www.amlett.com, www.vbripress.com/aml, DOI: 10.5185/amlett.2014.5680 Published online by the VBRI press in 2014

Microwave assisted synthesis of stable biofunctionalized silver nanoparticles using apple fruit (*Malus domestica*) extract

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Received: 03 January 2014, Revised: 28 March 2014 and Accepted: 11 April 2014

ABSTRACT

A simple and eco-friendly biosynthesis of silver nanoparticles (AgNPs) is reported here using apple fruit extract as reducing and capping media under microwave irradiation. AgNPs were characterized by UV–visible spectroscopy, XRD, FT-IR and TEM. The kinetics of reduction of aqueous silver ions during reaction with the apple fruit extract were monitored with the help of UV-visible spectroscopy. The XRD pattern of AgNPs was found agreeing with the *fcc* structure of Ag metal. Further, where TEM analysis exhibited formation of spherical shaped nanoparticles in the range of 10–45nm; FTIR analysis was carried out to identify the functional groups which were responsible for reduction/capping of AgNPs and conclude that the characterized AgNPs carry the potential for adoption in various medical and industrial applications. Copyright © 2014 VBRI press.

Keywords: Apple fruit extract; silver nanoparticles; microwave irradiation; antibacterial activity; antioxidant activity.

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Introduction

Study of metal nanoparticles (NPs) have become one of the great interest due to their wide range of applications in various fields; such as microelectronics, solar cells, energy storage, catalysts, sensors and medicine (cancer diagnosis, cancer therapy, drug delivery etc) **[1-4].** Further, silver and gold nanoparticles are found to have enormous biomedical applications. For example, silver sulfadiazine is well known and widely accepted silver preparative applied in human medicine and favorite antimicrobial agent for the treatment of serious burns. Many researches has proven that, when compared to fine particles; engineered NPs possess greater surface-to-volume ratio and carry functional groups on their surfaces, which results in improved biological activity both in-vitro and in-vivo conditions, making them a potential agent for biological application **[5,6].** Silver is also found its utilities for applications in antibacterial treatment of surgical instruments, water purification, food packaging, wound healing ointments, textiles, cosmetics **[7,8]** etc.

Over the last two decades, a number of silver and gold nanoparticles based therapeutic and diagnostic agents have been developed and found effective in treatment of cancer, diabetes, pain, asthma, allergy, infections, and so on **[9,10].** A number of researchers have synthesized AgNPs using different physical, chemical and biological methods; and

explored that when compared to physical and chemical synthesis there is greater demand and requirement for synthesizing AgNPs using biological sources like microbes, algae, fungi and plants, because of simplicity in their synthesis, being clean, biocompatible, non-toxic and biodegradability. Moreover, plant extracts are found very useful compared to microorganism synthesis, as it doesn't require any elaborate course of culturing and maintenance of the cells and are also less costly, environmentally benign even when large scale synthesis is carried out, and posses inherent medicinal properties of plants which becomes basis for their preparation **[11].**

In the earlier studies AgNPs is found to fabricate through action of metabolites present in different medicinal plant extracts like Neem (*Azadirachta indica*) leaves **[12],** *Aloe vera* **[13],** *Albizia adianthifolia* **[14],** *Ficus religiosa* **[15],** *Acalypha indica* **[16],** Banana (*Musa paradisiacal*) peels **[17],** *Coccinia grandis* **[18],** *Curcuma longa* **[19],** *Ocimum sanctum* **[20]** etc. In the above studies it is observed that phytochemicals (alkaloids, flavonoids, terpenoids, glycosides, poly phenols, tannins etc) present in the plant extracts are found to act as both reducing as well as capping agents in the synthesis of AgNPs, which helps to reduce the agglomeration of NPs and also improves their biological activity. Microwave heating method is considered particularly important as its use provides increased reaction kinetics, rapid initial heating which ultimately leads to higher yields **[21]** hence the same is adopted for the purpose of this study for synthesizing AgNPs using apple extract. Apple is accepted world wide as one of the important diffused fruit, which offers many health benefits due to the presence of high concentration of phytochemicals especially polyphenols, flavonoids, terpenoids etc. It is said that consumption of apple on regular basis reduces the risk of cancer, cardiovascular diseases, diabetes etc. It is also well known to posses very good antioxidant, cholesterol-lowering effects and antiproliferative activity **[22,23].** In this background, though there are a number of studies conducted using apple fruit extract; there are no studies conducted for synthesizing AgNPs using apple fruit extract under microwave irradiation, with faster reaction kinetics and clean process using green chemistry approach**.** AgNPs thus obtained are then characterized using UV–Vis spectra, XRD, FTIR and TEM analysis. Furthermore, the antioxidant and antibacterial activity of AgNPs is also evaluated.

