IJPSR (2013), Vol. 4, Issue 9 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 May, 2013; received in revised form, 14 June, 2013; accepted, 22 August, 2013; published 01 September, 2013

MICROWAVE-ASSISTED RAPID GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *SARACA INDICA* LEAF EXTRACT AND THEIR ANTIBACTERIAL POTENTIAL

Seema Garg

Amity Institute of Applied Sciences, Amity University, Noida, 201301, Uttar Pradesh, India

Keywords:

Nanoparticles, Microwave, *Saraca indica*, FTIR, Antibacterial potential

Correspondence to Author:

Seema Garg

Amity Institute of Applied Sciences, Sector - 125, Amity University, Noida, Uttar Pradesh, India

E-mail: sgarg2@amity.edu

ABSTRACT: The present study reports an environmentally friendly, microwave-assisted rapid green synthetic method using *Saraca indica* leaf extract makes a fast and convenient method for the synthesis of silver nanoparticles and can reduce most of the silver ions into silver nanoparticles within 180 sec of reaction time with the aid of microwave. The synthesized nanoparticles were characterized using UV-visible (UV-vis) spectrophotometer, Transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infra-red (FTIR) spectrometry. The nanoparticles were found to be spherical in shape and of 5 to 50 nm in size. The synthesized silver nanoparticles exhibited good antibacterial potential against both Gram positive and Gram negative bacterial strain as measured using well diffusion assay.

INTRODUCTION: Nanoparticles have gained tremendous attention of researchers in the past two decades due to their exceptional electronic, catalytic, optical, magnetic and other physical and chemical properties that are quite different from the bulk one [1].

Silver nanoparticles have proven useful in antibacterial clothing, burn ointments and as coating for medical devices because of their mutation-resistant antimicrobial activity [2].

To fulfill the growing need of environmental friendly nanoparticles, researchers are using microorganisms for the synthesis of various metal nanoparticles [3-5]. Unlike microbial nanoparticles synthesis, current method has an advantage of being simple, does not require much equipment and time [6-8].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(9).3615-19

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.4(9).3615-19

But now a day's, plant extract has been used as reducing and capping agent for the synthesis of nanoparticles which could be advantageous over microbial synthesis because there is no need of culturing and maintaining the cell. Plant leaf extract of onion [9], *Syzygium cumini* [10], basil [11], S*araca indica* [12], black pepper [13] and banana peel [14] had been used for the synthesis of gold and silver nanoparticles, which lead to formation of pure metallic nanoparticles of silver and gold and can be used directly. The chemical methods are extremely expensive and use toxic chemicals which may pose potential environmental and biological risks. Microwave-assisted route is selected for the synthesis to carry out the reaction fast, suppress the enzymatic action and to keep the process ecofriendly [15-17].

In this article, we have reported the microwave-assisted rapid green synthesis of stable silver nanoparticles using *Saraca indica* leaf extract and its antimicrobial potential. Extract of Saraca *indica* leaves has been reported to possess potent anthelmintic, analgesic, anti-microbial activity, CNS depressant, antiulcer, anti-inflammatory, larvicidal, anti-diabetic, shigellocidal, uterinetonic activity [18]. The antimicrobial potential of the synthesized silver nanoparticles was assessed against both gram classes of bacteria.

EXPERIMENTAL

Synthesis of Silver Nanoparticles: Freshly collected *Saraca indica* leaves were washed with deionized water. 100 ml double distilled water was added to the flask containing 5 g fine cut leaves and was exposed to microwave for 180 sec to make the aqueous extract of leaves rapidly and suppress the enzymes present in the solution. Then the raw extract obtained was filtered in hot condition with 11-micron mesh to remove fibrous impurities. The resultant clear extract was used for the synthesis of silver nanoparticles.

For reduction of Ag^+ ions, 10 ml aqueous *Saraca indica* leaf extract was added to 50 ml of 10^{-3} M aqueous $AgNO_3$ solution and the solution mixture was exposed to microwave radiation at a fixed frequency of 2450 MHz and power of 450 watts. Periodically, aliquots of the reaction solution were removed and subjected to UV-vis spectroscopy measurements. The synthesized nanoparticles were centrifuged at 8000 rpm for 10 min and subsequently re-dispersed in deionized water twice to get rid of any unbound biological molecules.

