GREEN SYNTHESIS AND ANTIBACTERIAL ACTIVITY STUDIES OF SILVER NANOPARTICLES FROM THE AQUEOUS EXTRACTS OF WRIGHTIA TINCTORIA

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ABSTRACT

Silver nano particles synthesised from the leaves of Wrightia tinctoria were characterised by following instrumental analysis - UV Vis spectroscopy, Scanning Electron Microscopy, Energy dispersive X-ray spectroscopy, Atomic force microscopy and X-ray diffraction. The interaction of the silver nanoparticle to the microorganism was studied in Pseudomonas aeruginosa and Bacillus subtilis through agar diffusion method, minimum inhibitory concentration, swarming motility assay and protein leakage assay. The nanoparticles were found to be more effective against the bacteria used in this study.

Keywords: Silver nanoparticles, Antibacterial activity, MIC, Protein leakage assay, Wrightia tinctoria.

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1. INTRODUCTION

The use of plants in medicine is an age hold practise documented well in Bible, Vedas, inscriptions etc., Plants are enriched with phytochemicals which confers wide range of bioactivities [1]. Plant secondary metabolites are organic compounds largely produced for various purposes like defence, communication, enzyme catalysis etc., although these are not directly involved in the growth and development of the plant secondary metabolites are used as drug excipients, food colourants, flavouring agents, perfumery and other industrial materials [2].

Wrightia tinctoria is a flowering plant of belonging to the Apocynaceae family [3]. This deciduous perennial plant producing milky white latex [4] has prominent therapeutic properties to cure piles, skin diseases especially psoriasis [5, 6]. The plant in a crude form was found effective to control infections of diarrhoea, dysenteric, helminth, dandruff etc [7, 8, 9]. Further they also possess febrifuge, aphrodisiac and anti-ulcer properties [10-12]. The beneficial effect of the plant is the result of diverse phytochemicals present in them. The seed pod has the terpene wrightial, vcyclo eucalenol and cycloartenol. Flavonoids like tryptanthrin, rutin, isatin, indigotin and indirubin are isolated from the leaf extracts while lupeol, campesterol, stigmasterol were extracted from the stem [13]. Compounds like tryptanthrin, indigotin, anthranilate, istin, indirubin and rutin are also reported from this species. Tryptanthrin and triacontanol are the compounds isolated from the leaf extract of *Wrightia tinctoria*[14,15]. The present study is focused on the green synthesis and characterisations of silver nanoparticles from the crude extracts of *Wrightia tinctoria* and tests it for antimicrobial effect on the Gram positive and Gram negative organisms.

2. MATERIALS AND METHODS

2.1. Preparation of Plant Extracts

Wrightia tinctoria leaf was collected and herbarium was made. It was then authenticated by Dr. P Jayaraman, Director, Plant Anatomy Research Center, Chennai. Dried plant sample was allowed to interact with boiling water to get the aqueous extract. The filtered contents are then stored in 4°C.

2.2. Synthesis of Silver Nanoparticle

The nano synthesis from metal halide was initiated by allowing the plant extract to reduce silver nitrate to silver nanomaterial in dark for 24 h. The formation of the nanomaterial was noted by the change in the colour of the solution to brown from a pale yellow. The particles were procured by centrifugation and lyophilised to be stored in 4°C [16].

2.3. Characterization of Silver Nanoparticles

Spectral data were obtained by subjecting the nanoparticle for instrument analysis to note the absorbance in UV-Visible spectroscopy (UV – 1800 Shimadzu, Japan), transmittance in FTIR (Alpha Bruker optic GmbH instrument) for the range 4000-400 cm⁻¹, size of the particle from Scanning Electron Microscopy (Zeiss, GeminiSem) and elemental makeup in EDX spectrometry, crystallinity in X-ray diffraction (XRD) unit for 20°– 80°at 2θ angles.

2.4. Interaction Studies carried out on Microorganisms

The response of the microorganism in a nano surrounding was tested on *Pseudomonas aeruginosa* and *Bacillus subtilis* for the concentration 2 µg/mL to 16 µg/mL and the results compared to Erythromycin [17]. The Minimum inhibitory concentration of the silver nanoparticle was undertaken through micro dilution method with the concentration range

(0.25 to 2 $\mu\text{g/mL}$). The interaction of the nanoparticle in the motility of the microorganism was assessed by Swarming motility assay for the concentration (0.5 $\mu\text{g/mL}$)[18]. Further the nanoparticle contact interaction on the microbes were tested by Protein leakage assay for *Pseudomonas aeruginosa* and *Bacillus subtilis*[19].