Experimental

Materials & Methods

Apple fruit was procured from local markets and washed with distilled water to remove dust. Silver nitrate ($AgNO_3$) was obtained from Merck Chemicals Ltd., Mumbai, India. The bacterial strains were procured from microbial type culture collection (MTCC), Chandigarh, India. Microwave oven (ONIDA, 2.45 GHz) was used for extracting the phytochemicals from apple fruit in water and also for the synthesis of AgNPs.

Apple fruit was pealed and 10 gm of its pulp was cut into small pieces and ground to paste; to which, 200 ml of distilled water was added and kept for microwave irradiation for about 180 sec to extract the biomolecules present in the fruit. The fruit extract was filtered by 0.2 µm membrane filter paper to remove the fibrous impurities in hot condition. This was stored at 4^0 C for further experimental requirements.

10 ml of the fruit extract was added to 50 ml of 10^{-3} mM $AgNO_3$ solution and the mixture was irradiated at a fixed frequency of 2.45 GHz at a regular interval of time for about 3 min (the experiment was done in duplicate for reproducibility); wherein, a change in color from colorless to light yellow and then to dark brown indicating the formation of AgNPs was observed.

Characterization

UV–Vis spectrum was recorded from periodically collected reaction mixtures, for a period of 20 sec to 420 sec to observe the surface Plasmon resonance peak. When the reactions were complete, the colloidal solution obtained were centrifuged and washed several times with distilled water to collect AgNPs present in the solution. Finally, the bio-capped AgNPs were dried in vacuum oven at 80^0 C for about 12 h to obtain the product in powdered form for further characterization.

AgNPs are characterized using X-ray diffraction (XRD) technique (Siemens X-ray diffractometer with Cu Kα radiation). A Fourier transform infrared spectrum (FT-IR, JASCO FT-IR–5300 model) was recorded for the sample by KBr method to know the organic residue adsorbed/biocapped on the surface of AgNPs. Transmission electron microscopic (TEM, PHILIPS CM-200) image was also obtained to observe the morphology, shape of AgNPs. For evaluating the reducing ability of fruit extract, we recorded the solution redox potential (E) and pH of reaction mixture using digital potentiometer and pH meter respectively.

Antioxidant and antimicrobial activity

The antioxidant activity of AgNPs is tested using DPPH (2, 2-diphenyl-1-picrylhydrazy) free radical scavenging assay. Different concentrations of AgNPs solution ranging between 20–200 µg/ml was added to 3 ml of 0.1 mM methanolic DPPH solution in a test tube. The mixtures were shaken briskly and allowed to react at room temperature in dark for 20 min and the absorbance was examined at 517 nm **[24]**; DPPH solution without AgNPs was served as the control and Butylatedhydroxy toluene (BHT) served as standard for the experiment. The radical scavenge activity of AgNPs was calculated using the following formula;

$$\% \text{ scavenging activity} = \frac{\text{Absorbacne control} - \text{Absorbance sample}}{\text{Absorbacne control}} \times 100$$

The antibacterial activity was tested using bacterial strains viz., *Escherichia coli* (*E.coli*, ATCC 25922), *Pseudomonas aeruginosa (P. aeruginosa,* ATCC 10145), *Agrobacterium tumifaciens* (*A. tumifaciens*, ATCC 4720) and *Bacillus subtillis (B.subtilis,* ATCC 6633). The test samples (AgNPs, fruit extract and the standard drug) with different concentrations (1.0, 0.5, 0.25 and 0.12 μg/ml) were dispersed in dimethyl sulfoxide and used for further experiment. Nutrient agar medium and petridishes were sterilized. About 20 ml of agar media was poured to

different petridishes and allowed for solidification; to which 50 µl of bacterial culture suspension was added and swabbed with the L shaped glass rod. A 6 mm diameter wells were punched carefully using a sterile steel cork borer and then 50 µl of the test samples of different concentrations were added to each labeled well. Later, the plates were incubated in incubator at 37^0 C for 24 h and at the inhibition zone; the well was measured using meter ruler and the mean values for each organism was recorded.

