Journal of Genetic Engineering and Biotechnology xxx (2018) xxx–xxx

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Contents lists available at ScienceDirect

Journal of Genetic Engineering and Biotechnology

journal homepage: www.elsevier.com/locate/jgeb

Original Article

Biosynthesis of silver nanoparticles formation from *Caesalpinia pulcherrima* stem metabolites and their broad spectrum biological activities

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ARTICLE INFO

Article history:
Received 5 November 2017
Received in revised form 21 December 2017
Accepted 29 December 2017
Available online xxxx

Keywords:
Silver nanoparticles
Characterization
Antibiofilm activity
Antioxidant activity
Cytotoxicity
Genotoxicity

ABSTRACT

The present work illustrates eco-friendly, rapid and cost effective method of AgNPs synthesis using *C. pulcherrima* stem extract. Initially, various physico chemical factors were optimized. Characterization was done by different spectroscopic and microscopic analysis. AgNPs were spherical in shape with an average size of 8 nm. AgNPs showed good synergistic antimicrobial, antibiofilm and antioxidant activity. The cytotoxicity effect against HeLa cancer cell line was dose dependent while genotoxic study revealed the non toxic nature of AgNPs at lower concentration. The results suggest that AgNPs from *C. pulcherrima* stem extract have great potential in biomedical applications.

1. Introduction

Nanoparticles, especially metal nanoparticles (silver, gold, copper, zinc, titanium, magnesium) are being applied in numerous fields because of their unique properties. They possess properties entirely different from the bulk metal from which they are synthesized. Their application includes diagnosis, wound healing, drug delivery, molecular imaging, water treatment, catalysis, cosmetics, clothing, food industry, sunscreens, etc. They also possess properties like antiviral, antimicrobial, antioxidant, anticancer, antidiabetic, analgesic, antidandruff, anticoagulant, antiinflammatory, antihelmintic, antiproliferative activities and also show properties like antigenotoxic, cytotoxic effect, etc. [13,11].

Today mankind is faced with two grave problems for which the cure is obscure; multidrug resistant microorganisms responsible for infectious diseases and oxidative stress generated free radicals responsible for innumerable diseases and disorders. The occurrence of cancer is also increasing steadily. Misuse or overuse of antibiotics has led to the development of resistance in the microorganisms and even second line of treatment has become questionable [20]. The cells have antioxidant mechanism to overcome the free radical generation but when this balance is shaken with over production of free radicals and reactive oxygen species, stress condition occurs which leads to many diseases and disorders [16]. Drugs are available to treat any of this pathological condition but their use is being questioned because of many disadvantages they pose like side effects, harmful nature, low efficiency, etc. Hence the need of the hour is new entities with novel mechanism of action. One therapy was the use of natural compounds or medicinal plant extracts, which proved quite successful but synthesis of metal nanoparticles using plant extracts is a novel approach to tackle the infectious disease causing microorganisms or oxidative stress related diseases or cancer [44].

Synthesis of metal nanoparticles using plant extracts is simple, easy and eco friendly. In general, the metal salt of a particular metal is reacted with plant extract and the metals are reduced to metal nanoparticles with the help of secondary metabolites present in the plant extract which act both as reducing and stabilizing agents. Any part of the plant can be used for the synthesis for eg. leaf, stem, flower, fruit, seed, root, bark, etc. [9].

In the present work, silver nanoparticles are synthesized using *Caesalpinia pulcherrima* stem extract. *Caesalpinia pulcherrima* is an ornamental plant with several medicinal properties and belongs to Caesalpiniaceae family. *C. pulcherrima* flower is known for is antiviral, antimicrobial, antioxidant, analgesic, anti inflammatory, anthelmintic activities [31]. The leaves are reported for

Peer review under responsibility of National Research Center, Egypt.
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https://doi.org/10.1016/j.jgeb.2017.12.003
1687-157X/

antimicrobial, antioxidant, antiulcer properties [23,48,39]. The stem extract is used for antiplasmodial, abortifacient, emmenagogue, cytotoxic activity [26,34].

In the present work, we report biosynthesis of AgNPs using the stem extract of *C. pulcherrima* and its various biological activities (synergistic antimicrobial, antibiofilm, antioxidant, cytotoxic and genotoxic) is reported perhaps for the first time.

2. Materials and methods

The fresh stem of *Caesalpinia pulcherrima* was collected from Rajkot, Gujarat, India. All the chemicals were obtained from Hi Media Laboratories and Sisco Research Laboratories Pvt. Limited, Mumbai, India. Ultra purified water was used for all the experiments. Extract preparation and optimization of different parameters was followed as described earlier [31].

2.1. Characterization and biological activity of synthesized silver nanoparticles

The AgNPs were characterized by FTIR analysis, XRD analysis, Thermogravimetric analysis, TEM analysis. Antimicrobial activity was measured by measuring the MIC and MBC values of AgNPs [36,1], synergistic antimicrobial activity [8,45] and antibiofilm activity [47] against eleven microorganisms. Four Gram positive bacteria (*Bacillus cereus, Staphylococcus aureus, Corynebacterium rubrum, Bacillus subtilis*), four Gram negative bacteria (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhimurium*) and three fungi (*Candida albicans, Candida glabrata, Cryptococcus neoformans*) were used for antimicrobial activity. The antioxidant activity of synthesized AgNPs was measured by five *in vitro* antioxidant assays. The antioxidant assays evaluated were 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH), Superoxide anion radical scavenging assay (SO), 2,2′-Azin o-bis-(3-ethyl)benzothiazoline-6-sulfonic acid radical cation scavenging assay (ABTS), Reducing capacity assessment (RCA), Ferric reducing antioxidant power assay (FRAP). The details of the method followed are as described earlier [10]. Cytotoxicity by the MTT assay and genotoxicity by comet assay [31]. Human cervical cancer cell line (HeLa) were used for MTT assay.

