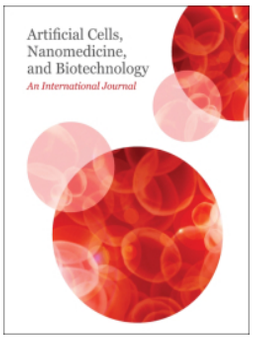

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Green biosynthesis of silver nanoparticles using *Althaea officinalis* radix hydroalcoholic extract

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Abstract

The objectives were to study the potential of *Althaea officinalis* radix in production of silver NPs, and the effect of the extract ethanol concentration on the produced NPs. Seventy and ninety-six percent hydroalcoholic extracts were prepared by percolation of the plant powder. The extract was concentrated by rotary evaporator and then freeze-dried. Silver ions were determined using atomic absorption analysis. The NPs were characterized by Nano-Zeta Sizer and TEM. Both of 70% and 96% of hydroalcoholic extracts of *A. officinalis* radix successfully synthesized spherical and poly-dispersed silver NPs. The conversion was fast and almost completed in 5 h.

Keywords: *Althaea officinalis*, green chemistry, green synthesis, hydroalcoholic extract, nanoparticle synthesis

Introduction

Development of reliable and eco-friendly processes for synthesis of metallic nanoparticles (NPs) is an important step in the field of application of nanobiotechnology and nanoscience. There are various methods for the synthesis of NPs, but most of them are environmentally unfriendly and expensive (Senapati 2005, Klaus-Joerger et al. 2001). Consequently, there is an ever-growing need to develop nontoxic and eco-friendly procedures for synthesis and assembly of NPs with the desired morphologies and sizes (Iravani 2011, Iravani et al. 2014, Iravani and Zolfaghari 2013, Korbekandi and Iravani 2012, 2013, Korbekandi et al. 2009, 2012, 2013). One of the options to achieve this objective is to use natural processes such as the use of microorganisms, enzymes, and biological systems.

One approach that shows immense potential is based on the phytosynthesis of NPs using plants (Iravani 2011, Iravani et al. 2014, Korbekandi et al. 2009). Actually, a great amount of effort has been done on the green synthesis of metal and metal oxide NPs using plants (Amin et al. 2012, Ankamwar et al. 2005, Iravani 2011, Iravani et al. 2014, Iravani and Zolfaghari 2013, Kaviya et al. 2011, Kora and Arunachala 2012, Nagati et al. 2012, Naik et al. 2013, Shankar et al. 2003, Yang et al. 2010). The use of plants in this area is rapidly developing due to their growing success and ease of formation of NPs. The objects of recent studies tend to provide a controlled and up-scalable process for synthesis of monodispersed and highly stable NPs (Iravani 2011, Korbekandi et al. 2009, Iravani et al. 2014). Furthermore, using plants for the synthesis of NPs can be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. It can also be suitably scaled up for large-scale synthesis of NPs (Iravani 2011, Korbekandi et al. 2009, Iravani et al. 2014).

Because of the valuable applications of silver NPs in the fields of electronic, material sciences, pharmaceutical sciences, and medicine, a rapid, simple, reliable, cost-effective, and eco-friendly green method for synthesis of these NPs is becoming increasingly important. Moreover, based on the above mentioned methods for synthesis of metal and metal oxide NPs, it seems that using plant extracts is one of the best methods. From the point of nanobiotechnology and green nanotechnology, this is a significant development that phytosynthesis of NPs using plants is simple and convenient to handle and it is believed that it has advantages over other biological syntheses. This

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is an ultra low cost technique for production of NPs as it does not involve any extreme operation conditions like high pressure or temperature in addition to very cheap cost of the biomaterials.

