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RESEARCH ARTICLE

BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES USING MEDICINAL PLANT (*Cassia italica*) LEAVES

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ABSTRACT

Biologically synthesized nanoparticles have been widely using in the field of medicine. Research in nanotechnology highlights the possibility of green chemistry pathways to produce technologically important nanomaterials. The synthesis, characterization and application of biologically synthesized nanomaterials have become an important branch of nanotechnology. Fresh leaves of *Cassia italica* was used for the synthesis of silver (Ag) nanoparticles. The present study revealed that the phytosynthesis of silver nanoparticles from 1mM $AgNO_3$ solution through the leaf extract of *C. italica* as reducing agent as well as capping agent. Characterization of newly synthesized silver nanoparticles was observed using UV-vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and High Resolution Transmission, Electron Microscope (TEM) studies. TEM image divulges that silver nanoparticles are quite poly-dispersed. The extra-cellular synthesis of Ag nanoparticles moves towards extracellular level. The above silver nanoparticles were found to be effective against *E. coli* and *C. albicans*. The effect of silver nanoparticles on the growth of bacteria and fungus varied. The important outcome of this study would help to formulate value added products in biomedical and nanotechnology based industries, when commonly available plants are properly screened medicinal plants.

INTRODUCTION

Nanotechnology involves tinkering work at atomic levels, tweaking and controlling substances 1, 00, 000 times smaller than a strand of human hair, to make useful materials and devices. It involves technology at the scale of one-billionth of a meter. The term 'NANO' is derived from Greek word "Dwarf" (Parthiban *et al*., 2010). Nanotechnology is foreseen to significantly influence science, economy and everyday life in 21st century and it may become one of the driving forces to the next industrial revolution. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Nanotechnology refers broadly to a field of applied science and technology, whose unifying theme is the control of matter on the atomic and molecular scale (Anima and Saravanan, 2009). The field of nanotechnology is one of the most active areas of research in modern material science. Silver has long been recognized as one of the nanoparticles having inhibitory effect on microbes present in medical and industrial process (Jose *et al*., 2005; Lok *et al*., 2007). The most important application of silver and silver nanoparticles is in medical industry such as typical ointments to prevent infection against burnt and open wounds (Ip *et al*., 2006). Silver nanoparticles have diverse applications both in *in vitro* and *in vivo* (Haes and Duyne, 2002) conditions. A number of approaches such as reduction of solutions (Goia and Matijevic, 1998), chemical and photochemical reactions in reverse micelles (Taleb *et al*., 1997), thermal decomposition of silver compounds (Esumi *et al*., 1990), radiation assisted (Henglein, 2001), electrochemical (Rodriguez-Sanchez *et al*., 2000), sonochemical (Zhu *et al*., 2000) and microwave assisted process (Pastoriza and Liz-Marzan, 2002) and recently via green chemistry route (Bar *et al*., 2009; Begum *et al*., 2009; Song and Kim, 2009) are available for the synthesis of silver nanoparticles.

Although there are many routes available for the synthesis of silver nanoparticles, biological synthesis using plant sources offers several advantages such as best in cost-effectiveness, non-toxic and eco-friendly agent (Aymonier *et al*., 2002; Sun and Xia, 2002). It could be advantageous over other environmentally benign biological processes, as this eliminates the elaborate process of maintaining cell culture. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. This traditional synthesis method is more convenient for pharmaceuticals and biomedical applications (Goodsell, 2004). Biosynthetic processes of nanoparticles would be more useful, if nanoparticles are produced extracellularly using plants or their extracts in a controlled manner according to their size, shape and dispersity (Kumar and Yadav, 2008). Although biosynthesis of gold and silver nanoparticles by the plants, *Alfalfa* (Gardea-Torresdey *et al*., 2003), *Azadirachta indica* (Shankar *et al*., 2004), *Emblica officianalis* (Ankamwar *et al*., 2005), *Tamarindus indica*

