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Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science S1 (2016) 253-257

Green Synthesis of Silver Nanoparticles from *Santalum album* Tender Leaf Extract and Evaluation of its Antioxidant Capacity

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Received 14th March 2016; Revised 5rd May 2016; Accepted 6th May 2016

ABSTRACT

In this study, biosynthesis of silver nanoparticles (AgNPs) using cell-free extract of Santalum album tender leaf was undertaken, and antioxidant capacity of the synthesized nanoparticles was evaluated. Ultraviolet-visible spectrophotometer analysis confirmed the production of AgNPs at 430-460 nm where the color change in the solution from light yellow to brown indicated the formation of AgNPs. The scanning electron microscope studies revealed the presence of nanoparticles of size ranging from 80 to 90 nm and energy dispersive X-ray confirmed the elemental composition. Fourier transform infrared analysis confirmed the capping of nanoparticles with organic residues such as, proteins, amino acids, and polyphenols present in the extract, which led to stabilization of nanoparticles. Plant extracts are known to contain polyphenols, flavonoids, tannins, which are known to be antioxidants. Therefore, an attempt was made to see if the biosynthesized AgNPs had better antioxidant activity compared to the extract by checking its antioxidant activities such as total phenolic content, total flavonoid content assay, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay, and total antioxidant capacity. Biocapped nanoparticles were found to retain its antioxidant capacity, but free radical scavenging ability was less than the extract.

Key words: *Santalum album, Silver nanoparticles, Biosynthesis, Antioxidant capacity.*

1. INTRODUCTION

Green synthesis of nanomaterials is an ecofriendly, clean approach for nanoparticles preparation where chemicals used are non-toxic and reducing agents used are renewable [1]. Nanoparticle synthesis using biological methods is called green synthesis. Researchers have attempted synthesis of nanoparticles of different sizes and shapes using a variety of biological materials [2]. In general, plants are known to contain antioxidants, which are defined as any substance, which when present at low concentration compared to that of the oxidizable substrate, inhibits or significantly delays the oxidation of that substrate. Many plant compounds such as phenolics, flavonoids, tannins act as antioxidants and they help in scavenging free radicals such as a hydrogen superoxide ($-H_2O_2$) free radicals, hydroxyl (−OH), and reactive oxygen species [3]. Free radicals play a major role in many degenerative diseases of aging such as cardiovascular disease, cancer, brain dysfunction, cataracts, and weak immune system. Synthesis of unfamiliar bioactive molecules from nature which has health-promoting prospective is a great deal of interest in recent years. Bioactive compounds such as antioxidants from different plant sources such as nutraceuticals are commercially promoted since it has the capability to deactivate the free radicals and hence have been shown to reduce the occurrence of various diseases [4]. Hence, the present work aims at trying to biosynthesize silver nanoparticles (AgNPs) using tender leaves of *Santalum album* AgNPs and evaluating its antioxidant ability by various assays.

2. EXPERIMENTAL

The AgNPs are synthesized using cell-free extract of *S. album* tender leaf by the reduction of $AgNO_3$. The details of the steps in the biosynthesis of AgNPs are given below.

2.1. Preparation of Cell-Free Extract

Tender leaves of *S. album* were washed in deionized water, then chopped and used for the preparation of cell-free extract. 10% (w/v) suspension was prepared by boiling the chopped leaves with required quantity of deionized water in the water bath for ½ h at 80°C. Whatman No. 1 filter paper was used to filter the cell-free extract, and the filtrate was then used for nanoparticle preparation (Figure 1). The AgNPs are synthesized using cell-free extract of *S. album* tender leaf by the reduction of $AgNO_3$.

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2.2. Synthesis of AgNPs

As reported in the literature [5-8], biosynthesis of nanoparticles was undertaken. 1 mM aqueous $AgNO_3$ was prepared in deionized water, and 25 ml of cell-free extract was then added to 225 ml of 1 mM $AgNO_3$ and mixed thoroughly. The resultant mixture is kept in a shaker at 120 rpm at room temperature. Color change in the resulting solution was measured at regular intervals by measuring absorbance at 360-700 nm in ultraviolet (UV) visible spectrophotometer. The reaction mixture was then centrifuged and the product, which was obtained was then washed thrice with deionized distilled water and finally with ethanol, and stored at −4°C for further analysis.

2.3. Characterization of AgNPs

2.3.1. UV-visible spectroscopic characterization of AgNPs

Color change is the solution takes place during the biosynthesis of nanoparticles which can be accounted for surface plasmon resonance (SPR) (Figure 2). The color change in the solution was measured at regular intervals by measuring absorbance at 360-700 nm using UV-visible spectrophotometer (UV1-Thermo Electronic Corporation, Merck).

