



GREENER SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *MURRAYA KOENIGII* LEAF EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

The green synthesis of nanoparticles from plant sources is simple, rapid and ecofriendly approach. In the present work synthesis and characterization of silver nanoparticles, and their antimicrobial effect on bacteria were studied. The novelty in this work, is the *Murraya koenigii* leaves present throughout the year and not an seasonable leaves and it has many medicinal properties. The synthesized silver nanoparticles loaded with activated carbon is used in adsorption studies and obtained good results. In the process of synthesizing silver nanoparticles using *Murraya koenigii* leaves extract, observed rapid reduction (i.e. within 20 minutes) of silver ions leading to the development of stable silver nanoparticles in the solution. The synthesized silver nanoparticles (AgNPs) were characterized by Ultraviolet-visible (UV-Vis) spectrometer, Fourier Transform Infrared spectroscopy (FTIR), X-ray diffraction study (XRD), SEM (Scanning Electron Microscopy) and antibacterial activity. Synthesized silver nanoparticles were confirmed by analyzing the excitation of surface plasmon resonance (SPR) using UV-Vis spectrometer at 433nm. The observed peaks in XRD pattern corresponding to (111), (200) and (311) planes. Disc diffusion method was used to determine the zone inhibition of the tested samples by using two gram positive and two gram negative bacteria and it has zone of inhibition from the range of 13.2mm to 23.4 mm. The silver nanoparticles are prepared by using *Murraya koenigii* leaves extract have potent antibacterial activity and cost wise is very cheap and ecofriendly to the environment.

KEYWORDS: Green synthesis, Silver nanoparticles, *Murraya koenigii* leaves, antibacterial.



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Received on: 27-02-2017

Revised and Accepted on: 15-04-2017

DOI: <http://dx.doi.org/10.22376/ijpbs.2017.8.2.p292-298>

INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern material sciences. Green synthesis pathways are cost effective and do not use toxic chemicals, high pressure and temperatures^{1,2,3}. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Various metals have been used in a synthesis of nanoparticles which are useful in the field of medicine, catalysis, electronics, optics, photonics, biolabelling, optoelectronics, photography and surfaced enhanced Raman scattering (SERS) detection⁴. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organisms at low concentrations without any side effect⁵ however biocompatible inert nano materials have been find their way in cancer diagnosis and delivery of anticancer drugs⁶. In small concentrations, silver is safe for human cells, but lethal for microorganisms⁷. Silver (Ag) in the nano scale form exhibits remarkably unusual physicochemical and biological activities, thus has been widely applied in the health care sector^{8,9}. In particular, the outstanding antimicrobial properties of Ag NPs have led to the development of a wide variety of nanosilver products, including nano silver-coated wound dressings, contraceptive devices, surgical instruments and implants¹⁰⁻¹¹. Apart from these antimicrobial activities, Ag NPs are also known to possess antifungal, anti-inflammatory, antiviral, anti-angiogenesis and antiplatelet properties^{12,13,14}. Additionally, more recent developments have seen Ag NPs used in room spray, laundry detergent and wall paint formulations as well as in the textile industry for clothing manufacturing^{15,16,17,18}. Many reports are available on the biogenesis of silver nanoparticles using several plant extracts, *Azadirachta indica* (neem)¹⁹, *Aloe vera*²⁰, *Carrica papaya*²¹, *Osmium sanctum*²², *Saururus chinensis*²³, *Calotropis procera*²⁴, *Eucalyptus hybrida*²⁵, *Tinospora cordifolia*²⁶, *Coriandrum sativum*²⁷. *Murrayakoenigii*, commonly known as curry leaf or karpatta in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species²⁸. In traditional system of Medicine, it is used as antiemetic, anti diarrhoeal, dysentery, febrifuge,

blood purifier, tonic, stomachic, flavoring agent in curries and chutneys. The oil is used externally for bruises, eruption, in soap and perfume industry²⁹. Curry leaves is found to be effective as antioxidant, antidiabetic, antibacterial, antihypertensive, cytotoxic and also in the treatment of bronchial respiratory difficulties. In view of the importance of *Murraya koenigii* and silver nanoparticles, the present study has been planned to synthesize and investigate the antibacterial activity of silver nanoparticles synthesized from the aqueous leaf extracts of *Murray koenigii* using silver nitrate against some bacteria. Further the synthesized nanoparticles were tested for antibacterial activity using disc diffusion method, for this two gram positive and two gram negative bacteria was employed.