Althaea officinalis (marshmallow) belongs to Malvaceae family, native to Europe, United States of America, and parts of Asia and is cultivated throughout the world. The flowers are terminal and axillary, with short peduncles, each bearing one, two, or three flowers. The petals are pale pink, reddish pink, and rarely, white, in color. This plant widely used traditionally for the treatment of the irritation of oral and pharyngeal mucosa (which is associated with dry cough), mild gastritis, skin burns, and for insect bites. It is also used in catarrh of the mouth and throat, gastrointestinal (GI) tract and urinary tract complains, as well as for inflammation, ulcers, abscesses, burns, constipation, and diarrhea. This plant possessed antimicrobial, anti-inflammatory, immunomodulatory, demulcent and soothing, antittusive and many other nutriceutical and pharmacological effects (Al-Snafi 2013). The extract of *A. officinalis* radix has high content of valuable flavonoid glycosides, phenolic acids, tannins, and flavonoids and has exhibited strong total antioxidant activity (Sadighara et al. 2012, Moalemi et al. 2012). Several investigations reported that these biomolecules and phytochemicals were involved in production of metal and metal oxide NPs (Dubey et al. 2009, Gnanasangeetha and SaralaThambavani 2013, Gunalan et al. 2012, Jain et al. 2009, Xin et al. 2010). It seems that the plants with high content of antioxidant compounds are one of the best candidates for nanoparticle synthesis. Thus, the objectives of this work were to study the potential of *A. officinalis* (marshmallow) radix in production of NPs, and the effect of the extract ethanol concentration on the produced NPs.

Materials and methods

Preparation of the plant extract

A. officinalis (marshmallow) radix was freshly collected from Isfahan Agricultural and Natural Resources Research Center (Herbarium number: 2627). After washing, air drying at room temperature and powdering them, the powder was screened using a17-mesh sieve. Hydroalcoholic extract was prepared by percolation (about 48 h). Two hundred gram of the plant powder was extracted with hydroalcoholic solution (70% and 96%, 500 mL) using a 2-Liter percolator. The extract was concentrated in rotary evaporator to 50 mL, and then freeze-dried (Christ®, Martin Christ; Germany).

Preparation of the reaction mixtures

The reaction mixtures contained (final concentrations): $AgNO_3$ (1 mM) as the substrate, concentrated and freeze-dried plant extract (equivalent to 100 g plant powder) as the biocatalyst, and phosphate buffer (pH = 7, 100 mM) as the medium in the reaction mixture (50 mL). The aforementioned ingredients were added in appropriate amounts into Duran® bottles (100 ml) and were incubated (70 rpm) at room temperature.

Characterization and analysis

Transmission electron microscopy analysis

Transmission electron microscopy (TEM) was performed on selected samples in order to investigate the process of formation of silver NPs and study the sizes and shapes of them. Samples for TEM were prepared by drop-coating the silver nanoparticle solutions onto carbon-coated copper grids. Micrographs were obtained using EM 900 ZEISS transmission electron microscope.

Dynamic light scattering analysis

Particle size distribution of NPs was analyzed using Nano-Zeta Sizer (ZEN 3600, Malvern Nano®, UK).

Atomic absorption analysis

In order to quantify the substrate (Ag^+) and calculate the conversion of it to Ag^0 NPs, Ag^+ ions were determined using atomic absorption analysis (AA) by Ag lamp at 328 nm. The samples (1 mL) were centrifuged (Sigma D-37520, Germany, 2240 *g*, 45 min) before the analysis. Zeeman technique, pyro-coated tube with platform, pretreatment temperature of 650°C, and atomization temperature of 1600°C were used.

Results and discussion

Visual inspection

When *A. officinalis* (marshmallow) radix extract was exposed to Ag^+ ions ($AgNO_3$, 1 mM), the color of the reaction mixture turned to dark brown, which was considered as the formation of silver NPs. The appearance of dark brown seems to be due to excitation of surface plasmon resonance in the NPs.

Monitoring the synthesis of silver NPs

To quantify the substrate (Ag^+) and calculate the conversion of it to Ag^0 NPs, Ag^+ ions were determined using AA analysis. Seventy percent hydroalcoholic extract of *A. officinalis* radix transformed 99.5% of $AgNO_3$ to Ag NPs after 96 h (Figure 1). The reaction was almost complete (conversion% = 97.4) after 5 h and the conversion rate was maximum (0.94 mmole h^{-1}) between hour 0 and 1.