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(Ankamwar *et al*., 2005), *Aloe vera* (Chandran *et al*., 2006), *Cinnamomum camphora* (Huang *et al*., 2007), *Hibiscus rosa-sinensis* (Mukherjee *et al*., 2008), *Capsicum annuum* (Harekrishna *et al*., 2009), *Carica papaya* (Devendra *et al*., 2009), *Magnolia kobus* and *Diopyros kaki* leaf (Song and Kim,2009), *Rose* leaf (Dubey *et al*., 2010a) and *Acalypha indica* leaf (Krishnaraj *et al*., 2010) *Euphorbia hirta* (Elumalai *et al*., 2010), *Coriandrum sativum* (Sathyavathi *et al*. (2010), *Pongamia pinnata* (Rajesh *et al*. (2010), *Cassia auriculata* leaf (Udayasoorian *et al*., 2011), leaf broth of *Ocimum sanctum* (Mallikarjuna *et al*. (2011), *Saururus chinensis* leaf (Nagajyoti *et al*. (2011), *Garcinia mangostana* (Ravichandran *et al*., 2011) have been reported, the potential plant as biological material for the synthesis of nanoparticles is yet to be fully explored. *Cassia italica* Lam. ex F.W. Andrews is a medicinal herb. It is a small herb belongs to the family, Caesalpinaceae. This herb is being used to treat number of aliments. The form of this plant is sprig on the ground and their leaf has purgative effect in the humans and it is used for the treatment of constipation (Waltenberger *et al*., 2008). *C. italica* leaves are also used in the treatment of gas troubles and skin diseases. Although this plant is considered as undesirable one, but to the best of our knowledge we are the first to report its use in synthesizing silver nanoparticles, which can provide a new platform for making a value added medicine in future in nanotechnology based industries.

MATERIALS AND METHODS

Preparation of leaf extract

The fresh (matured) leaves of *Cassia italica* were collected from Sathiyanathapuram, Theni District, Tamilnadu, India. These leaves were subjected to Nanoparticle synthesis that was carried out in Kongunadu Arts and Science College, Coimbatore and University of Madras, Chennai. The mature, undamaged and disease free leaves were selected and washed thoroughly with sterile double distilled water (DDW) and surface sterilization was made with 0.1% $HgCl_2$ for 2 to 3 min under the hood of laminar air flow chamber. 25gm of sterilized leaf samples were taken and cut into small pieces. Finely pieces of cut leaves were put in a 500ml conical flask containing 100ml of sterile double distilled water (DDW). It was boiled for 5min and filtered. The filtrate was stored at 4ºC for further usage.

Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles**.** 5ml of leaf extract was added to 100ml of 1mM $AgNO_3$ (99.99%) aqueous solution in 250ml conical flask that was kept at room temperature. The flask was thereafter put into a shaker (150rpm) at 30ºC for a period of 48hrs to facilitate reaction.

UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + leaf extract) was recorded through visual observation. The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquots (1ml) and subsequent measuring was carried out using UV-visible spectroscopy. Thus UV-visible spectra of these aliquots were obtained from Elico UV-visible spectrophotometer.

X-ray Diffraction (XRD) measurements

The complete bioreduced sample was concentrated in concentrator at 60ºC to reduce the volume of the reaction mixture. To remove any free biomass residue or compound that is not capping the ligand of the nanoparticles, the residual solution of 30ml after reaction was centrifuged at 10,000rpm for 10min and the resulting suspension was redispersed in 2ml sterile distilled water. The centrifugation and redispersion process was repeated thrice to remove the water soluble biomolecules such as proteins and secondary metabolites. Thereafter, the purified suspension was freeze dried to obtain dry powder. XRD measurements of purified silver nanoparticle solution casted onto the glass substrate was carried out using X' Pert Pro X-ray (PAN alytical BV, The Netherland) instrument operating at a voltage of 40 vK and current of 20Ma. The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum. The XRD pattern was measured by drop coated films of $AgNO_3$ on glass plate and employed with X-ray diffractometer (PAN X-ray diffractometer) of characteristic Co-kα1 radiation (λ = 1.78 A°) in the range of 20° to 90° at a scan rate of 0.05°/min with the time constant of 2seconds.

