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Biosynthesis of silver nanoparticles using leaf extract of *Satureja hortensis* treated with NaCl and its antibacterial properties

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ABSTRACT

Bio-nanotechnology is a rapidly growing scientific field of producing Nano sized particles by using biological systems. In this study the biosynthesis of silver nanoparticles (Ag NPs) using leaf extract of *Satureja hortensis* treated with different concentration of NaCl (0, 50, 100 and 150 μM) was reported. In addition, the nanoparticles were assessed against two gram-positive and one gram-negative bacteria. The biosynthesized Ag NPs were characterized using FESEM, XRD, UV/vis spectroscopy and related to the size, shape and morphology of the nanoparticles as revealed by FESEM. FTIR spectrum indicated various functional groups effective on Ag NPs biosynthesis. In each treatment, the plant extract showed color change from yellow to brownish-red after Ag NPs biosynthesis. The surface Plasmon resonance found at 450 nm confirmed the formation of Ag NPs. The highest rate of Ag NPs biosynthesis was related to 150 μM treatment. FESEM images revealed relatively spherical shape ranged from 2.9 to 3.4 nm. FTIR results expounded the functional groups of plant extract responsible for the bio-reduction of silver ions and their interaction between them. Ag NPs biosynthesis by 150 μM treatment showed the smallest size (2.9 nm) and thus the most antibacterial activity especially against *Bacillus subtilis*. Our results revealed that aromatic bicyclic monoterpenes have the most effective role in the biosynthesis process in 150 μM treatment. To the best of our knowledge no similar study has been reported.

1. Introduction

Recently, biological methods of nanoparticle biosynthesis using enzyme [1], microorganism [2], plant or plant extract [3] have been investigated as possible eco-friendly alternatives to chemical and physical procedures. Due to their exclusive properties, Ag NPs have more applications in many areas such as pharmaceutical components [4], chemical sensing and bio-sensing [5], catalysts in chemical reactions [6], optical elements [7], and electrical batteries [8]. Moreover, many of previous studies have shown antibacterial properties of nanoparticles such as silver nanoparticles [9,10]. Although antibiotics are used to kill harmful bacteria nowadays, inappropriate use of these materials has enabled bacteria to increase resistance to them, resulting emergence of resistant bacteria [11]. So to the opinion of the researchers, using of Ag NPs can be a good alternative to antibiotics [12]. Biosynthesis of Ag NPs by plant extracts have already been reported by numerous previous researchers [13–17]. The exact mechanism for this biosynthesis is still unknown. However, there have been several reports that have revealed the presence of biomolecules in plants such as terpenes, phenols and tannins act as effective reducing and capping agents for converting Ag^{+} to Ag° [18–20]. Some environmental and nutritional factors are known to influence the quantity and quality of plants biomolecules such as light, temperature, culture medium compositions and etc. [21]. Salinity is one of the factors that can changes quantity and quality of secondary metabolites and enzyme activity in plants [22]. Many gene networks and metabolic processes in plant affected by osmotic and ionic components of salt. Such responses depended mainly on the salt concentration, the duration of exposure of the plant to the salt and also innate salt tolerance of the plant [23]. However, numerous of previous studies have shown increase of bioactive compounds in treated plants with different concentration of NaCl [22,24,25]. Science the major biomolecules of plants are involved in nanoparticles biosynthesis [26,27], so the change in the quantity and quality of them can affected the process.

Satureja hortensis (*Summer savory*) is annual plant belonging to the lamiaceae family. *S. hortensis* is native to southern Europe and in parts of north America, south and west Asia, including Iran [28]. *S. hortensis* contain a wide range of chemical composition including carvacrol, terpinene cymene, caryophyllene and etc. [29]. This plant is used to treat of many diseases such as cramps, muscle aches, nausea,

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indigestion and diarrhea [30], also has specified its antifungal, antibacterial and antioxidant properties [31]. Science the medicinal plants contained a high amount of bioactive compounds; there are many reports on the biosynthesis of Ag NPs by using them [32–35]. To the best of our knowledge, there is no report of Ag NPs biosynthesis using aqueous extract of *S. hortensis* leaf extract treated with different concentration of NaCl. In this study, Ag NPs biosynthesis has been investigated using leaf extract of *S. hortensis* treated with different concentration of NaCl. Antibacterial property of silver nanoparticles depends on the size of them [36]. So, reduce the size of nanoparticles is one of the reasons for their increased antibacterial properties [37]. Accordingly, the aim of this particular research is providing useful information regarding the mechanism by which nanoparticles are biosynthesized by plants bioactive molecules followed by effective factors on the biosynthesized Ag NPs characteristic such as size, shape, stability and crystallinity. Moreover, we discuss the changes in antibacterial properties of the biosynthesized nanoparticles because of the NaCl treatments, which is different from already reports.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *S. hortensis* purchased from the company of Pakanbazr (Isfahan, Iran), were surface sterilized by immersion in 70% ethanol for 2 min and then 5% sodium hypochlorite for 5 min, followed by three times rinsing in sterile water after each step. The seeds were next soaked in a dilute solution of benomyl and transferred to plastic pots containing peat moss (Klasmann-Deilman, potgrond H) under equal greenhouse condition at Imam Khomeini International University (Qazvin, Iran) in February 2015. At six leaf stage, plant thinning carried out so that the remaining 20 numbers of the plants in each pot. The remaining plants were subjected to different concentrations of NaCl. Four levels of salinity (0, 50, 100 and 150 μM) were used in this study which were prepared using distilled water. Salt treatment was initiated at six leaf stage every 3 days and continued until early of flowering phase. In each pot, 700 ml saline water was applied and the control plants received 700 ml of distilled water. Every 15 days all of the plants were irrigated with 700 ml distilled water to leach out any accumulated salts in the peat moss. The experiment was organized in a factorial randomized complete block design with three replications. At late vegetative stage (flowering initiation) the leaves were harvested and immediately frozen in liquid nitrogen separately. Then, the samples stored frozen at −80 °C until use.

