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A Study of Synthesis and Characterization of Silver Nanoparticles from *Ocimum basilicum*

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Abstract: Plants used in folk and traditional medicines have been accepted as therapeutic drug development in modern medicine. Although many studies have been focused on Lamiaceae family, but only few studies on medical effects of purple basil have been performed. The synthesis of nanoparticles by using biological sources is gaining attention due to its cost effective, ecofriendly and large scale production possibilities. In the present study *Ocimum basilicum* 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. *Ocimum basilicum* showed great capability to synthesize silver nanoparticle at optimum temperature conditions. The UV absorption peak at 428 nm clearly indicates the synthesis of Ag NPs. FTIR studies confirmed the biofabrication of the silver nanoparticle 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: Basils, Green synthesis, Nanoparticles, Silver nanoparticles, *Ocimum basilicum*

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Introduction

The *Ocimum* genus belongs to the Lamiaceae family (Omer *et al*., 2008) which includes approximately 150 species (Javanmardi *et al*., 2002). The species have variation in phenotype, oil content, composition and possibly bioactivity (Simon *et al*., 1999). Although the taxonomy of basil is complicated by the existence of numerous botanical varieties within the species that may not differ significantly in morphology, a system of standardized descriptors, which include volatile oil, has been proposed by Paton and Putievsky (1996). This permits easy identification of the different forms of *O. basilicum*. Dark Opal is one of the basil cultivars that are a rich source of anthocyanin. Basils are highly marketable herbs, not only for culinary purposes but also for their ornamental value. Inter-specific hybridization and polyploidy are common within *Ocimum* genus (Harley *et al*., 1992) and purple types such as 'Dark Opal', is a possible hybrid between *O. basilicum* and *O. forskolei*, which has lobed-leaves, with a sweet basil plus clove-like aroma (Darrah, 1974). The anthocyanins present in purple basils have been analyzed using high performance liquid chromatography, spectral data and plasma-desorption mass spectrometry. Fourteen different anthocyanins have been identified by this analysis (Phippen and Simon, 1998). Apart from role of anthocyanins as pigments there are many functions performed by these flavonoid compounds in these plants (e.g. UV protection, defense against pathogens and pests, protecting DNA) and numerous scientific studies have shown that these active compounds act also as antioxidant (Horbowicz *et al*., 2008).

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 1-100 nm and 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).

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). Considering the chemical and immense pharmacological properties of *Ocimum basilicum*, the present study was aimed to explore the biosynthesis of silver nanoparticle and its characterization.

Materials and Methods

Collection of plant samples:

Mature plants of *Ocimum basilicum* (family Lamiaceae) 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; Matthew, 1983) with standard references (Kirtikar and Basu, 1993).

Preparation of Ocimum basilicum extract:

The whole plant was shade dried and pulverized. 100 g of the powder was soaked in 150 ml 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.

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 h. 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 (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 colour. *Ocimum basilicum* colour was red before addition of $AgNO_3$, 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 *O. basilicum* were used to perform this study. 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. Figure 1 shows the UV absorption peaks of *O. basilicum*. UV-Vis spectra shows the peaks approximately at 421 nm, 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 (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 *Ocimum basilicum* 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 which shows high-density AgNPs

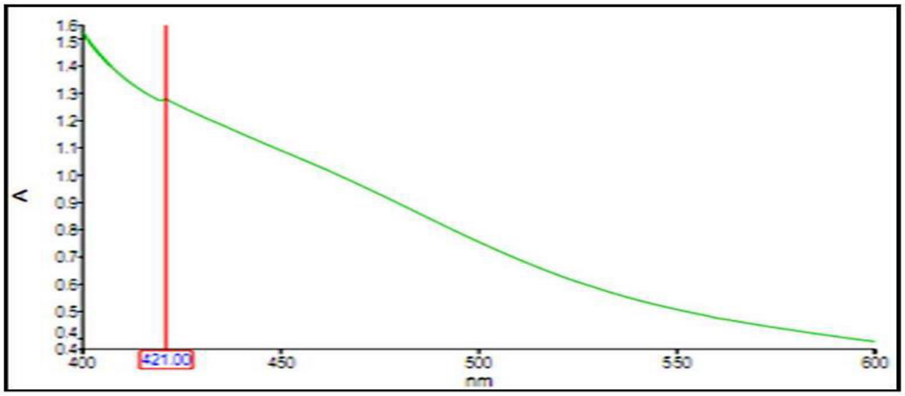

Fig. 1: UV-Visible spectrum of Ag NPs.