Ninety six percent hydro-alcoholic extract of *A. officinalis* radix transformed 98.1% of $AgNO_3$ to Ag NPs, after 96 h (Figure 2). The reaction was almost complete (conversion% = 97.4) after 5 h and the conversion rate was maximum (0.73 mmole h^{-1}) between hour 0 and 1.

DLS analysis

Seventy percent hydroalcoholic extract

Three peaks were seen in dynamic light scattering (DLS) analysis of the reaction mixture after 50 h, therefore the NPs were polydispersed (Figure 3). Single NPs with the diameter of about 279 nm were the most frequent ones (46.1%) and the mean size was 112 nm (Z-Average). The second most frequent (31%) particles were 4.1 nm (smallest NPs). The aggregates with the diameter of 5026 nm were 23% of the NPs. The poly-dispersity index (PDI) was 1. On increasing the time of reaction (96 h), the aggregates disappeared (Figure 4).

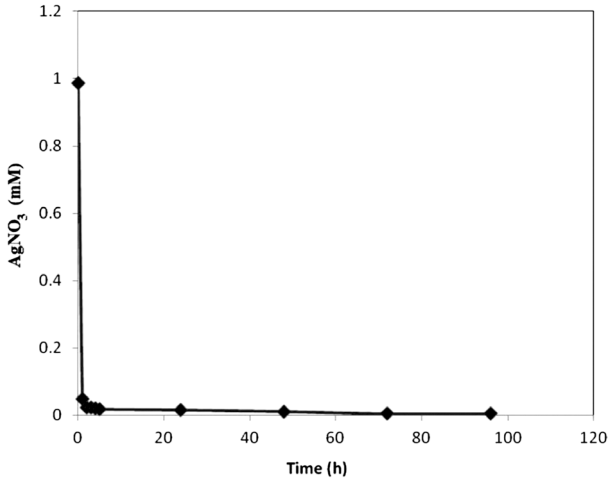

Figure 1. Time course of silver nitrate conversion to silver NPs by 70% hydroalcoholic extract of *A. officinalis*.

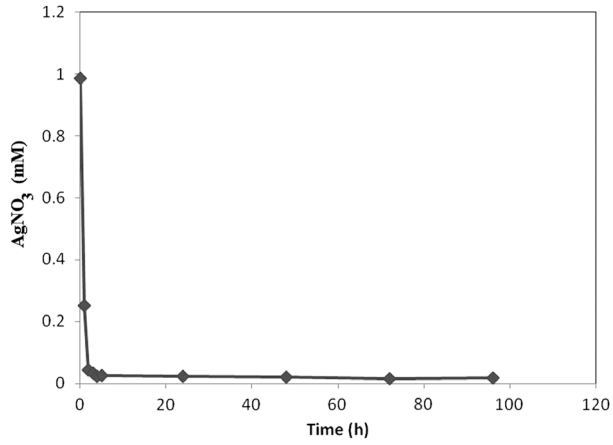

Figure 2. Time course of silver nitrate conversion to silver NPs by 96% hydroalcoholic extract of *A. officinalis*.

Ninety-six percent hydroalcoholic extract

Three peaks were seen in DLS analysis of the reaction mixture after 50 h, therefore the NPs were poly-dispersed (Figure 5). Single NPs with the diameter of 420 nm were the most frequent ones (88.3%) and the mean size was 440 nm (Z-Average). The second most frequent (10.1%) particles were 2.22 nm (smallest NPs). The aggregates with the diameter of 5560 nm were 1.6% of the NPs (Figure 5). The PDI was 0.331 (near to optimal value). On increasing the time of reaction (96 h, Figure 6), the peak related to 2.22 nm disappeared and the areas under the curves of the other peaks increased.

TEM analysis

TEM images of the silver NPs synthesized by using 70% alcoholic extract of *A. officinalis* after 24 h biotransformation

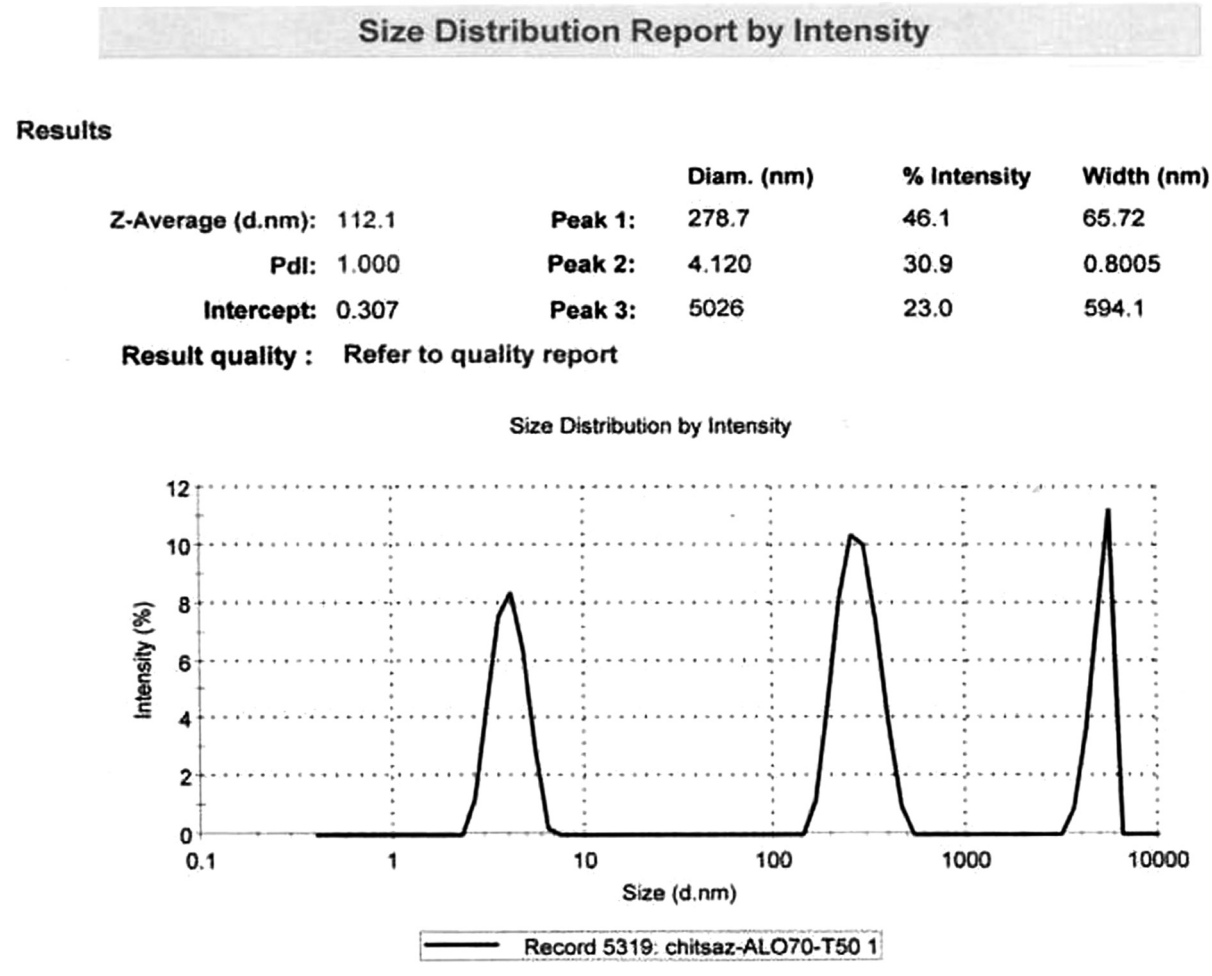

Figure 3. Particle size analysis of the produced NPs by 70% hydroalcoholic extract of *A. officinalis* after 50 h biotransformation.