3. Results and discussion

3.1. Optimization of different parameters

Green synthesis of AgNPs, involves addition of plant extract to silver nitrate solution and incubating the reaction mixture for definite time at room temperature. The phytoconstituents present in the plant extract reduce silver to silver nanoparticles. In order to achieve, good AgNPs, it is essential to optimize different procedure parameters involved like boiling time of extract preparation, extract concentration, $AgNO_3$ concentration, pH and incubation time of reaction mixture, etc. These parameters vary with the plant extract and plant part used; thus it is essential to optimize these conditions as also reported by other researchers [50,32]. The first indication of AgNPs formation is the colour change that occurs when plant extract is added to silver nitrate solution due to surface plasmon resonance. In the present work also, initially when 6 ml stem extract was added to 40 ml 1 mM $AgNO_3$ and incubated at room temperature, the colourless solution changed to brown colour indicating the formation of AgNPs (Fig. 1a). Moteriya et al. [33] reported such colour change effect for different plants.

3.2. UV–Visible spectroscopic analysis of AgNPs

UV–Vis spectroscopy is an important tool to study the formation of metal nanoparticles in aqueous medium. The synthesized AgNPs show characteristic absorption maxima in the visible region in the range of 350–750 nm. Further, the peak size and peak intensity clearly indicate the number and size of nanoparticles formed; broader peak indicates larger particle formation and narrow peak indicates smaller size of the particles [51] while the intensity of absorption peak indicates the number of particles formed. In other words, the peak intensity is directly proportional to number of particles formed [43]. This selection criterion was used for optimizing various parameters for synthesizing AgNPs, from stem extract of *C. pulcherrima.*

The first parameter optimized was boiling time of plant extract preparation. The stem extract was boiled for 5, 10 and 15 min. and then 6 ml stem extract was added to 40 ml 1 mM $AgNO_3$. The absorption intensity was higher in 10 min boiled stem extract as compared to 5 and 15 min boiled extract (Fig. 1b). Hence, 10 min boiling time was finalized for the preparation of the stem extract. This is in contrast to AgNPs synthesized using flower extract of the same plant; when flower extract was used for the synthesis of AgNPs, 5 min boiling for extract preparation gave the best results [31].

After confirming the boiling time of extract preparation, the extract concentration was optimized.

The next parameter optimized was by the addition of extract concentration to the reaction mixture. 10 min boiled different extract concentration (1.5, 3, 6, 9 and 12 ml) was added to 40 ml 1 mM $AgNO_3$. In 12 ml extract concentration higher absorbance intensity was observed (Fig. 1c). Absorbance intensity increased with increasing extract concentration because the availability of biomolecules required for the reduction of silver ions to silver nanoparticles is more and results in the formation of more AgNPs. Elavazhagan and Arunachalam [14] used 15 ml extract while [43] used 10 ml extract concentration for AgNP synthesis. Hence, 12 ml extract concentration was finalized for the preparation of AgNPs from the stem extract of *C. pulcherrima*.

The concentration of silver nitrate also has a tremendous effect on the size of synthesized AgNPs. 10 min boiled 12 ml extract concentration was added to 40 ml different concentrations (0.5 mM, 1 mM, 1.5 mM, 2 mM) of $AgNO_3$. 0.5 mM $AgNO_3$ containing reaction mixture did not show any peak indicating no formation of AgNPs; while 1.0, 1.5 and 2.0 mM $AgNO_3$ containing reaction mixtures showed characteristic peak at 410 nm indicating formation of AgNPs (Fig. 1d). However there are several reports that higher concentration of silver nitrate produces larger particle size [6]. Hence, 1 mM $AgNO_3$ concentration was finalized for the synthesis of AgNPs pH of the reaction mixture affects and also plays an important role in the formation of nanoparticles. In order to evaluate the effect of pH on AgNPs formation, 12 ml 10 min boiled stem extract was added to 40 ml 1 mM silver nitrate and the reaction mixture was adjusted with different pH (6, 7, 8, 9, 10). At pH 6 and pH 7, the absorbance peak was broader and intensity was less (Fig. 1e), indicating less number of particle formation with larger size. As the pH increased, from pH 8 to pH 10, the absorption peak becomes narrowed and the intensity also steadily increased; again clearly indicating smaller size and more number of the particles formed. Best particle formation occurred at pH 10. Using alkaline pH for AgNPs synthesis is also reported by [22].

The effect of reaction time on the biosynthesis of AgNPs was evaluated at various time intervals (30 min, 60 min, 2 h and 24 h). The characteristic maximum absorbance peak of AgNPs was observed at 410 nm at various time intervals. No change in absorption peak or intensity was found after 24 h (Fig. 1f). Kumar et al. [5]

Please cite this article in press as: Moteriya P, Chanda S. Biosynthesis of silver nanoparticles formation from *Caesalpinia pulcherrima* stem metabolites and their broad spectrum biological activities. Journal of Genetic Engineering and Biotechnology (2018), https://doi.org/10.1016/j.jgeb.2017.12.003