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Original Article

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACT OF *BASELLA ALBA* AND THEIR *IN-VITRO* ANTIOXIDANT POTENTIALS

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ABSTRACT

Objective: To synthesize silver nanoparticles (AgNps) from *Basella alba*(B. alba) aqueous extract using 3 mM $AgNO_3$ solution, to characterize the resulted AgNps and also to study the antioxidant activity by DPPH and reducing assay.

Methods: 150 ml of aqueous extract was mixed with 600 ml of 3 mM silver nitrate solutions and kept at room temperature for 1hr. A change from straw yellow to golden yellow was observed and absorption spectra were obtained by UV-VIS spectrometer. The resulted nanoparticles (NPs) were characterized by FT-IR spectrum, SEM-EDS, TEM and XPS techniques. The purity and nature of the particles were observed through TG-DSC analysis. Further, Antioxidant activity was performed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method and by Ferric chloride reducing assay.

Results: UV-Vis spectral analysis shows a maximum absorption peak at 435 nm. FT-IR spectrum indicated the formation of reducing and capping agents in AgNps. The synthesized silver nanoparticles was spherical in shape and its size ranges from 22.6 to 25 nm. The synthesized silver nanoparticles possess excellent antioxidant activity by various methods. The phenol carboxylic acid present in *B. alba* leaf extract acted as a reducing agent which was identified by HPTLC technique. A Protein present in the B. alba extract was responsible for the capping of bio-reduced silver nanoparticles.

Conclusion: It has been demonstrated that *Basella alba* plant extract could be used as a proficient green reducing agent for the synthesis of AgNPs. Further studies will be focused towards the mass scale production of formulation.

Keywords: Biosynthesis, *Basella alba*, Silver nanoparticles, Antioxidant activity.

INTRODUCTION

The field of Nanotechnology is the most active area in modern research. Researchers have found that metal nanoparticles (NPs) are exceedingly proficient with astonishing benefits in various fields such as electronics, electromagnetic, cosmetic and biomedical applications [1], [2]. Metal NPs have been used extensively as an antibacterial agent in food storage, textile coating and in a number of environmental applications [3]. NPs were synthesized through various methods such as chemical, photochemical, electromagnetic and biological techniques [4], [5]. Synthesis of NPs through biological technique provides advancement over physical and chemical methods as it is less toxic, cost effective and eco-friendly [6], [7]. Many reports have been published in the literature on green synthesis of AgNPs using several plants such as *Catharanthus roseus* [8], *Ocimum sanctum* [9], *Bryophyllum pinnatum* [10].

In the present research work, we report the green synthesis of AgNPs using *B. alba (Linn.)* aqueous extract.

B. alba (family: Basellaceae), a perennial vine found in the tropics where it is widely used as a leafy vegetable. It has been used in Indian traditional medicines as an aspirant, rubefacient and for catarrh infections [11]. Leaves of *B. alba* are helpful for the treatment of hypertension, malaria and anemia [12-13]. It also has antifungal, anti-inflammatory and analgesic activities [14-15]. The Literature review revealed that the *B. alba* species were rich in ascorbic acid, carbohydrates, proteins, flavonoids and phenols [16]. Green synthesized AgNPs also possesses good antioxidant activity [17].

The objective of the study focuses on the synthesis of silver nanoparticles and its characterization and also to prove the silver nanaoparticles is having potential antioxidant activity.

MATERIALS AND METHODS

Reagents

Silver nitrate, DPPH purchased from Merck, Germany. All other reagents are of AR grade.

Synthesis of silver nanoparticles

About 150g of fresh leaves of *B. alba* was weighed and washed with de-ionized water before use. The crushed leaves were mixed with 1000 ml of de-ionized water and allowed to stand for 6 hrs at room temperature. The mixture was filtered. 150 ml of collected filtrate was mixed with 600 ml of 3 mM silver nitrate solution. The colour of the solution gets changed from straw yellow to golden yellow indicates the formation of silver nanoparticles. The reduced solution was centrifuged at 7000rpm for 15 minutes. The centrifugation process was repeated for two to three times to remove any impurities adsorbed on the surface of silver nanoparticles. The dried powder was used for the experimental work.