TEM analysis

Morphology and size of the silver nanoparticles were investigated by using TEM images. 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 TEM grid was allowed to dry overnight.

FT-IR measurement

FT-IR measurement of sample was performed using Nicolet Avatar Model FT-IR Spectrophotometer in a diffuse reflectance mode at a resolution of $4cm^{-1}$ in KBr pellets.

RESULTS

Use of environmentally benign materials like plant extracts (or) drugs of plant origin (Parashar *et al*., 2009) for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications, as they do not contain toxic chemicals at any levels of the synthesis protocol. The extracellular synthesis of silver nanoparticles occurred during the exposure of *C. italica* leaf extract to 1mM aqueous silver nitrate solution. The complete reduction of silver ions was observed after 48hrs of reaction at 30ºC under continuous shaking condition. The colour change in reaction mixture was observed during the incubation period, because the formation of silver nanoparticles is able to produce particular colour in the reaction mixtures due to their specific reaction properties. The appearance of dark yellowish-brown colour is a clear indication of the formation of silver nanoparticles in the reaction mixture (Fig.1). UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. Fig. 2 shows the UV-Visible spectra recorded from the reaction medium after 48hrs. Absorption spectra of silver nanoparticles formed in the reaction media have absorption peak at 450nm. Broadening of peak indicates that the particles are poly-dispersed. So, silver nanoparticles can be used as carriers of drugs originated from *C. italica*. The

Fig 1. Formation of silver nanoparticles
a – Leaf extract, b – Leaf extract + AgNO3 after 48 hours

