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Nano silver particle synthesis using leaf extract of pharmaceutical plant *Thymus vulgaris*

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Abstract

Nanotechnology is significantly influencing science and economy in the 21st century. plant mediated synthesis of nanoparticle is a green chemistry approach that interconnects nanotechnology and plant biotechnology. Silver nano particles are promising agents in bionanotechnology, because of their unique activity against unfavorable processes in bioscience such as undesirable microbial growth. This study presents a safe method of silver nanoparticles (Ag NPs) preparation by green synthesis approaches that have advantages over contractual methods involving chemical agents associated with environmental toxicity. Green synthesis of nanoparticles will became is very importance field in nanotechnology. In this study leaf extract of *Thymus vulgaris* were used to synthesize silver nanoparticles.The silver nanoparticles were characterized by UV-Visible, X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FT-IR) and transmission electron microscopy (TEM) techniques.TEM analysis of silver nanoparticles showed formation of spherical shapes in the size range 9–78 nm. The characterized nanoparticles of *Thymus vulgaris* potential for various medical and industrial applications.

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Introduction

Nanotechnology is one of the most active fields of research in recent various sciences. In the last decate, both science and industry focussed on the production of nanoparticles. Synthesis of nanometal particles, is one of the most important fields of study in modern material science advisable to their high specific surface (Ahmad and Sharma , 2012). Silver nanoparticles have been widely used in medical application, surgical industruments and orthopaedics (Jain *et al*., 2009), anti-HIV agents (Elechiguerra *et al*., 2005), anti-bacterial activity (Edwards-Jones V. 2009.; Montleiro *et al*. 2009), optics (Kamat PV. 2002),water treatment, filters, sensors, catalysts, and vascular prosthesis. Even increasing applications of silver nanoparticles in biomedical field are due to the anti bacterial, anti fungal (Kim *et al*., 2008), anti viral and anti angiogenesis (cancer therapy) properties. Many different ways have been used to reduce silver nitrate to silver nanoparticles. In the most of the ways were used toxic and chemical compounds (Daniel *et al*., 2004). In recently years, plants extract (involve phytochemicals) is used to synthesis of silver nanoparticles because they are safe , cost effective without any toxic materials (Rupiasih *et al*., 2013.; Saxena *et al*. 2010). Plants leaf extracts, that are rich in polyphenols like flavonoids, are strong reducing agents for the synthesis of silver and gold nanoparticles.

In this study we report an eco-friendly method to synthesis silver nanoparticles using the extract of *Thymus vulgaris*, a pharmaceutical plant that rich in flavonoid compounds, for synthesis of silver nanoparticles due to reductive and capping power of the phytochemicals.

Material and method

Chemicals

Silver nitrate ($AgNO_3$, 99.99%) was procured from sigma Aldrich chemicals.

Preparation Leaf Extract of Thymus vulgaris

Thymus vulgaris leaves werepicked from the east Azerbaijan region in Iran.*Thymus vulgaris* leaveswere completely washed in water and shade dried .*Thymus vulgaris*leaves were usedto make the water boiled extract, 20 g of *Thymus vulgaris* dried leaves cut into fine pieces, grinded into obtains powder and were boiled into 100 ml deionized water for 5 minutes and filtered. The extract was stored at 4°c for more experiments.

Synthesis of silver nanoparticles

First 1mM silver nitrate ($AgNO_3$) aqueous solution wasprepared. Then 10 ml of Thymus *vulgaris*leaf extract was added into 100 ml of silver nitrate solution (1Mm).The volume ratio of *Thymus vulgaris* to silver nitrate solution was (1:10). *Thymus vulgaris* leaf extract was used for reduction $AgNO_3$ into silver nanoparticles and were kept on a shaker at 120 rpm in dark at room temperature.

Characterization

Visual observation

The color change in reaction mixture (silver nitrate solution + *Thymus vulgaris* extract) was seized through visual observation (fig.1.).

UV-Vis Spectroscopy

The bio reduction of AgNO3 was corroborated using measuring the UV-Visible double beam spectrophotometer (CARY 100 conc), after 1hour, 2and 24 hours. The UV-Vis spectral analysis was done with a resolution of 1nm between 300 and 800 nm (fig.2.).

FTIR (Fourier Transform Infrared Spectroscopy) analysis

sample of 30 ml of the reaction mixture , was centrifuged at 5000 rpm for 10 minutes and the obtained suspension was disperesed in 2 ml sterile deionized water. The centrifuging and redispersing process was done three times. Then the suspension was freeze dried to get dried powder. At the end the dried biomass was analyzed using FTIR (fig.3.).

XRD (X-ray diffraction) analysis

The Ag nanoparticles of reaction solution was purified by repeated centrifugation at 5000 rpm for 20 min

and the resulting pellet of silver nanoparticles was dispersedin 10 ml de-ionized water.This process repeated and the purified pellet wasair dried on a sterile slide and carried out the X-ray analysis.The scanning was done in the region of 2θ from 0° to 80° at 0.02° $min^{-1.}$ SNPs crystalline nature was analyzed by X-ray diffractometer,operated at 40 Ma currentand 40 Kv voltages with Cu-Kα radiation (fig.4.).

