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# Synthesis of silver nanoparticles using methanol and dichloromethane extracts of *Pulicaria gnaphalodes* (Vent.) Boiss. aerial parts

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## Abstract

The objectives were to study the potential of *Pulicaria gnaphalodes* (Vent.) Boiss. aerial parts in production of nanoparticles and the effect of the extraction solvent on the produced nanoparticles. Methanol and dichloromethane extracts were prepared by percolation of the plant powder. Both the extracts of *P. gnaphalodes* (Vent.) Boiss. successfully produced small and polydispersed nanoparticles with low aggregates in early hours of the biotransformation. Methanol extract produced spherical and many single nanoparticles, whereas dichloromethane produced porous polyhedral and more aggregated nanoparticles. Methanol extract of this plant seems to be quiet useful for industrial scale production of nanoparticles.

**Keywords:** bioreduction, green chemistry, methanol and dichloromethane extracts, nanoparticle synthesis, *Pulicaria gnaphalodes* (Vent.) Boiss., silver NPs

## Introduction

Various important applications of silver nanoparticles (NPs) have led to an increase in the need for the production of these NPs. Different methods were developed to obtain silver NPs of different morphologies and sizes, including laser ablation, gamma irradiation, electron irradiation, chemical reduction, photochemical methods, microwave processing, and thermal decomposition of silver oxalate in water and in ethylene glycol. But in recent years, phytosynthesis of silver NPs by plants was reported by researchers (Chahardooli et al. 2014, Gade et al. 2014, Mariselvam et al. 2014, Sun et al. 2014, Suresh et al. 2014, Umoren et al. 2014, Velmurugan et al. 2014). These green methods can be considered as possible eco-friendly alternatives to chemical and physical approaches. Thus, using screened plants

with high production capability and controlling the reaction conditions, well-characterized NPs can be obtained by making the synthesis rates faster or comparable with those of chemical and physical approaches. Phytosynthesis of silver NPs can potentially be used in various areas, including pharmaceuticals, cosmetics, foods, and medical applications (Korbekandi and Iravani 2012, Iravani 2011, Iravani et al. 2014a, 2014b, Iravani and Zolfaghari 2013, Korbekandi and Iravani 2013, Korbekandi et al. 2009, 2012, 2013).

*Pulicaria* is a plant genus in the family of Asteraceae. It comprises about 100 species distributed from Europe to North Africa and Asia, particularly around the Mediterranean region (Khan et al. 2013). They are commonly used as herbal tea, flavoring agent, and medicinal plant. *P. gnaphalodes* (Vent.) Boiss. is a persistent plant of about 10–30 cm high, with gold-yellow flowers, which grows on sandy, stony places in the Asia (Shariatifar 2012). Kakkosh-Biabani (*Pulicaria gnaphalodes* Linn) is a plant which grows wild in abandoned areas such as Iran, Iraq, Pakistan, India, Turkey, and Saudi Arabia (Mozaffarian 1996). This plant is a worldwide known folk medicine for severe dehydration, inflammation, etc. (Shariatifar 2012). The extracts of *P. gnaphalodes* (Vent.) Boiss. contains high amounts of phenolic compounds and antioxidant activity, and thus can be considered as a potential source for green synthesis of silver NPs. Herein, the objective was to study the potential of *Pulicaria gnaphalodes* (Vent.) Boiss. aerial parts for the production of silver NPs and the effect of the extraction solvent on the produced NPs.

## Materials and methods

### Preparation of the plant extract

*Pulicaria gnaphalodes* (Vent.) Boiss. aerial parts were freshly collected from Isfahan Agricultural and Natural Resources

Research Center (Herbarium number: 1362). After washing, air-drying at room temperature, and powdering them, the powder was screened using a 17-mesh sieve. Methanol and dichloromethane extracts were prepared by percolation. The plant powder (200 g) was extracted with methanol and dichloromethane (500 mL) using a 2-L percolator for 48 h. The extract was concentrated in rotary evaporator to 50 mL and powdered through freeze-drying (Christ®, Martin Christ; Germany).

### Preparation of the reaction mixtures

The reaction mixtures contained (final concentrations)  $\text{AgNO}_3$  (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 microscopic 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 suspensions onto carbon-coated copper grids. Micrographs were obtained using EM 900 ZEISS transmission electron microscope.

