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Green Synthesis of Silver Nanoparticle Using *Moringa oleifera* Lam Extract for Pharmacological Activity

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Bhusnure O.G[1]*, Jadhav P.P[1], Hindole S.S., Gholve S.B.[1], Giram P.S.[2], Kuthar V.S.

1. Channabasweshwar Pharmacy College, Dept of Quality Assurance, Latur (MS), India

2. Channabasweshwar Pharmacy College, Dept of Pharmacology, Latur (MS), India

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ABSTRACT

Nanobiotechnology gives emphasis to the synthesis of nanoparticles using living organisms such as microorganisms, plant extracts or plant biomass in an eco-friendly way. Among the various agents used for nanoparticle synthesis, plants have found important application. The biomolecules found in plants induce the reduction of Ag+ ions from silver nitrate to silver nanoparticles (AgNPs). The aqueous leaf extract of *Moringa oleifera* Lam was used as reducing and stabilizing agent for the synthesis of silver nanoparticle. Synthesized nanoparticle is confirmed by the change of color from transparent yellow to dark brown which indicates the formation of silver nanoparticles. FTIR spectra was used to monitor the quantitative formation of silver nanoparticles. The plant based route could be considered to be an environmentally friendly, safe and economic biological method for the silver nanoparticle production.

INTRODUCTION

In the present scenario, nanotechnology is an important enabling active area of research in modern material sciences. Nanoparticles deals with the synthesis and control of matter in scales less than 1µm, normally from 1 to 100 nanometers (nm) [1]. Nanoparticles show completely new or improved properties and have wide scope for their diversified application based on specific characteristics such as size, distribution and morphology. Silver nanoparticles have found various and important applications for their bactericidal and fungicidal activity[2]. Antimicrobial effect is due to blockage of respiratory enzyme pathways, alterations of microbial DNA and the cell wall[3]. Historically, the synthesis of metallic nanoparticles utilized chemical reducing agents such as hydrazine, sodium citrate and sodium borohydride to create uniform suspensions[4]. But chemical method is harmful in some way as the chemicals used are toxic, flammable, low synthesis rate etc. In current phase, green synthesis of nanoparticles is exploited to improve and also to protect the environment by the use of chemicals. Raveendran et al. 2003 suggested three important factors which should be considered for the synthesis of nanoparticles: solvent choice, the use of reducing agent and the use of non-toxic material for nanoparticle stablisation[5]. Recently, biological entities serving as both reducing and stabilizing agents for green synthesis of metallic nanoparticles[6]. Utilizing biological organisms such as microorganisms[7], enzymes[8] and plant extract or plant biomass could be an excellent alternative to chemical and physical methods for the production of nanoparticles in a cheap and eco-friendly manner compared to physical and chemical methods.

Synthesis of nanoparticles using plants can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell culture[9]. The microbial enzymes and secondary metabolites with antioxidant or reducing properties are usually reducing metal compounds into their respective nanoparticles. Plants have been reported to be used for synthesis of metal nanoparticles of gold and silver and of a gold-silver-copper alloy [10-14]. Colloidal silver is of particular interest because of its distinctive properties such as good conductivity, chemical stability, and catalytic and antibacterial activity[15-16]. In the present study, we found that plant extract prepared from *Moringa oleifera* Lam can be used for synthesis of silver NPs under bright conditions. The objective of the present study was the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of medicinal plants and evaluation of synthesized silver nanoparticles against animal toxicity.

MATERIAL AND METHODS

EXPERIMENTAL

Silver nitrate was purchased from Merck Chemicals. All glasswares were sterilized with nitric acid and further with distilled water and dried in oven before use. *Moringa oleifera* Lam leaves were collected from college campus in the month of March (2017).

PREPARATION OF LEAF EXTRACT

The fresh leaves were washed several times with running tap water and after that with distilled water. Around 20 g of leaves were weighed and boiled for 1h in 100 mL double distilled water at 60°C and then the extracts were filtered through whatman filter paper. Then the filtered extract was stored in refrigerator at 4°C for further use in synthesis of silver nanoparticles.

ONE POT GREEN SYNTHESIS OF SILVER NANOPARTICLES

100 mL (1mM) aqueous solution of silver nitrate was prepared in volumetric flask. Then 1.0, 2.0, 3.0, 4.0 and 5.0 mL of leaf extract was added separately to 10mL aqueous silver nitrate solution kept in separate beakers at room temperature. The solution was kept in dark chamber until colour of solution changes to yellow to dark yellow. After 15 min, the solution turns yellow to yellow-red or dark brown indicating the formation of silver nanoparticles.

Sample	Plant extract (ml)	$AgNO_3$ solution (ml)
A	10	-
1A	1	10
2A	2	10
3A	3	10
4A	4	10
5A	5	S10

STRUCTURAL STUDIES

FTIR has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules which is used to search the chemical composition of the surface of the silver nanoparticles and identify the biomolecules for capping and efficient stabilization of the metal nanoparticles There were many functional groups present which may have been responsible for the bio-reduction of Ag+ ions. The band intensities in different regions of the spectrum for plant extract and silver nanoparticles were analyzed and are shown in Figure.

D. ACUTE TOXICITY STUDIES

Animal Activity

Selection of animals, caring and handling

The wistar rats (wistar strain 150-200 g) of either sex were used. After randomization into various groups, animals were accustomed for a period of 10 days under standard husbandry condition.

Room temperature: 24 ± 3°c

Relative humidity: 50 ± 20%

12 hrs dark and light cycle.