TEM (Transmission Electron Microscopy)

After the reaction, a sample of solution were prepared by repeated centrifugation at 5000 rpm for 20 min and the resulting pellet of silver nanoparticles was redispersed in 10 ml of distilled water Thereafter the purified nanoparticles solution were sonicated and then were placing a drop of solution on carbon coated copper grids and allowing water to evaporate. Transmission Electron Microscopy was operated at 100 kV voltages (fig.5.)

Results

The color change in reaction mixture was showed through visual observation(fig.1.).The formation of silver nanoparticles was confirmed by UV visible Spectrophotometer at 1 hour, 2 and 24 hours after reaction at wavelengths 425, 435, 475 (Fig. 2.). Fourier Transform Infrared Spectroscopy showed the amines and secondary metabolites existing in the pharmaceutical plant -*Thymus vulgaris*- leaf extract were responsible in the bioreduction and stabilization of silver nanoparticles (fig.3.).The crystalline nature of silver nanoparticles was corroboratedfrom the X-ray diffraction analysis. The XRD pattern showed the characteristic Bragg peaks of (111), (200), (220), (311), (222)cubic crystal structure (Fig.4.). The broadening of the Bragg peaks demonstratesthe formation of nanoparticles.TEM technique was used to visualize the shape and size of silver nanoparticles.The TEM micrograph showed the synthesized silver nanoparticles(fig.5.). It is observed that most of the silver nanoparticles were spherical in shape and the particles are highly polydispered and the nanoparticles size ranged from 9 nm to 78 nm.

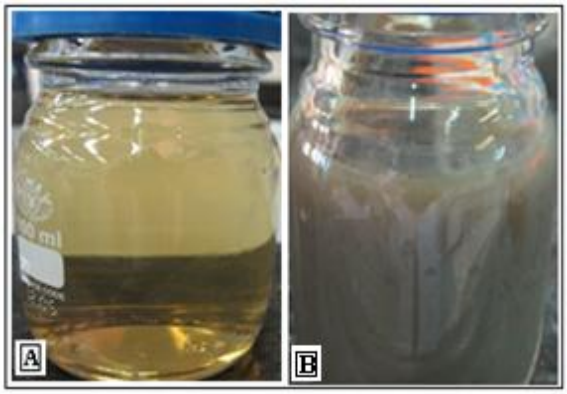

Fig. 1. A: Beforereaction (silver nitrate solution + *Thymus vulgaris* extract), B: after reaction color changed (silver nitrate solution + *Thymus vulgaris* extract).

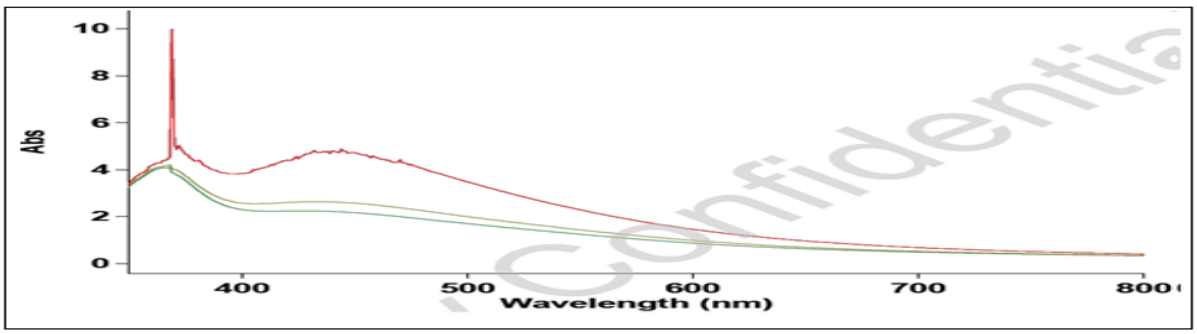

Fig. 2. UV-Vis absorption spectra of Ag nanoparticles synthesized by *Thymus vulgaris* leaf extract after 1 hour, 2hours and 24 hour.

Discussion

The green synthesis of nanoparticles using extract of plants provides a safe and eco-friendly method. Visual observations, UV–Vis spectroscopy, FTIR, XRD and TEM techniques certified the formation ofsilver nanoparticles using leaf extract of *Thymus vulgaris*. The shape and size of the nanoparticles synthesized through bioreduction using plant extracts were

forcefully dependent on the process parameters such as concentration of plant extract, concentration of silver nitrate, mixing ratio of plant extract to silver nitrate solution, interaction time, temperature and pH of the solution. In this method of synthesis silver nanoparticles does not use any toxic materials for reduction of silver nitrate to silver nanoparticles. The main advantages of this method are safe, eco-friendly, cost effective, easy and compatible for biomedical and pharmaceutical applications.

