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BIOSYNTHESIS OF SILVER NANOPARTICLES USING TRACHYSPERMUM AMMI AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITIES

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ABSTRACT

Bio-chemical reduction method was employed to synthesise the silver nanoparticles (Ag NPs) by aging the mixture of the aqueous extract of the *Trachyspermum Ammi* (TA, Ajwain) seeds and $AgNO_3$ solution at different time interval. Reaction time of $AgNO_3$ and TA could accelerate the reduction rate of Ag^+ and affect AgNPs size and concentration of NPs. X-ray diffraction (XRD) studies confirms the formation of pure AgNPs that crystallized into the cubic shape particles of size 36nm. Surface plasmon resonance band centred at 420-430 nm was recognised as the first exitonic peak of UV-Vis absorption spectra that confirmed the presence of the AgNPs. FTIR results TA supported AgNPs showed decrease in intensity of peaks at 3497 and 1695 cm^{-1} with respect to the pure TA indicating the involvement of NH_2, O-H, carbonyl group and C=C stretching in formation of TA-AgNPs aggregates. The C-O-C and C-N stretching suggested the presence of the bonding between the phytochemicals and AgNPs. TA extract play a role of capping and reducing agent for converting silver ions into silver nanoparticles. Pronounce effect of the aging on AgNPs concentration and particle size, was exhibited by the system. As synthesized AgNPs was characterized using transmission electron microscopy (TEM), UV- Vis spectrophotometer (UV-Vis), Fourier transformation infrared (FTIR) spectroscopy and XRD. Study demonstrated the inside view of the most probable mechanism of the biosynthesis of AgNPs and the potent antagonistic activity against bacteria, which possess potential applications in medicine and pharmaceutical fields.

KEYWORDS: Biogensis, surface plasmonic resonance, Fourier transformation infrared spectroscopy, silver nanoparticles, antagonistic activity

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INTRODUCTION

Nanotechnology plays a vital role in engineering and manipulating of the size particles at the nano level ranging from approximately 1-100 nm.[1] The importance of the nanotechnology in a variety of fields such as food, health care and feed, cosmetics, biomedical science, environmental health, chemical industries, drug and gene delivery, power science, electronics, mechanics, and space industries,[2] has been already established. It also has extensively been used for the treatments of diabetes,[3] cancer,[4] allergy, [5] inflammation, [6] and infection, [7] etc. In recent years the trend of the green synthesis of NPs is growing due to the number of its advantages over chemical synthesis methods such as simplicity, mild reaction conditions and cost effectiveness.[8] Moreover, it is compatible for biomedical and food applications and this technique eliminates the use of high pressure, temperature, energy and toxic chemicals. [9, 10] The growing need of environmental friendly production of nanoparticles forced researchers to choose the green way for their fabrication. Various metallic nanoparticles, [11] because of their remarkable properties over their bulk counter parts, used in the variety of applications. [12] Biological methods are more beneficial than the most popularly used photochemical reduction, chemical reduction, electrochemical reduction, heat evaporation, etc. [13] In biological method, the plant extract has been used as reducing and capping agent for the production of nanoparticles [14] due to their reducing properties. [15] The change in properties of the nanoparticles such as size, distribution, and morphology of the nanoparticles are clearly observed with biomaterial. [16] Various nanoparticles like gold, silver, copper, iron, palladium, zinc, quantum dots (CdS, ZnS) are synthesized using variety of biochemicals. Silver nanoparticles are selected for the study among the above mentioned nanoparticles because of their several unbeatable properties such as optical, chemical, electronic, photo electro chemical, catalytic, magnetic, antibacterial, and antimicrobial activity. Silver nanoparticle acts as antimicrobial agent can be used in medical applications such as blood collecting vessels, coated capsules, band aids, biological labeling, etc.[23] The silver is non-toxic to animal cells and highly toxic to bacteria, and other microorganisms (E-coli, Pseudomonas aeruginosa, Staphylococcus aureus, etc). Therefore, nanosilver is considered as a safe, effective and valuable bactericidal metal to be used for medical purpose. [24-26] In present work, we used biogenic method for synthesis of silver nanoparticle, which are usually non-toxic, low cost, usage less amount of chemicals, environmental friendly workable at mild temperature and pressure conditions. Plants in general used for green synthesis of AgNPs are, *Zea mays* [27], *Azadirachta indica* (Neem) [28], *Medicago sativa* (Alfa alfa) [29,30], *Aloevera* [31], *Emblica officinalis* (Amla) [32], *Capsicum annuum* [33], *Geranium* sp. [34,35], *Diopyros kaki* [36], *Magnolia kobus* [37] and *Coriandrum* sp. [38], etc. Different parts of plant like leaf, stem, flower, seed and skin of the fruits were already used for the synthesis of AgNPs. Plant extract-coated nanoparticles, has medical advantageous, can be used in drugs and cosmetic applications. [39] *Trachyspermum Ammi* (TA) belongs to the family of *Apiaceae* plants, commonly known as Ajwain. TA is a climbing herbaceous annual plant found in throughout India and has been served as an antibacterial and carminative drug in the traditional medicines. [40] TA consists of the fatty acids, proteins, flavonoids and alkaloids that promotes antioxidant, antimicrobial, anti inflammatory and immune stimulant activity.[41] The current investigation focused on the synthesize of AgNPs, using the aqueous seeds extract of TA at different experimental conditions and their application in antibacterial activity against the *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* at room temperature. Work will contribute in establishing the importance of plant sources and implementing green chemistry in synthesis of nano metal particles for the future research.

