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Phytogenic synthesis of silver nanoparticles, optimization and evaluation of *in vitro* antifungal activity against human and plant pathogens

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ABSTRACT

An attempt was made to synthesis of biocompatible silver nanoparticles from ten different *Cassia* spp. Among them, *Cassia roxburghii* aqueous leaf extract supported the synthesis of highly efficient and stable AgNPs. The synthesis of AgNPs was optimized at different physico-chemical condition and highly stable AgNPs were synthesized with 1.0 mL of *C. roxburghii* leaf extract, pH 7.0, 1.0 mM $AgNO_3$ and at 37 °C. The synthesized AgNPs were characterized by XPS, DLS and ZETA potential. DLS and ZETA potential analysis, the average AgNPs size was 35 nm and the zeta potential was −18.3 mV. The AgNPs exhibit higher antifungal activity when compared with the conventional antifungal drug amphotericin B against all the tested human fungal pathogens such as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium* sp., *Candida albicans* and the plant pathogens such as *Rhizoctonia solani*, *Fusarium oxysporum* and *Curvularia* sp. Scanning electron microscope (SEM) analysis showed distinct structural changes in the cell membranes of *C. albicans* upon AgNPs treatment. These results suggest that phytosynthesized AgNPs could be used as effective growth inhibitors in controlling various human and plant diseases caused by fungi.

1. Introduction

Nanotechnology is a multidisciplinary field which has currently advanced avenues in various fields such as engineering, physics, chemistry and biology (Wilson et al., 2002). The faith of nanotechnology is to derive particles of nanoscale which are further called nanoparticles. Silver nanoparticles (AgNPs) are a metal which constantly exhibits adverse effects on microbes such as inhibition and to the extent of inactivation (Nasrollahi et al., 2011). The AgNPs synthesized by using various biological sources such as herbs, plants and biological organisms display excellent properties in various fields such as non-linear optics and intercalation materials for electrical batteries, as optical receptors, catalysis in chemical reactions, biolabelling and as antibacterials (Sanghi and Verma, 2009). In recent years, plant mediated synthesis of nanoparticles is gaining importance as this method is facile and rapid (Sathishkumar et al., 2012). The preparation of AgNPs should consider imperative parameters such as uniform size and shape for self assembly mechanism and physio-chemical properties. The AgNPs with high surface stability deliver the drug or an artificial protein to target cells that can be lethal to cells. In earlier studies AgNPs were reported to exhibit good antifungal activity (Fayaz et al., 2009; Madhu Gupta et al., 2012). *Aspergillus* sp. and *Candida* sp. are the major rational species for mycosis infection (Chandan Singh et al., 2012).

The growth of phytopathogenic fungi are controlled by synthetic fungicides; however, the uses of these are increasingly restricted due to their harmful effects on human health and the environment (Harris et al., 2001). The phytopathogenic fungi such as *R. solani* and *F. oxysporum* are known to cause the disease and attribute to a loss of 5–60% yield (Mishra, 2006). The increasing demand of production and regulations on the use of agrochemicals and the emergence of resistant pathogen to the products employed, justifies the search for novel active molecules and new control strategies.

Very recently, in our laboratory, AgNPs were synthesized using *C. roxburghii*. These nanoparticles have shown better antibacterial activity against human pathogenic bacteria (Balashanmugam, 2015; Balashanmugam and Kalaichelvan, 2015). Hence, in this

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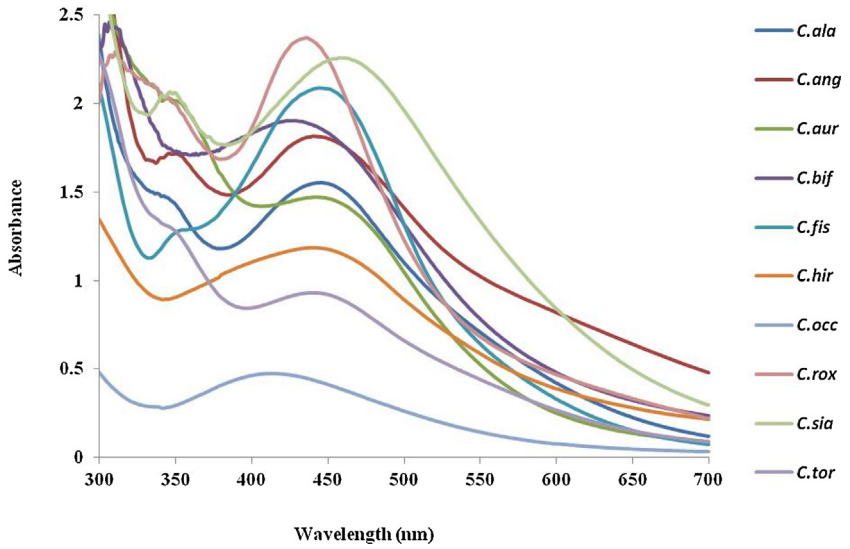

Fig. 1. UV–vis spectrum of AgNPs synthesized from *Cassia* sp.