2.3.2. Fourier transform infrared (FTIR) spectroscopic studies

Biosynthesized nanoparticles are known to be capped with proteins, amino acids, and polyphenols, which can be determined by FTIR [9,11]. The functional groups present in the phytoconstituents found in *S. album* tender leaf extract and their involvement as capping agents in the biosynthesized AgNPs was determined by recording the IR on Bruker Alpha.

2.3.3. Scanning electron microscope (SEM) and energy dispersive X-ray (EDX) analysis

Biosynthesized nanoparticles assume different shapes and sizes [5-8], which are identified by SEM or TEM. Size and morphology of AgNPs were examined by SEM JOEL JSM 6380 LA by sputtering, coupled with EDX for elementary composition. The sputtered samples were analyzed for its microstructure at 20 KV. SEM specimens preparation was done by taking small amount of nanoparticles powder dispersed on carbon tape.

2.4. Antioxidant Assays

2.4.1. Determination of total phenolic content (TPC) [12]

Various volumes (0-1.0 ml) of gallic acid (250 µg/ml) were taken, and 1 ml of extract and AgNPs solution was also taken as sample in the test tubes. 5 ml of Folin–Ciocalteu reagent was added to the test tubes which were then incubated for 5 min at room temperature. 4 ml of aqueous sodium carbonate was then added to all the tubes and incubated again for 15 min at same temperature condition. The absorbance was then measured at 765nm using the UV-visible spectrophotometer.

2.4.2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [12]

Various volumes (0-1.0 ml) of BHT (10 mg/ml) were taken as standards, and 1 ml of extract and AgNPs solution was taken as samples in the test tubes. It was made up to 3 ml by adding double distilled water. 1 ml of DPPH was added, and the tubes were incubated for 30 min in the dark. The absorbance was measured at 517 nm using the UV-visible spectrophotometer. Here, ethanol was used to set zero base.

% Inhibition = (Control OD − Sample OD/Control OD) × 100

2.4.3. Total antioxidant capacity [13]

Various volumes (0-1.0 ml) of ascorbic acid (500 µg/ml) were taken as standards and were made up to 1 ml by adding double distilled water. 1 ml of extract and AgNPs solution was taken as samples in the capped test tubes. To all the tubes, 10 ml of reagent solution was added, and the tubes were incubated at 95°C for 90 min in water bath. Allowing it to cool, the

Figure 1: (a) Tender leaves and (b) extract of *Santalum album*.

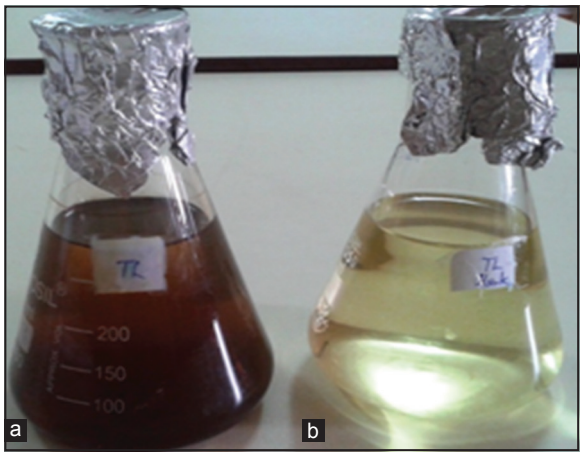

Figure 2: Conical flask confirming nanoparticle synthesis (left) control (right).

absorbance was then measured at 695 nm using the UV-visible spectrophotometer.

2.4.4. Total flavonoid content assay [14]

To 1 ml of extract and AgNPs solution, 4 ml of double distilled water was added. 0.3 ml of sodium nitrite solution (5%) was then added to all test tubes. Then, it was incubated at room temperature for 5 min. 0.3 ml of aluminum chloride (10%) was then added to all the tubes followed by 2 ml of 1 M NaOH. Then, the volume was made up to 10 ml with double distilled water, and absorbance was measured at 510 nm spectrophotometrically. Double distilled water was taken as blank, and the same procedure was followed.

3. RESULTS AND DISCUSSION

3.1. Characterization of Synthesized Nanoparticles

3.1.1. Characterization using UV-visible spectrophotometer

Color change of the solution from light yellow to brown indicated the formation of AgNPs. UV-visible spectrophotometer confirmed the production of AgNPs at 430-460 nm (Figure 3).

3.1.2. Characterization using SEM and EDX

SEM studies revealed the presence of nanoparticles of size 80-90 nm and EDX confirmed the elemental composition (Figure 4). The SPR peaks observed for AgNPs produced by *S. album* are comparable to the literature reports [5]. AgNPs synthesized using *Aloe vera* extract had an average size of 20 nm [6]; AgNPs synthesized using *Candida albicans* had an average size of 30 nm [7]. AgNPs synthesized using *Mentha piperita* leaf extract had an average size of 90 nm and those synthesized using *Cyanodon dactylon* leaf extract had an average size of 8-10 nm [8]. Compared to the earlier reports [8], AgNPs synthesized in this study from the different extracts of *S. album* were found to be quite large.

3.1.3. Characterization using FTIR

Capping of nanoparticles with organic residues, such as proteins and amino acids, presents in the extract lead to the stabilization of nanoparticles which was confirmed by FTIR.

Figures 5 and 6 show the FTIR measurements of *S. album* tender leaf extract and AgNPs synthesized from *S. album* tender leaf extract. AgNPs was found to have C=C (1616 cm^{-1}) stretching, Br, OH (3281 cm^{-1}) functional groups. AgNPs synthesized using tender leaf extract may potentially possess rocking vibration of CH_3 (665 cm^{-1}), C=C (1616 cm^{-1}), C=O (1754 cm^{-1}) stretching, C-H stretch in CH_3 (2989 cm^{-1}) and CH_2 (2848 cm^{-1}), Br, OH (3281 cm^{-1}) functional groups on the surface. The most common rocking vibration found between the extract and the AgNPs were found to be C=C1616 cm^{-1} and Br, OH 3281 cm^{-1}.

Earlier studies of IR spectra of biologically synthesized nanoparticles have shown the presence of biomolecules on their surface [9,10]. From this study also, it is evident that the AgNPs synthesized are capped with organic residues such as proteins and amino acids. It is well-known fact that amines of the proteins facilitate the binding to Ag nanoparticles, and therefore, stabilization of AgNPs through surface bound proteins occurs.

3.2. Antioxidant Assays

Phytochemicals, such as phenolics, flavonoids, and tannins, have the ability to inhibit free radical that are extensively produced and hence act as antioxidants (Table 1) [15,16].

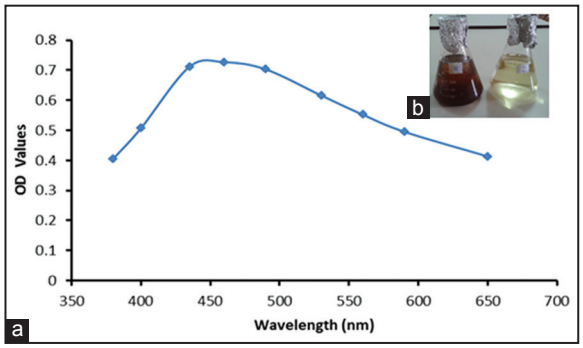

Figure 3: (a) Visual observation of ultraviolet-visible spectra of biosynthesized silver nanoparticle (AgNPs), (b) conical flasks confirming AgNPs synthesis.

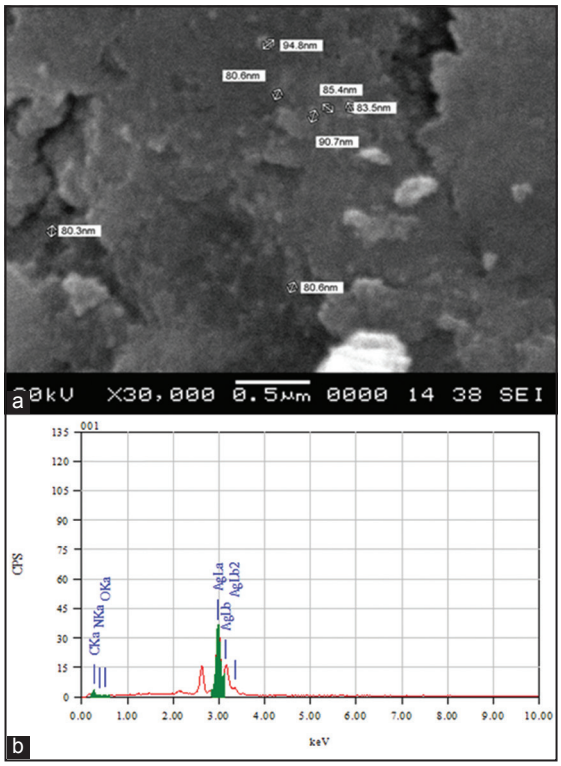

Figure 4: (a) Scanning electron microscope image of silver nanoparticles (AgNPs), (b) energy dispersive X-ray image of AgNPs.

