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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(1): 612-615

www.thepharmajournal.com
Received: 02-11-2018
Accepted: 06-12-2018

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Study of synthesis and characterization of silver nanoparticles from *Tinospora cordifolia*

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Abstract
The nanoparticle is gaining attention due to its cost effective, ecofriendly and large scale production possibilities. In this present study *Tinospora cordifolia* 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. Silver nanoparticles were characterized by UV–vis spectrophotometer, SEM, XRD, AFM and FTIR spectroscopy. *Tinospora cordifolia* leaf showed great capability to synthesis silver nanoparticle 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. The silver nanoparticles have great pharmacological activity.

Keywords: Green synthesis, characterization, silver nanoparticles, *Tinospora cordifolia*

1. 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) [23]. 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) [12, 21]. 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) [19].
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) [7], electronic, optics (Evanoff and Chumanoy, 2005) [2] and biotechnological area (Soloviev, 2007) [2]. 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) [11].

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) [19], 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) [5] 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) [24]. 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) [20].

Tinospora cordifolia (Willd.) Miers, (Guduchi) is one of the important dioecious plants belongs to the family Menispermaceae. In Hindi, the plant is commonly known as Giloe which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young. In Ayurveda, it is designated as Rasayana drug recommended to enhance general body resistance, promote longevity and as antistress and adaptogen. This significant plant is also mentioned in important pharmacopoeias. Phytochemistry of *T. cordifolia* belongs to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. *T. cordifolia* is widely used in folkloric veterinary medicine and traditional Ayurvedic medicine in India for its, anti-inflammatory, immuno-modulatory, anti-pyretic activity, antioxidant, anti-diabetic, anti-allergic and anti-arthritic activities and various other medicinal properties. Almost all the parts of the plant are documented to be useful in ethnobotanical surveys conducted by ethnobotanists. Details of various important aspects such vernacular names of *T. cordifolia* and its important ethnobotanical, Ayurvedic properties, pharmacological and phytochemistry have been published (Mathew George *et al*., 2016) [10]. Keeping this in view, the present study has been undertaken to investigate the phytoconstituents present in ethanolic leaves extract of *Tinospora cordifolia*. Considering the chemical and immense pharmacological properties of *Tinospora cordifolia*, the present study was aimed to explore the biosynthesis of silver nanoparticle and its characterization.

2. Materials and Methods

2.1 Collection of plant samples

For the present study, the mature green leaves of *Tinospora cordifolia* belongs to family Menispermaceae were collected from in and around area of Thanjavur District, Tamil Nadu, South India. The plant was identified with the help of manual of Tamil Nadu and Karnatic flora (Gample, 1967 and Matthew, 1983) [3, 9] with standard references (Kirtikar and Basu, 1993) [6].

2.2 Preparation of *Tinospora cordifolia*

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.

2.3 Biosynthesis 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.

2.4 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 spectrometer. 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.

3. Results and Discussion

The silver nanoparticle solution has dark brown or dark reddish in colour. In *Tinospora cordifolia* 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 *Tinospora cordifolia* 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 *Tinospora cordifolia*. 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) [23]. 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
Tinospora cordifolia 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 *Tinospora cordifolia* 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 by cannonball 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 *Tinospora cordifolia* 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) [23] 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 synthesized AgNPs shows that they have a uniformly packed surface with height 0.703μm.