High Performance thin layer chromatography analysis (HPTLC)

Before HPTLC analysis, the secondary metabolites present in herbal extract as well as synthesized AgNPs were identified by various chemical tests such as Mayer's, Shinoda's test, Folin-ciocalteu and Million's reagent [18]. Phenol carboxylic acid present in the herbal and AgNPs was identified by HPTLC (CAMAG, Linomat5, Switerland) technique using Benzene: Methanol: glacial acetic acid (9:1.9:0.8v/v) as mobile phase which was scanned densitometrically at UV 254 nm using CAMAG TLC scanner3.

Characterization of silver nanoparticles

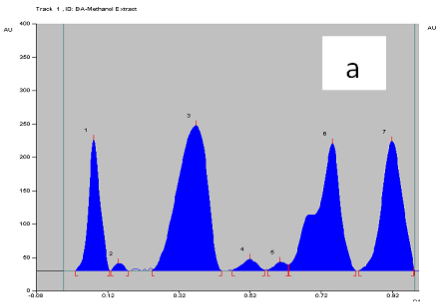
The bio-reduced AgNPs were monitored using UV-Visible spectrophotometer. The spectra was recorded on a PerkinElmer (Lambda 25 model, USA) spectrophotometer in the wavelength region of 200-800 nm at a resolution of 1 nm. FT-IR spectra of the extract and synthesized silver nanoparticles were recorded at room temperature using PerkinElmer spectrum one spectrometer, USA by KBr pellet technique on the range of 4000-400 cm^{-1} with the spectral resolution of 4cm^{-1}. Purity of AgNps was characterized by TG-DSC analysis. Approximately 5mg of the sample was taken in aluminum cup holder and heated up to 1000°C at the rate of 10°C/min using TG-DSC (SDT Q600 model, TA instrument, USA). Scanning electron microscope (SEM) analysis was performed

using FE-SEM (JEOL JSM 6701-F model, USA). Thin film of the sample was prepared on a carbon coated copper grid by using a pinch of the sample. The film on the SEM grid was then allowed to dry and the SEM images were observed. In addition, elemental analysis was done by Energy dispersive spectroscopy (EDS). The morphological features of nanoparticles were confirmed by TEM (Transmission Electron Microscope 2100F, JEOL Japan) using an accelerated voltage of 200kv. XPS technique was carried out on a K-Alpha instrument supplied by Thermoscientific, USA. The sample material was placed in a thin film sample holder and accelerated at 50ev. The antioxidant activity of synthesized nanoparticles was carried out by DPPH method and reducing assay [19, 20].

RESULTS

High Performance Thin Layer Chromatography (HPTLC)

The metabolites present in the extract and the synthesized AgNPs were identified by HPTLC fingerprinting technique which was shown in Figure1-a, b. The resulted chromatogram shows corresponding R_f values at 0.43, 0.49, 0.63, 0.73, 0.80 and 0.93 that signifies the presence of different phenol carboxylic acids [21].



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	0.3	0.08	196.1	23.33	0.13	0.5	4644.1	12.39	unknown *
2	0.13	0.2	0.15	11.2	1.33	0.18	0.0	218.2	0.58	unknown *
3	0.25	1.7	0.37	217.6	25.89	0.44	0.1	13612.7	36.32	unknown *
4	0.47	0.6	0.52	17.5	2.09	0.56	1.3	537.6	1.43	unknown *
5	0.57	1.8	0.61	13.3	1.59	0.63	9.4	331.5	0.88	unknown *
6	0.63	9.3	0.76	190.6	22.68	0.82	0.4	9821.2	26.21	unknown *
7	0.83	0.6	0.92	194.1	23.10	0.98	4.3	8311.6	22.18	unknown *