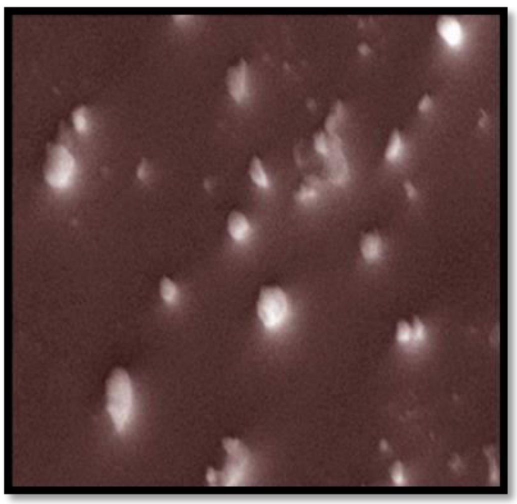

Fig. 2: SEM image of Ag NPs.

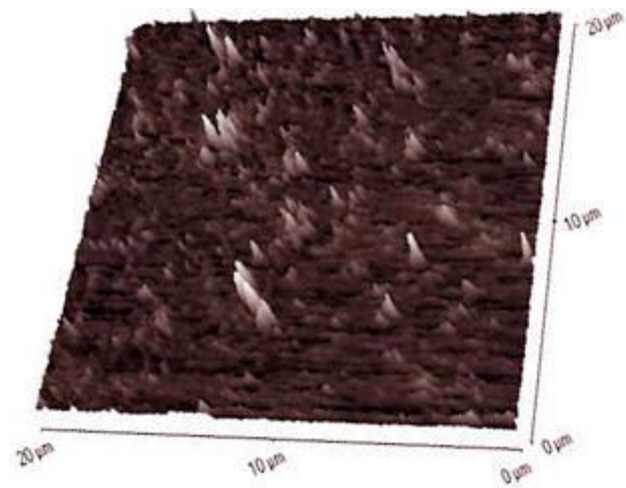

Fig. 3: AFM image of Ag NPs.

synthesized bycannonball leaf extract. It was shown that relatively spherical and uniform AgNPs were formed with diameter of 13 to 61 nm. 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 *Ocimum basilicum* mediated synthesized silver nanoparticle shows that they have a uniformly packed surface with height 0.837 mµm. Figure 3 shows the 3D AFM images of the plant extract mediated synthesized nanoparticles. Pradhan (2013) reported green synthesis of silver nanoparticles by the help of green plants which is 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 synthesize 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