MATERIALS AND METHODS

(1) Materials

Aqueous extract of TA seeds was used to synthesize AgNPs, using the method mentioned elsewhere. [42] Dry seeds were washed thoroughly with distilled water to make them free form dust particles and surface contamination and dried in sunlight for week long time. Afterwards 2 gm dried seeds were soaked in to 50 mL of pure deionised water for 24 h. The extract was filtered using whatmann filter paper No.42. The filtrate was centrifuged at 2000 rpm for 20 min, and the suspended solid was used for further analysis.

(2) Chemicals

Analytical grade chemicals from different suppliers such as $AgNO_3$ (silver nitrate, ≥99.0%, Merck), $Zn(NO_3)_2.6H_2O$ (zinc nitrate hexahydrate 98%; Sigma Aldrich), were used as purchased without any further purification. All solutions were prepared with deionised water (DIW).

(3) Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using the following procedure. Firstly, the aqueous 10^{-3} M $AgNO_3$ solution was prepared in 250 mL of deionised water. Secondly, 2.5 mL of concentrated TA seeds extract was added drop wise into the 25 mL $AgNO_3$ solution that kept at room temperature for after 2 minutes, 7h, 24h, 7day's, 21day's etc, respectively. During aging of $AgNO_3$ in TA extract, electron transfer from the solution to the Ag^+, was responsible for the electrical conduction in colloids and final conversion of Ag^+ ions into Ag nanoparticles. Reverse process occurs in oxidation process, where electrons get lost during reaction.

(4) Optical Properties of Ag Nanoparticles

Ultraviolet-visible spectroscopy (UV-Vis) refers to the absorption of light in the UV-visible spectral region i.e. 200-900 nm that directly affects the perceived colour of the involved chemicals. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. UV-Vis absorption spectrum was taken using a (LABINDIA UV-Visible 3000^+) spectrophotometer, where the cuvette path length was set to 1.0 cm and DIW was used for background subtraction. UV-VIS absorption spectra have been proven a sensitive tool to get the information about the formation of silver nanoparticles. Because an intense absorption peak of silver nanoparticles exhibited in certain region (~ 420 nm) that attributed to the surface plasmonic resonance (it describes the collective excitation of the conduction electrons in a metal) excitation. This technique can characterize the silver dendrites, colloids and surfaces**.**

(5) pH Analysis

The pH was determined by using digital pH meter (Systronics; Model-ERMA). In making of the AgNPs process the pH of TA extract and $AgNO_3$ solution gradually became acidic (pH=7 to pH=5) within 24h time that revealed in the form of the reduction in pH of studied sample.

(6) XRD Analysis

The redispersed silver nanoparticles were kept in an oven at 60°C for 24h in order to obtain the dry powdered AgNPs of high purity. As synthesized silver nanoparticles, were used for phase identification and the crystallinity check using powder XRD. The diffracted intensities were recorded from 35 º to 90 º of 2 theta angles. XRD analysis was performed using an X'Pert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu K_α radiation.

(7) FTIR Analysis

In order to collect solid biomass of the seed residue or synthesised compound, the corresponding solution of 100 ml was centrifuged at 20,000 rpm for 10 min. This was followed by the drying of the AgNPs at 60°C. As obtained sample of the synthesised silver nanoparticles, was subjected to FTIR analysis in the range of 400 to 4000 cm^{-1} using Bruker -Tensor Model FTIR spectrophotometer in the diffuse reflectance mode at a resolution of $4cm^{-1}$ in KBr pellets.

(8) TEM Analysis

The TEM measurement was done using JEOL model 1200Ex microscope operated at an accelerating voltage of 80kV. Samples were prepared by placing 3-4 drops of the well dispersed Ag nanoparticles in alchohol on a 300-mesh carbon coated Cu grid (EM sciences) and allowing the liquid to evaporate in air. For Ag nanoparticles the particle size distribution was based on 30 randomly selected particles. The TEM image was taken with very high resolution and MATLAB analysis gives the pixel depth of the image equal to 24 bits and the image format as JPEG. The TEM Images have been taken at National Chemical Laboratory, Pune, India.

RESULTS

(1) Optical Properties with UV Visible Spectroscopy

Biosynthesis of the silver nanoparticles was confirmed on the basis of the colour developed in solution during the course of reaction (transparent to pink) and change in pH (7 to 5) of solutions. The intensity of colour interprets the degree of bio-reduction of AgNPs due to addition of reducing agent. As the plant extract of TA seeds mixed in the aqueous solution of the silver ion complex, it started to change the colour (from transparent to deep reddish violet) due to reduction of silver ion, which may be the indication of formation silver nanoparticles, as shown in Figs 1 (a) and 1 (b). Colour of silver colloid is attributed to surface plasmon resonance (SPR) phenomena arising due to the collective oscillation of the free conduction electrons induced by an interacting electromagnetic field. [43] Therefore, the formation of AgNPs was primarily recorded on the basis of the surface plasmonic resonance/first exitonic peak in UV-visible spectroscopy.

(a)  (b)

Figure 1
(a) Pure TA seed aqueous extract and filtered extract of TA (b) Synthesis of TA-Ag NPs at different aging time such as after 2 minutes, 7h, 24h, 7day's, and 21day's.

The deep reddish violet coloured sample powder was dissolved in DIW and sonicated for10 min, was taken in cuvette and exposed to UV-visible radiation for monitoring of the absorbance with respect to the DIW. Surface plasmon resonance phenomena, attributed to the first exitonic peak at different wavelength for different nanoparticles solution. It is reported in the literature [44-53] that typical AgNPs shows the characteristic SPR at the wavelength in the range of 380-440nm. Fig 2 exhibited the SPR peak at the wavelength of 422 nm, which verified the presence of AgNPs in the solution. This is absent in neat TA extract solution. The SPR absorbance is sensitive to the nature, concentration, size and shape of the particles present in the solution and also depends upon their inner particle distance and the surrounding media.

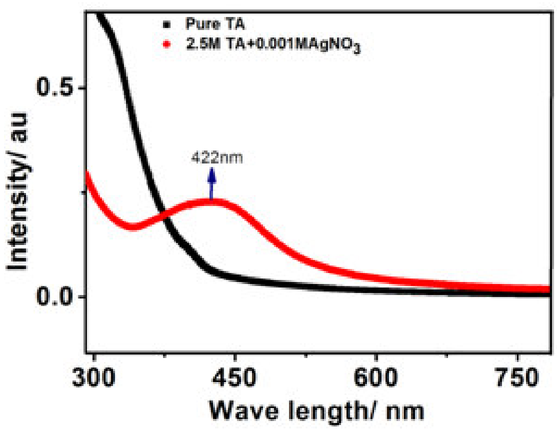

Figure 2
UV-visible spectrum of pure TA extract (black) and silver nanoparticles (red).

(2) XRD Pattern

XRD patterns of the synthesized AgNPs are shown by Fig 3, where four major peaks appeared. The peak position explains crystal parameters along with the translational symmetry, namely size and shape of the unit cell, whereas the peak intensities gives the details about the electron density inside the unit cell. These XRD patterns correspond to the cubic crystal structure which is in agreement with the standard JCPDS file No. 00-004-0783 and indicates that the synthesized nanoparticles are crystallised in a pure form without any impurities. The Braggs reflections observed in the XRD pattern at 2θ = 38.142°, 44.514°, 65.146° and 77.274° (Fig 3) which can be indexed to the (200), (220), (311) and (222) planes of pure silver, respectively. A strong diffraction peak was ascribed to the 32.23 facets of silver. The results thus illustrate that AgNPs was crystallized in face centered cubic symmetry of space group Fm-3m (Space group number: 225) along the point group m3m (O_h). Williamson-Hall plot (sin θ *Vs* β cos θ, where, θ in radians) was used to determine the particle size of the NPs, i.e. 36 nm.

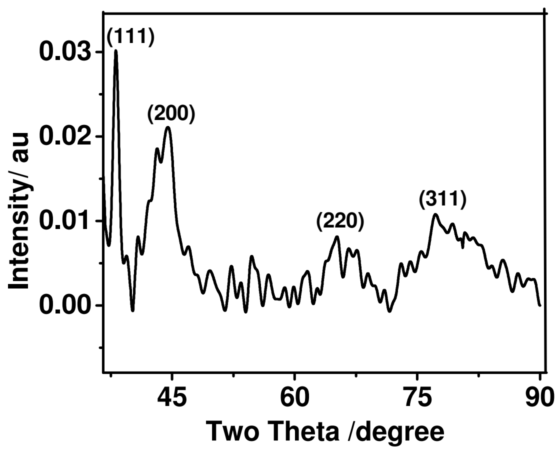

Figure 3
XRD Plot of Silver Nanoparticles

(3) FTIR Analysis

The FTIR measurements were carried out to identify the possible bio molecules present in dispersion of AgNPs that was responsible for the reduction of the Ag^+ ions. The spectrum as shown in the Fig.4 indicates the major peak at 3428.45 cm^{-1} (pure TA) resembles the presence of the intermolecular hydrogen bond in pure TA where, sharp and well defined peaks at 3723.95 cm^{-1} and 3664.86 cm^{-1} attributed to the intra-molecular hydrogen bond in AgNPs. [54] In addition to this, other peaks were obtained at 1695 cm^{-1}, 1047 cm^{-1} and minor peaks at 2876 cm^{-1}, 1539 cm^{-1}, 676 cm^{-1}, are corresponding to alkene C=C stretch, alcoholic C–O stretch, asymmetric and symmetric stretching and wagging vibrations of CH_2 group, NH_2, O-Ag-O stretching, which plays a major role in the synthesis of TA mediated silver nanoparticles. These bonds tethered AgNPs with organics present in TA and reduce Ag^+ ion.

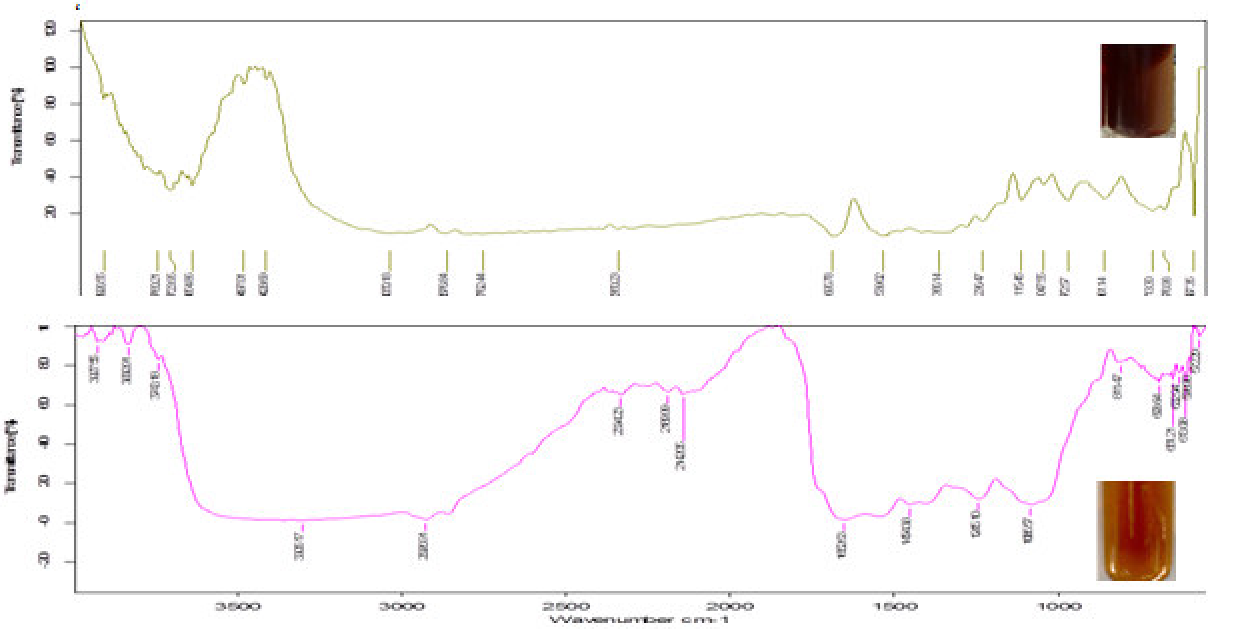

Figure 4
FTIR Spectrum of Pure Ajwain Extract (Pink) and Silver Nanoparticles (Black)

(4) TEM Analysis:

TEM image shown in Figs 5a and 5b reveals that there is formation of poly-disperse spherical particles with non uniform distribution and bar plot revealed the particle size distribution of AgNPs i.e. between 1-10 to 30-40, respectively. It was revealed that the nanoparticles formed were of different sizes and particle size was found to be 8nm, 14nm, 24nm and 36nm and the mean size of about 22nm which lies in the nano range. Difference in average particle size obtained from XRD and TEM observed because edges of particles are not well defined in TEM images.

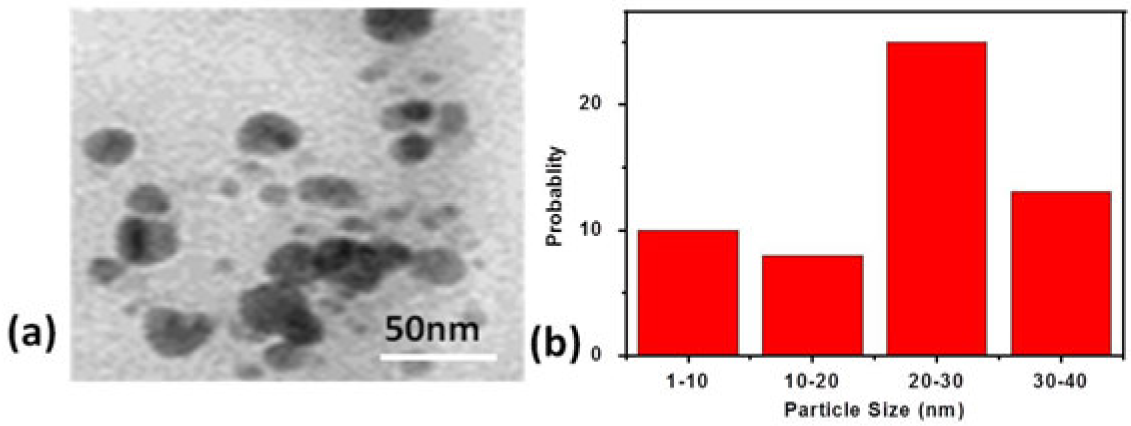

Figure 5
(a) TEM image of poly-disperse silver nanoparticles
(b) Average particle sizes of silver nanoparticles

(5) Antagonistic activity of silver nanoparticles against bacteria

The TA seeds reduce the silver salts and produced silver nanoparticles by stabilizing and capping the nanoparticles with the plant peptides. The antimicrobial activity of the nanoparticles is thus enhanced due to the presence of plant proteins and phytochemicals. The antimicrobial activity of silver nanoparticles was studied against Bacillus subtilis, Staphylococcus aureus and Escherichia coli and Table 1 represents the comparative activities in terms of inhibition zone i.e 17, 16 and 14, respectively. Study demonstrated that the AgNPs show best antagonistic activity of silver nanoparticles against Bacillus subtilis.

Table 1
Antagonistic activity of silver nanoparticles against bacteria

Organism	Zone of inhibition (mm)
Bacillus subtilis	17
Escherichia coli	16
Staphylococcus aureus	14

CONCLUSION

The present green synthetic method is a low cost approach of synthesizing AgNPs at ambient condition. The size and structure of obtained NPs were characterized by FTIR, TEM, UV absorption, and XRD. Our results have shown that the ajwain seeds aqueous extract is the easy, economic and eco-friendly way to synthesize metallic nanoparticles. The antagonistic activity of silver nanoparticles was studied. It was experimentally evident that the Ag nanoparticles found effective against the bactericidal activity of the Bacillus subtilis, Staphylococcus aureus and Escherichia coli. Thus, this study proves to be an effective and economical method. Moreover; this plant mediated synthesis method represents a considerable improvement in the preparation of AgNPs because of various advantages such as reduced reaction time, no need of additional capping agent, and better control over their size and shape. There are bulks of the investigations which have been carried at research laboratories in small scale whereas there are no reports on pilot plants or industrial scale fabrication of nanomaterials using natural products. However, we believe that there are good opportunities for developing large scale synthesis using greener processes.

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