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BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *MURRAYA KOENIGII*

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ABSTRACT
The nanoparticle is a novel way to synthesis nanoparticles by using biological sources. It is gaining attention due to its cost effective, ecofriendly and large scale production possibilities. In this present study *Murraya koenigii* was taken to investigate their potential for synthesizing silver nanoparticle. The silver nanoparticles synthesized were confirmed by its change of colour to dark brown due to the phenomenon of surface plasmon resonance. Ag NPs were characterized by UV–vis spectrophotometer, SEM, XRD, AFM and FTIR spectroscopy. *Murraya koenigii* leaf showed great capability to synthesis Ag NPs at optimum temperature conditions. The UV absorption peak at 428nm clearly indicates the synthesis of Ag NPs. The SEM, TEM and AFM studies were helpful at deciphering their morphology and distribution. DLS and Zeta potential studies validated the size and charge of the nanoparticles in the colloidal system without any aggregation. FTIR studies confirmed the biofabrication of the Ag NPs by the action of different phytochemicals with its different functional groups present in the extract solution. The XRD patterns confirmed the purity, phase composition and nature of the synthesized nanoparticles. The silver nanoparticles have great pharmacological activity.

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

INTRODUCTION

In recent days nanotechnology has induced great scientific advancement in the field of research and technology. Nanotechnology is the study and application of small object which can be used across all fields such as chemistry, biology, physics, material science and engineering. Nanoparticle is a core particle which performs as a whole unit in terms of transport and property (Sonali Pradhan, 2013). As the name indicates nano means a billionth or 10^{-9} unit. Its size range usually from 1-100nm due to small size it occupies a position in various fields of nano science and nanotechnology. Nano size particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material. So the main aim to study its minute size is to trigger chemical activity with distinct crystallography that increases the surface area (Osaka *et al*., 2006; Sinha *et al*., 2009). Thus in recent years much research is going on metallic nanoparticle and its properties like catalyst, antibacterial activity, the data storage capacity (Sharma *et al*., 2009).

The biological synthesis of nanoparticle is a challenging concept which is very well known as green synthesis. The biological synthesis of nano material can solve the environmental challenges like solar energy conservation, agricultural production, catalysis (Kumar *et al*., 2011), electronic, optics (Evanoff and Chumanoy, 2005) and biotechnological area (Soloviev, 2007). Green synthesis of nanoparticle are cost effective, easily available, eco friendly, nontoxic, large scale production and act as reducing and capping agent in compared to the chemical method which is a very costly as well as it emits hazardous by-product which can have some deleterious effect on the environment. Biological synthesis utilizes naturally occupying reducing agent such as plant extract, microorganism, enzyme, polysaccharide which are simple and viable which is the alternative to the complex and toxic chemical processes (Du *et al*., 2009). Plants can be described as nano factories which provide potential pathway to bioaccumulation into food chain and environment. Among the different biological agents plants provide safe and beneficial way to the synthesis of metallic nanoparticle as it is easily available so there is possibilities for large scale production apart from this the synthesis route is eco-friendly, the rate of production is faster in comparison to other biological models such as bacteria, algae and fungi. From the various literature studies it can be stated that the amount of accumulation of nanoparticle varies with reduction potential of ions and the reducing capacity of plant depends on the presence of various polyphenols and other heterocyclic compounds (Nair *et al*., 2010).

Nanoparticle of gold, silver, copper, silicon, zinc, titanium, magnetite, palladium formation by plants has been reported. Colloid silver nanoparticle had exhibited distinct properties such as catalytic, antibacterial (Sharma *et al*., 2009), good conductivity and chemical stability. Silver nanoparticles have its application in the field of bio labelling, sensor, antimicrobial, catalysis, electronic and other medical application such as drug delivery (Jong and Borm, 2008) and disease diagnosis. The size dependent use of silver nano particles as carrier molecules in applications, such as drug delivery, diagnostics, nanobiosensors, etc are increasing with the advancement in technology (Xiangling Ren *et al*., 2005). To meet the commercial demand of nano particles, three main objectives are low cost, environmental compatibility and non toxicity. Studies have already been conducted to synthesize nanoparticles from different parts of plants (Siavash Iravani, 2011).