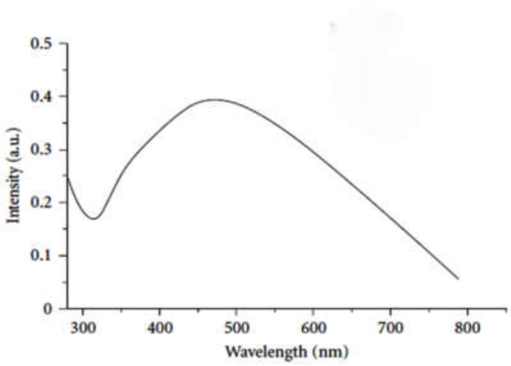

Fig. 2. UV-Vis Spectra of reduction of Ag ions to Ag nanoparticles

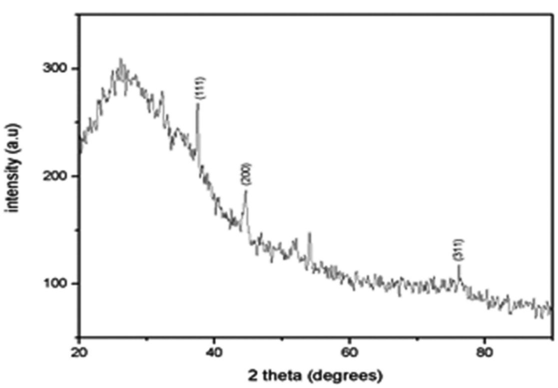

Fig. 3. XRD pattern synthesized Ag nanoparticles

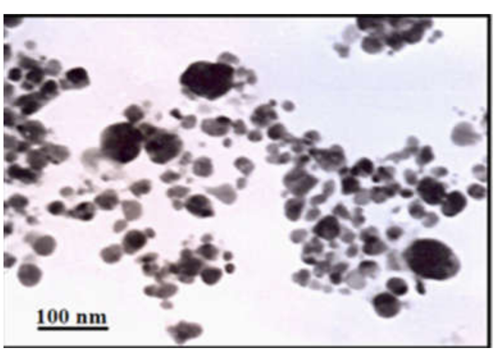

Fig. 4. TEM image of synthesized Ag nanoparticles

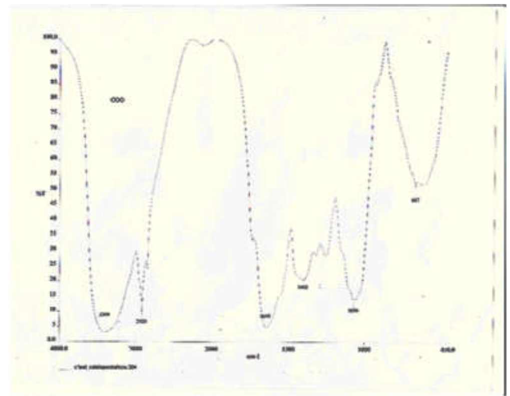

Fig. 5. FT-IR spectrum of Ag nanoparticles

uniform distribution of silver nanoparticles reveals that these particles would help for the availability of the drugs through out the system (body). The biosynthesis of silver nano structure by employing *C. italica* leaf extract was further demonstrated and confirmed by characteristic peaks observed in the XRD image (Fig. 3). The XRD spectrum showed three distinct diffraction peaks at 37.6°, 44.7° and 76.3°, which are indexed the (111), (200) and (311) of the cubic face-centered silver. The obtained data was matched with the Joint Committee on Powder Diffraction Standards (JCPDS) file No.03-0921. The average grain size of the silver nanoparticles formed in the process was estimated from the Debye-Scherrer equation [(d = (kλ×180)/ β Cos θβ Π) by determining the width of the (111) Bragg's reflection, where "k" is Scherrer constant, λ is the wavelength of the X-rays, β and θβ are full width half maximum of the Bragg angle]. The estimated mean size of the particles was 6.2nm. The silver nano particle of 6.2nm size has the ability to distribute the *C .italica* extract through out the system.

The Transmission Electron Microscope (TEM) image has shown the distribution of individual silver particles as well as the formation of number of aggregates. The morphology of the silver nanoparticles was predominately spherical and aggregated into larger irregular structure with no well-defined morphology observed in the micrograph (Fig. 4). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (proteins secreted by plant leaf extracts). The TEM image shows the distribution of the high density silver nanoparticles synthesized/organized by the *C. italica* leaf extract. Further it is confirmed by the development of silver nanostructures as a result of interaction between the particles and extract. The wave number or frequency (cm^{-1}) of absorption band or peak assigned to the type of vibration, intensity and functional groups of the silver nanoparticles synthesized/organized using *C. italica* leaf extract are shown in Fig. 5. Different functional groups were involved in reduction of silver ions to silver nanoparticles. The peaks in the region of 3400 to 3200cm^{-1} and 3000 to 2850cm^{-1} were assigned to O-H stretching of alcohol and phenol compounds and CH (CH_2) stretching - lipids and proteins, respectively. The peaks in the region of 1645 to 1450cm^{-1} correspond to C=C of carbonyl OH (bend) alcohols. The peaks at the region of 1070 to 667cm^{-1} correspond to C-O bond of the carbonyl groups or alkynes, aliphatic compounds. FT-IR analysis reveals that the carbonyl group from amino acid residues and proteins have stronger ability to bind metal indicating that the proteins could possibly form a covering layer around the metal nanoparticles (*i.e.,* capping of silver nanoparticles) to prevent agglomeration and thereby stabilized in the medium.

DISCUSSION

Development of new, easy, reliable and eco-friendly technologies/methods help in endorsing extra interest in the synthesis and application of nanoparticles, which are good and beneficial for mankind (Bhattacharya and Gupta, 2005). In this context, the utilization of biological systems for nanoparticles synthesis is notable alternative in advanced multifaceted approaches. Biological systems have shown the ability to interact with metal ions and reduce them to form metallic nanoparticles (Beveridge *et al*., 1997). Nanotechnology is a fast emerging discipline not only in physics and chemistry but also in the field of biology. In view of the tremendous applications of nanotechnology, there is a fillip among scientists to carry out research in this most vital discipline. Chemists are highly interested in synthesizing nanoparticles through different dimensions employing many of the precious metals. The present study was conducted to synthesis nanoparticles, especially in silver, using the extract of an un-exploited plant *C. italica*. Similar results were reported by Leela and Vivekanandhan (2008) and Vivekanandhan *et al*. (2009) in many plant species. As the *C. italica* leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from watery to yellowish brown due to the reduction of silver ion, which indicated the formation of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasma vibrations in skin nanoparticles (Kelly *et al*., 2003; Shankar *et al*., 2004).