MATERIALS AND METHODS

The AR grade silver nitrate (AgNO₃) was purchased from Sigma-Aldrich chemicals. The leaves of *Murraya koenigii* were collected from the Coimbatore district of Tamilnadu, India.

Preparation of *Murraya koenigii* leaves extract

The fresh leaves were washed several times with tap water and then with distilled water. 25gm of the leaves were dried, cut in to fine pieces and boiled with 100 mL of distilled water for 3 mins. The crude extract was then passed through Whatmann No.1 filter paper and the filtrate was stored at 4°C for further use.

Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, 10 ml of the *Murraya Koenigii* leaf extract was added to 90 mL of 1 mM aqueous AgNO₃ solution in a conical flask for the reduction of silver ions, and kept at room temperature. To avoid the unnecessary photochemical reactions of silver nitrate the overall reactions was carried out in dark condition. The color change of reaction mixture was observed from light yellow to dark brown indicates the formation of silver nanoparticles and it was confirmed by UV-Vis spectroscopy. The solution was centrifuged twice at 10,000 rpm for 10 minutes. The pellet thus obtained was washed with distilled water and stored at 4°C for further use.





(c) *Murraya koenigii* leaves aqueous extract (d) *Murraya koenigii* leaves after adding Silver nitrate

Figure 1

(a-d) Picture of leaves, silver nitrate and silver nanoparticles

Characterization of Silver nanoparticles

UV-Vis spectral Analysis

UV-Vis spectroscopy analysis was used for monitoring the synthesis of silver nanoparticles and it is a powerful tool for the characterization of colloidal particles. The reaction mixture was diluted 20 times and used for UV-Visible spectroscopy analysis. The spectrum was measured by using UV-visible 2450 (Shimadzu) spectrophotometer model from 200 to 800 nm.

Fourier Transform Infra Red Spectroscopy

The FTIR result analysis is used to determine the functional groups on the *Murraya koenigii* leaf powder and their involvement in the synthesis of silver nanoparticles. FTIR spectrum of the sample mixed with KBR powder, in a mortar and pressed in to a pellet for measurement. FTIR measurements were done using Shimadzu spectrophotometer in the range 400-4000 cm^{-1} .

X-ray diffraction studies

X-ray diffraction (XRD) measurement was carried out to determine the crystallographic structure and crystallite size (grain size) based on the angle of diffraction of the X-ray beam by the atoms in the crystalline planes. The air dried silver nanoparticles were coated on to XRD grid and analyzed using X-ray diffractometer (X'Pert-Pro) operated at a voltage of 40 kV and a current of 30 mA with Cu K α radiation. The scanning was done in the region of 2 θ from 10° to 80°.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using JEOL JSM-6390 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes.

Antibacterial activity

Collection and maintenance of test organisms

The organisms used were clinical isolates of *Staphylococcus Aureus*, *Salmonella paratyphi*, *Staphylococcus albus* and *Escherichia coli* was collected from Coimbatore medical college, Coimbatore. They were collected in McCartney bottles containing nutrient agar slants.

Preparation of inoculum

The inoculums for the experiment were prepared in fresh Nutrient broth from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth (if excessive or by further incubation to get required turbidity

Each Petri dish is divided into 4 quadrants, in 3 quadrants nano particles discs such as I (100mcg), II (200mcg), III (300mcg) discs (discs are soaked overnight in nanoparticles solution) and one quadrant for Standard Ciprofloxacin 2mcg, are placed in each quadrant with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4°C or at room temperature for 1 hour for diffusion. Incubate at 37°C for 24 hours. Observe the zone of inhibition produced by different Antibiotics.

RESULTS

UV-Visible spectral analysis

The UV-visible spectroscopy analysis of silver nanoparticles using *Murraya koenigii* leaf extract showed peak at 433nm. The broadening of maximum absorption peak at 433 nm indicates that the particles were polydispersed.