#### Dynamic light scattering (DLS) analysis

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

#### Atomic absorption analysis

In order to quantify the substrate ( $\text{Ag}^+$ ) and calculate the conversion of it to  $\text{Ag}^0$  NPs,  $\text{Ag}^+$  ions were determined using atomic absorption (AA) analysis by Ag lamp at 328 nm. Atomic absorption spectrophotometer (Perkin-Elmer Zeeman 3030, Germany) with pyro-coated tube and platform, pretreatment temperature of 650°C, and atomization temperature of 1600°C was used. The samples (1 mL) were centrifuged (Sigma D-37520, Germany, at 2240 g, for 45 min) before the analysis.

## Results and discussion

### Bioreduction of $\text{Ag}^0$ to $\text{Ag}^+$ by methanol extract

Methanol extract of *Pulicaria gnaphalodes* (Vent.) Boiss. aerial parts transformed 100% of  $\text{AgNO}_3$  to Ag NPs after 96 h (Figure 1). The reaction was almost complete (conversion%, 96) after 5 h, and conversion rate was maximum ( $0.959 \text{ mmole h}^{-1}$ ) between hour 0 and 1.

### Bioreduction of $\text{Ag}^0$ to $\text{Ag}^+$ by dichloromethane extract

Dichloromethane extract of *P. gnaphalodes* (Vent.) Boiss. aerial parts transformed 99.7% of  $\text{AgNO}_3$  to Ag NPs after 96 h (Figure 2). The reaction was also complete (conversion%,

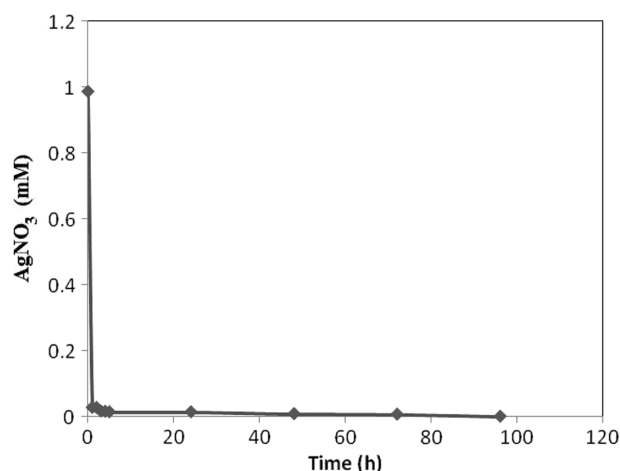


Figure 1. Time course of silver nitrate conversion to silver NPs by methanol extract of *P. gnaphalodes* (Vent.) Boiss.

98.6%) after 5 h, and conversion rate was maximum ( $0.951 \text{ mmole h}^{-1}$ ) between hour 0 and 1.

### DLS analysis

#### Methanol extract

Three peaks were seen in DLS analysis of the reaction mixture after 2 h; therefore, the NPs were polydispersed (Figure 3). Single NPs with diameter of 19.1 nm were the most frequent ones (70.8%), and the mean size was 7.2 nm (Z-Average). The NPs with smallest diameter were 0.91 nm (26.3%). The polydispersity index (PDI) was 0.368 (near to optimal value). The average diameter was increased by increasing the reaction time (72 h), and the NPs were aggregated further (Figure 4).

#### Dichloromethane extract

Three peaks were seen on DLS analysis of the reaction mixture after 2-h biotransformation; therefore, the NPs were polydispersed (Figure 5). Single NPs with diameter of 20.3 nm were the most frequent ones (40.3%), and the mean size was 8.4 nm (Z-Average). The second most frequent (38.2%) particles were with diameter of 0.97 nm (smallest

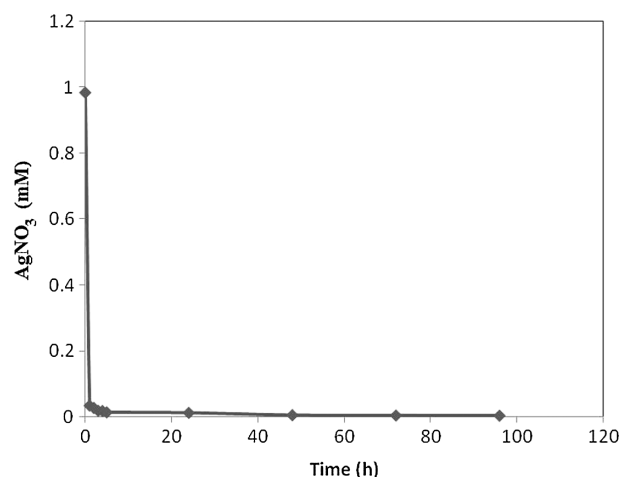


Figure 2. Time course of silver nitrate conversion to silver NPs by dichloromethane extract of *P. gnaphalodes* (Vent.) Boiss.