Analysis of Antibacterial Activity: Agar well diffusion method was used to evaluate the bactericidal activity of silver nano-colloid solutions. Sterile nutrient agar medium was poured into sterile petri plates and allowed to solidify. The petri plates were incubated at 37°C for 24 hours to check for sterility. The medium was seeded with the organism culture (1ml) by pour plate method. Bores were made on the medium using sterile borer. 10, 20, 40 and 80 µg/ml of silver nanoparticles was added to the respective bores. The petri plates were kept in refrigerator at 4°C for 30 min for diffusion. After diffusion the petri plates were incubated at 37°C for 24 hours and zone of inhibition were observed and measured.

RESULTS AND DISCUSSION: As soon as *Saraca indica* leaf extract was mixed in aqueous solution of silver nitrate, the reduction of pure Ag^+ ions to Ag^0 was monitored by measuring UV-vis spectrum of the reaction media at regular intervals. The color of silver nanoparticles is seen dark brown, is due to the excitation of the surface plasmon vibration in metal nanoparticles. UV-vis spectra were recorded as function of reaction time. The metal ions reduction occurs very rapidly and most of the reduction of Ag^+ ions was completed in 210 sec. The intensity of the color of reaction mixture increases evenly with time of microwave exposure. Absorbance intensity increases steadily as a function of reaction time and it is observed that the surface plasmon peak occurs at 420 nm with a slight shift in the vertex of the peak towards shorter wavelength (blue shift) and fixed at 405 nm (**Fig. 1A**).

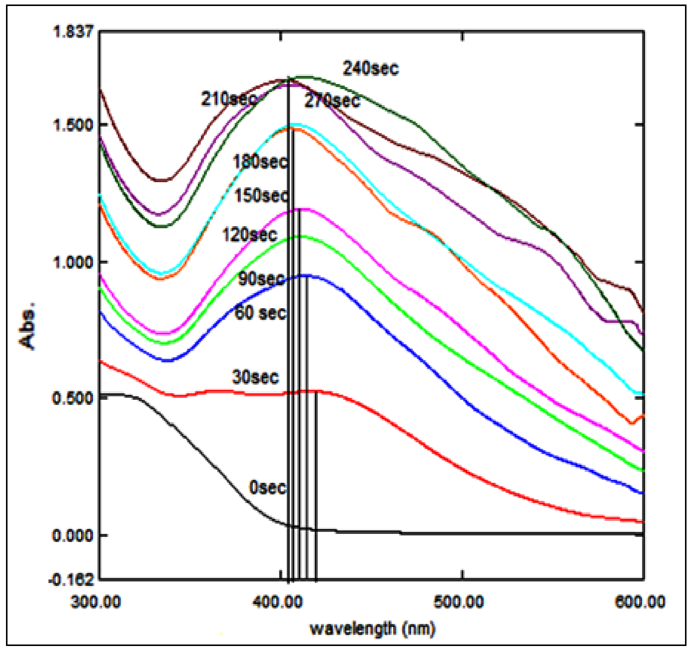

FIG. 1(A): UV-VIS SPECTRA OF BIO FUNCTIONALIZED MICROWAVE ASSISTED SILVER NANOPARTICLES AT VARIOUS TIME INTERVAL

The peak height gradually increases from 0 to 210 sec which shows the gradual formation of nanoparticles and blue shift reflects the formation of smaller nanoparticles. The microwave assisted method is much faster than the earlier conventional studies with other biological routes. The time required for the conventional synthesis of silver nanoparticles from other plants was 2–4 h [19, 20] and from bacteria was 24–120 h [6-8] and thus are rather slow. The X-ray diffraction (XRD) analysis showed diffraction peaks corresponding to fcc structure and crystallinity of silver nanoparticles. Intense peaks were observed at 38.3^0, 44.5^0, 64.6^0, and 77.5^0 (**Fig. 1B**), corresponding to 111, 200, 220, and 311 Bragg's reflection, respectively (JCPDS, silver file no. 04-0783).