UV-Visible absorption spectra of silver nanoparticles were formed in the reaction media has absorbance peak at 450nm. Broadening of peak indicates that the particles are poly-dispersed (Geethalakshmi and Sarada, 2010). It is generally recognized that UV-Visible spectroscopy on be used to examine the size and shape of the controlled nanoparticles in aqueous suspension (Wiley *et al*., 2006). The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within a hour of reaction, making it one of the fastest bio-reducing methods to produce Ag nano structures that have been reported till date (Shiv Shankar *et al*., 2003; Begum *et al*., 2009; Sathyavathi *et al*., 2010; Udayasoorian *et al*., 2011).

Shankar *et al*. (2004) reported that nanoparticles synthesized using plant extracts were surrounded by a thin layer of some capping organic material from plant leaf broth. These nanoparticles appear to have assembled into very open, quasi-linear superstructures rather than a dense closely packed assembly (Shankar *et al*., 2003). The Fig. 4 also reveals that nanoparticles are not in physical contact, but are evenly separated. The image also clearly shows that the particles of approximately 100nm thickness are surrounded by the leaf broth and developed into nanoparticles, which can be assigned to bio-organic compounds present in the leaf broth. In the FT-IR analysis, the presence of high number of hydroxyl stretching alcohol confirmed that the bioreduction of Ag^+ ions into silver nanoparticles occurred due to the formation of capping of plant extract material. IR spectra can be assigned to the N-H stretching frequency arising from the peptide linkages present in the proteins of the extract (Mukherjee *et al*., 2008). The presence of secondary capping materials with the silver nanoparticles may be assigned to bio-organic compounds derived from leaf extracts (Rajesh *et al*., 2009). The flavonoid and terpenoid constituents of the leaf extract are believed to be the surface active molecules stabilizing the nanoparticles (Shankar *et al*., 2003). Yin *et al*. (2006) has reported the presence of prenylated flavonoid derivatives, pongaflavanol and tunicatachalcone in *Pongamia pinnata*, whereas the latter one is proposed as biogenetic precursor of the former one. Therefore, it reflects that water soluble heterocyclic compounds such as flavones are the reducing and capping ligands of the nanoparticles.

Black tea leaf extracts are known to contain more amounts of flavonoids and polyphenols. It was found that the reduction of metal ions was accompanied by oxidation of polyols (Begum *et al*., 2009). It was reported that terpenoids are believed to be the surface active molecules stabilizing the nanoparticles and reaction of the metal ions is possibly facilitated by reducing sugars and terpenoids present in the neem leaf broth (Shankar *et al*., 2004). Presently, silver nanoparticles are finding a variety of applications starting from biological tagging to electronic devices (Rao *et al*., 2003). Li *et al*. (2007) reported that *Capsicum annum* extract is known to contain a number of biomolecules such as proteins, enzymes, polysaccharides, aminoacids and vitamins, which would be responsible for the synthesis of silver nanoparticles.

Our hypothesis is that several factors together determines the nanoparticle synthesis, including the plant source, the phyto-constituents in the crude leaf extract, the concentration of silver nitrate, temperature and other than these, even the pigments in the leaf extract. The bio-reduction of the aqueous Ag^+ ions by the leaf extract of the plant, *C. italica* leading to the formation of silver nanoparticles is fairly well-defined. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Biological methods for nanoparticle synthesis using plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods (Mohanpuria *et al*., 2008).

Nanoparticles may have different effects on human health relative to bulk material from which they are produced (Albrecht *et al*., 2006). Increase in biological activity of nanoparticles can be beneficial or detrimental or both. Many nanoparticles are small enough to have an access to skin, lungs and brain (Koziara *et al*., 2003; Oberdorster *et al*., 2004). In conclusion, the bio-reduction of aqueous Ag+ ions by the leaf extract of the *C. italica* plant has been demonstrated. The capabilities of the other plant part such as leaf as a capping and reducing agent is not tested and not well defined. In the present study we found that leaves can also be good source for synthesis of silver nanoparticles. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). From a technological point of view, these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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