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	0.4	0.08	62.1	12.38	0.11	8.5	1154.7	5.80	unknown *
2	0.16	0.1	0.21	22.3	4.44	0.25	0.2	709.4	3.57	unknown *
3	0.39	5.7	0.43	15.1	3.01	0.44	11.5	393.7	1.98	unknown *
4	0.49	12.0	0.49	13.6	2.71	0.54	0.2	277.6	1.40	unknown *
5	0.61	0.2	0.63	13.0	2.59	0.64	9.8	147.7	0.74	unknown *
6	0.65	18.7	0.73	51.0	10.17	0.75	41.9	2500.5	12.57	unknown *
7	0.75	42.0	0.80	124.7	24.85	0.84	80.1	4655.3	23.40	unknown *
8	0.86	80.3	0.93	199.9	39.85	0.98	4.4	10052.6	50.54	unknown *

Fig. 1: HPTLC fingerprinting of phenolic acids present in *B. alba* extract (a) synthesized AgNPs (b)

UV-Visible spectral analysis

Figure 2 shows UV-Vis spectra of aqueous extract of *B. alba* with 3 mM silver nitrate solution. The colour of the solution changes from straw yellow to golden yellow due to reduction of silver ion, which indicates the formation of silver nanoparticles. UV-Visible absorption spectra of silver nanoparticles showed a maximum absorbance of 435 nm.

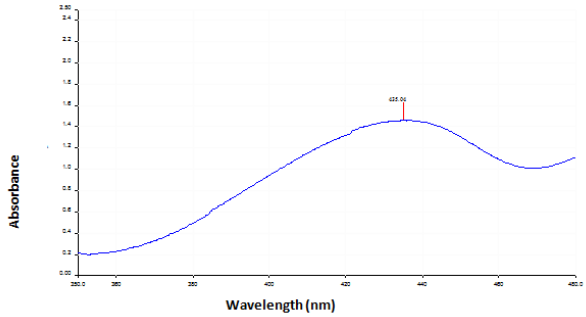


Fig. 2: UV-Vis spectra of synthesized nanoparticles

FT-IR Spectra

The functional groups present in the extract and AgNPs were characterized by FT-IR spectrum. Figure 3-a shows the spectral result of *B. alba* extract which gives peaks at 2323.81 cm^{-1}, 1664.28 cm^{-1}, 1373.38 cm^{-1}, 1329.32 cm^{-1} were assigned to C=N stretching, C-O stretching (phenols), C-N stretching (aromatic tertiary amines) whereas interaction of bio-molecules with NPs show peaks at 1628.33 cm^{-1}, 1522.48 cm^{-1}, 1384.21 cm^{-1} and 1048.84 cm^{-1} corresponds to C=N stretching, aromatic C=C stretching, C-O stretching (phenols) and C-N vibration (as shown in Figure 3-b). These absorbance peaks related to flavonoids, proteins, phenolic acids.

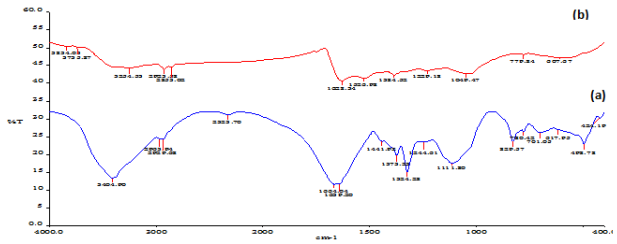


Fig. 3: FT-IR spectrum of bio-molecules in extract (a) and silver nanoparticles (b)

TG-DSC analysis

The purity and thermal stability of AgNPs was detected by TG-DSC analysis (Fig 4). The reported melting point 960.54°C through this analysis was closely related to metallic silver which indicates the purity. The loss in mass of about 16.32 mg proved that the metallic core is surrounded by bio-molecules. TGA plot suggested that the weight loss in the temperature range of 0°C-100°C is due to moisture. Degradation pattern of organic compounds was between 100°C-750°C. There was no degradation above 750°C that accounts for the weight of silver.

