Study on Medicinal Plant Extracts Mediated Synthesis of Silver Nanoparticles by Their Reducing Potential

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Abstract- *Nanotechnology involves the use of materials with components that have dimensions less than 100 nm. Application of nano-sized materials is an emerging area of nanotechnology. Among various metal nanoparticles, silver nanoparticles gain special interest due to its remarkable properties. Silver nanoparticles are usually ranging from 1-100 nm size. The demand for green synthesis of nanoparticles increases day by day due to the drawbacks of chemical synthesis. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Hence, the present work provides information on biological synthesis of silver nanoparticles by using various medicinal plants leaf extracts. The formation of silver nanoparticles was characterized through UV-Visible spectroscopy, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM).*

Keywords- Synthesis, Silver Nanoparticles, Plant Extracts, Reduction

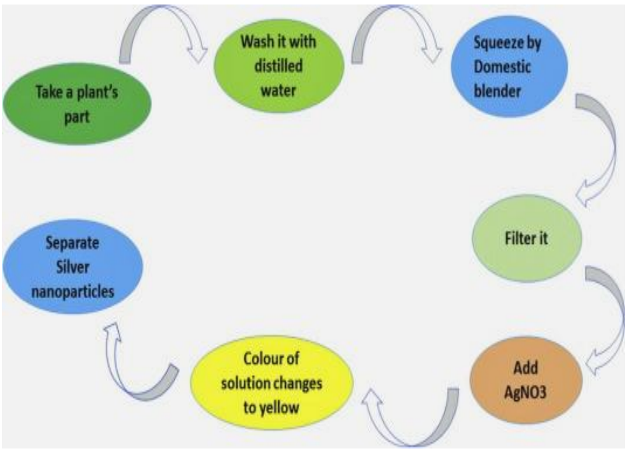
I. INTRODUCTION

The development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles generate a large amount of hazardous byproducts. Thus, there is a need for green chemistry that includes a clean, non toxic and environment friendly method of nanoparticles synthesis. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration. Biosynthesis of nanoparticles is a type of bottom up approach which employs a biological system or its components for the formation of nanoparticles, where the main reaction is reduction of raw metal into nanoparticles. Biological entities in synthesis of nanoparticles may vary from simple prokaryotic bacteria to eukaryotes such as fungi and plants. Compared to microorganisms, plants have better advantages wherein plant mediated synthesis is a one-step protocol towards synthesis whereas microorganisms during the course of time may lose their ability to synthesize nanoparticles due to mutations. Further preservation of microorganisms and maintenance of cultures in active form are very laborious and time consuming. While in plants it is easy and safe with one step protocol towards synthesis, hence research on plants has expanded rapidly [3, 6]. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process o maintaining cell culture. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracelluary using plants or their extracts and in a controlled manner according to their size, shape and dispersity [4]. The aqueous silver nitrate solution, after reacting with geranium (*Pelargonium graveolens*) leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles (16 to 40 nm) [9]. Silver nanoparticles were synthesized by treating silver ions with *Capsicum annuum* L. leaf extract, the crystalline phase of the nanoparticles changed from polycrystalline to single crystalline and their size increased with increasing reaction time. Five hours reaction time led to spherical and polycrystalline shaped nanoparticles (10 ± 2 nm) [5]. The use of plants in synthesis of nanoparticles has become one of the popular alternatives for conventional methods. Hence, the present study was carried out to synthesis of silver nanoparticles through biological method using the medicinal plant leaf extract.

II. MATERIALS AND METHODS

Medicinal plants

The following medicinal plants were collected and used for the synthesis of silver nanoparticles.



Method of Ag nanopartciles synthesis using plant extracts

Synthesis of silver nanoparticles

The fresh and young leaf samples of medicinal plants were collected and washed thoroughly with sterile double distilled water (DDW) and finally surface sterilized with 0.1 % $HgCl_2$ for 2 - 3 min under the hood of laminar air flow. Twenty gram of sterilized leaf samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW. After that the mixture was boiled for 5 min and filtered. The extract was stored in 4 ^{0}C. Silver nitrate was used as precursor of synthesizing the silver nanoparticles**.** Five ml of leaf extract was added to 100 ml of 1 mM $AgNO_3$ (99.99 %) aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (150 rpm) at 30^0 C and reaction was carried out for a period of 48 h. The colour change in reaction mixture was recorded through visual observation. The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquot (1 ml) and subsequently measuring UV-vis spectra of the solution. UV-vis spectra of these aliquot was monitored as a function of time of reaction on Elico UV-vis spectrophotometer (model S3-159) operated at a resolution of 1 nm.