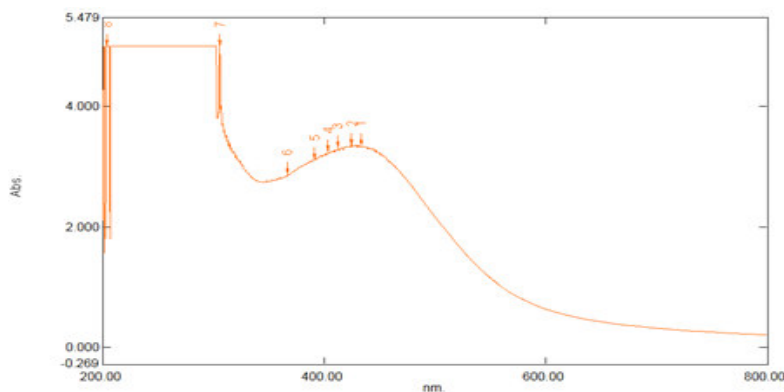


Figure 2
UV-Vis absorption spectra of silver nanoparticles synthesized from Murraya Koenigii leaves extract

Fourier Transform Infra Red Spectroscopy

The functional groups of green synthesized AgNPs were recorded by FTIR analysis and the results are shown in Fig.3. The FTIR result analysis showed sharp absorption peaks located at about 3446.79 and 1637.56 cm^{-1} were assigned to stretching vibrations. The band appearing at 3446.79 cm^{-1} is assigned for O-H stretching

vibration indicating the presence of polyphenols in the reducing agent. The band at 1637.56 cm^{-1} indicates the presence of -NH stretching vibration of the amide I band of proteins. The band at 2056.12 may be due to C=O stretching vibrations of the carbonyl functional group in ketones, aldehydes and carboxylic acids.

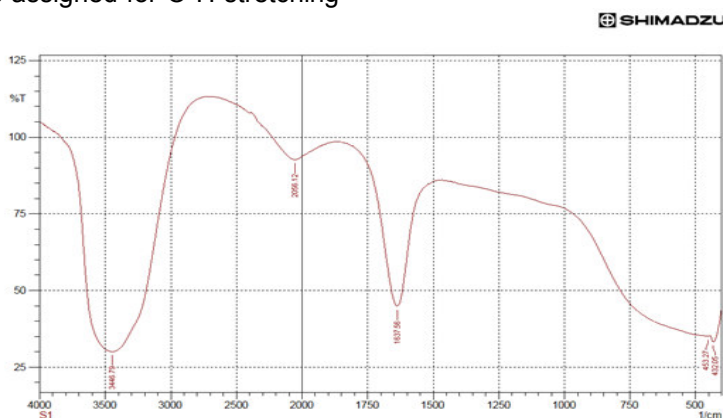


Figure 3
Fourier Transform Infrared spectra of silver nanoparticles synthesized from Murraya Koenigii leaves extract

XRD Analysis

The XRD analysis confirmed the presence of silver colloids in the sample. The number of Bragg reflections was observed at $2\theta = 32.23^\circ$, 46.27° and 77° . These Bragg reflections clearly indicated the presence of (111), (200), (311) corresponding to set of lattice planes which

may be indexed as the band for face centered cubic(FCC)crystal structure of silver. The XRD pattern thus clearly shows that the silver nanoparticles synthesized by the present green method are crystalline in nature.

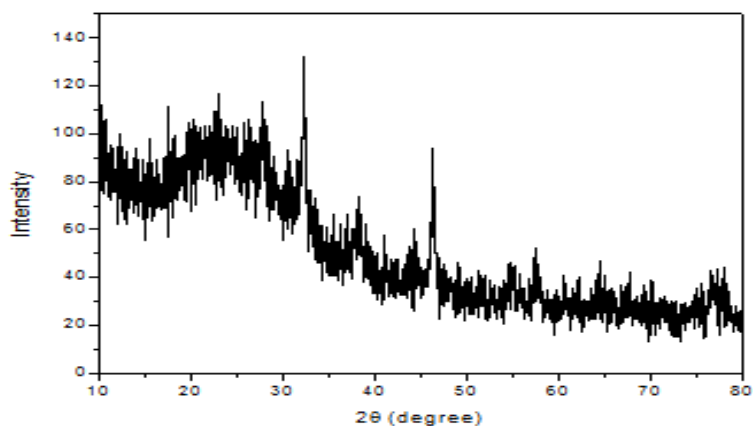


Figure 4
XRD analysis of silver nanoparticles synthesized from Murraya Koenigii leaves extract

SEM Analysis of silver nanoparticles

The synthesized nanoparticles from *Murraya koenigii* leaf extract mediated silver nitrate solution were observed by SEM for identification of the silver

nanoparticle surface. From the SEM images it is evident that the morphology of silver nanoparticles was spherical shaped and well distributed without aggregation (Figure 5).