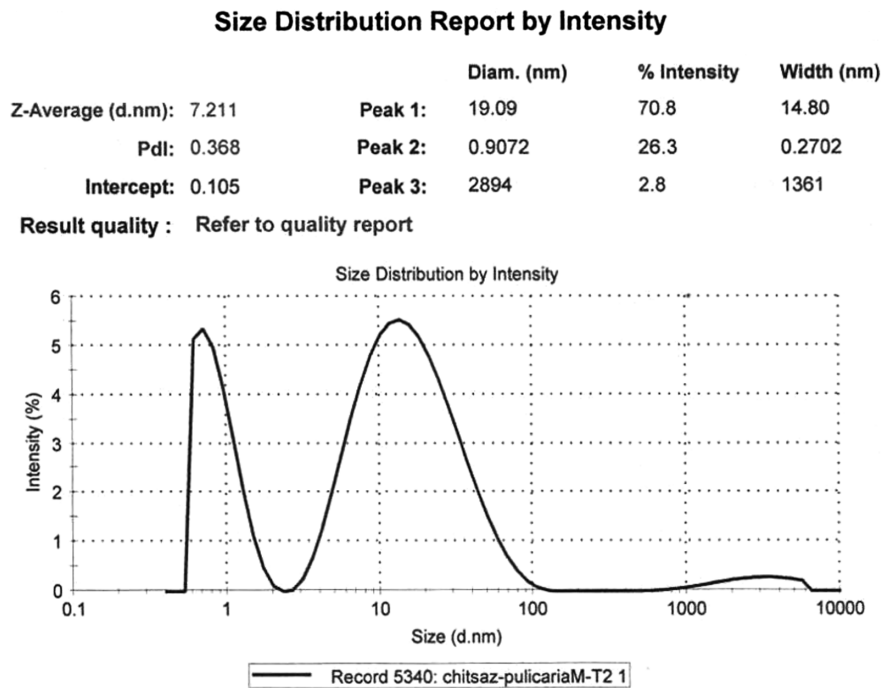


Figure 3. Particle size analysis of NPs produced by methanol extract of *P. gnaphalodes* (Vent.) Boiss. after 2 h of biotransformation.

NPs). By increasing the reaction time (96 h, Figure 6), the average size of the NPs was increased to 535 nm and the percentage of the aggregated NPs (73.2%) was increased further.

TEM analysis

TEM images of the NPs produced with 100% methanol extract of *P. gnaphalodes* (Vent.) Boiss. after 24 h of biotransformation were shown in Figure 7. The silver NPs produced were spherical. Furthermore, TEM images of the produced

NPs by dichloromethane extract of *P. gnaphalodes* (Vent.) Boiss. after 24 h of biotransformation were shown in Figure 8. The produced NPs were porous polyhedral, and in some places aggregated in assemblies.

Effect of the extraction solvent

Biotransformation time courses, bioconversion percentages, and bioreduction rates of Ag<sup>+</sup> to Ag<sup>0</sup> NPs by both the extracts (Figures 1 and 2) were almost the same. By comparing the NPs shapes and size distributions, produced by methanol