3. RESULTS AND DISCUSSION

3.1. Characterization of Silver Nanoparticles

The initiation of metallic silver formation from silver nitrate solution by reduction process was accounted by the change in colour of the solution to shady brown colour after being in contact with the plant extract for 24 h. The formation of silver nanoparticles was characterized by exposing to UV – visible spectrometry which showed the spectral peak between 250 nm - 400 nm (Fig. 1) [20]. The FTIR analysis of the sample showed peaks at 3270 cm^{-1} indicating the presence of O-H stretching, peaks at 2623 cm^{-1} depicting C-H stretching. Peak at 1044 cm^{-1} indicating C-N amines and N=O symmetry for a peak at 1358 cm^{-1} (Fig. 2) [21]. The nanoparticles when exposed to the scanning electron microscopy highlighted the particles in the size range 30 – 60 nm (Fig. 3). The elemental silver confirmation was done in EDX analysis which gave a peak at 3keV (Fig. 4). Diffraction points of 38°, 44.5°, 64.81°, 77.43° corresponding to (111), (200), (220), (311) planes confirmed the crystalline structures in XRD (Fig. 5) analysis [22].

Figure 1 UV Visible spectroscopy of crude silver nanoparticles

Figure 2 Fourier Transform InfraRed (FT-IR) spectroscopy of crude silver nanoparticles

Figure 3 Scanning Electron Microscopy (SEM) of crude silver nanoparticles

Figure 4 Energy Dispersive X-Ray spectroscopy (EDX) of crude silver nanoparticles

Figure 5 X-Ray Powder Diffraction (XRD) of crude silver nanoparticles

3.2. Antibacterial Activity of Silver Nanoparticles

The ability of the silver nanoparticles to inhibit the growth of both Gram-positive (*Bacillus subtilis*), and Gram-negative (*Pseudomonas aeruginosa*) bacteria is shown in (Fig 6). *Pseudomonas aeruginosa* had a clear zone of inhibition for all the study concentrations with the highest of 1.4cm for 16 μ g. (Table 1). *Bacillus subtilis* had 1.4 cm for 16 μ g. The minimum concentration to inhibit *Pseudomonas aeruginosa* was 0.5 μ g whereas it was less than 0.25 μ g for *Bacillus subtilis* (Table 2). These studies on the antibacterial effect could be appropriately used in industrial applications, therapeutic formulations and environmental remediation studies [23, 24].

Figure 6 Antibacterial activity of crude silver nanoparticles against a) *Pseudomonas aeruginosa*
b) *Bacillus subtilis*

Table 1: Antibacterial activity of crude silver nanoparticles against the micro organisms

Concentration (μg)	Zone of Inhibition (cm)	
	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
2	0.9	1.2
4	1.0	1.3
8	1.0	1.2
16	1.4	1.4
Positive control (Erythromycin)	1.0	1.6
Negative control (Solvent)	-	-

Table 2: Minimum Inhibitory Concentration (MIC) of crude silver nanoparticle

S.No	Bacterial species	Concentration ($\mu\text{g/mL}$)			
		2	1	0.5	0.25
1	<i>Bacillus subtilis</i>	+	+	+	+
2	<i>Pseudomonas aeruginosa</i>	+	+	+	-

3.3. Swarming Motility and Protein Leakage Assay of Silver Nanoparticles

The interaction of the nanoparticle onto the membrane of the organisms was analysed by protein leakage assay (Fig 7). The nanoparticle initially did not destabilise the membrane of *Pseudomonas aeruginosa* but upon 12 h, the particles interferes with the organism resulting in seepage of cellular contents which is evident from the protein assay. The nanoparticle did not have much effect on *Bacillus subtilis*. However, the silver nanoparticles arrested the motility of both *Pseudomonas aeruginosa* and *Bacillus subtilis*. There are several reports for the efficient antibacterial activity of silver nanoparticles against bacteria[25-29].

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(a)

(b)

Figure 7 Protein leakage assay of crude silver nanoparticles a) *Pseudomonas aeruginosa*
b) *Bacillus subtilis*

(a)

(b)

Figure 8 Swarming motility of crude silver nanoparticle against a) *Pseudomonas aeruginosa*
b) *Bacillus subtilis*

4. CONCLUSION

The silver nano particles produced from the aqueous extracts of *Wrightia tinctoria* were crystalline particles having the size of 30-60nm. The nanoparticles were effective against both Gram positive and Gram negative microorganisms and significantly interfered with the motility of the microbes. With more studies on the safe dosage and administration, these particles can be used as an effective antimicrobial agent.

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All the authors have made direct contribution on idea creation and research work. All the authors are involved in manuscript preparation.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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