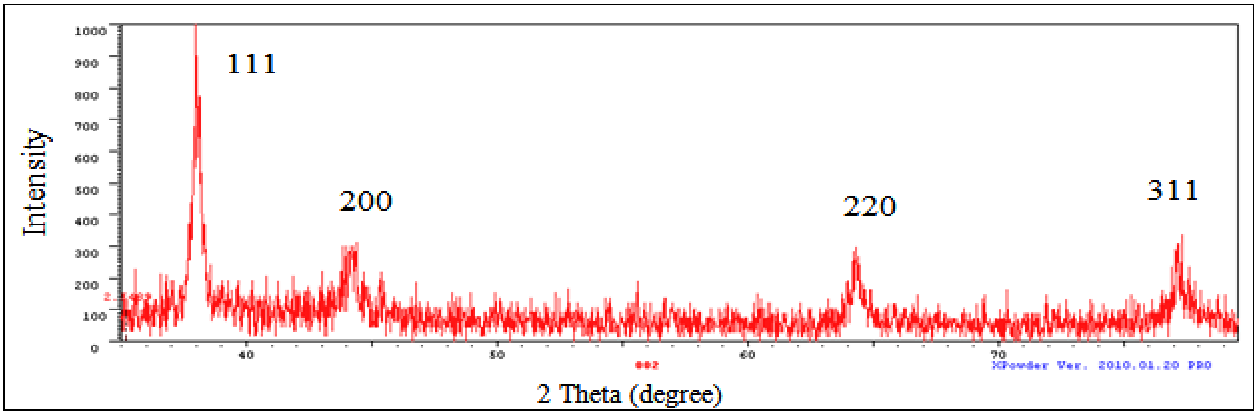

FIG. 1(B): XRD SPECTRUM OF BIO-SYNTHESIZED SILVER NANOPARTICLES

The particle size and shape is confirmed with drop coated TEM grids. The particles are almost in spherical shape with diameters in the range of 5 to 50 nm and are well dispersed (**Fig. 2A**).

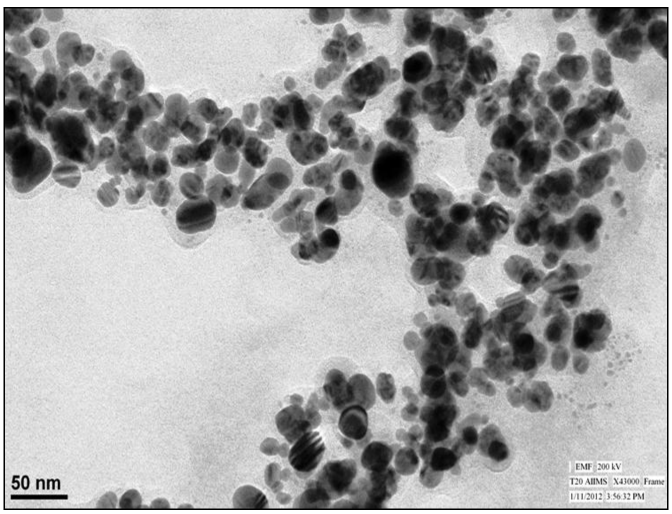

FIG. 2 (A): TEM IMAGE OF BIO-SYNTHESIZED SILVER NANOPARTICLES

The Fourier transform infra-red spectroscopy (FTIR) measurements of synthesized silver nanoparticles were carried out to identify the possible interaction between protein and silver nanoparticles. Results of FTIR study showed sharp absorption peaks located at about 1635 cm^{-1} and 3430 cm^{-1} (**Fig. 2B**). Absorption peak at 1635 cm^{-1} may be assigned to the amide I bond of proteins arising due to carbonyl stretch in proteins, and peaks at 3430 cm^{-1} are assigned to OH stretching in alcohols and phenolic compounds [21].

The absorption peak at 1635 cm^{-1} is close to that reported for native proteins [22], which suggest that proteins are interacting with biosynthesized nanoparticles and also their secondary structure were not affected during reaction with Ag^+ ions or after binding with silver nanoparticles [23]. These IR spectroscopic studies confirmed that carbonyl group of amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nanoparticles and acting as capping agent to prevent agglomeration and providing stability to the medium [24]. These results confirm the presence of possible proteins acting as reducing and stabilizing agents for silver nanoparticles.