Table 1
UV–vis absorption peak of phytosynthesized AgNPs from different *Cassia* sp.

S. no	Plant name	Wavelength (nm) AgNPs
1	*Cassia alata* L.	445
2	*Cassia angustifolia* L.	440
3	*Cassia auriculata* L.	450
4	*Cassia biflora* L.	425
5	*Cassia fistula* L.	445
6	*Cassia hirsute* L.	440
7	*Cassia occidentalis* L.	415
8	*Cassia roxburghii* DC.	430
9	*Cassia siamea* L.	455
10	*Cassia tora* L.	445

study, we aimed to evaluate the potential of phytosynthesized AgNPs against different human and plant pathogenic fungi.

2. Materials and methods

2.1. Chemicals

All the analytical grade chemicals were purchased from the Sigma-Aldrich Co. (St Louis, MO, USA). Amphotericin B and Potato Dextrose Agar (PDA) medium were procured from the Hi-media Lab Pvt. Ltd Mumbai, India. The human pathogenic fungi such as *A. niger*, *A. fumigatus*, *A. flavus*, *Penicillium* sp. and *C. albicans*, and phytopathogenic fungi such as *R. solani*, *F. oxysporum* and *Curvularia* sp. were obtained from the Fungal Culture Collection Facility at the Centre for Advanced Studies in Botany, University of Madras, Chennai.

2.2. Collection of experimental plants

Ten different healthy and matured leaf samples of *Cassia* sp. such as *C. alata* L., (*C.ala*) *C. angustifolia* L., (*C.ang*) *C. auriculata* L. (*C.aur*) *C. biflora* L. (*C.bif*) *C. fistula* L., (*C.fis*) *C. hirsute* L., (*C.hir*) *C. occidentalis* L.,(*C.occ*) *C. roxburghii* DC.,(*C.rox*) *C. siamea* L.,(*C.sia*) and *C. tora* L. (*C.tor*) were chosen in the present study to synthesize AgNPs. The leaf samples of *C. auriculata* L. and *C. biflora* L. were collected from the Pachaiyappa's College, Chennai and rest of the samples were collected in and around, Guindy campus, University of Madras, Chennai, in January 2012.

2.3. Preparation of plant extract

Four gram of the ten different *Cassia* sp. leaf powder samples was taken separately and mixed with 100 mL of glass distilled water. The extraction procedure was followed as mentioned in the earlier study (Balashanmugam and Kalaichelvan, 2015).

2.4. Screening of Cassia sp. for phytosynthesis of AgNPs

Silver nanoparticles screened from 10 different leaf samples of *Cassia* sp. One mL of the leaf aqueous extract was taken and added to 9.0 mL of 1.0 mM $AgNO_3$. The reaction was performed in dark condition at room temperature for 24 h in a static condition and recorded for any change in colour (Balashanmugam and Kalaichelvan, 2015). The synthesized AgNPs were analyzed under UV–vis spectrophotometer (HITACHI U-2900) from 300 nm to 700 nm. In addition, the samples were visualized and recorded for any precipitation or agglomeration of nanoparticles. Among the samples tested, the AgNPs synthesized with *C. roxburghii* showed good stability than the other tested species. Therefore, *C. roxburghii* leaf extract were chosen for further studies.

2.5. Qualitative phytochemical analysis

Qualitative tests were performed to establish the phytochemical of aqueous leaf extract of *C. roxburghii*. Alkaloids (Evans, 1997), carbohydrates (Ramakrishnan et al., 1994), glycosides (Evans, 1997), saponins (Kokate, 1999), phenolic (Evans, 1997), proteins (Gahan, 1984) and aminoacids (Yasuma and Ichikawa, 1953), were detected by standard methods.

2.6. Optimization of phytosynthesis of AgNPs

2.6.1. Effect of different pH

To 1.0 ml of *C. roxburghii* leaf aqueous extract 9.0 mL of 1 mM $AgNO_3$ was added and maintained at different pH (4, 5, 6, 7, 8, 9 and 10) at 37 °C. The absorbance of the resulting solutions was measured between 300 and 700 nm using HITACHI U-2900 spectrophotometer after 24 h. The pH 7.0 showed high synthesize and good stability of AgNPs. Therefore this pH was chosen for further studies.