Murraya koenigii, commonly known as curry leaf or kari patta in Indian dialects, belonging to Family Rutaceae which represent more than 150 genera and 1600 species (Satyavati et al., 1987). *Murraya koenigii* is a highly valued plant for its characteristic aroma and medicinal value. It is an important export commodity from India as it fetches good foreign revenue. A number of chemical constituents from every part of the plant have been extracted. The most important chemical constituents responsible for its intense characteristic aroma are P-gurjunene, P-caryophyllene, P-elemene and O-phellandrene. The plant is rich source of carbazole alkaloids (Kumar et al., 1999). Bioactive coumarins, acridine alkaloids and carbazole alkaloids from family Rutaceae were reviewed (Ito, 2000). *M. koenigii* is widely used in Indian cookery for centuries and have a versatile role to play in traditional medicine. The plant is credited with tonic and stomachic properties. Green leaves are eaten raw for cure of dysentery, diarrhoea and for checking vomiting (Nadkarni, 1976). Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders. *M. koenigii* contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. Considering the chemical and immense pharmacological properties of *Murraya koenigii*, the present study was aimed to explore the nanoparticle biosynthesis of silver and its characterization in ethanolic extract of *Murraya koenigii*.

MATERIALS AND METHODS

Collection and Identification

Murraya koenigii was collected from Thanjavur District. The plant was authenticated by Director, Plant Anatomy & Research Center, Chennai and the voucher specimen is deposited in our laboratory.

Preparation of ethanolic extract of *Murraya koenigii*

The whole plant was shade dried and pulverized. 100gm of the powder was soaked in 150ml of ethanol (w/v) for 3-5 days with intermediate shaking. This was filtered through a fine cheese cloth and the filtrate was pooled after 3 days of repeated extractions. The filtrate obtained was evaporated to dryness using rotary evaporator. The concentrate was lyophilized and used for the study.

Bio synthesis of silver Nanoparticles

To the ethanol extract, silver nitrate solution was added slowly drop wise in a molar ratio of 1:2 under vigorous stirring, and the stirring was continued for 12 hrs. The precipitate obtained was filtered and washed thoroughly with deionized water. The precipitate was dried in an oven at 100°C and ground to fine powder using agate mortar. The powder obtained from the above method was calcined at different temperatures.

Characterization of nanoparticles

The pure sample was analyzed for UV–vis absorption and optical band gap (Eg) using UV–Vis spectrophotometer (a Lambda 25-Perkin Elmer). The functional group of Nanoparticles was examined by using FTIR spectrometer (Perkin-Elmer 1725X). The shape and size of the sample were characterized by using field emission scanning electron microscope (FESEM) (JSM-6360LA). Size distribution and the average size of the nanoparticles were estimated on the basis of FESEM image.

The size distribution or average size of the synthesized silver nanoparticles were determined by dynamic light scattering (DLS) and zeta potential measurements were carried out using DLS (Malvern, UK). The air dried nanoparticles were coated onto X-Ray Diffraction (XRD) grid and analyzed for the formation of silver nanoparticle by Philips X-Ray Diffracto meter with Philips PW 1830 X-Ray Generator operated at a voltage of 40kV and a current of 30mA with Copper Potassium alpha radiation.

RESULTS AND DISCUSSION

The silver nanoparticle solution has dark brown or dark reddish in colour. In *Murraya koenigii* before addition of $AgNO_3$, its colour was red but after its treatment with $AgNO_3$, its colour changes to dark brown which indicated the formation of silver nanoparticles. This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles. Silver nanoparticles with their unique chemical and physical properties are proving to be an alternative for the development of new phamacological agents. Silver nanoparticles have also found diverse applications in the form of wound dressings, coatings for medical devices and silver nanoparticle impregnated textile fabrics, etc. (Rai *et al*., 2009). A detailed study on the biosynthesis of silver nanoparticles by *C. auriculata* were used to carry this out, and reported in this work.

The silver nanoparticles exhibit yellow brownish colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Probin Phanjom *et al*., 2012).