XRD measurement and determination of crystalline size of silver nanoparticles

The sample was drop-coated onto aluminum plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample on plate was prepared. The XRD measurement was performed on a Shimazdu, model LabX-XRD-6000 instrument operated at a voltage of 20 to 30 keV and a current of 30 mA with Cu K α radiation with a wavelength of 1.5418 Å.

Average crystallite size of silver was calculated using the Scherrer's formula,

$$D = k\lambda / \beta\cos\theta$$

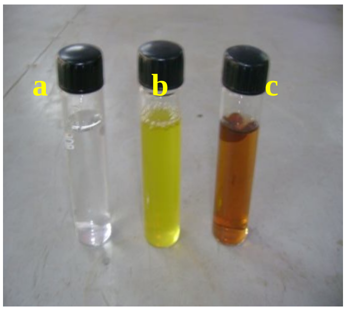
D- Average crystallite size: K- Constant: λ- X-ray Wavelength: β- Angular FWHM of the XRD peak at the diffraction angle: θ- Diffraction angle.

Scanning Electron Microscopy (SEM) analysis

The thin film of the samples were prepared on a small aluminum plate by just dropping a very small amount of the sample on the plate, extra solution were removed using a blotting paper and then the film on the plate was allowed to dry for overnight. The SEM analysis was performed on a JEOL, model JSM-6390 instrument operated at an accelerating voltage of 20 keV and counting time of 100 s.

III. RESULT AND DISCUSSION

The extracellular synthesis of silver nanoparticles occurred during the exposure of plant leaf extracts to 1 mM aqueous silver nitrate solution. The complete reduction of silver ions was observed after 48 h of reaction at 30^0 C under room 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 properties. The appearance of dark yellowish-brown colour is a clear indication of the formation of silver nanoparticles in the reaction mixture (Fig.1). The colour exhibited by metallic nanoparticles is due to the coherent excitation of all the "free" electrons within the conduction band, leading to an in-phase oscillation and is known as Surface Plasmon Resonance-SPR [1].



(a) 1 mM $AgNO_3$ solution

(b) Leaf extract

(c) Leaf extract + $AgNO_3$ after 48 h of reaction

Fig 1. Optical photograph

UV-vis spectroscopy analysis showed that the SPR absorbance band of silver nanoparticles synthesized using different plant leaf extract centered at 424 to 450 nm (Table 1) and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength (Fig. 2). The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [7].

Table 1. UV-Vis absorption band of silver nanoparticles

Reaction Mixtures (Aqueous Silver Nitrate + Plant Extract)	Wavelength at which the SPR band of silver nanoparticles occur
Coleus aromaticus	435 nm
Adhatoda vasica	442 nm
Baliospermum montanum	450 nm
Calotropis procera	440 nm
Nerium oleander	445 nm
Ocimum sanctum	424 nm
Cassia auriculata	450 nm
Murraya koenigii	438 nm
Delonix elata	448 nm
Hibiscus rosasinensis	430 nm

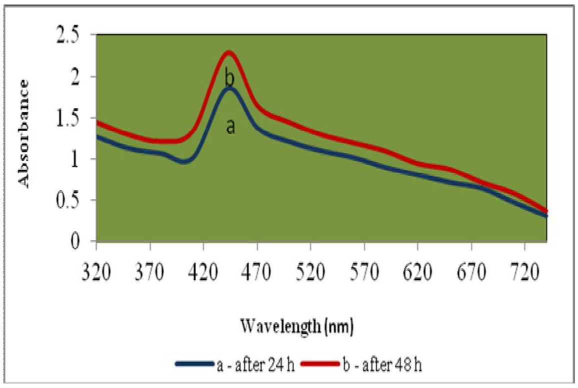


Fig. 2. UV-Vis spectra of aqueous solution of silver nitrate with leaf extract

XRD pattern obtained for synthesized silver nanoparticles showed characteristic peaks near the 2θ value of near 38^0 (Table 2). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature (Fig.3). A Bragg reflection corresponding to the (111) sets of lattice planes are observed which may be indexed based on the face-centered cubic (fcc) structure of silver [3].