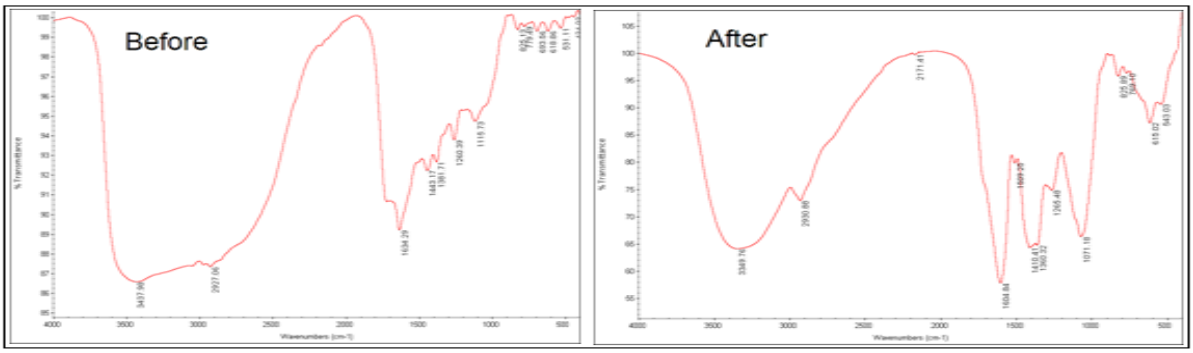

Fig. 3. FTIR spectra of *Thymus vulgaris* recorded before and after synthesis of SNPs.

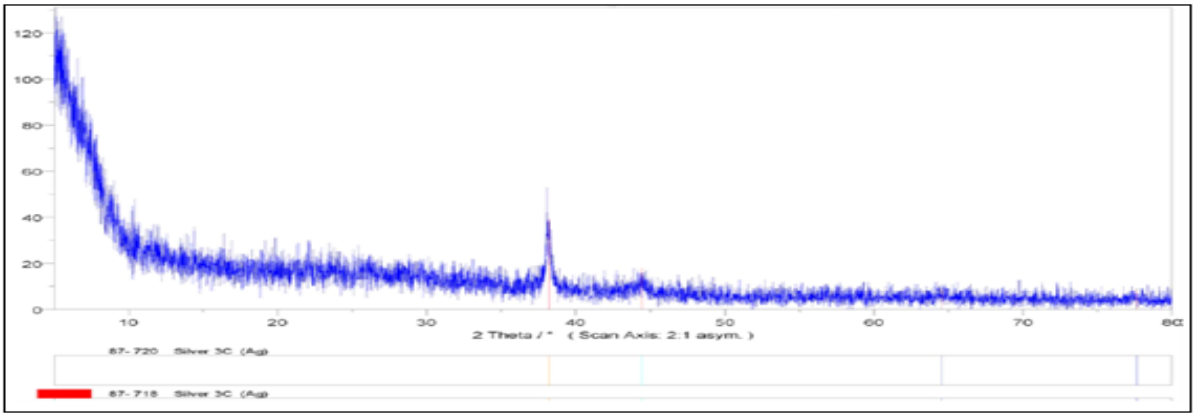

Fig. 4. XRD pattern of silver nanoparticles synthesized using *Thymus vulgaris*.

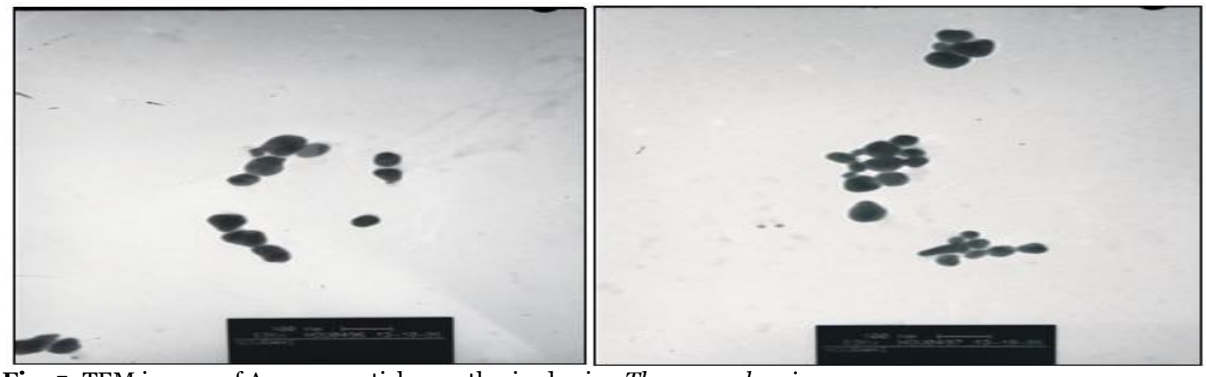

Fig. 5. TEM images of Ag nanoparticles synthesized using *Thymus vulgaris*.

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