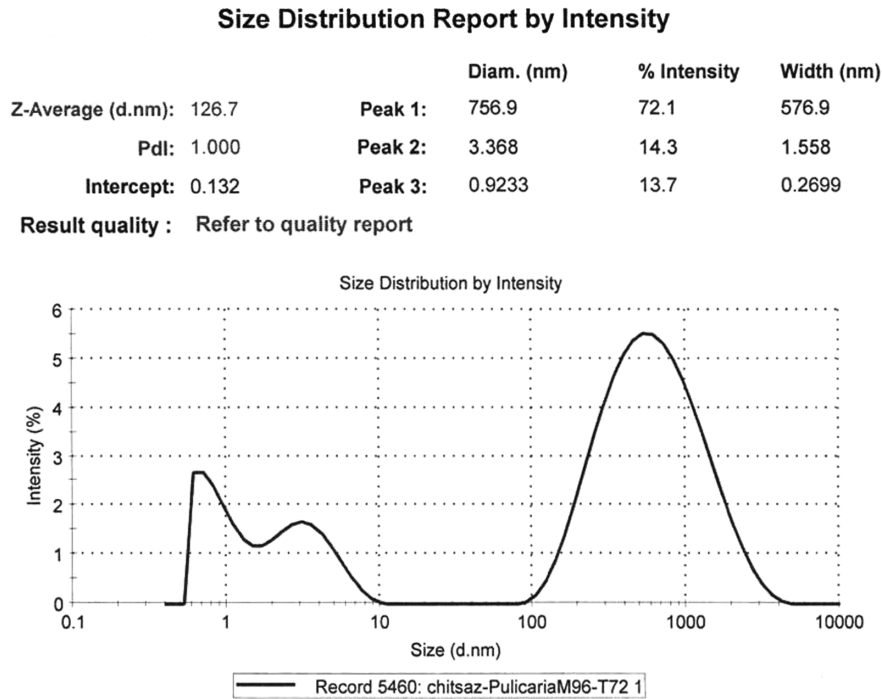


Figure 4. Particle size analysis of NPs produced by methanol extract of *P. gnaphalodes* (Vent.) Boiss. after 72 h of biotransformation.

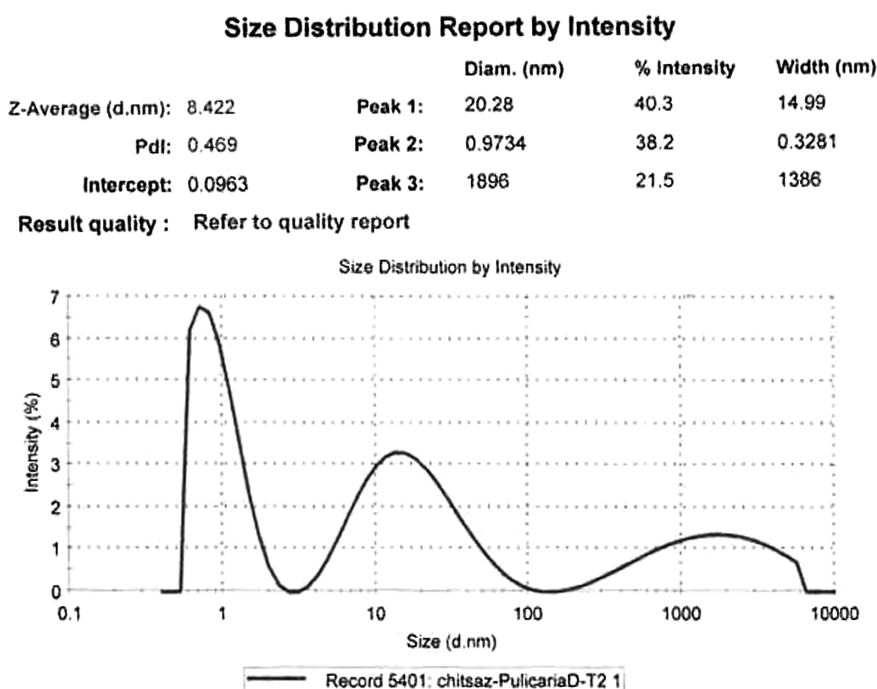


Figure 5. Particle size analysis of NPs produced by dichloromethane extract of *P. gnaphalodes* (Vent.) Boiss. after 2 h of biotransformation.

(Figures 3, 4, and 7) and dichloromethane (Figures 5, 6, and 8) extracts of *Pulicaria gnaphalodes* (Vent.) Boiss., it can be seen that methanol extract produced spherical, smaller, and less aggregated spherical silver NPs.