Results and discussion

Formation of AgNPs

As known, AgNPs in its aqueous medium exhibit yellowish-brown color due to the excitation of surface plasmon resonance **[25] Fig. 1** shows the UV–Vis. spectra of reaction mixture after microwave irradiated at different intervals of time. The color changes from light-yellow (t = 20 sec) to yellowish-brown (t = 120 sec) and then to dark-brown (t = 420 sec) indicates the formation of AgNPs. The characteristic absorption peak at 420 nm in UV–vis spectrum confirmed the formation of AgNPs. The intensity of Plasmon resonance band gradually increased and slightly shifted towards longer wavelength as a function of irradiation time. This is a clear indication of the growth of particle size. SPR patterns, characteristics of metal nanoparticles strongly depend on particle size, stabilizing molecules or the surface adsorbed particles and the dielectric constant of the medium **[17]**.

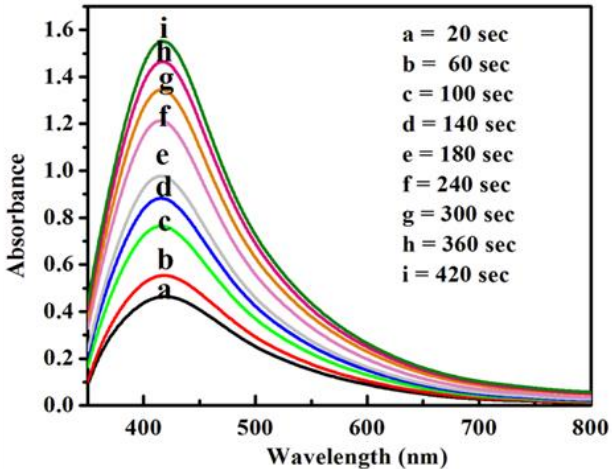

Fig. 1. UV–Vis. spectra of AgNPs showing the SPR peak at 430 nm.

The pH and *E* of the reaction mixture (**Fig. 2**) exhibits the reduction process, $Ag^+ \rightarrow Ag^0$. The initial redox potential and pH indicates the reducing ability of the plant extract i.e., phytochemicals responsible for metal ion reduction. This procedure is very useful and effective in choosing a suitable plant material for synthesizing AgNPs rather than selecting the plant randomly. The initial E = 0.060 V increased to 0.095 V after 400 sec of microwave irradiation. The initial pH 5.8 of the reaction mixture decreased to pH 5.0 due to the release of H^+ ions during the oxidation of the plant phytochemicals simultaneously reducing metal ion. A similar behavior was found in AgNPs synthesis using *Acacia farnesiana* seed extra**ct [26]**.

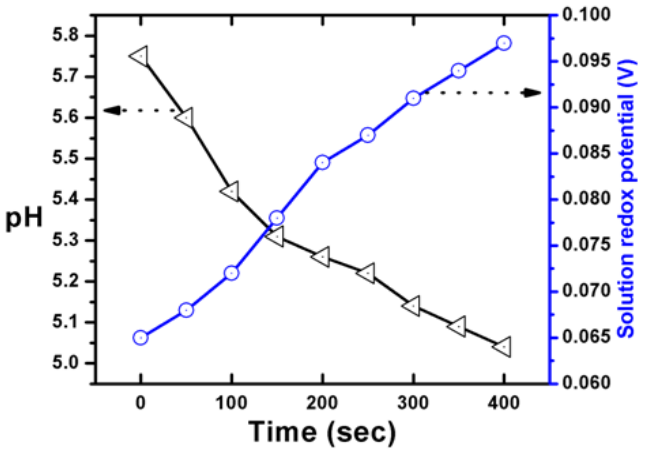

Fig. 2. Variation of pH and redox potential of the reaction mixture as a function of irradiation time.