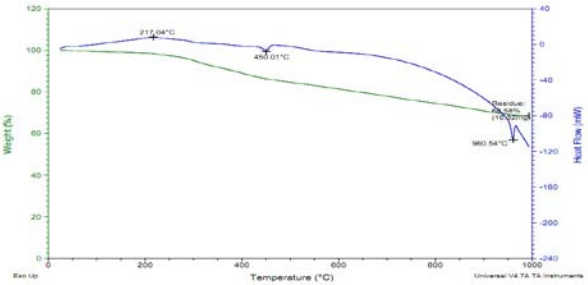


Fig. 4: TG-DSC curve of synthesized nanoparticles

SEM and EDS studies

According to Scanning electron microscope (SEM), the size of the agglomerated nanoparticles ranges from 22.6 to 25 nm (as shown in Figure 5-a). Energy dispersive spectroscopy (EDS) analysis confirmed the presence of elemental silver signals of the nanoparticles (Figure 5-b). Strong signals of metallic silver and other elemental weak signals (O, N) are recorded in the energy of 3Kev. The morphology and coating of bio-molecules over the nanoparticles was observed by transmission electron microscope (TEM). Representative TEM image of AgNPs shows the particles were in nano range with a mean diameter of 24.79 nm and the particles were spherical in shape (Figure 5-c,d).

XPS analysis

The XPS spectrum displays silver signal of the nanoparticles (Figure 6). The general scan spectrum shows the presence of strong Cls, Ols, Nls, and Ag3d core levels (shown in Figure 6-a). The Ag3d core level spectrum (shown in Figure 6-b) is resolved into two spin-orbit components, which occurred at binding energy of 368.0 and 375.0ev respectively.

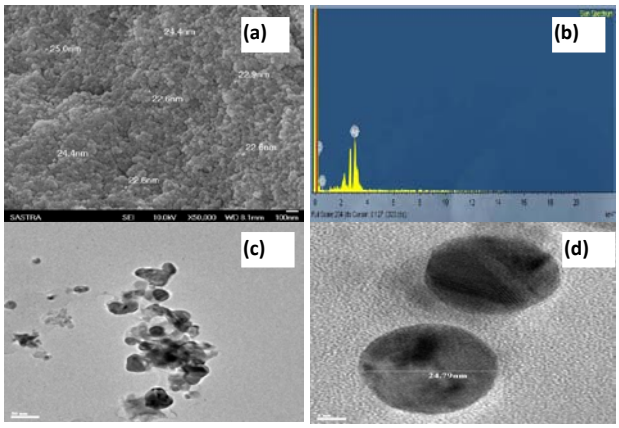


Fig. 5: SEM images of synthesized AgNps (a) with EDS image (b) and typical TEM micrograph of Synthesized silver nanoparticles(c, d)

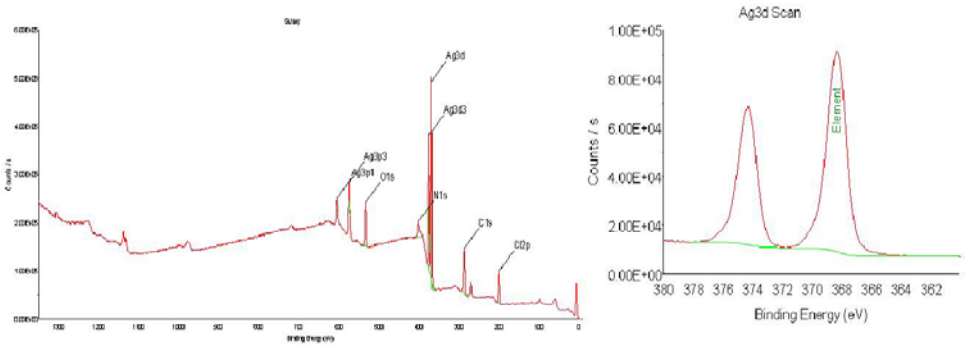


Fig. 6: XPS survey scan (a) Ag3d scan (b) core level spectra recorded from silver nanoparticles

Anti-oxidant Activity

The antioxidant activity of Silver nanoaparticles was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) and reducing assay. As shown in Figure 7, DPPH free radical scavenging activity of synthesized AgNPs showed a potent inhibitory effect when compared with Gallic acid as a standard ranging from 7.81µg/ml to 1000µg/ml. The percentage inhibition of free radical gets increased with increase in concentration of substrates. The IC_{50} value of nanoparticles was found to be 62.5µg/ml. Figure 7 also shows the reducing ability of silver nanoparticles compared with ascorbic acid (Vitamin c) as standard. The reducing power of nanoparticles was found to be effective and increased with an increase in concentration.