Previously, Khan et al. (2013) used the aqueous extract of aerial parts of *P. glutinosa*, which was collected from local fields in Saudi Arabia. They soaked small pieces of the plant in deionized water and refluxed them for 3 h. The solution was filtered and dried at 50°C under reduced pressure in a rotary evaporator to give a powder of dark brownish color. They stirred the reaction mixture at 90°C for 2 h and

produced spherical nanosilver particles with the size range of 40–60 nm. Comparing our work with the study of Khan et al. (2013), we used the species, *P. gnaphalodes*, grown in Iran, while they used *P. glutinosa* from Saudi Arabia but both of us used the aerial parts from the same genus. In our case, the bioreduction reaction was almost finished in 5 h, at room temperature, but Khan et al., using aqueous extract, spent 20 h at this condition to complete the reaction; then, they used 90°C for the reaction mixture. As we mentioned earlier, methanol extract of *P. gnaphalodes* produced dense spherical NPs, but dichloromethane extract synthesized

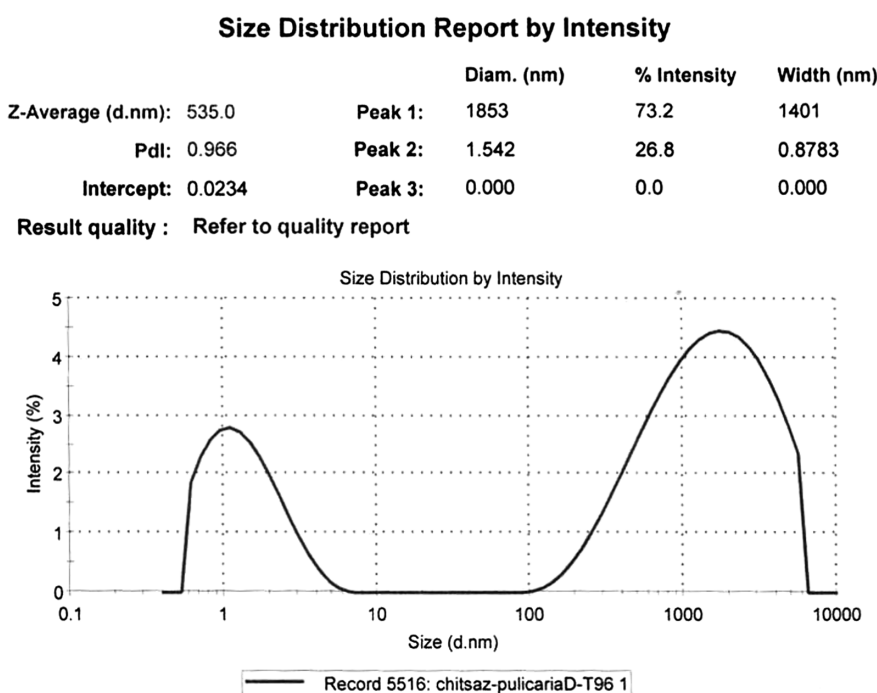


Figure 6. Particle size analysis of NPs produced by dichloromethane extract of *P. gnaphalodes* (Vent.) Boiss. after 96 h of biotransformation.



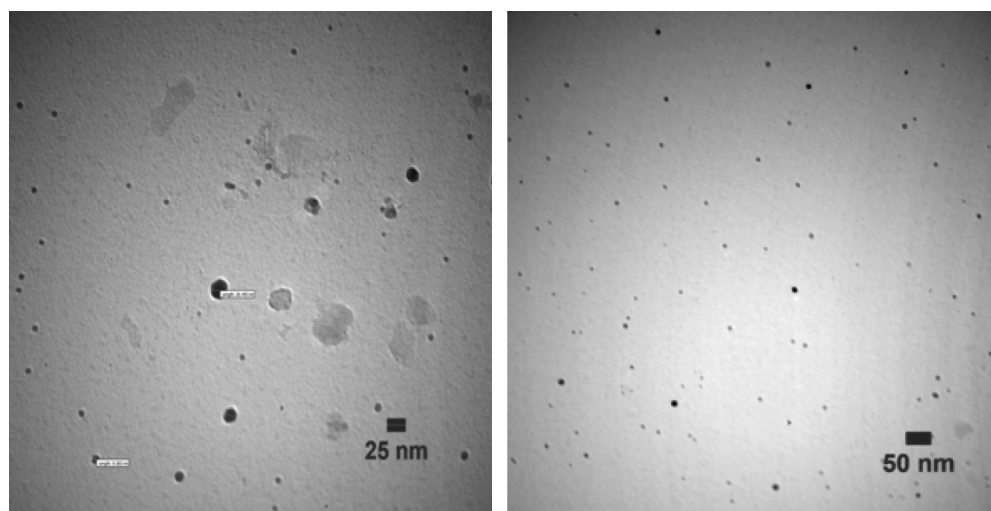


Figure 7. TEM micrographs of the produced NPs by methanol extract of *P. gnaphalodes* (Vent.) Boiss. after 24 h of biotransformation.

porous polyhedral NPs. Using aqueous extract of *P. glutinosa*, Khan et al. produced spherical NPs, as well, which confirms the effect of extraction solvent on the produced NPs. After 5-h bioreduction, our methanol extract produced nanosilver particles with the mean size of 7.2 nm, and dichloromethane extract synthesized NPs with the mean size of 8.4 nm. However, using the aqueous extract and 2 h reaction, Khan et al. produced nanosilver particles with the size range of 40–60 nm. It was reported that less aggregated, small, and spherical silver NPs are more effective for most applications (Khan et al. 2013).