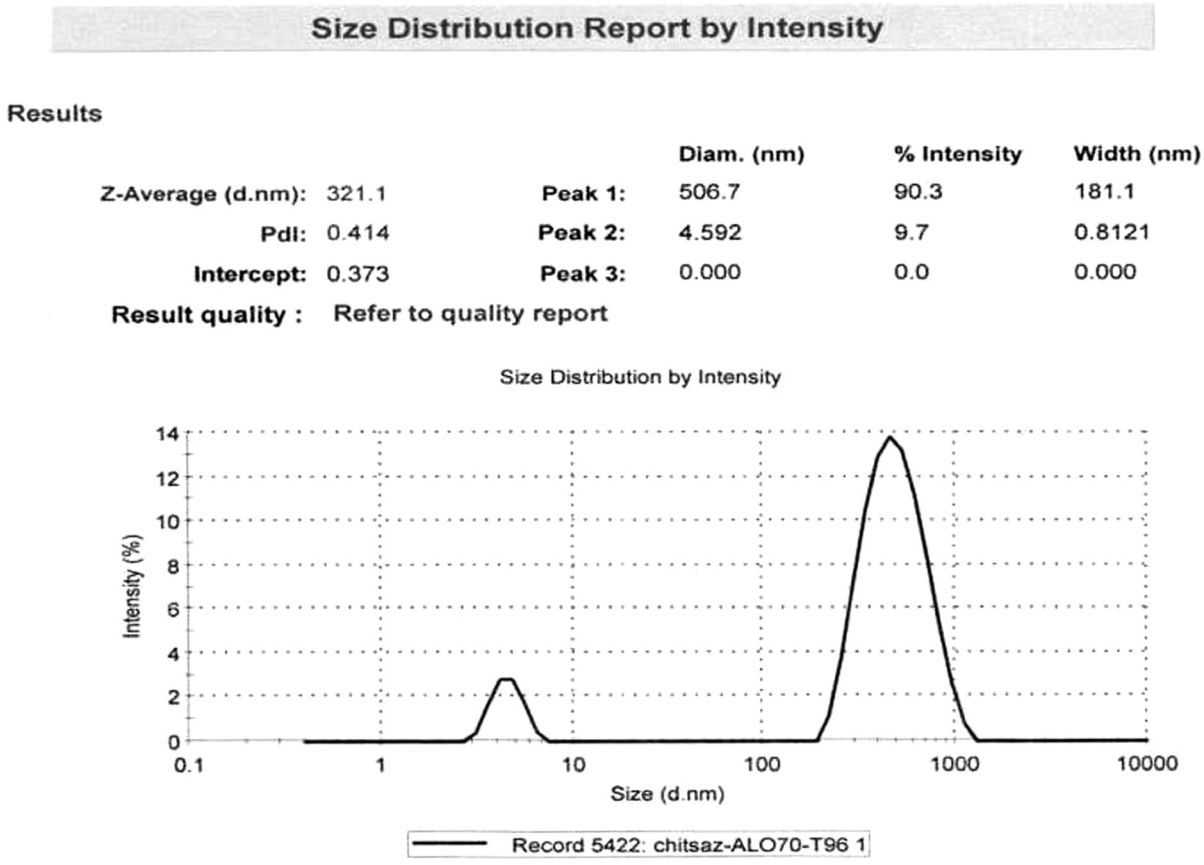

Figure 4. Particle size analysis of the produced NPs by 70% hydroalcoholic extract of *A. officinalis* after 96 h biotransformation.

were shown in Figure 7. The produced NPs were spherical. Figure 8 shows TEM images of silver NPs produced by the phytoreaction of silver nitrate solution with 96% alcoholic extract of *A.* officinalis, 24 h after the start of the reaction. These NPs were spherical, as well. Both of 70% and 96% hydroalcoholic extracts of *A. officinalis* produced quite small (as small as 2.2 nm), spherical, and poly-dispersed NPs with low aggregates (as low as 1.6%).

