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Synthesis of silver nanoparticles using aqueous extract of *Dolichos lablab* for reduction of 4-Nitrophenol, antimicrobial and anticancer activities

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ABSTRACT

In this paper, we report for the first time an eco-friendly method for the preparation of silver nanoparticles (Ag NPs) using an aqueous leaf extract of *Dolichos lablab* as reducing and capping agents using UV–vis, FT-IR, XRD, FE-SEM, EDX and TEM. The crystallinity, phase purity, size and morphology of Ag NPs were investigated by UV–vis, FT-IR and XRD. The spectroscopic and microscopic results revealed the formation of Ag NPs. The morphology of Ag NPs is found to be spherical with an average diameter of 9 nm. As prepared Ag NPs effectively reduced the 4-Nitrophenol to 4-Aminophenol in the presence of $NaBH_4$. The Ag NPs showed potent antimicrobial activity against *Escherichia coli* and *Bacillus subtilis*. Ag NPs also exhibited anticancer activity against human liver cancer (Hep G2) cell line.

1. Background

During the past decade, nanomaterials have overwhelmed attention to researchers due to their potential technological applications such as commercial goods: cosmetics, water treatment, sensors, biomedical, energy, drug and gene delivery, and bioimaging devices [1–4]. More recently, metal nanoparticles have received great attention for many technological applications due to their small size and unique physical and chemical properties. Among all metal nanoparticles, Ag NPs have emerged as potential material due to their applications in catalysis, antimicrobial, antifungal, anticancer, and water treatment [5–7]. In general, Ag NPs have been prepared by various chemical and physical methods, such as, chemical reduction [8], photochemical reduction [9], electrochemical reduction [10], and heat evaporation [11]. However, all these methods employ toxic reducing and capping agents for preparation of aggregated free Ag NPs, which limits their technological biomedical applications [12]. In order to overcome the limitation, Ag NPs have been prepared via green synthesis route using green precursors such as plants [1], fungi [13], bacteria [14], actinomycetes [15], yeasts and algae [16]. Among, all green synthesis methods, plant mediated synthesis has emerged as a potential route for synthesis of

Abbreviations: DMEM, Dulbecco's modified eagle's medium; EDX, energy dispersive spectroscopy; FBS, fetal bovine serum; FE–SEM, field emission scanning electron microscopy; FT–IR, Fourier transform infrared; Hep G2, human liver cancer; IC_{50}, half maximal inhibitory concentration; MIC, minimum inhibitory concentration; NCCLS, National Committee on Clinical Laboratory Standards; NP, Nitrophenol; OD, Optical diffraction; RPM, Rounds per minute; SAED, Selected area electron diffraction; TEM, Transmission electron microscopy; UV–vis, Ultraviolet visible; XRD, X-ray diffraction

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nanomaterial due to the ease of scale up, cost-effective, and safe for human therapeutic applications. However, in microbes assisted green synthesis, it is very difficult to maintain cell cultures at aseptic conditions, the biohazards and elaborative process. Moreover, the phytoconstituents present in plants such as proteins/enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins could be act as reducing and capping agent for preparation of aggregated free and smaller size metal nanoparticles [16,17]. Biosynthesis of metal nanoparticle is an environmentally friendly method without the use of harsh, toxic, high temperature, pressure and expensive chemicals [18]. In plant mediated synthesis, the phytoconstituients present in plant such as proteins/enzymes, amino acids, polysaccharides, alkaloids, alcoholic compounds, and vitamins acts as reducing, stabilizing and capping agents for formation of aggregate free metal nanoparticle [16,19]. Numerous plants extracts have been employed for synthesis of Ag NPs including *Punica granatum* L [20], *Syzygium alternifolium* [21], *Aegle marmelos* [22], and *Medicago sativa* [23]. Based on literature review made, there were no reports for synthesis of Ag NPs using an aqueous leaf extract of *Dolichos lablab*.

