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Biosynthesis of Silver Nanoparticles by Irradiation Technique from *Baliospermum montanum* Leaf Extract and Identification of their Antimicrobial Activity

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***Abstract*—The most interesting attractive feature of nano-scale material is possessing incredible properties of 1 nm -100 nm dimension which brings so many industrial and biomedical applications, particularly their ability to pass through a biological cell membrane for delivering information, such as drug delivery by minimizing any adverse effect on the non-targeting cell. In this work, an attempt was made to report a novel one-pot, microwave assisted method for the synthesis of silver nanoparticles using the leaf extract of *Baliospermum montanum* as both reducing and capping agent and its antimicrobial potential was tested against some selected pathogenic bacterial strains.**

In the present study, oven dried leaves (40º C) of *Baliospermum montanum* was used to synthesize silver nanoparticles with 1mM $AgNO_3$ using the microwave-assisted method. The colour changes in the reaction mixture were observed (i.e. pale yellow to ruby red colour) during the incubation period. UV- Visible spectroscopy revealed the formation of stable silver ions at 437 nm. XRD (X-Ray Diffraction), SEM and FTIR were used to characterize the synthesized nanoparticles. XRD studies corroborated that the biosynthesized nanoparticles are crystalline silver. SEM image indicated that the silver nanoparticles are quite polydispersed. FT-IR implies a different peak 1543.57cm^{-1}, 1641.48 cm^{-1}, 2920.34 cm^{1} and 2171.08 cm^{-1} indicating the presence of C= C, C = O, sp^3CH and C ≡ N, confirms the presence of proteins and secondary metabolites (phenolic compounds etc.). Amide groups, enzymes, and aldehyde groups may be responsible for the reduction and stabilization of the nanoparticles. Antibacterial activity of biogenic silver nanoparticles was evaluated by disc diffusion method, it indicates a maximum inhibition against *V. cholerae, K. pneumoniae* and *S. aureus* with 14 mm, 13 mm and 16 mm respectively while *E. coli* shows a minimum range of 8 mm. Moreover, *K. pneumoniae* showed an incredible activity even beyond the antibiotic (Streptomycin) used as a standard. The synthesized nanoparticles showed maximum inhibition zone against all the studied pathogenic bacteria in compared with plant extract. This is a simple, green method for the rapid and facile synthesis of silver nanoparticles appears to be cost-effective, ecofreindly when compared to the conventional methods.

***Index Terms*—Antibacterial, *Baliospermum montanum,* Silver nanoparticles**

I. INTRODUCTION

Nanomaterials have generated immense interest in recent times because of their promising applications in various areas of Science and Technology. One of the interesting features of nano-scale material is 1 nm -100 nm dimension which brings so many industrial and biomedical applications [1], particularly their ability to pass through a biological cell membrane such as drug delivery by minimizing adverse effects on the non-targeting cell. Moreover, some application were identified in life sciences, such as antibacterial [2], and viral activity, as labelling material, sequencing, catalyst and as therapeutic agent in genetic effect [3]. Several methods are employed such as chemical and biological methods [4]. However, in this method, the main objective of reducing agent is to reduce the silver ions which lead to transforming into metallic silver (Ag^0) then followed by colloidal silver nanoparticles [5], but the chemical reagents used as reducing and capping agent are usually toxic and lead to environmental pollution. Thus, recently biosynthetic methods employing either plant extract, microorganism like fungi, bacteria, actinomycets [6]. Thus, silver nanoparticles playing a major role in the field of nanotechnology and nanomedicine [7].

II. MATERIALS AND METHODS

Plant Sample Collection

The fresh leaves of *Baliospermum montanum* plant were collected from the greenhouse of PRIST University campus, Vallam, Thanjavur, Tamil Nadu, India.