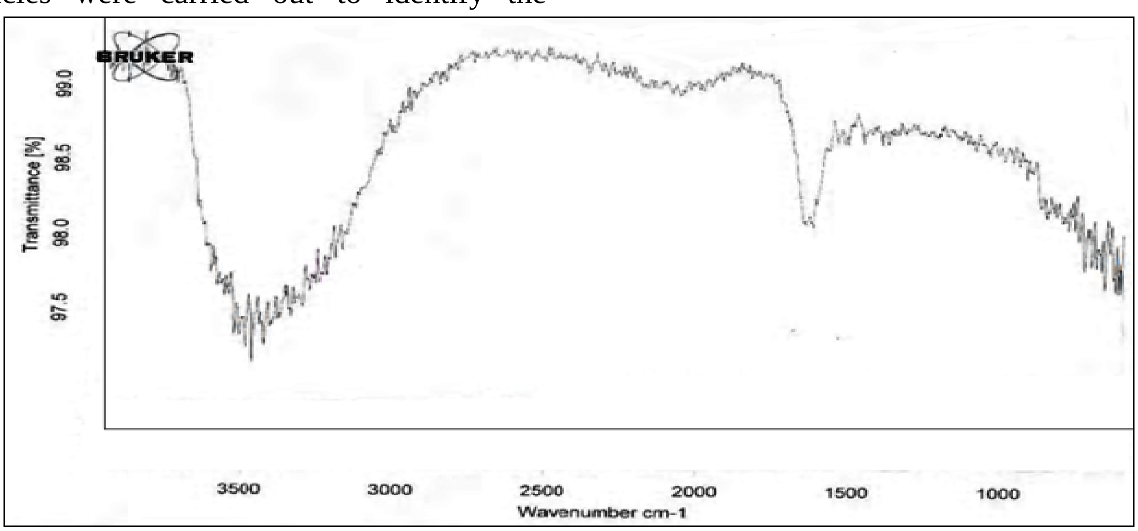

FIG. 2(B): FTIR ABSORPTION SPECTRA OF BIOSYNTHESIZED SILVER NANOPARTICLES

Antibacterial Activity: A good zone of inhibition was observed for both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacterial strains tested. The zone of inhibition increased with increasing concentration of silver nanoparticles (**Table 1**). From the data it is inferred that the synthesized silver nanoparticles were effective for both type of microbes.

Moreover, the zone of inhibition observed around both the bacterial strains is found to be reproducibly of the same size. Nano silver is more active towards Gram positive bacterial strain as compared to Gram negative and it was reasoned that factors other than membrane structure might be playing the role [25]. We have noticed that silver nanoparticles synthesized using *Saraca indica* leaf extract produce sensitivities towards both *E.coli* and *S. aureus*.

TABLE 1: ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES AGAINST *E. COLI* & *S. AUREUS*

Concentration (µg/ml)	Average zone of inhibition (mm) *E. coli*	Average zone of inhibition (mm) *S. aureus*
10	13	13
20	16	15
40	18	17
80	20	19

CONCLUSION: Rapid green synthesis of silver nanoparticles from *Saraca indica* leaf extract using a simple, fast and efficient microwave-assisted route of spherical shaped, fcc structure with diameter range of 5 to 50 nm has been envisaged. The formation of silver nanoparticles with the microwave-assistance is the fastest methodology available till today. No chemical reagent or surfactant was required in this synthesis. Color change occurs due to surface plasmon resonance during the reaction with the ingredients present in the plant leaf extract results in the formation of silver nanoparticles which is confirmed by UV-Vis, XRD, FTIR and TEM.

Silver nanoparticles, so obtained, are stable for more than 3 months. Investigation of the antibacterial effect of nano sized silver colloidal solution against *E. coli* and *S. aureus* reveals high efficacy of silver nano particles as a strong antimicrobial agent which can be useful in pharmaceuticals and in cosmetic industry. Further development of suitable dosage of silver nanoparticle formulation is being carried out in our laboratory.

ACKNOWLEDGMENTS: I heartly acknowledge Dr Amrish Chandra, Dr Sangeeta Tiwari, Dr.Sunita Rattan, Dr B. Shukla and Dr. A. K.Chauhan of Amity University, Noida, UP, India for their support and providing facilities for the fulfilment of this study. I also acknowledge Dr. Richa Krishna, for her support.

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How to cite this article:

Garg S: Microwave-assisted rapid green synthesis of Silver Nanoparticles using *Saraca indica* leaf extract and their antibacterial potential. *Int J Pharm Sci Res* 2013: 4(9); 3615-3619. doi: 10.13040/IJPSR. 0975-8232.4(9).3615-19