Recently, Ag NPs have emerged as potential antimicrobial agents and a broad-spectrum against different fungal agents [24,25]. Bagherzade et al. had reported that biosynthesized Ag NPs using *Crocus sativus* L. wastages showed significant antibacterial effect against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella flexneri* and *Bacillus subtilis* [26]. Vivek et al. [27] synthesized spherical shaped with 20–100 nm sized Ag NPs using leaf extract of *Annona squamosa* for treatment of human breast cancer cell (MCF-7) IC_{50} of 50 ¼g/mL after 24 h. Similarly, Jacob et al. [28] reported green synthesis of spherical Ag NPs with 17.6–41 nm in size using 1 mM $AgNO_3$ solution and leaf extract of *Piper longum* as reducing and capping agents that had exhibited excellent cytotoxic effect on Hep-2 cells.

Nitrophenols have emerged as major water pollutant due to its toxic nature, non biodegradable, and other health problems for both aquatic and human [29]. The best approach to removal of 4-Nitrophenol from water is catalytic reduction into non toxic 4-aminophenol, which is also starting materials for various drugs [30,31]. In this paper, we investigated synthesis, and applications of Ag NPs by using leaf extract of *Dolichos lablab* for reduction of 4-Nitrophenol, antimicrobial and anticancer activities.

2. Materials and methods

2.1. Materials

Analytical grade $AgNO_3$ (99.9% purity), NH_3 solution (28–30%) and NaOH pellets were purchased from Merck, India and used without further purification. *Escherichia coli, Bacillus subtilis* and Hep G2 cell line, MTT assay, DMEM, FBS and DMSO were received from Sigma–Aldrich. The leaves of *Dolichos lablab* L. were collected from Andhra University campus, Visakhapatnam. The plant was authenticated by Dr. S. B. Padal, Voucher specimen number - AU (AUH) 22232, in Andhra University Herbarium, Botany Department, Andhra University, Andhra Pradesh, India.

2.2. Preparation of aqueous leaf extract of Dolichos lablab

The leaves were washed with double distilled water to remove dust particles and were shed dried at ambient temperature for 15 days. Finally, the dried leaves were milled into powder using a mixer grinder. The powder was allowed to pass through 2 mm sieve mesh and was preserved in an airtight polyethylene plastic bag at 4–10 °C for further use. 1 g of *Dolichos lablab* leaf powder was placed into 250 mL Erlenmeyer flask containing 100 mL double distilled water and heated at 70 °C for 20 min and then allowed to cool to room temperature, filtered with Whatman no. 42 filter paper and the resultant extract was stored in a refrigerator at 4 °C for further use.

2.3. Synthesis of silver nanoparticles

Ag NPs was synthesized using leaf extract of *Dolichos lablab* as reducing and capping agents. 1:5 ratio of leaf extract of *Dolichos lablab* and 1 mM $AgNO_3$ solution were added in 250 mL round bottomed flask, under sonication for 15 min. Then, 10 mL (28–30%) of NH_3 solution was added to the reaction mixture and then the reaction mixture was refluxed for 1 h under constant magnetic stirring. The color of the reaction medium was turned to amber coloured solution, confirms formation of Ag NPs, **Figure S1**† (Supplementary file) [32]. As formed Ag NPs was centrifuged and washed with double distilled water three times to remove any unreacted plant materials.

2.4. Optimization parameters of green synthesis of silver nanoparticles

In order to prepare size control, aggregate free Ag NPs, the reaction was carried at different experimental parameters such as concentration of $AgNO_3$ (1, 3, 5, 7, 10 mM), concentration of plant extract (1%, 3%, 5%), volume of plant extract (1–6 mL), and temperature (50, 60, 70, 80 °C). As a final point, the optimized Ag NPs was synthesized with 1 mM $AgNO_3$, 1% (w/v) plant extract, 1 ml volume of plant extract, 80 °C, and 1 h reaction time.

2.5. Characterization of silver nanoparticles

The optical property of Ag NPs was analyzed using UV–vis (spectrophotometer UV–2600 SHIMADZU). Fourier transform infrared (FT–IR) spectra was recorded using SHIMADZU-IR PRESTIGE – 2 spectrometer. The X-ray diffraction (XRD) measurement was