Synthesis of Silver Nanoparticles

The fresh *Baliospermum montanum* leaves were collected, washed thoroughly 2 - 3 times with sterile water, dried, powdered and then used for extraction. About 2 grams of dried leaf powder were ground to fine paste with 20 ml of distilled water using mortar and pestle. It was centrifuged at 10,000 RPM for 10 minutes. Ten ml of the leaf extract was added to 90 ml of aqueous solution of 1 mM Silver nitrate solution ($AgNO_3$). Then it was heated by microwave assisted irradiation for 8 minutes. The Particles were characterised using the following techniques below:

UV-Visible Spectroscopy Analysis
X-ray Diffraction (XRD) Measurements
FTIR (Fourier Transform Infrared Spectroscopy) Analysis
SEM Analysis Of Silver Nanoparticles

Antibacterial Assay

Antibacterial activity of the silver nanoparticles was assessed using the standard agar disc diffusion method with 6 mm diameter of Whatmann No.1 filter paper discs [8]. (Table:2)

III. RESULTS AND DISCUSSION

Biosynthesis Of Silver Nanoparticles

Therefore, due to the reduction of silver ion complex by the reducing agent (leaf extract) in the mixture, then the color was changed from pale yellowish color to ruby red (Fig. 1), thus the silver nanoparticles synthesized was confirmed [9].

UV-Visible Spectra Analysis

Ag nanoparticles were detected by UV-Vis spectrophotometer in the range of 200-1100 nm. The maximum absorption UV-Vis Spectra taken twice at 24 h interval *i.e* right after the AgNPs has been synthesized and the next following day showed a peak of 437 nm and 443 nm respectively (Fig.2). This indicates a gradual increase of silver nanoparticles synthesis within the time interval due to surface plasmon resonance and dielectric constant of the medium [10].

Fig. 1. Photographs of (a) $AgNO_3$
(b) Leaf extract + Plant extract before heating
(c) Leaf extract + $AgNO_3$ after 5 m of reaction

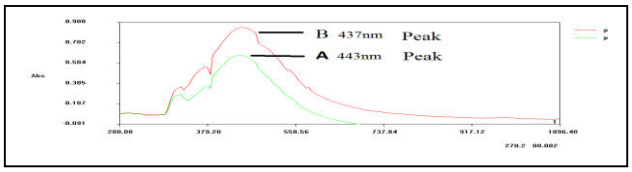

Fig 2. UV-Vis spectra of reduction of Ag ions to Ag nanoparticles (A) 24 hours intervals (B) Right after the AgNPs been synthesized

X-ray Diffraction (XRD) Measurements

The XRD pattern of dried leaf powder is shown in Fig. 3.a. as a control. The XRD pattern showed eight intense peaks of 2θ values ranging from 27.94 to 76.84. XRD pattern of AgNPs showed peak near the 2θ value of 32.37° (Fig.3 b). The peaks observed in the spectrum at 2θ values of 27.94°, 32.37°, 46.36° and 54.89° corresponds to 111, 200, 220, and 311 planes for silver, respectively. Which indicates crystalline nature [11]. The average estimated particle sizes were calculated by Debye-Scherrer formula. An average size was 25.3 nm and shown in Table.1

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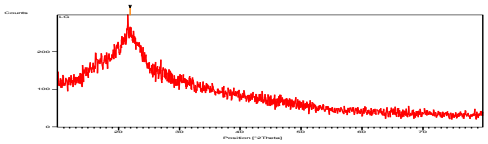


