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Extracellular biosynthesis of silver nanoparticles using *Cassia fistula* extract and *in-vitro* antimicrobial studies

P. Balashanmugam[1*], R. Caral Dinesh[2], V. Manivasagan[2] and N.G. Ramesh Babu[2], P.T. Kalaichelvan[1]
[1]Centre for Advanced Studies in Botany, University of Madras, Chennai, India - 600025.
[2]Department of Biotechnology, Adhiyamaan college of Enginering, Hosur, India – 635109.

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ABSTRACT

The synthesis of extracellular silver nanoparticles by biological method is one of the easier and convenient than that of the physical or chemical method of synthesis. The silver nanoparticles are synthesized using the dried plant leaves of *Cassia fistula* treated with silver nitrate solution. Silver nanoparticles are synthesized due to the reduction of the silver ions. The synthesized silver nanoparticles are characterized in different ways such as UV-Visible Spectrophotometer, XRD analysis, EDX analysis, HR-TEM analysis. The characterized silver nanoparticles are studied for their antibacterial and antifungal activities against different pathogenic microorganisms. From the antimicrobial study, it has been found that the synthesized silver nanoparticles are susceptible to *Pseudomonas aeruginosa* and *Curvularia lipolytica* which shows maximum zone of inhibition. The plant material mediated synthesis of silver nanoparticles is comparatively faster and less expensive and has wide application in antibacterial and antifungal therapy in modern medicine.

KEY WORDS: *Cassia fistula,* Biosynthesis, Silver nanoparticles, Anti-microbial activity

INTRODUCTION

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology [1]. The synthesis of silver nanomaterials or nanoparticles is extensively studied by using chemical and physical methods, and the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology [2]. Biological synthesis provides a wide range of environmentally acceptable methodologies with low cost of production and less time [3].

Nanosized inorganic particles, of either simple or composite nature, display unique physical and chemical properties and represent an increasingly important material in the development of novel nanodevices which can be used in numerous physical, biological, biomedical and pharmaceutical applications [4]. A number of recent advances offer the possibility of generating new types of nanostructured materials with designed surface and structural properties [5] .Resistance of bacteria to bactericides and antibiotics has increased in recent years [6]. Some antimicrobial agents are extreme irritants and toxic and there is much interest in finding ways to formulate new types of safe and cost-effective biocidal materials [7]. Nanoparticles are defined as particulate dispersions or solid particles ranging from 10-1000 nm [8]. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained [9]. Nanocapsules are systems in which the drug is confined to a cavity surrounded by unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed [10]. In recent years, biodegradable polymeric nanoparticles, have been used as potential drug delivery devices [11].

***Corresponding author.**
P. Balashanmugam
Centre for Advanced Studies in Botany,
University of Madras,
Chennai- 600025, India .

Cassia fistula Linn (Cassia) family Caesalpiniaceae commonly known as Amulthus and in English popularly called “Indian Laburnum” has been extensively used in Ayurvedic system of medicine for various ailments. It is widely used in traditional Indian medicial systems and has been reported to possess hepatoprotective, antiinflammatory, antifungal and also wound healing and antibacterial properties. It is known as a rich source of tannins, flavanoids and glycosides. The innumerable medicinal properties and therapeutic uses of *C.fistula* as well as its phytochemical investigations prove its importance as a valuable medicinal plant [12]. *C.fistula*, has a high therapeutic value and it exerts an antipyretic and analgesic effect. Besides, it has been found to exhibit antiinflammatory and hypoglycaemic activity. In Indian literature, this plant has been described to be useful against skin diseases, liver diseases and in the treatment of haematemesis, pruritus, leucoderm and diabetes [13].

Biosynthetic methods have been investigated as an alternative to chemical and physical ones [14]. The synthesized nanoparticles are characterized by different methods such as UV-Vis Spectroscopy, High-resolution transmission electron microscope (HR-TEM) analysis [15], Energy Dispersive X-ray Spectroscopy (EDX) and X-ray diffraction analysis (XRD).