Characterization of AgNPs by XRD

The XRD pattern as observed in **Fig. 3** confirms the formation of AgNPs with *fcc* structure (JCPDS 89-3722) **[27].** The diffraction peaks observed at 37.1^0, 44.3^0, 64.5^0 and 77.1^0 can be assigned for (111), (200), (220) and (311) planes, respectively. Some minor peaks were also observed as marked in the diffraction pattern. For instance, a peak at about 55^0 is assigned for (220) of Ag_2O [**28**] and the other peaks are assumed to be the crystalline phase of organic moiety originated from the plant extract **[29].** In fact, this organic phase can be easily removed by washing AgNPs with mixture of ethanol/acetone. Further, the broad peaks indicate the formation of nanoparticles here. The crystallite size of AgNPs was calculated to be 15 nm using the Scherer's equation, $d = K\lambda/\beta\cos\theta$ where, K-shape factor between 0.9 and 1.1, λ-incident X-ray wavelength (Cu Kα = 1.542 A°), β-full width half maximum in radians of the prominent line (111) and θ-position of that line in the pattern.

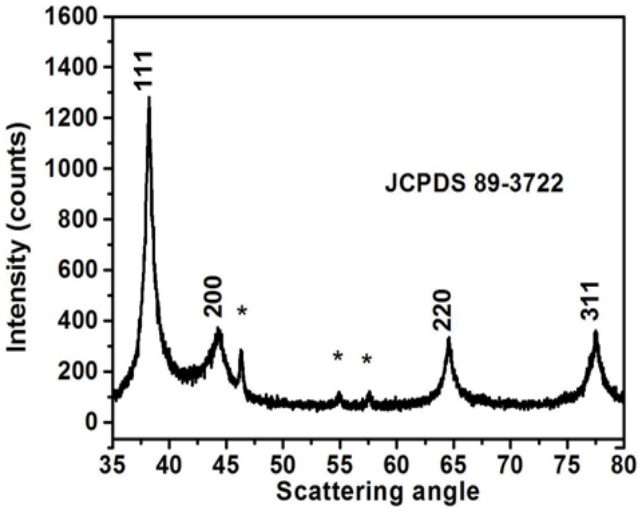

Fig. 3. XRD pattern of AgNPs.

Characterization of AgNPs by TEM

The TEM image and the selected area electron diffraction (SAED) pattern of the AgNPs are exhibited in **Fig. 4**. The particles are in the nano-regime with spherical shape. Most

of the particles are in the range of 15-20 nm and are in agreement with XRD data. Also, SAED image confirms the *fcc* structured AgNPs. TEM also reveals that all nanoparticles are well separated showing no agglomeration. A careful observation indicates a thin layer of amorphous matter on the surface of AgNPs, which may be due to the capping of biomolecules **[30].**