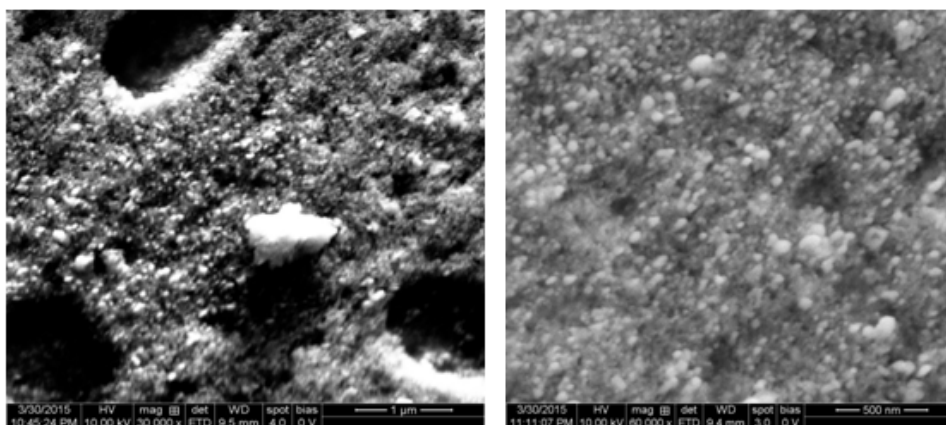


Figure 5

(a - b) SEM images of silver nanoparticles synthesized from *Murraya koenigii* leaves extract

Antibacterial activity

The synthesized nanoparticles were tested against four bacterial strains namely *Staphylococcus Aureus*, *Salmonella paratyphi*, *Staphylococcus albus* and *Escherichia coli*. The results were given in the table

1.the zone of inhibition of *S. aureus*, *S. paratyphi*, *S. albus* and *E. coli* were 21.2mm, 13.3mm, 23.4mm and 18.6 mm respectively, and results are comparable with standard Ciprofloxacin.

Table 1
Antibacterial activity of Silver nanoparticles from *Murraya koenigii* leaves extract

Tested Organisms	Zone of inhibition (mm)	Ciprofloxacin (2µg/ml)
<i>S. Aureus</i>	21.2	23.1
<i>S. paratyphi</i>	13.2	26.4
<i>S. albus</i>	23.4	24.5
<i>E. coli</i>	18.6	27.3

DISCUSSIONS

Reduction of silver ion in to silver nanoparticles during exposure to the *Murraya koenigii* leaf extract could be observed by colour changes. Silver nanoparticles exhibited dark yellowish-brown color in aqueous solution due to excitation of surface plasmon vibrations in the silver nanoparticles³⁰. The nanoparticles were primarily characterized by UV-Visible spectroscopy, and proved to be very useful techniques for the analysis of nanoparticles. The absorption spectra of the silver nanoparticles formed in the reaction media had an absorbance peak at 433nm and a broadening of the peak indicated that the particles were polydispersed. FTIR spectrum were carried out to identify the possible molecules responsible for the reduction of Ag⁺ ions and represents the capping of the bioreduction and stabilization of AgNps present in the *Murraya koenigii* leaf extract. XRD is commonly used for determining the chemical composition and crystal structure of a material; therefore detecting the presence of silver nanoparticles in plant tissues can be achieved by using XRD to examine the diffraction peaks of the plant. The XRD pattern showed three intense peaks in the whole spectrum of 2θ ranging from 10 to 80. In this experiment the X-ray diffraction pattern of synthesized silver nanoparticles matches the FCC structure of the silver with the broad peaks at 32.23°, 46.27° and 77°. These are corresponding to (111), (200), (311) planes

respectively. This results clearly indicates that the silver nanoparticles formed by the reduction of Ag⁺ ions by *Murraya koenigii* leaf extract are crystalline in nature. The SEM image showed relatively spherical shaped nanoparticles. In order to find the biological activity of synthesized nano particles we employed antibacterial activity by disc diffusion method, two gram positive bacteria *S. aureus*, *S. paratyphi* and two gram negative bacteria *S. albus* and *E. coli* were used. All tested bacteria showed moderate to good antibacterial activity from the range of 13.2mm to 23.4mm zone of inhibition and results were comparable to the standard Ciprofloxacin. *The S. Aureus* and *S. albus* have good antibacterial activity with maximum zone of inhibition 21.2mm and 23.4 respectively; the findings are good agreement with literature^{31,32}.

CONCLUSION

This work demonstrates the synthesis of nanoparticles using of plants is quite novel method leading to green chemistry route and it has wide advantages. Further it has potent antibacterial activity against all tested bacteria and is useful for medicinal field.

CONFLICT OF INTEREST

Conflict of interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript