Green synthesis of silver nanoparticles from *Citrus medica* peels and determination of its antioxidant activity

S C Satia, Sumita* Ankit S Bartwal & Alok Kumar Agarwal

*Department of Chemistry, H.N.B. Garhwal University, Srinagar Garhwal 246 174, India

bDepartment of Chemistry, Chinmay Degree College, Haridawar 249 403, India

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*Corresponding author (E-mail: ringwalsumit93@gmail.com)

**Citrus medica** commonly known as Nimboo, is an evergreen aromatic tree of Rutaceae family, with height of 4-5 m, commonly found in the Garhwal Himalayan region. Leaves are generally long, ovate – lanceolate, 10-12 cm long. Flowers auxillary, 1.5-2 cm across, sweet scented. Fruits yellow, ovoid-subglobose, rough or warty, rind thick, pulp loose, aromatic, sweet & sour, juice sacs small, slender, seeds numerous, oval, commonly found in uncommon forest edges of outer Himalaya. Present study deals with the synthesis of Silver nanoparticles (AgNPs) from *Citrus medica* peels by green synthesis method, which does not involve any harmful chemicals. On mixing of AgNO₃ solution and plant extract in respective ratios, the reduction of Ag⁺ ions take place due to presence of some water-soluble organic compounds. The reduction of Ag⁺ ion is observed with the help of UV-Vis absorption spectroscopy and the synthesized AgNPs were characterized by X-ray diffraction method followed by SEM, EDAX and HR-TEM studies. The results of UV-Vis absorption study show a strong absorption band of silver nanoparticles (AgNPs) at λ<sub>max</sub> 440 nm. The X-ray diffraction analysis confirmed that the synthesized AgNPs are cubic crystal solid. The SEM analysis of AgNPs shows average size of 1.35 μm, while the EDAX analysis confirmed the significant presence of silver with carbon, oxygen, chlorine and Iron as other contaminants. This analysis is followed by TEM which shows that the water-soluble AgNPs, have approximate size 53nm. The characterized AgNPs was also analysed for antioxidant activity. The IC₅₀ value for BHT has been calculated as 6.4 mg/ml while for the AgNPs it is 19.3 mg/ml.

**Keywords:** Silver nanoparticles, Green synthesis, *Citrus medica*, Antioxidant activity.

1 Introduction

Microscopic particle with size usually ranging from 1 to 100 nanometers (nm) are known as nanoparticles and they have been studied in a different branch of science called Nanoscience and Nanotechnology. Nanomaterials are very efficient tool in solving many health and environmental issues. To reduce generated hazardous waste green synthesis method is an important demand of mankind. By following 12 principles of green chemistry one can observe minimal harsh effects by products and can maximize the efficiency of chemical reactions using eco-friendly solvents and nontoxic chemicals<sup>13</sup>. The nanoparticles have characteristic and considerably different chemical, physical and biological properties compared to its macro scaled counterparts<sup>4</sup>. Noble metal nanoparticles have been very effective and useful in some areas such as catalysis, electronics, optics, environment, photography, biological labeling, photonics and Surface-Enhanced Raman Scattering (SERS) detection<sup>57</sup>. Over the past few decades silver nanoparticles are more frequently synthesized nanoparticle due to their unique physicochemical properties (with a high ratio of surface area to mass), electrical, optical, catalytic, particularly antimicrobial and antibacterial activities. Silver nanoparticles are also reported to possess antifungal, anti-inflammatory, antiviral, anti-angiogenesis and antiplatelet, activities. They all confer to them a major advantage for the development of alternative products. e.g. multidrug-resistant microorganisms. Nowadays, Silver nanoparticles (AgNPs) are used extensively as antibacterial agents in the field of medicines, implantable biomaterial, water treatment, molecular imaging, diagnosis and treatment of cardiovascular diseases, wound healing, drug delivery and clotting<sup>8</sup>. *Citrus medica* belongs to family Rutaceae is an evergreen aromatic tree, 4-5 m high, commonly found in the uncommon forest edges of outer Himalaya or cultivated Sub –Himalayan tracts, Garhwal to Sikkim, Indomalaysia, Pakistan and many parts of European countries like Greece and Italy<sup>9</sup>. The fruits of the plant are used as medicine in
sickness, pulmonary troubles, intestinal ailments, scurvy. The essential oil obtained from fruits and flavedo was regarded as antibiotic. Juice obtained from fruits is used for treating condition like nausea, vomiting and excessive thirst and also used in various Ayurvedic preparations and syrup\textsuperscript{10}. Essential oil (monoterpene hydrocarbon –limonene), 5-6% citral and dependence isolated from fruit coat (peels) and the bark of the plant are important components for the perfume industries\textsuperscript{11}. Flavonoids, glycosides (hesperidin), vitamin-C, tetranortriterpenoids (limonin, luminol, and nomlinic acid) have been isolated from the alcoholic extract of the leaves of the plant\textsuperscript{12}.

2 Materials and Methods

2.1 Chemicals

\textit{Citrus medica} was collected from Ukhimath, Rudraprayag (1317 meter asl) and Mandal Gopeshwar Chamoli (1550 meter asl). Their botanical authentication established with the aid of taxonomists and treaties on regional flora and comparison of specimens of with herbaria which is (GUH 15217). Silver nitrate (AgNO\textsubscript{3}) was purchased from Sigma-Aldrich, double-distilled water was used to perform experiment.

2.2 Preparation of peals extract

Fresh peels of \textit{Citrus medica} were collected and shade dried for 15 days, crushed, well dried, washed thoroughly with double distilled water. 10 gm of finely crushed \textit{Citrus medica} peels were transferred into a 250 ml beaker containing 100 ml double-distilled water, mixed well and boiled for 60 mins at 60-70 °C temperature. Extract was filtered through whatman filter paper No. 1 and the filtrate was collected in 250 ml Erlenmeyer flask and stored at refrigerator for further uses\textsuperscript{13-14}.

2.3 Preparation of silver nanoparticles

5mM aqueous solution of silver nitrate (AgNO\textsubscript{3}) was prepared and 100 ml of plant extract was added to 150 ml of 5mM AgNO\textsubscript{3} solution (ratio 2:3). This experiment was performed at room temperature. In the solution of plant extract and AgNO\textsubscript{3}, the bio-reduction of Ag\textsuperscript{+} ion was confirmed by using UV–Visible spectrophotometer after 24 h. When color change was observed it confirmed formation of AgNPs\textsuperscript{15}, and resulting solution was centrifuged at 5000 rpm for 25 min and this process was repeated for three times. Finally, nanoparticles were collected and kept for further characterization\textsuperscript{16}.

3 Results and Discussion

3.1 UV-Visible study

The peels extract of \textit{Citrus medica} was added to 5 mM solution of AgNO\textsubscript{3}, after adding extract the color of mix solution changed from colorless to yellowish brown in about 60 mins. The brightness of color is increases with time. The reaction takes up to 75 min for completion at room temperature. Formation of AgNPs using 5mM solution of AgNO\textsubscript{3} was confirmed using UV–visible spectral analysis. AgNPs show UV–Visible peak at 440 nm (as shown in Fig. 1) which is characteristics peak for AgNPs\textsuperscript{16}.

3.2 XRD study

The observed XRD patterns of AgNPs from \textit{Citrus medica} peels extract is shown in Fig. 2. The XRD patterns shows cubic structure of the prepared AgNPs nanoparticles and have been found in good agreement with the Inorganic Crystal Structure Database-(ICSD)
data of AgNPs with peaks corresponding to (111), (200), (220) and (110). Interplanar spacing (d) values for the above-mentioned planes are 2.34400 Å, 2.03000 Å and 1.43540 Å, the observed XRD pattern shows the synthesized nanoparticles are crystalline in nature. Thus, the XRD pattern proves to be strong evidence in favor of the presence of silver nanocrystals. XRD pattern confirmed the cubic phases of the silver nanocrystals.

3.3 SEM results

Figure 3 shows that the observed Scanning Electron Micrographs (SEM) of the prepared AgNPs nanoparticles. The SEM photographs show that the prepared samples have an average grain size of about 1.35 µm but a few have larger grains that exceed by 10 µm.

3.4 EDX analysis

The EDX spectrum (Fig. 4) was analyzed to determine the purity of the synthesized AgNPs. It shows the peaks for the presence of Ag, C, O, Fe and Cl. The Ag peak could be originated from AgNPs, and other peaks of C, O, Fe and Cl from the heterocyclic compounds of the Citrus medica Peels extract. Wight % of different elements present in AgNPs confirms that Ag has maximum percentage 44.16% which clear the synthesis of AgNPs. Presence of carbon 23.72 % and oxygen 29.46 % is may be viscous nature of AgNPs.

3.5 TEM analysis

Transmission Electron Microscopy (TEM) analysis confirmed the synthesis of nanocrystalline silver particles, as shown in (Fig. 5) The nanoparticles predominately adopt a spherical morphology and are often agglomerated into small aggregates, comprising of 5–6 particles each, as (Fig. 5 (b)) illustrated. The average gain size of AgNPs is found to be 53 nm and which is ranging from 34 nm to 133 nm.

3.6 Anti-oxidant activity

DPPH 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential of the AgNPs was determined using the modified method\textsuperscript{17}. Sample had prepared 10 ml solution of AgNPs by dissolving 10 mg of AgNPs in 10ml methanol and then we divided resulting solution in three different parts of 1ml, 2ml and 3ml. The absorbance of stable DPPH was recorded at 517nm \textsuperscript{18}. The DPPH was used as a control. Calculation of % inhibition from the absorbance of sample and negative control using the equation

\[
\% \text{Inhibition} = \frac{(\text{control OD} - \text{Sample OD})}{\text{Control OD}} \times 100
\]

Using % inhibition against concentration IC\textsubscript{50} value was further determined.

3.7 DPPH assay

DPPH is well known free radical and its activity depend on its ability to accept hydrogen or electron from metal. The reducing ability of DPPH on the
AgNPs was monitored by observing color change. The DPPH % inhibition activity of AgNPs when compared with the standard, BHT (Fig. 6). DPPH values were increased in a dose dependent manner. The recorded value of % inhibition for the lowest concentration of the AgNPs (1 mg/ml) was 82% and this value change to 81% when the concentration was increased to (2 mg/mL) and value increased to 84% when concentration was (3 mg/mL). This result confirmed that AgNPs has comparable antioxidant activity in comparison of BHT. The antioxidant activity of AgNPs may be due to presence of functional groups present in the peel extract of *C. medica*. The IC$_{50}$ value for BHT has been calculated as 6.4mg/ml while for the AgNPs it is 19.3 mg/ml.

4 Conclusions

On the basis of above studies, we conclude that the AgNO$_3$ solution and plant extract mixed in ratio 2:3, the reduction of Ag$^+$ ions take place due to presence of some water-soluble organic compounds. The reduction of Ag$^+$ ion is observed with the help of colour change observation in which colour changes light pink to yellowish brown, then in UV-Vis absorption spectroscopy the absorption peak appears at 440nm. The synthesized AgNPs were characterized by X-ray diffraction method followed by SEM, EDX and TEM studies. The X-ray diffraction analysis confirmed that the synthesized AgNPs are cubic crystal solid. The SEM analysis of AgNPs shows average size of AgNPs cluster is 1.35 $\mu$m, while the EDX analysis confirmed the significant presence of silver with carbon, oxygen, chlorine and iron as other contaminants in the AgNPs. This analysis is followed by TEM analysis which shows that the water-soluble AgNPs, have approximate size 53nm. The characterized AgNPs were also analysed for antioxidant activity. The calculated IC$_{50}$ value for BHT was 6.4mg/ml while for AgNPs it is 19.3 mg/ml.

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