Table 2. Diffraction angle of synthesized silver nanoparticles

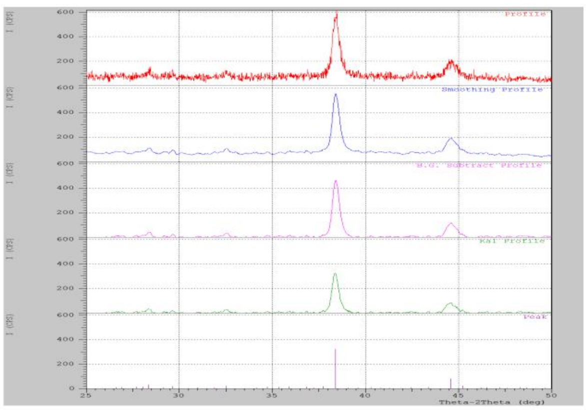
Plant leaf extracts	Diffraction angle of silver nanoparticles at 2θ value (degree) corresponding to lattice plane of (111)
Coleus aromaticus leaf extract	38.38
Adhatoda vasica leaf extract	37.47
Baliospermum montanum leaf extract	38.43
Calotropis procera leaf extract	38.04
Nerium oleander leaf extract	38.11
Ocimum sanctum leaf extract	37.86
Cassia auriculata leaf extract	38.02
Murraya koenigii leaf extract	38.05
Delonix elata leaf extract	38.76
Hibiscus rosasinensis leaf extract	38.12

Crystallite size of silver nanoparticles as estimated from the Full width at half maximum (FWHM) of the (111) peak of silver using the Scherrer's formula exhibited average particles size of 20-70 nm (Table 3).

Table 3.Crystalline size of synthesized silver nanoparticles

Plant leaf extract	θ value (degree)	d - spacing (Å)	FWHM (degree)	Intensity (CPS)	Average Particle size (nm)
Coleus aromaticus	19.06	2.358	0.242	66.0	63.33
Adhatoda vasica	18.74	2.397	0.370	23.0	41.35
Baliospermum montanum	19.22	2.340	0.660	29.0	23.24
Calotropis procera	19.02	2.363	0.280	109.0	54.57
Nerium oleander	19.05	2.359	0.500	14.0	30.65
Ocimum sanctum	18.83	2.374	0.296	84.0	51.75
Cassia auriculata	19.01	2.364	0.735	47.0	20.84
Murraya koenigii	19.03	2.363	0.270	26.0	56.76
Delonix elata	19.38	2.321	0.703	33.0	21.83
Hibiscus rosasinensis	19.19	2.343	0.428	78.0	35.83

SEM image has shown individual silver particles as well as a 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 presence of secondary materials capping with the silver nanoparticles and this capping may be assigned to bio-organic compounds from leaf extracts [8].



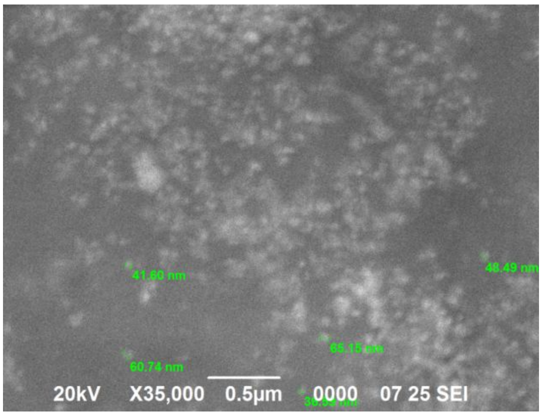


Fig 3. XRD pattern of Ag nanoparticles
Fig. 4. SEM image of Ag nanoparticles

CONCLUSION

The synthesis methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. An increasing attention towards green chemistry and utilization of plant extracts for metal nanoparticles synthesis lead to the development of environment-friendly techniques. A benefit of silver nanoparticles synthesis by using plant extracts is that it's economical, energy efficient, cost-effective and safe to environment. Synthesis of nanoparticles using plants has several advantages over other biological organisms which overcome the time-consuming process of growing microbial cultures and maintenance. Green synthesized silver nanoparticles play a significant role in the area of nanotechnology Therefore, utilization of plant extract for the synthesis of silver nanoparticles has potential impact in coming decades.

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