Fig. 3 b. XRD dried leaf powder

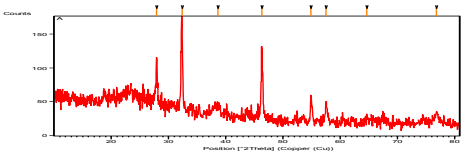


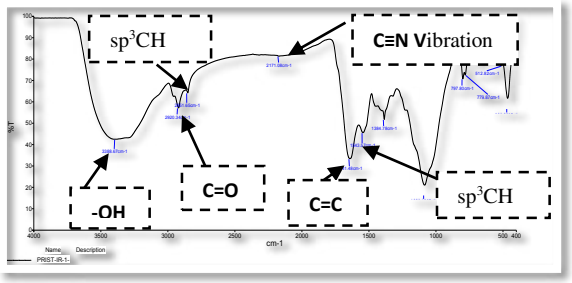
Fig. 3 a. XRD patterns of AgNPs

TABLE.I. CRYSTALLINE SIZE OF SILVER NANOPARTICLES

Plant extract	θ value (degree)	d- spacing (Å)	FWHM (degree)	Intensity (CPS)	Average Particle size (nm)
Baliospermum montanum* leaf silver *nanoparticles	24.96	2.36	0.5966	40.00	25.3

FTIR (Fourier Transform Infrared Spectroscopy) Analysis

However, by focusing the range between 1500 toward the left, reveals the following peaks 1543.57cm^{-1} 1641.48 cm^{-1} 2920.34 cm^{-1} 2171.08 cm^{-1} 3388.67 cm^{-1} and their functional groups C=C, C=O, sp^{3}CH, C≡N stretching vibration [12], –OH which is in alcohol and phenolic compound [13], respectively, this indicated that despite the heating and binding of silver nanoparticles with the plant extract, their secondary metabolites remain the same as well as the proteins structures (Fig. 4).



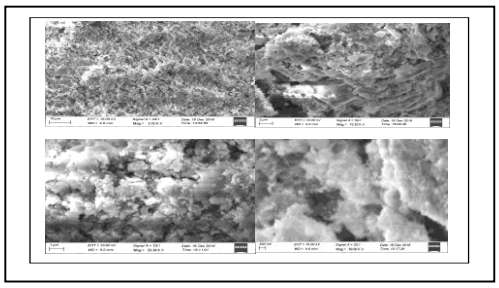


Fig. 4. *FTIR* spectrum AgNPs Fig. 5. SEM Image at different magnification (a) 3.02KX (b) 10.20KX (c) 30.00KX (d) 50.00KX

SEM Analysis of Silver Nanoparticles

The observed morphology of the AgNPs was predominately cuboidal and rectangular, uniform, polydispersed and aggregated into a larger irregular structure with no well-defined morphology (Fig.5 a, b, c & d). AgNPs were formed with a diameter of 25 to 85 nm. The particles were assembled due to the interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bound to the Ag nanoparticles [14].

Antibacterial Activity Analysis

TABLE. II. ANTIBACTERIAL ACTIVITY OF SYNTHESIZED NANOPARTICLES FROM THE LEAF EXTRACT *BALIOSPERMUM MONTANUM*

Bacterial Species	**Zone of inhibition in mm**					
	Plant extract (10 µl)	**$AgNO_3$ (10 µl)**	**AgNPs (10 µl)**	**AgNPs (20 µl)**	**Standard Antibiotics (Streptomycin**	**Control**
Vibrio cholerae	10	15	11	14	15	Nill
E.coli	Nill	7	Nill	8	23	Nill
Staphylococcus aureus	Nill	12	10	13	15	Nill
Klebsiella pneumonia	Nill	9	8	16	14	Nill

Regarding antimicrobial activity, it was found that the entire bacterial species used, showed a different respond against the disc prepared with the various concentration (Table 2) Therefore, the AgNPs was found more actively against the bacteria compared to the silver nitrate, in case of *Klebsiella pneumonia* was even greater than that of the standard antibiotic disc. Additionally, it was discovered in another study using a different species [9]. However, using other plant shows a massive activity against the *E.Coli* [15] , in contrast to the current study.

IV. CONCLUSION

In this study, simple, low cost, a clean, nontoxic and rapid approach for the bio reduction and synthesis of silver nanoparticles was adopted. From the point of view of nanotechnology, this is a significant advancement to synthesize of silver nanoparticles economically. The AgNPs size were in the range of 25 to 85 nm with an average size of 25.3 nm. However, this investigation reveals the high efficacy of AgNPs as a strong antibacterial agent. This can be useful in food industries, cosmetic industries, and medicines.

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