Peaks observed at 1718.28 cm-1 may be due to the presence of carbonyl group. Peak observed at 1600 cm^{-1} region indicates aromatic C-C stretching. Peak observed at 1114.11 cm^{-1} may be due to the presence of C-H bend for alkanes. Peak observed at 863.3 cm-1 may be due to the presence of CH in plane bend due to para substituition. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

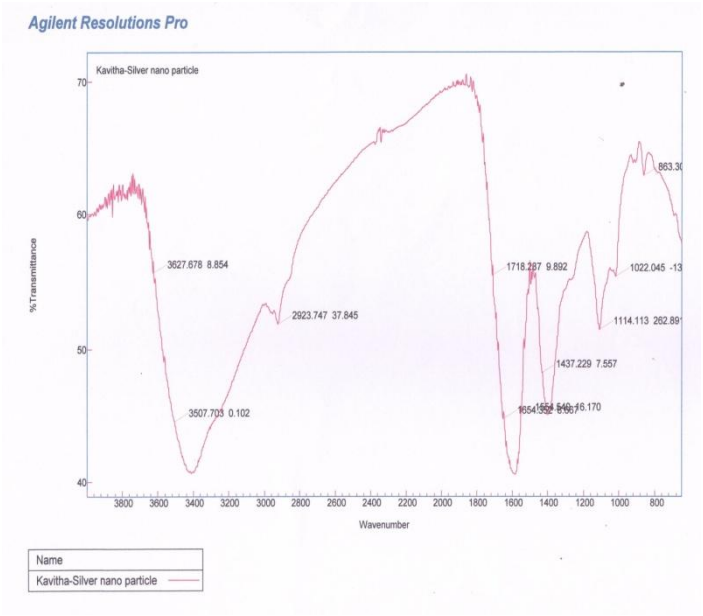

Figure 3: FT-IR spectrum of silver nanoparticles

Antimicrobial Activity of Green Synthesized Silver Nanaoparticles

The antimicrobial activity of green synthesized AgNPs was performed by agar- well diffusion method.

Table 4: Antimicrobial acitivity of silver nanoparticles

Test microorganism	Zone of inhibition in mm			
	Standard	Control	50µg/ml	100µg/ml
Staphylococcus aureus	26	-	16	20
Escherichia coli	28	-	15	19
Aspergillus niger	26	-	4	8

Figure 4: Antimicrobial activity of silver nanoparticles

Green synthesized silver nanoparticles of roots of *Morinda pubescens* aqueous extract were found to be highly toxic against different pathogenic bacteria. Silver nanoparticles are very effective against microorganisms because of their enormously high surface area. The maximum zone of inhibition was found out with the *S.aureus* at the concentration of 100µg/ml. Smaller silver nanoparticles having the large surface area available for interaction would give more bactericidal effect than extracts.

CONCLUSION

The present study is a attempt for green synthesis of green and ecofriendly biosyntheisi of silver nanoparticles with the aid of aqueous extract of roots of *M. pubescens*. 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 system.

REFERENCES

1. Kirtikar and Basu. Indian Medicinal Plants, Part II, Sushindra Nath, basu, M.B, at the Panini Office, Allahabad, 268-514
2. Sebastien Mongrand *et al.* chemotaxonomy of the Rubiaceae family based on Leaf Fatty acid composition. Phytochemistry,2005, (66): 549-559
3. Bioactive Iridoid glycosides isolated from *Morinda tinctoria* (Roxb) root exhibit therapeutic efficacy. Nat.Prod. Resour. Repos.2012, (3) : 349-356
4. Karunai Selvi and B. Balagengatharathilakam. Isolation and screening of Endophytic Fungi from Medicinal Plants of Virudhunagar district for Antimicrobial activity. International Journal of Science and nature,2014, 5(1): 147-155
5. V.V. Sivarajan and Indira Balachandran. Ayurvedic Drugs and Their Plant Sources. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi,342-345
6. Prajapati, purohit, Sharma, Kumar. A hand Book Of medicinal Plants, A Complete Source Book. Agrobios(India) Jodhpur, 349
7. McGaw L J et al. Invitro Anthelmintic, Antibacterial, Cytotoxic effects of Extracts from plants used in South African. Ethnoveterinary Medicine; Open Up; 2007, (2) 1-5
8. Diflo Manual of Dehydrated Culture Media and Reagents for Microbial and Chemical Laboratory Procedures. 4th edition. 1953. 32, 33, 238, 338
9. McGaw LJ, Van der Merwe D, Eloff JN. Invitro 86iscose86c86ic, antibacterial and 86iscose86c effects of extracts from plants used in South African. Ethnoveterinary Medicine. Open UP. **2007**; (2) 1-5
10. Farahnaz Nariman, Fereshteh Eftekhar, Zohreh Habibi, Sadegh Massarrat, Reza Malekzadeh. Antibacterial Activity of Twenty Iranian Plant Extracts against Clinical Isolates of Helicobacter pylori. *Iranian Journal of Basic MedicalSciences*. 2009. Vol. 12/ 2: 105-111
11. Varaprasad Bobbarala, Prasanth Kumar Katikala, K. Chandrasekhar Naidu and Somasekhar Penumajji. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger* F2723. *Indian Journal of Science and Technology* .2009;2 /4. ISSN: 0974- 6846.
12. Omer Erturk. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants, Biologia, Bratislava. *Cellular and Molecular Biology*.2006; 61/3: 275—278. DOI: 10.2478/s11756-006-0050-8.
13.
14. Ravikumar Rajendran, Nithya Ganesan, Satheesh Kumar Balu, Srinivasan Alagar, Premkumar Thandavamoorthy, Devaki Thiruvengadam. Green Synthesis, Characterization, Antimicrobial and Cytotoxic Effects of Silver Nanoparticles Using *Origanaum heracleoticum* L., Leaf Extract. International Journal of Pharmacy and Pharmaceutical Sciences, 2015, Vol 7, Issue 4 : 288-293
15. Ramesh Kumar K et al. Biosynthesis of Silver Nanoparticles from *M. tinctoria* Leaf Extract and their Larvicidal Activity against *Aedes aegypti Linnaeus* 1762, Nanomedicine and Nanotechnology, 2014,5(6) 1-5
16. N. Kannan et al. Preparation of Silver nanoparticles using leaf and fruit extracts of *Morinda coreia* Buck. Ham – A green approach. Journal of scientific and Innovative Research2014, 3(3): 315-318
17. R. Kirubha and G. Alagumuthu. *Morinda tinctoria* fruit assisted biosynthesis of Silver nanoparticles. Asian Journal of Pharmaceutical and Clinical Researh, 2013, 6(4): 60-64
18. K. Ramesh kumar et al. Synthesis of Eco- friendly Silver nanoparticles from *Morinda tinctoria* leaf extract and its larvicidal activity against *Culex quinquefascitus*
19. Jancy Mary E and Inbathamizh L. Green synthesis and Characterization of Nano silver using leaf extract of *M. pubescens*. Asian Journal of Pharmaceutical and Clinical Research. 2012, 5: 159-16.
20. Vanaja *et al*. Degradation of Methylene Blue Using Biologically Synthesized Silver nanoparticles. Hindawi Publishing Corporation, 2014.