The UV absorption peak of silver nanoparticles ranges from 400 nm – 450 nm. The UV absorption peak of silver nanoparticles range was from 400 nm – 450 nm. Fig. 1 shows the UV absorption peaks of *M. koenigii*. UV-Vis spectra shows the peaks approximately at 421nm, clearly indicating the formation of spherical silver nanoparticle in the plants extracts. The occurrence of the peak at 421 nm is due to the phenomenon of surface Plasmon resonance, which occurs due to the excitation of the surface plasmons present on the outer surface of the silver nanoparticles which gets excited due to the applied electromagnetic field (Sonali Pradhan, 2013). Silver nanoparticles exhibited yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Jancy Mary and Inbathamizh, 2012).

A scanning electron microscope was employed to analyze the shape of the silver nanoparticles that were synthesized by green method. SEM analysis showed that the *M. koenigii* have tremendous capability to synthesize silver nanoparticles which were roughly spherical in shape (Fig. 2) and were uniformly distributed. The formation of spherical shaped silver nanoparticle extracted through *M. koenigii* whose size ranging in between 20 nm to 149 nm was confirmed by SEM. Preetha Devaraj *et al*. (2013) observed SEM image shows high-density AgNPs synthesized bycannonball leaf extract. It was shown that relatively spherical and uniform AgNPs were formed with diameter of 13to61nm. The SEM image of silver nanoparticles was due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (Priya *et al*., 2011). The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements.

AFM was used to analyze the particle morphology (shape, size). AFM image of *M. koenigii* mediated synthesized silver nanoparticle shows that they have a uniformly packed surface with height 0.837mµm. Fig. 3 shows the 3D AFM images of the plant extract mediated synthesized nanoparticles. Sonali Pradhan (2013) previously similar reported green synthesis of silver nanoparticles by the help of green plants is a very cost effective, safe, non-toxic, eco- friendly route of synthesis which can be manufactured at a large scale. *H. sinensis*, *C. maxima, M. oleifera, A. indica* and *A.calamus* showed great capability to synthesis AgNPs at optimum temperature conditions. AFM was used to analyses the particle morphology. AFM image of *H. Siniesis* mediated synthesised AgNPs shows that they have a uniformly packed surface with height 0.703µm.

Dynamic light scattering (DLS) is a technique used to determine the size, size distribution profile and poly disparity index of particles in a colloidal suspension. Fig. 4 shows the DLS and zeta potential graph of *M. koenigii* which has an average size of 75.32nm and the particles carry a charge of -7.14 mV. Poly disparity index (PDI) is a measurement for distribution of silver nanoparticle with from 0.00 to 0.5. PDI greater than 0.5 values indicates the aggregation of particles. It was clear that the silver nanoparticle synthesized from the *M. koenigii* extracts does not aggregate at all. Zeta potential measures the potential stability of the particles in the colloidal suspension. Silver nanoparticles generally carry a negative charge. The silver nanoparticles synthesized from the *M. koenigii* showed negative charge and were stable at room temperature.

FTIR gives the information about functional groups present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the biofabrication of silver nanoparticles mediated by the *M. koenigii* extracts. Fig. 5 shows the FTIR spectrum of *M. koenigii* mediated synthesized silver nanoparticle, the silver nitrate salt and dried leaves petal extract, in AgNO3 peaks were observed at 3697cm-1, 1761cm-1,1390cm-1,831cm-1which are associated OH stretching, C=C stretching, CH stretching, NH stretching respectively. In this plant *M. koenigii* leaf extracts peak were observed which are associated OH stretching, CH stretching, C=N stretching, N-H stretching, CN stretching, C-Cl stretching. In the synthesized silver nanoparticle from *M. koenigii* peaks were observed which are associated with NH stretching, C=O stretching, N-O stretching, CH2 and CH3 deformation, C-O stretching and halogen group presence. The presence of peaks at 3749 cm−1 and 1523 cm−1 indicate the –NH2 symmetric stretching and N–O bonds in nitro compounds (Saranya Raju and Rajakumar, 2017). They indicates the presence of ethanols and phenols (O-H), carboxylic acids and its derivatives (C=O) and Chloroalkanes (CX) respectively (Kumar *et al*., 2011). The bonds or functional groups such as -C-O-C-, –C-O- and -C=C- are derived from heterocyclic compounds. The amides I bond derived from the proteins are the capping ligands of the nanoparticles (Raut *et al*., 2009).