2.2. Extraction and biosynthesis of Ag NPs

All chemicals were purchased from Merck or Aldrich. Frozen collected leaves were used for preparation of *S. hortensis* leaf extract. The aqueous extract solutions of plant leaves were prepared separately for each treatment. 10 gr of finely cut plant leaves were boiled in 100 ml of distilled water for 5 min. After cooling, the obtained extract was filtered through Whatman paper No.1 and filtered extract was stored at 4 °C. In order to biosynthesis of Ag NPs, 10 ml of the resulted aqueous extract solution was added to 90 ml of aqueous solution of 1 mM of $AgNO_3$. The resulted aqueous solution was kept at room temperature with constant rotation. The addition of the plant extract to $AgNO_3$ aqueous solution leads to turning initially yellowish solution to brownish-red and finally deep brown indicating the formation of Ag NPs.

2.3. Characterization study of biosynthesized Ag NPs

The synthesized nanoparticles indicated by UV–visible spectroscopy, were carried out in a UV–vis spectrophotometer (Labomed, UV-win5, Germany), operating in a wavelength from 300 to 600 nm. Spectroscopy was performed for 150 min with intervals period of 15 min from the beginning time of reaction. To study the effect of salinity on the speed of the biosynthesis of nanoparticles, the charts related to increase of the concentration versus time-dependent were drawn for different treatments and compared. Also for Experimental design and statistical analysis was carried out in the form of factorial experiments according to a completely randomized design and with three replications, and were evaluated statistical analysis related to the effect of salinity on the biosynthesis of silver nanoparticles on the basis of obtained the maximum optical density of the silver nanoparticles by UV–vis spectrophotometer device for 10 times and with 3 replications for each treatment. The data were subjected to ANOVA analysis of variance. Comparison between means to determine significant differences ($p \leq 0.05$) was performed using the Duncan ¯s multiple range test. Correlation between variables was determined with Pearson ¯s correlation coefficient test, considering a confidence level of 95% ($p \leq 0.05$). All statistical analysis was performed using the IBM SPSS software version 21.0. The SOV table and chart were drawn by Microsoft office Excel 2010 software. Bars showing the same letter are not significantly different at $p \leq 0.05$.

X-ray powder diffraction patterns of Ag NPs were obtained by X´pert Pro MPD diffractometer made in Holland. The morphology and size of the Ag NPs were determined by Field Emission Scanning Electron Microscopy (HITACHI S-4160, Japan). Fourier Transform Infrared spectroscopy (FTIR) measurements were carried out separately for each treatment to find out the compound responsible for the synthesis of Ag NPs. FTIR spectra of Ag NPs were taken with potassium bromide pellets (1:100) on a Broker Tensor 27 spectrophotometer. The spectra were recorded in the wavenumber rang of 400–4000 Cm^{-1} and analyzed by subtracting the spectrum of pure KBr (Potassium Bromide). Average particles size of biosynthesized Ag NPs was determined by Dynamic Light Scattering (DLS) technique (brookhaven, Zetaplus, Canada).

2.4. Assessment of antibacterial activity of Ag NPs

In order to examine the antibacterial activity of the biosynthesized Ag NPs Kirby-Bauer disc diffusion method against bacterial species *Bacillus subtilis*, *Bacillus vallismortis* (gram-positive) and *Escherichia coli* (gram-negative) was used. Luria-Bertani medium was prepared and sterilized at 121 °C. About 25 ml of the medium was transferred aseptically into each sterilized plate. The bacterial strains were spread on the petri plates using pipette. Later, the soaked discs of 6 mm diameter with different samples ($AgNO_3$, distilled water and the biosynthesized Ag NPs) were placed on agar plates, followed by incubation for 24 h in 37 °C. Zone of inhibition was measured with a meter ruler around each disc in mm and recorded.

3. Results and discussion

3.1. Biosynthesis and characterization Ag NPs

Biosynthesis of Ag NPs using 1 mM $AgNO_3$ was added to leaf extract of the treated plants is shown in Fig. 1. Color change from yellow to brownish-red after addition of $AgNO_3$ and stirring at room temperature was due to excitation of the surface Plasmon resonance [38,39].

The Characteristics silver surface Plasmon resonance (SPR) bands were detected at the wavelength of 450 nm (Fig. 2) that is in good agreement with the reported spectra in the literature for the silver nanoparticles [40]. The results revealed that incubation time affects the Ag NPs formation [41]. As the time duration increased, the nanoparticle synthesis also increased. Ag NPs biosynthesis was initiated nearly within 15 min. The completion of Ag NPs biosynthesis was reduced after 2.5 h as identified in Fig. 2. After this time, the precipitation of Ag NPs occurred due to the instability of the nanoparticles. Agglomeration of Ag NPs showed the larger size of nanoparticles. So the optimum time duration for the formation of Ag NPs was 2.5 h. It was also found that the treatments had affected processes of Ag NPs