MATERIALS AND METHODS

Preparation of the extract

Cassia fistula leaves were collected from Centre for advanced Studies [CAS] in Botany, University of Madras, Guindy, Chennai. 100 ml of double distilled water was added to 4g of dried *C. fistula* leaves. The mixture was boiled for about 15mins, cooled and filtered through Whatman No.1 filter paper. The extract was stored in a refrigerator.

Synthesis of silver nanoparticles

1 mM of silver nitrate solution was prepared. 9 ml of the aqueous filtrate extract of *Cassia fistula* was mixed with 1ml of 1 mM silver nitrate solution. The reaction mixture was kept in dark room for 12 hrs.

Characterization of silver nanoparticles

The silver nanoparticles were characterized by using UV-Vis Spectrophotometer Hitachi U–2900 for the conformation of the reduced silver ions by the plant extract. The morphological analysis of the particle was done with High resolution transmission electron microscopy (HR-TEM). The HR-TEM micrograph image were recorded on T-30, S TWIN instrument on carbon coated copper grids. The particles were collected in powdered form and used for EDX analysis. The formation and quality of the compounds were checked by XRD (X- Ray diffraction) spectrum.

Antibacterial activity

Antibacterial activity of the synthesized silver nanoparticles was determined using the agar well diffusion method. The antibacterial activity of silver nanoparticles was observed against *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and *Klebsiella pneumoniae.* The nutrient agar medium was poured into petriplate and allowed to solidify. Fresh bacterial cultures were spread and wells were made on the solidified agar. The wells were loaded separately with silver nitrate solution, silver nanoparticles, plant extract and the positive control (Streptomycin). The plates were incubated at 37ºC for 24 hours and observed for the zone of inhibition.

Antifungal activity

The antifungal activity of synthesized silver nanoparticles was determined using pathogenic microorganisms such as *Aspergillus niger, Aspergillus flavous, Curvularia liopolytica.* These microorganisms were swabbed on the sterile Potato Dextrose Agar plates. The wells were filled with silver nitrate solution, silver nanoparticles, plant extract and the positive control (Griesofluvin) separately. The plates were incubated at 37ºC for 72 hrs and observed for the zone of inhibition.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles

The reduction of silver nitrate into silver nanoparticles was observed by the color change from light yellow to dark brown after the incubation of 12 hours under dark conditions at room temperature (Fig 1). This resulted due to the surface plasmon resonance phenomenon.

Fig 1. a) *Cassia fistula* aqueous extract b) Synthesized silver nanoparticles from *Cassia fistula*

UV-Visible spectroscopy

The formation and stability of silver nanoparticles was monitored on UV-Visible spectrophotometer. The UV-Vis spectrum showed surface plasmon resonance (SPR) peak bands centered at 440 nm [16] .In this study, UV- Vis Spectra were recorded for the plant extract of *C.fistula* treated with silver nitrate after 12 hrs of incubation. The synthesized silver nanoparticles exhibit a strong peak at 435 nm indicating the formation of silver nanoparticles in the reaction mixture (Fig 2).

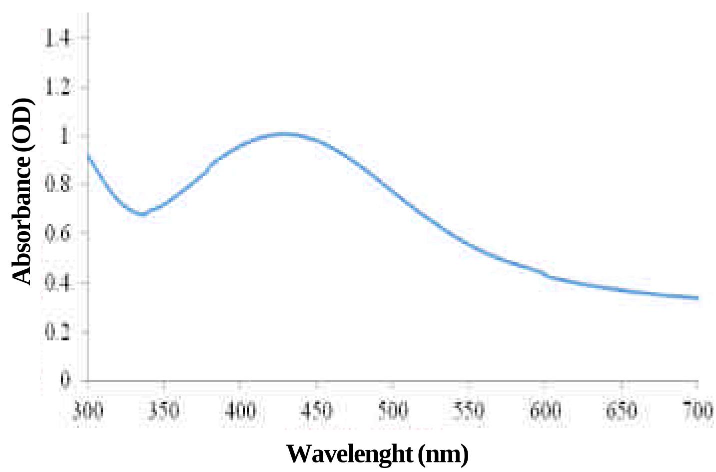


Fig 2.UV-Vis Spectra of Synthesized silver nanoparticles from *Cassia fistula* extract

HR-TEM analysis

The biologically synthesized silver nanoparticles using the plant extract of *Cassia fistula* were confirmed by High-resolution transmission electron microscope. HR TEM is carried out for the structural morphology and crystallinity. The aliquot of silver nanoparticles solution was placed in a drop coated grid and the sample was allowed to dry. The HR-TEM images were recorded at different magnification to find the individual particles. The synthesized silver nanoparticles were spherical and the average size was found to be 20 nm (Fig 3).

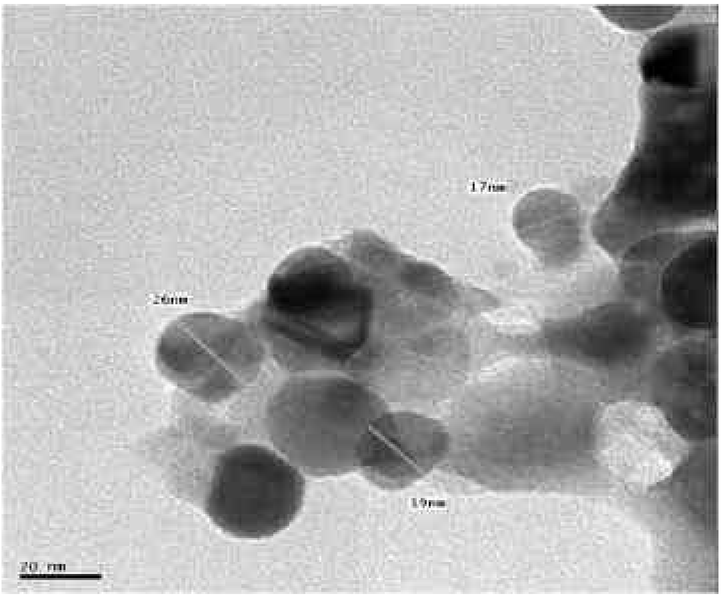


Fig 3. HR-TEM image of the synthesized silver nanoparticles

EDX analysis

Energy Dispersive X-ray Spectroscopy is an analytical technique used for the analysis of elements. EDX analysis is done for the further characterization of the synthesized silver nanoparticles. The presence of silver nanoparticles was confirmed by EDX analysis and mostly showed strong signal energy peaks for silver atoms in the range 2- 4 KeV [17]. (Fig 4).

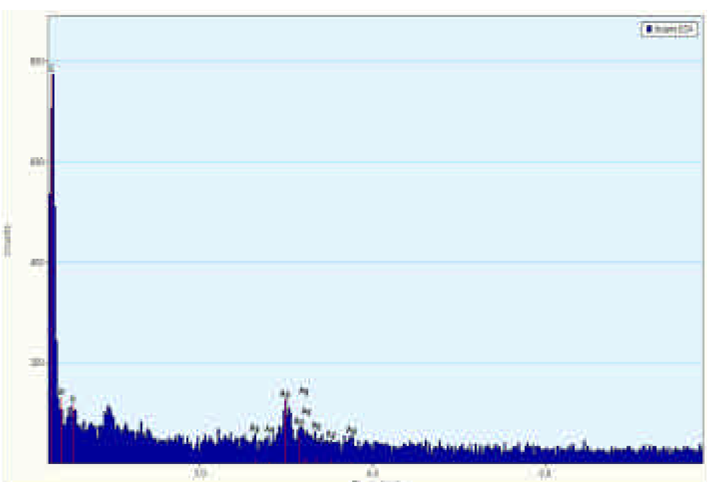


Fig 4: EDX analysis of the synthesized silver nanoparticles

XRD analysis

XRD investigates crystalline material structure, including atomic arrangement, crystalline size and imperfections. XRD clearly illustrates that silver nanoparticles synthesized from *Prosopis juliflora* leaf are crystalline in nature [18]. The X – ray diffraction pattern of silver nanoparticles produced by the extract of *Cassia fistula* exhibit intense peak at 38.3^0, 44.2^0, 64.5^0 in the whole spectrum of 2θ value ranging from 20 to 70 and the lattice plane was observed at 111, 200, and 220 respectively. XRD clearly illustrates that silver nanoparticles are crystalline in nature, and the size of the silver nanoparticles were found to be 20 nm (Fig 5).

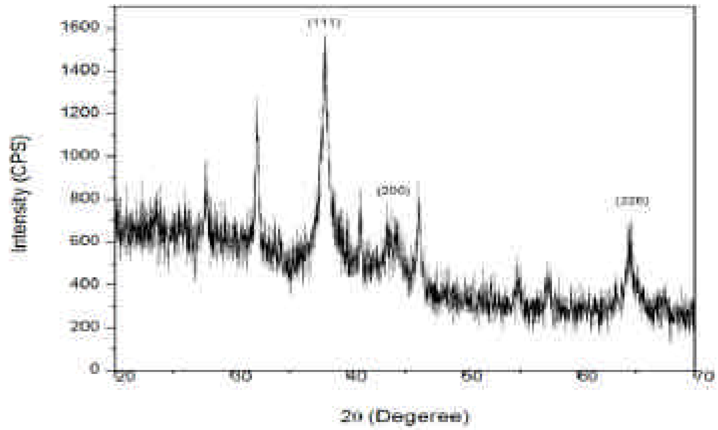


Fig 5: XRD pattern of synthesized silver nanoparticles

Antibacterial activity of Silver nanoparticles

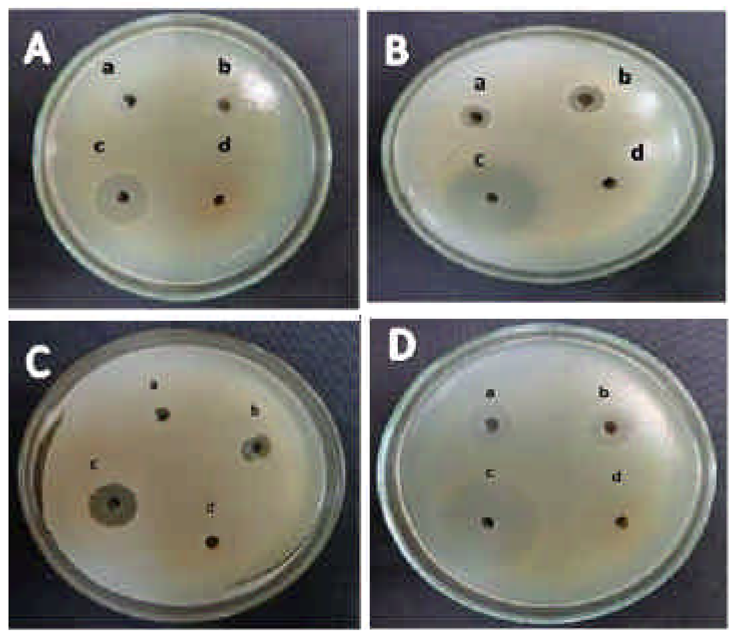


Fig 6. Antibacterial activity of synthesized silver nanoparticles A) *Klebsiella pneumoniae* B) *Staphylococcus aureus* C) *Bacillus subtilis* D) *Pseudomonas aeruginosa*