XRD analysis is used to determine the phase distribution, crystallinity and purity of the synthesized nanoparticles particles. Fig. 6 shows the XRD patterns of *M. koenigii*. It was concluded that the nanoparticles were crystalline in nature having cubical shape with no such impurities. Logeswari *et al*. (2013) reported the XRD pattern for silver nanoparticles synthesized using commercial plant powders. The silver nanoparticles synthesized were calculated by the particle size ranges of the silver at 48 nm, 34 nm, 43 nm and 33 nm, corresponding to *S. cumini*, *C. sinensis*, *S. tricobatum* and *C. asiatica* respectively.

Synthesis of silver nanoparticles by using leaf extract of *M. koenigii* medicinal plant has been demonstrated in present investigation. The reduction of Silver ions and their capping were achieved by the organic molecules present in the leaf extract. The UV-Vis, SEM, AFM, FTIR, XRD results revealed that the Silver nanoparticles were spherical in shape and ranging from 30 to 40 nm in size. The elemental nature and purity of the sample was confirmed by the spectrum report. The silver nanoparticles showed good pharmacological activity.

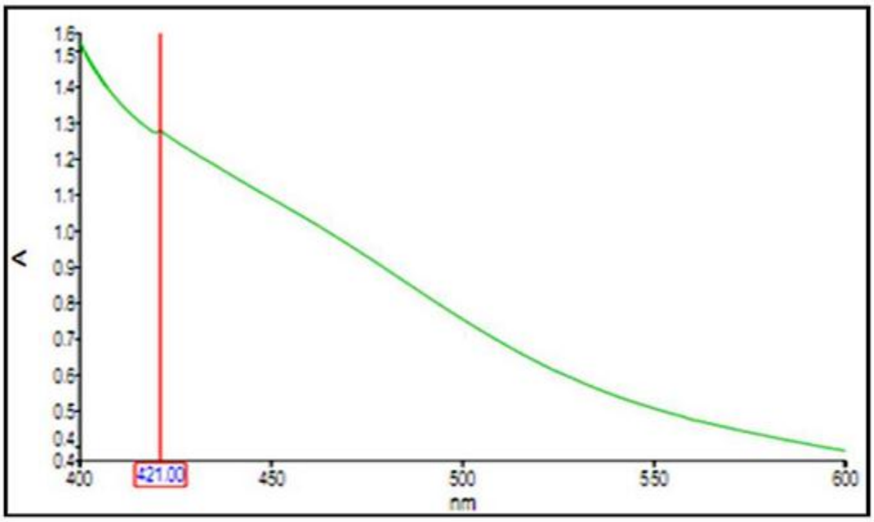

Fig. 1: UV-Visible spectrum of Ag NPs.

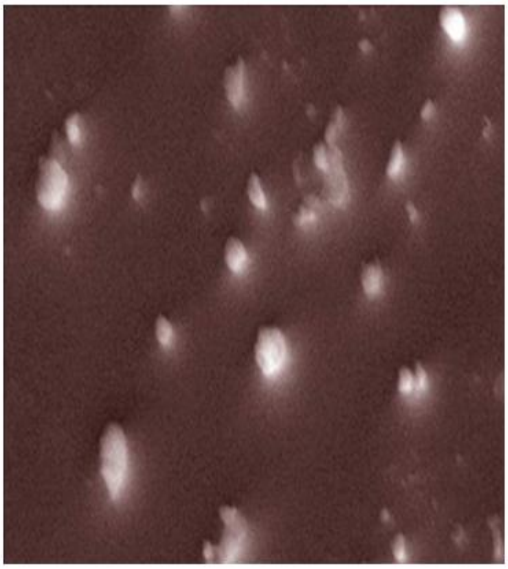

Fig. 2: SEM image of Ag NPs.