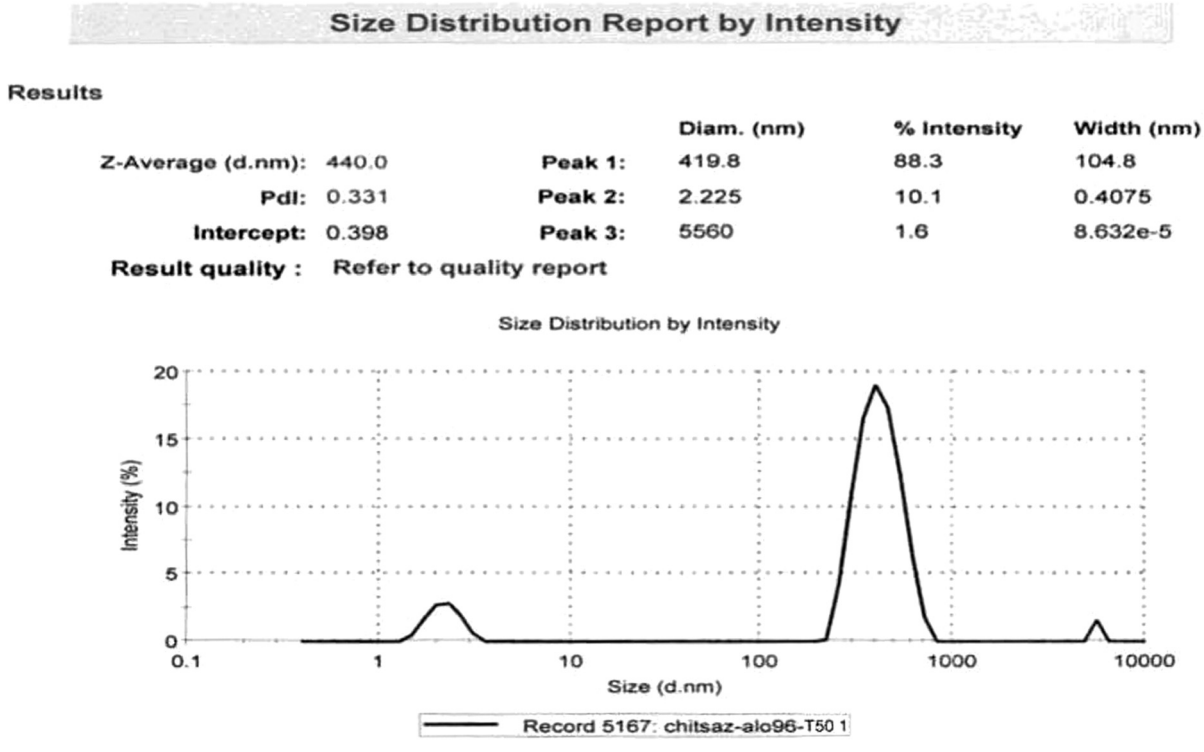

Figure 5. Particle size analysis of the produced NPs by 96% hydroalcoholic extract of *A. officinalis* after 50 h biotransformation.