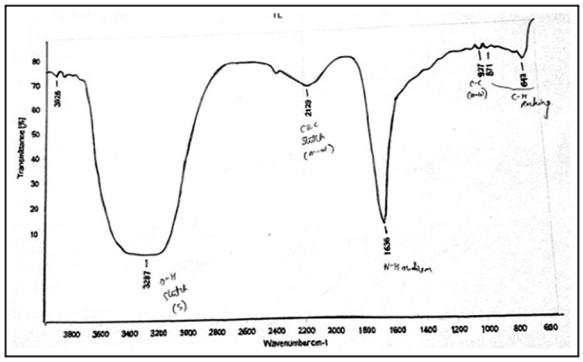

Figure 5: Fourier transform infrared of *Santalum album* tender leaf extract.

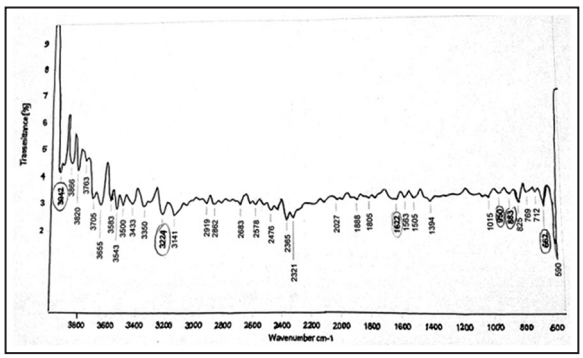

Figure 6: Fourier transform infrared of silver nanoparticles produced from tender leaf extract.

3.2.1. TPC

The TPC for AgNPs is 28.2 mg gallic acid equivalent (GAE)/g, which is almost close to that of extract that is 33.3 mg GAE/g extract. This proves that the nanoparticles can be effectively used as an antioxidant. Phytochemicals, such as phenolics, flavonoids, and tannins, present in the extract have the ability to inhibit free radical that are extensively produced and hence act as antioxidants. It is evident from the literature that the reducing components present in the extract are mainly responsible for antioxidant activity. Hence, the biocapped nanoparticles have retained their antioxidant capacity.

3.2.2. DPPH free radical scavenging assay

Percentage inhibition is more in extract, i.e., 30.02 compared to synthesized nanoparticles which are 9.7 this may be because the biosynthesized nanoparticles are coated with flavonoids but extract has other components in it which have the radical scavenging ability. From the literature, it is evident that flavonoid chemical structure is the governing factor for inhibiting the free radical mediated events [17]. Furthermore, flavonoids inhibit free radical generation, generally methylation of hydroxyl groups of flavonoids decreases their radical scavenging capacity. This is the reason for obtaining comparatively less inhibition for AgNPs compared to extract [18].

Table 1: Results of antioxidant assays.

Antioxidant assays	Extract	AgNPs
TPC (mg GAE/g extract)	33.33	28.2
Total flavonoid content (mg QE/g extract)	24	67
Reducing power assay (mg AAE/g extract)	48.16	28.5
Total antioxidant capacity (mg AAE/g extract)	12.4	7.56

TPC=Total phenolic content, QE=Quercetin equivalent, AAE=Ascorbic acid equivalent, AgNPs=Silver nanoparticles

3.2.3. Total antioxidant capacity

The TPC reduced to 7.56 mg ascorbic acid equivalent (AAE)/g for AgNPs, which is less than that of extract that is 12.4 mg AAE/g extract. Total antioxidant capacity is the antioxidants ability present in different samples to clean harmful free radicals. Since the nanoparticles may be coated with flavonoids and it is evident from the literature that O-methylation of hydroxyl groups of flavonoids decreases their free radical scavenging capacity. This is the reason for getting the low value of total antioxidant capacity in nanoparticles compared to that of the extract.

3.2.4. Total flavonoid content assay

The flavonoid content for AgNPs was 67 mg quercetin equivalent/g extract and that of extract is 24 mg AAE/g extract. This proves that the nanoparticles are coated with flavonoids, and hence, higher value was obtained for the assay of nanoparticles coated with flavonoids.

4. CONCLUSION

By employing economical and ecofriendly biological approach using cell-free extract of leaf of *S. album* AgNPs capped with proteins and other organics were synthesized. The organic reducing agents present in the cell-free extract were capable of reducing the silver ions to their corresponding neutral atoms, which then nucleates to become the nanoparticles. The SPR peaks observed for AgNPs were comparable to the literature reports. The SEM studies revealed the presence of nanoparticles of size 80-90 nm. The purity of the sample and its elemental composition was confirmed by EDX. The antioxidant assays proved that AgNPs can be effectively used as an antioxidant agent.

5. ACKNOWLEDGMENTS

The author Nayak Sneha gratefully acknowledges the support from NMAMIT, Nitte. The author would like to thank the Department of Metallurgy, NITK, Surathkal, for kind support in characterizing the nanoparticles.

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****Bibliographical Sketch***

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