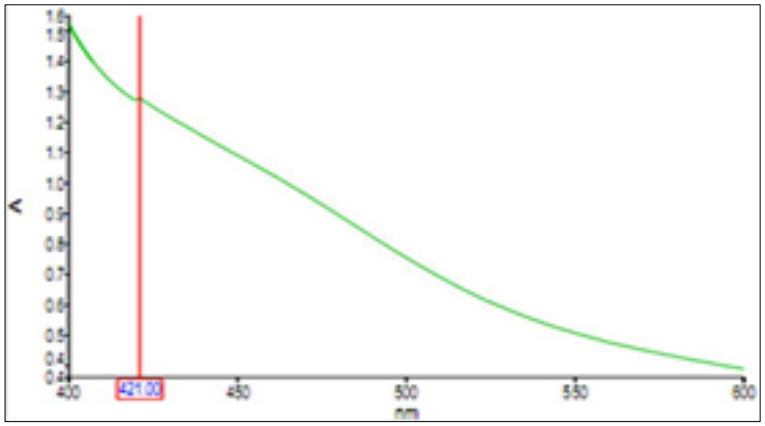

Fig 1: UV-Visible spectrum of Ag NPs

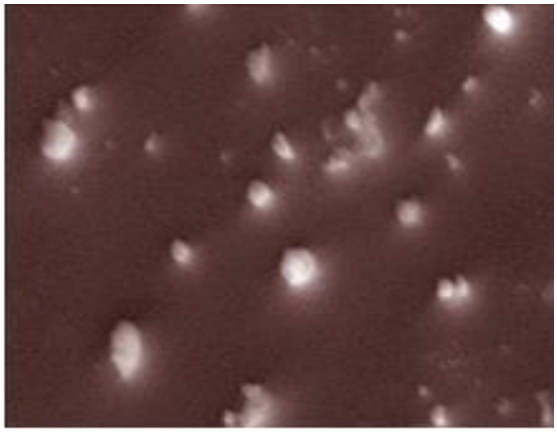

Fig 2: SEM image of Ag NPs

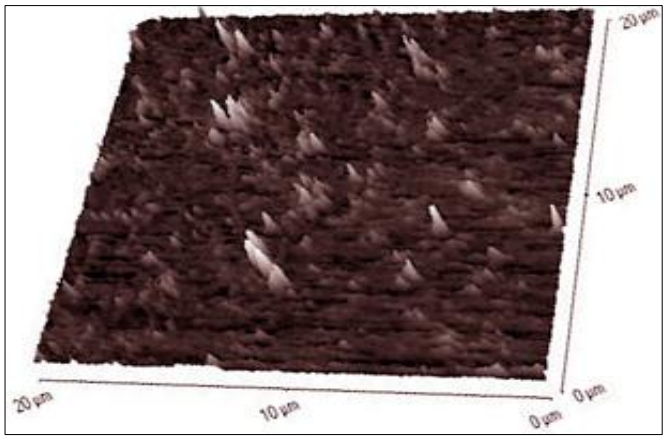

Fig 3: AFM image of Ag NPs

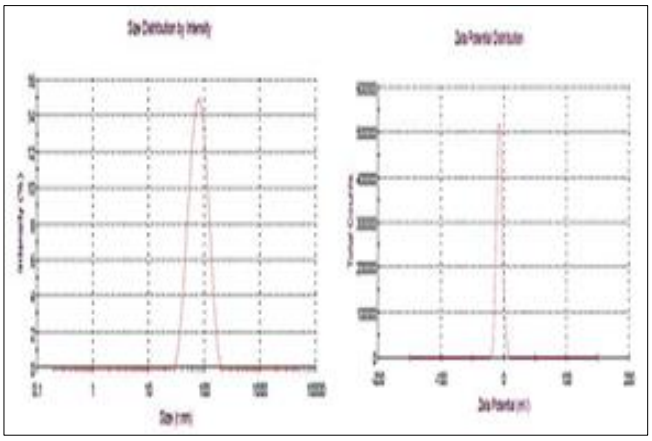

Fig 4: DLS and Zeta potential graph of Ag NPs

4. Conclusions

Nowadays herbs are extensively used for the research purpose and it possesses more than one chemical entity so it has been widely used for the research investigations. The plant based nanoparticles have the effective dosage response and minimal side effects when compared to the synthetic compounds Phytochemical screening of *Tinospora cordifolia* leaves reveals it as a valuable medicinal plant with numerous medicinal properties. Synthesis of silver nanoparticles by using leaf extract of *Tinospora cordifolia* 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, DLS and Zeta potential 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 against the cancer, arthritis, diabetes mellitus.

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