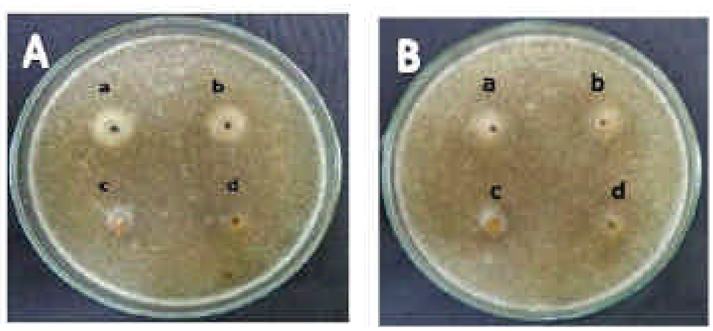
(a) – Silver nitrate, (b) – Silver nanoparticles, (c) – Positive control (Streptomycin), (d) – Plant extract

Table 1 Antibacterial activity of Silver nanoparticles

Bacteria	Zone of Inhibition in Diameter (mm)			
	Silver nanoparticles	Silver nitrate	Positive control	Plant extract
Pseudomonas aeruginosa	15	12	28	Nil
Staphylococcus aureus	11	9	27	Nil
Bacillus subtilis	8	5	15	Nil
Klebsiella pneumoniae	10	8	18	Nil

In this study, *Pseudomonas aeruginosa* showed maximum inhibition at 15 mm (Fig 6.D) and *Bacillus subtilis* showed minimum inhibition of 8 mm (Fig 6.C). *Staphylococcus aureus* showed inhibition at 11 mm (Fig 6.B) and *Klebsiella pneumoniae* showed inhibition at 10 mm (Fig 6.A). The plant extract shows no inhibition in all pathogenic bacteria whereas the silver nitrate showed maximum inhibition growth in *Pseudomonas aeruginosa* at 12 nm and in positive control (Streptomycin) showed 28 mm (Table 1). [19].

Antifungal activity of Silver nanoparticles



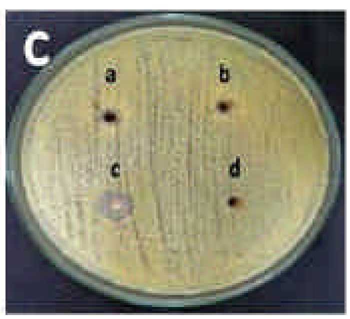


Fig 7
A) *Curvularia lipolytica*
B) *Aspergillus niger*
C) *Aspergillus flavous*
(a) – Silver nanoparticles,
(b)–Positive control (Griesofluvin)
(c) – Silver nitrate,
(d) – Plant extract

Table 2 Antifungal activity of silver nanoparticles

Fungal Species	Zone of Inhibition in Diameter (mm)			
	Silver nanoparticle	Silver nitrate	Positive Control	Plant Extract
Curvularia lipolytica	12	7	16	Nil
Aspergillus niger	10	7	12	6
Aspergillus flavous	8	10	8	Nil

Silver nanoparticles showed maximum inhibition of 12 mm in *Curvularia lipolytica* (Fig 7.A) and minimum of 10 mm inhibition in *Aspergillus niger* (Fig 7.B), where *Aspergillus flavous* showed inhibition at 8 mm (Fig 7.C) Silver nitrate showed maximum growth inhibition in *Aspergillus flavous* at 10 mm where as the plant extract showed no zone of inhibition except *Aspergillus niger* showed 6 mm. The positive control (Griesofluvin) showed the maximum inhibition of 16 mm for *Curvularia lipolytica*.

CONCLUSION

From the current study, it was found that synthesized silver nanoparticles showed zone of inhibition of various pathogenic organisms. The biological synthesis of silver nanoparticles is the most convenient method when compared to the physical and chemical method of synthesis. Further, the biological approach appears to be cost efficient and is suitable for developing a biological process for large-scale production. In future, silver nanoparticles can be used in medicine for cancer treatment, drug delivery and sensors.

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