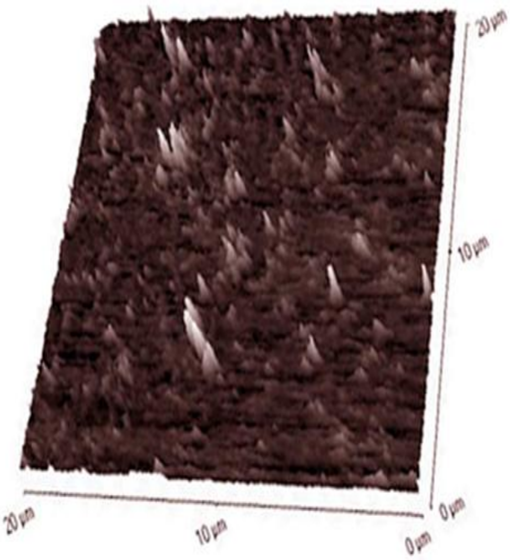

Fig. 3: AFM image of Ag NPs.

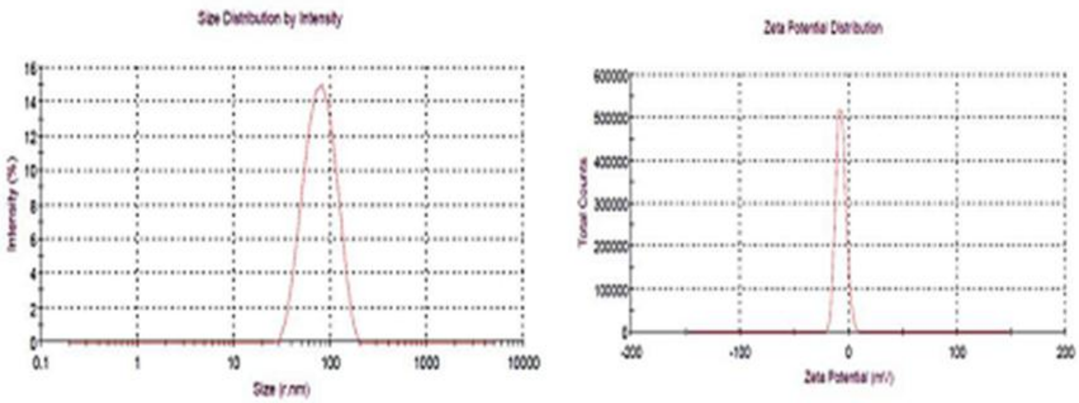

Fig. 4: DLS and Zeta potential graph of Ag NPs.

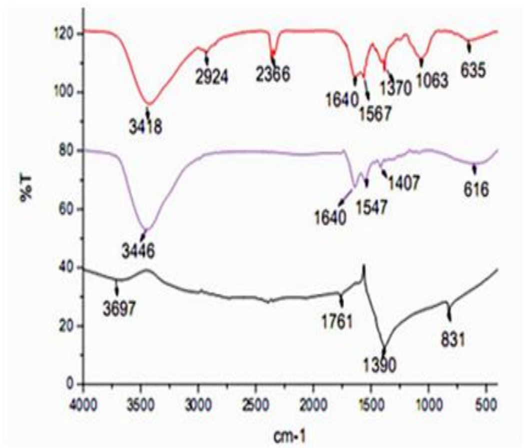

Fig. 5: FTIR spectrum of Ag NPs.

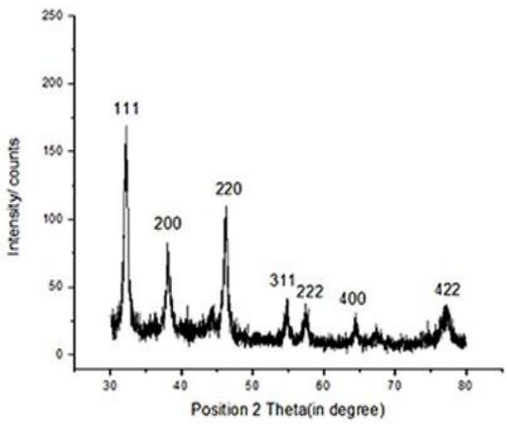

Fig. 6: XRD patterns of Ag NPs.

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