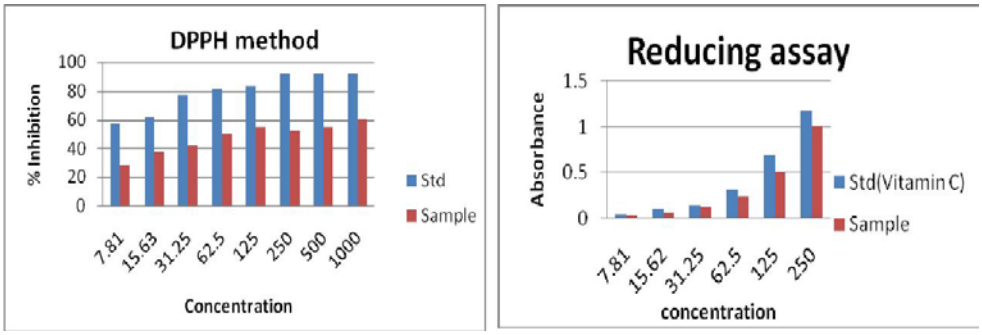


Fig. 7: Histogram showing Inhibitory activity and reducing assay of synthesized AgNPs

DISCUSSION

Green synthesis always proved to be efficient, less toxic and eco-friendly because this process reduces or eliminates the used and generation of hazardous substance [22]. In this study, green synthesis was made from the extract of *Basella alba* using silver nitrate solution. The silver ion gets reduced into silver nanoparticles by the reducing agents present in plant extract which was observed by the change in colour. The formation of golden yellow colour might be due to the excitation of the surface Plasmon vibration of the synthesized nanoaparticles which was confirmed by the maximum absorbance at 435 nm using UV-VIS spectral analysis. The corresponding bio molecules in the extract were identified by HPTLC technique. The chromatogram of the extract and AgNps indicates the presence of different bio active molecules. which was shown in Figure1. The reducing and capping

of AgNps was closely related to the functional groups of secondary metabolites in the extract which was characterized by FTIR spectra [23]. SEM and TEM analysis is commonly used for determining the morphology of the size and shape of nanoparticles. The strong signal from Energy dispersive spectroscopy (EDS) analysis at 3Kev confirms the presence of metallic silver and weak signal of C, O was also found.

Most of the phenols shown to contain high level anti-oxidant activity. Phenolic compounds present in plants acting as antioxidant or free radical scavengers due to their OH groups, which contribute directly to the antioxidative action [24]. In this study a rapid, simple & inexpensive method to measure antioxidant capacity was the use of free radical, 2,2-Diphenyl-1-picryl hydrazyl (DPPH). When DPPH reacts with anti-oxidative compound it donates hydrogen and gets reduced. The change in colour from purple to yellow indicates the reducing activity. The IC_{50} value of NPs was found to be 62.5µg/ml. IC_{50} values represent the concentration of drugs that is required for 50% inhibition *in vitro* and the result indicates that AgNps has high reducing activity.

CONCLUSION

We have established that the synthesis of silver nanoparticles using natural resources is an alternative method for chemical synthesis due to its less toxicity and cost effective. *Basella alba* plant extract could be used as a proficient green reducing agent for the synthesis of AgNPs. The metallic nanoparticles formation was due to the reduction of silver ion into metallic silver which was facilitated by phenol carboxylic acids present in *B. alba* extract. The characterization from UV-Visible analysis, SEM and TEM supports the stability of biosynthesized silver nanoparticles. Next part of the work is being focused on the mass scale production of formulation.

CONFLICT OF INTERESTS

Declared None

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