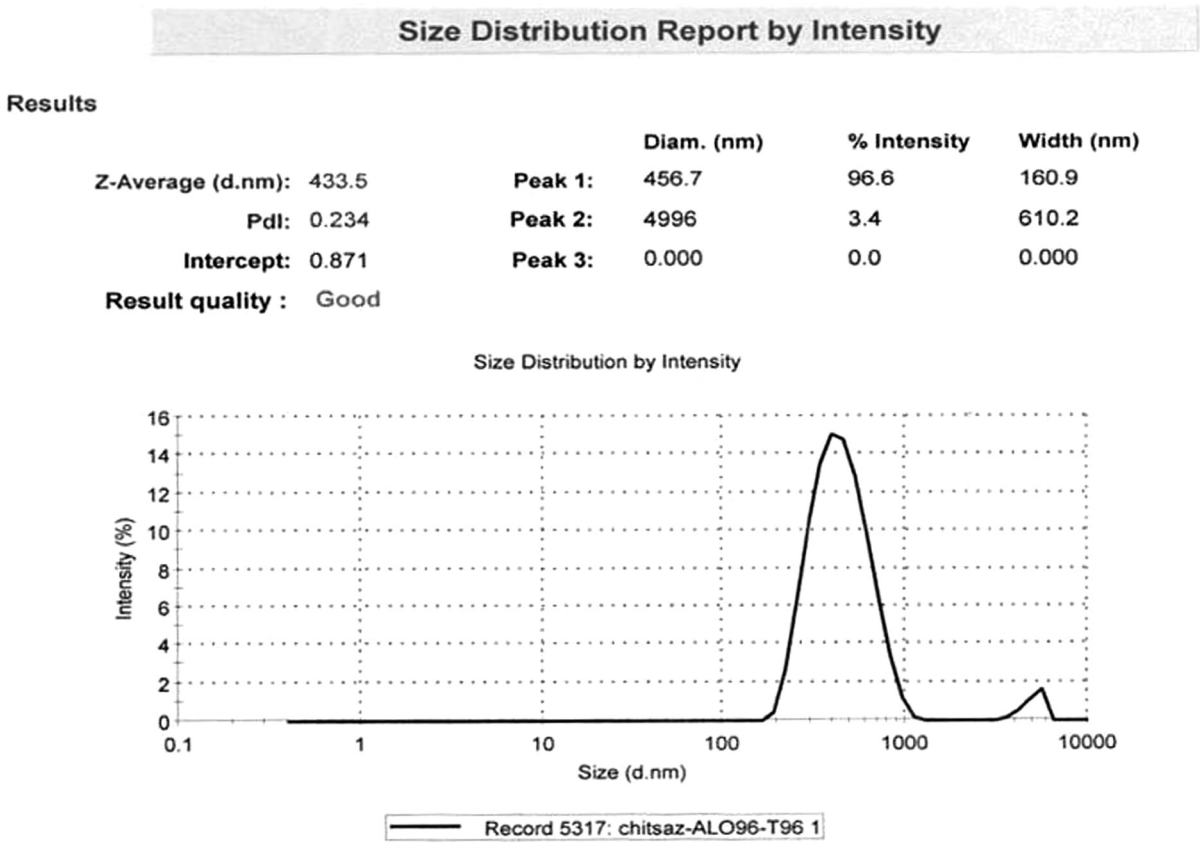

Figure 6. Particle size analysis of the produced NPs by 96% hydroalcoholic extract of *A. officinalis* after 96 h biotransformation.

Effect of ethanol concentration of hydroalcoholic extract on the produced NPs

Biotransformation time-courses, bioconversion percents, and bioreduction rates of Ag^+ to Ag NPs by both of the extracts (Figures 1 and 2) were almost the same. Comparing the NPs shapes, produced by 70% (Figure 7) and 96% (Figure 8) hydroalcoholic extract of *A. officinalis*, it can be seen that 96% hydroalcoholic extract produced more and denser silver NPs, which might be interpreted that 96% hydroalcoholic solution can extract the nanoparticle producing compounds better than 70% hydroalcoholic solution. Comparing the NPs size distribution, it can be found that 70% alcoholic extract produced NPs with smaller average size than 96% alcoholic extract, but lower percentage of aggregates were produced by 96% alcoholic extract (after 50–96 h of reaction).

Mechanistic aspects

It has been reported that the extract of *A. officinalis* exhibited strong antioxidant activity in different antioxidant tests (Elmastas et al. 2004). The antioxidant activity is accounted for approximately 69% of the activity of the reference compound α-tocopherol (Kardosová and Machova 2006). *A. officinalis* radix contains valuable flavonoid glycosides, phenolic acids, tannins, and flavonoids and has exhibited strong total antioxidant activity, as well (Moalemi et al. 2012). Flavonoids and phenolic compounds are responsible for antioxidant activity in plants

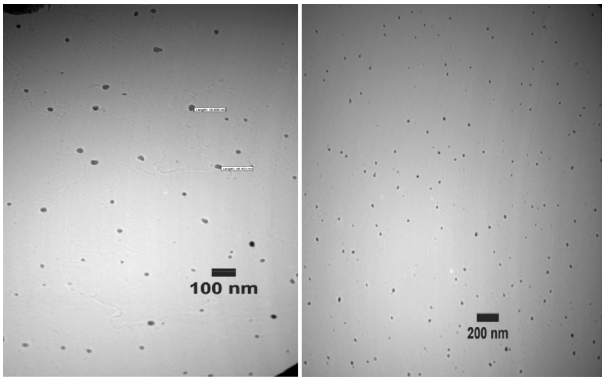

Figure 7. TEM micrographs of the produced NPs by 70% hydroalcoholic extract of *A. officinalis* after 24 h biotransformation.