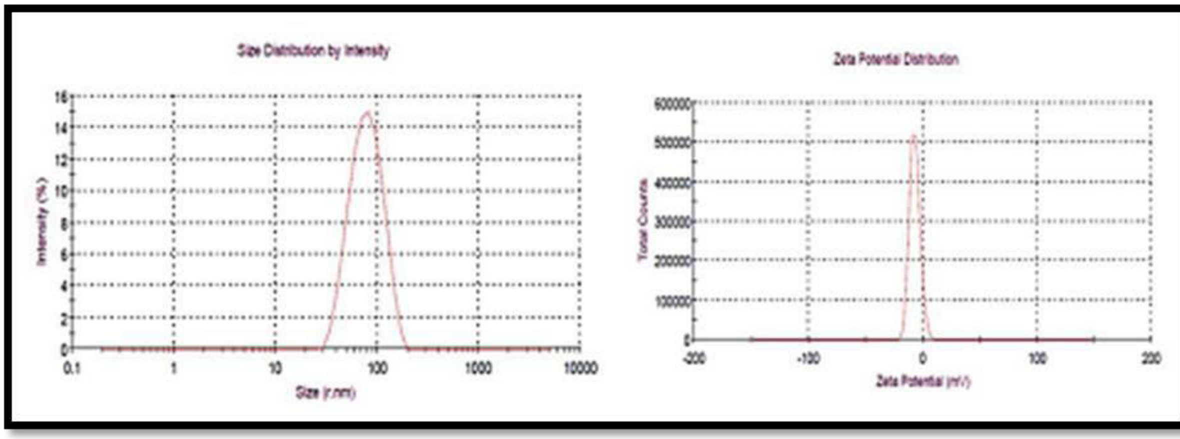

Fig. 4: DLS and Zeta potential graph of Ag NPs.

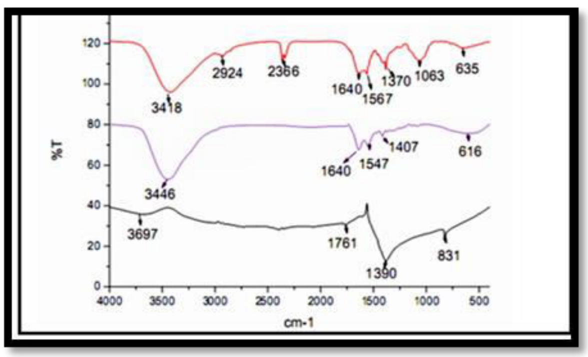

Fig. 5: FTIR spectrum of Ag NPs.

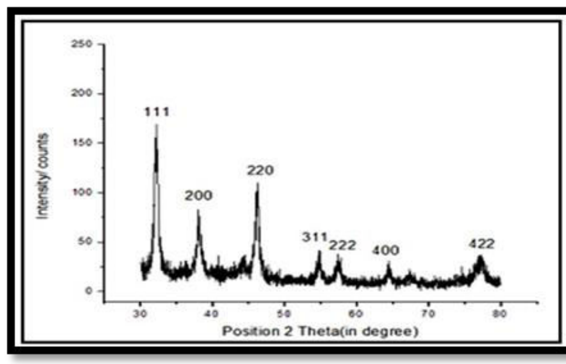

Fig. 6: XRD patterns of Ag NPs.

uniformly packed surface with height 0.703 µm.

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. Figure 4 shows the DLS and zeta potential graph of *Ocimum basilicum* which has an average size of 75.32 nm 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 *Ocimum basilicum* 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 *Ocimum basilicum* 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 *Ocimum basilicum* extracts. Figure 5 shows the FTIR spectrum of *Ocimum basilicum* mediated synthesized silver nanoparticle, the silver nitrate salt and dried leaves petal extract, in $AgNO_3$ peaks were observed at 3697cm^{-1}, 1761 cm^{-1}, 1390 cm^{-1}, 831 cm^{-1} which are associated OH stretching, C=C stretching, CH stretching, NH stretching, respectively. In *Ocimum basilicum* 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 *Ocimum basilicum* peaks were observed which are associated with NH stretching, C=O stretching, N-O stretching, CH_2 and CH_3 deformation, C-O stretching and halogen group presence. The presence of peaks at 3749 cm^{-1} and 1523 cm^{-1} indicate the $-NH_2$ 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. Figure 6 shows the XRD patterns of *Ocimum basilicum*. 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 *Ocimum basilicum* 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.

Conclusion

The plant based nanoparticles have the effective dosage response and minimal side effects when compared to the synthetic compounds. Phytochemical screening of *Ocimum basilicum* reveals that it is a valuable medicinal plant with numerous medicinal properties. Synthesis of silver nanoparticles by using *Ocimum basilicum* 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, TEM, 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 against the cancer, arthritis and diabetes mellitus.

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