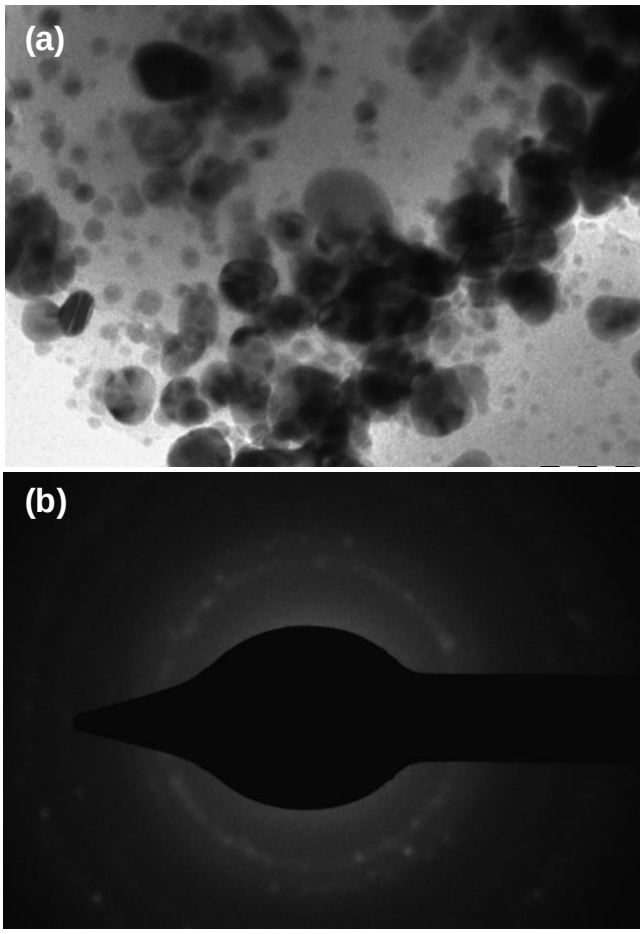

Fig. 4. TEM image (a) and SAED pattern (b) of AgNPs.

Characterization of AgNPs by FTIR

FTIR measurements were carried out to recognize the potential biomolecules present in fruit extract which are responsible for the reduction and capping of NPs. **Fig. 5** exhibits the FTIR spectra of AgNPs. The peak at 3427 cm^{-1} was assigned to O–H stretching of polyphenols, flavonoids, which is the main constituent of the apple. The peak at 1627cm^{-1} corresponds to amide I arising due to carbonyl stretch in proteins. It is well-known that proteins can bind to AgNPs through free carboxylate group. The presence of bands at 1627cm^{-1} confirms that carboxylate group of proteins interacted with the AgNPs **[31].** A peak at 2922 cm^{-1} corresponds to aldehydic C–H stretching; weaker band at 1725cm^{-1} is ascribed to ketone and ester group. The peak at 1381 cm^{-1} corresponds to germinal methyl group and a peak near 1019 cm^{-1} is assigned to C–N stretching vibrations. All these prominent peaks indicate the presence of flavonoids, terpenoids and polyphenols of the fruit extract which are responsible for the reduction/stabilization of AgNPs **[32, 33].**

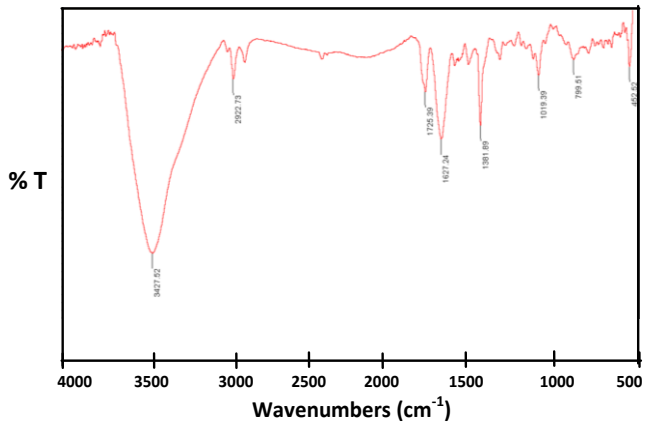

Fig. 5. FT-IR spectra of AgNPs.

Antioxidant and Antibacterial activity of AgNPs

DPPH as a stable free radical at room temperature accepts an electron or hydrogen to become stable diamagnetic molecule and shows a characteristic absorption at 517 nm and its color, changes from violet to yellow upon reduction. As observed in **Fig. 6**, the antioxidant property of bio-capped AgNPs is very much comparable to that of BHT. The antioxidant property of apple fruit extract alone was found to be less effective when compared to AgNPs; further, AgNPs along with bio-capped phytochemicals (mainly polyphenols) are responsible for potential antioxidant activity.

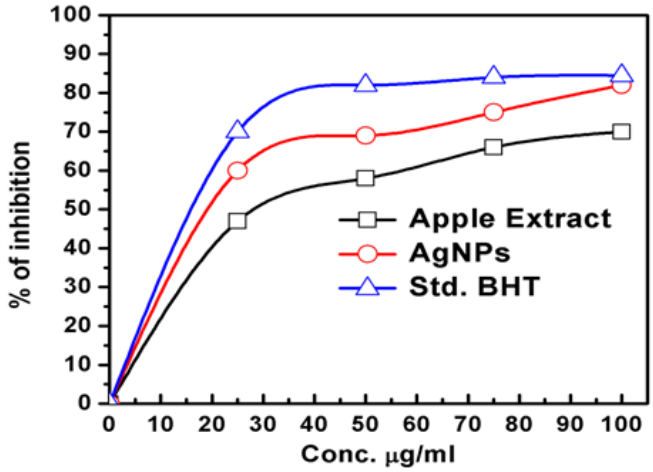

Fig. 6. Antioxidant activity of AgNPs.