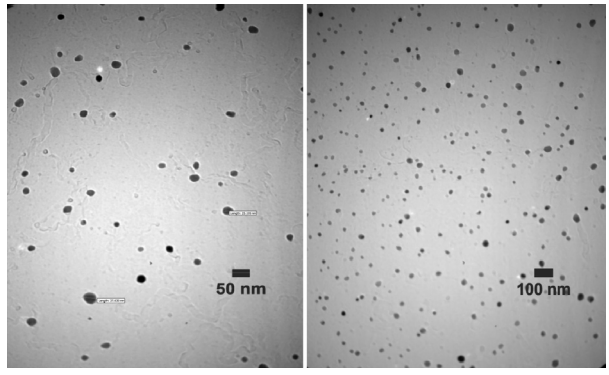

Figure 8. TEM micrographs of the produced NPs by 96% hydroalcoholic extract of *A. officinalis* after 24 h biotransformation.

(Sakai et al. 1996). Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolic compounds possessing hydroxyl and carboxyl groups may inactivate iron ions by chelating and suppressing the superoxide-driven Fenton reaction, which is believed to be the most important source of reactive oxygen species (ROS). Furthermore, polyols components and heterocyclic components were believed to be responsible for reduction of metal ions and stabilization of metal NPs, respectively (Dubey et al. 2009, Gopalakrishnan et al. 2012, Pavani et al. 2012, Satyavani et al. 2011, Singh et al. 2011, Vanaja and Annadurai 2012, Xin et al. 2010). In addition, it was reported that water soluble carbohydrates, proteins, and amino acids were responsible for bioreduction and stability of silver NPs (Sadighara et al. 2012, Rajasekharreddy et al. 2010, Moalemi et al. 2012, Iravani and Zolfaghari 2011, 2013, 2014, Dubey et al. 2009, Gopalakrishnan et al. 2012, Pavani et al. 2012, Satyavani et al. 2011, Singh et al. 2011, Vanaja and Annadurai 2012, Xin et al. 2010). Many compounds were extracted from different parts of *A. officinalis*, these included pectins, starch, mono-, and di-saccharide saccharose, uucilage, flavonoids (Hypolaetin-8-glucoside, isoquercitrin, kaempferol, caffeic, p-coumaric acid), coumarins, scopoletin, phytosterols, tannins, asparagine, and many amino acids. The demulcent effects of Radix Althaea are due to its high content of polysaccharide hydrocolloids, which form a protective coating on the oral and pharyngeal mucosa, soothing local irritation and inflammation (Ali Shah et al. 2011, Blumenthal et al. 2000). Therefore, it seems that such phytochemicals and antioxidant compounds were involved in the green synthesis of silver NPs using *A. officinalis* radix extract. However, more efforts still need to be done to understand the effect of parameters regarding the effects of various biomolecules and phytochemicals available in the extract of *A. officinalis* radix for phytosynthesis of silver NPs.

Conclusion

The exploitation of various plant materials for the biosynthesis of NPs is considered a green technology. Both of 70% and 96% hydroalcoholic extracts of *A. officinalis* radix successfully produced spherical and poly-dispersed NPs. Results from TEM and DLS analysis demonstrated that by increasing the time of reaction, the NPs were aggregated further. The conversion was fast and almost completed in 5 h. Therefore, this plant's radix and the extraction method seem to be quite attractive for industrial scale production of NPs. The ability to synthesize silver NPs rapidly by this method is exciting and represents an important advance in making them viable alternatives to the more popular chemical methods. This environmentally friendly method of biological silver nanoparticle synthesis can potentially be applied to various products that directly come in contact with the human body, such as cosmetics, foods, and consumer goods, besides medical applications.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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