All the animals were fed with rodent pellet diet and water was allowed ad-libitum under strict hygienic condition. The animals were fasted overnight prior to the experiment. Fixed dose method as per OECD Guideline No. 425, given by CPCSEA was adopted for toxicity studies. The study was conducted by prior permission of institutional animal ethical committee (IAEC registration no. 731/Po/Re/2002/CPCSEA, approval no. CBPC/IAEC/2016-17/11). The Wistar rats were divided in to control and test group each containing 6 animals. The test groups of rats were administrated with the dose of 25, 200, 500 &2000 mg/kg of silver nanoparticles. Carefully observe all the rats for any sign of toxicity in the first four hours, after the administration of extracts and daily following that for the period of 14 days.

RESULTS AND DISCUSSION

Synthesis and FTIR Spectra Analysis: The green synthesis of silver nanoparticles through herbal extracts was carried using aqueous silver nitrate solution. The method utilizes a non-toxic, agent which functions as both reducing and stabilizing agent during synthesis. The mechanism of the reaction involves the reduction of aqueous metal ion with plant leaves extract. Plant extracts color changes after the completion of the reaction.

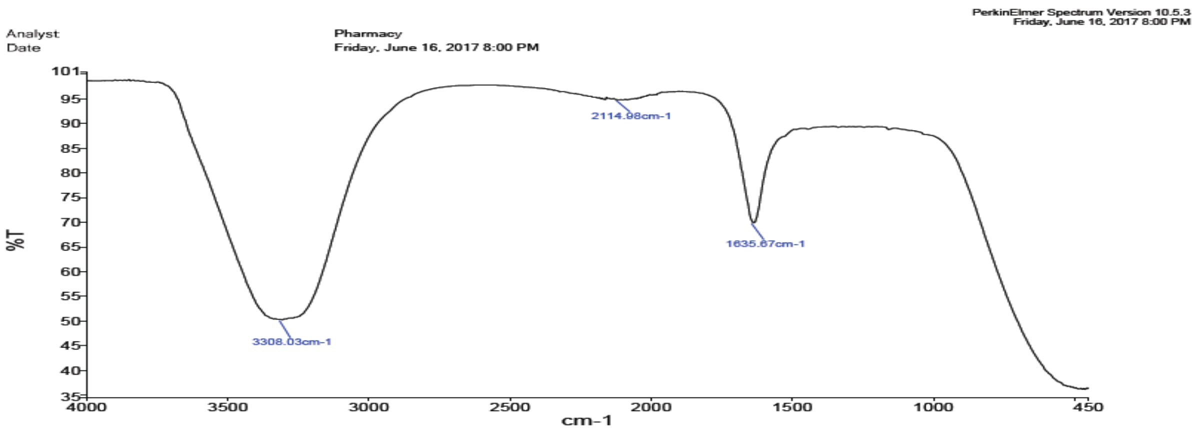

Fig. 1: FTIR spectra of Moringa Oleifera Lam extract

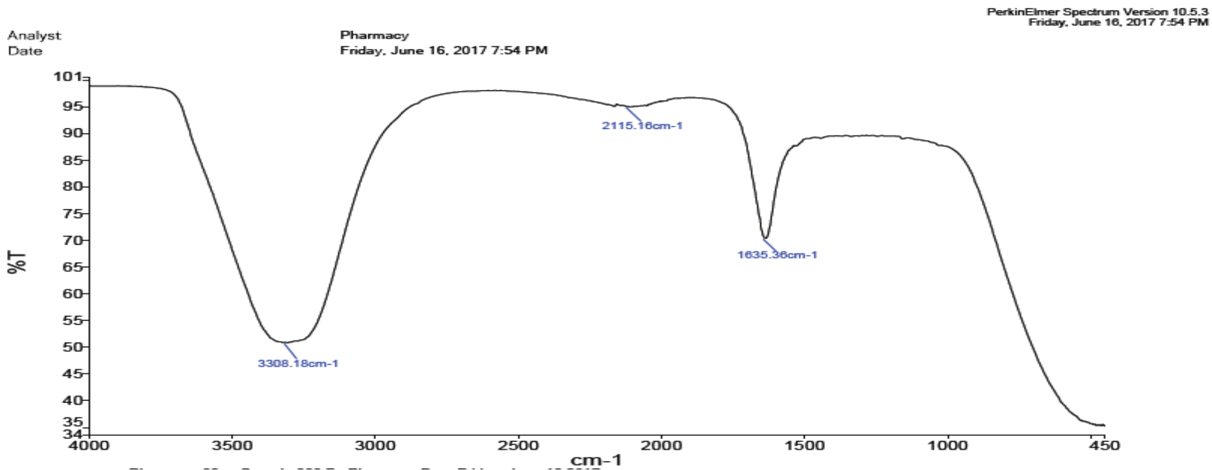

Fig. 2: FTIR spectra of synthesized silver nanoparticle

Fig. 3 Synthesis of silver nanoparticle indicated by change in colour

ACUTE TOXICITY STUDY

Wistar rat (1)	Wistar rat (2)	Wistar rat (3)	Wistar rat (4)	Wistar rat (5)	Wistar rat (6)
100 mg/kg	200mg/kg	500mg/kg	1000mg/kg	1500mg/kg	2000 mg/kg
Mortality not observed	Mortality not observed	Mortality not observed	Mortality not observed	Mortality not observed	Mortality not observed

CONCLUSION

This work indicates that important herbal extract could be used as an efficient and potential green material for the reliable synthesis of silver nanoparticles. The synthesized phyto nanoparticles is safe to use as per the conducted acute toxicity studies. Thus, this phyto nanoparticles has the potential for the development of drugs for various diseases and also useful in biomedical application.

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