The antibacterial activity of AgNPs was tested against viz., *B. subtilis*, *E. coli*, *A. tumifaciens* and *P. aeruginosa* under well-plate method [**34, 35**]. The activity of the AgNPs was observed to be mainly dependent upon its concentration (i.e., 1.0>0.5>0.25>0.12 µg/ml), as the concentration of test samples (AgNPs/fruit extract alone) increased; the activity (as observed in **Fig. 7**) also increased the AgNPs exhibiting higher antibacterial activity as compared to apple fruit extract, against all the tested bacteria and found very effective against *E. coli*; though a precise mechanism of inhibitory action of AgNPs on microorganisms is very hard to establish.

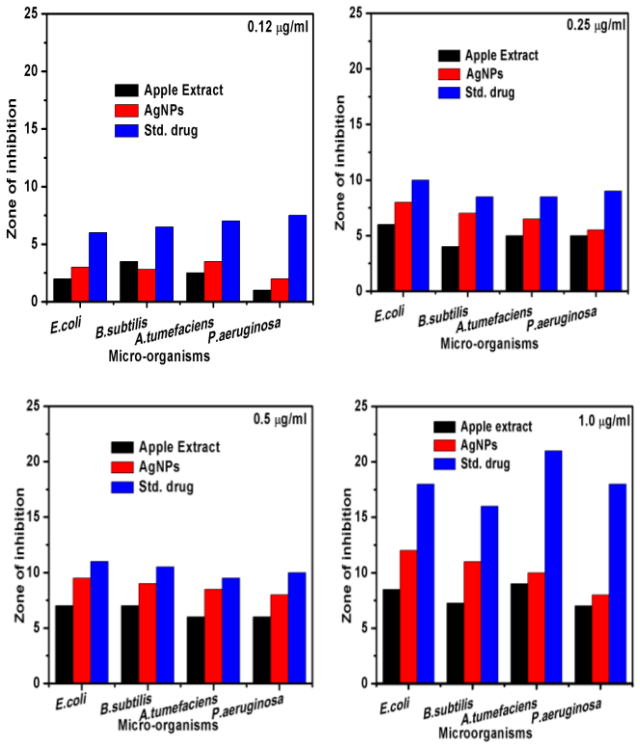

Fig. 7. Antibacterial activity of AgNPs.

A number of theories are proposed by various researchers to know the mode of action of AgNPs against microorganisms; precision to its effectiveness has been very nascent; some of their important contributions tough are cited below;

- AgNPs have positive charge, when attached with the negative charged microorganisms by the electrostatic attraction in the cell wall membrane and penetrate inside the cell,
- AgNPs when linked with thiol groups of cell wall resulted in the production of reactive oxygen species and disrupting the cell and;

AgNPs closely coupled with cell wall of bacteria by forming 'pits' finally affects the permeability, and cause cell death [**30**].

Conclusion

The biofunctionalized AgNPs of near spherical shape are found successfully synthesizing under microwave irradiation using apple fruit extract as a reducing agent. The increase in redox potential and decrease in pH of the reaction mixture indicates the presence of biomolecules in the reaction process. These nanoparticles showed characteristic UV–Vis absorption peak at 420 nm;. Detailed structural analysis of AgNPs is obtained by using XRD, FTIR, TEM techniques. AgNPs are spherical in shape, poly-dispersed and stable at room temperature. Further, the antimicrobial activity is also found to be effective against *E. coli* and exhibits very good antioxidant property. Thus it can be concluded that apple fruit extract can be useful as cheap and eco-friendly bio-resource for synthesis of AgNPs with antibacterial activity.

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