### Mechanistic aspects

The extract of *P. gnaphalodes* contains high amounts of phenolic compounds and antioxidant activity, and it can be used as a potential source of antioxidant compounds for food and pharmaceutical industry (Kamkar et al. 2013, Shariatifar 2012). Five compounds including one phenol acid (salicylic acid), two clerodane diterpenoids (salvifolin and salvicin) and two flavonoids (pulicarin and giperoside) were isolated from alcoholic extract of the aerial parts of *P. gnaphalodes* (Vent.) Boiss. (Eshbakova 2011). Members

of the genus *Pulicaria* were investigated extensively all over the world, and the chemical literature survey shows the presence of flavonoids, sesquiterpenoids, diterpenoids, and sesquiterpenoid lactones (Shaiq Ali et al. 1999). Phytochemicals such as phenolic and flavonoid compounds are the largest group of antioxidant compounds, and they scavenge peroxide, lipid peroxyl, and hydroperoxide, and chelate metals (Sakai et al. 1996). Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolic compounds possess hydroxyl and carboxyl groups, which may inactivate iron ions by chelating and additionally suppressing the superoxide-driven Fenton reaction, which is believed to be the most important source of reactive oxygen species (Iravani and Zolfaghari 2011, 2013, 2014).

Bioreduction of silver ions to yield NPs using plant extracts such as Geranium leaf broth (Shankar et al. 2003), Neem leaf broth (Shankar et al. 2004), and Aloe vera plant extracts (Chandran et al. 2006) has been reported. It seems that the basis of all metal NPs synthesis methods is the bioreduction of metal ions by reducing agents. Accordingly, it might be interpreted that the aforementioned phytochemicals and

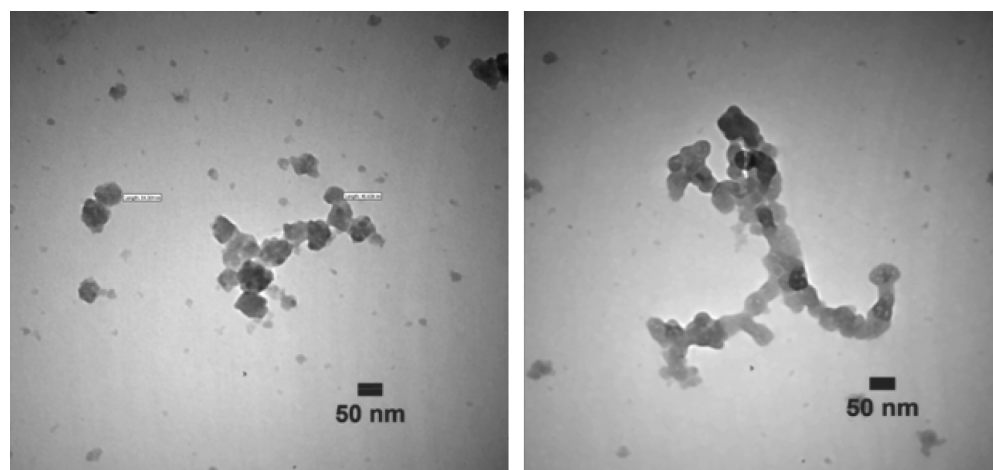


Figure 8. TEM micrographs of the produced NPs by dichloromethane extract of *P. gnaphalodes* (Vent.) Boiss. after 24 h of biotransformation.

phenolic compounds have the potential role in phyto-reduction of silver ions and formation of silver NPs.

## Conclusion

Both the extracts of *P. gnaphalodes* (Vent.) Boiss. successfully produced small and polydispersed NPs with low aggregates in early hours of the biotransformation. The optimum conditions for the reaction mixture were as follows (final concentrations): AgNO<sub>3</sub> (1 mM) as the substrate, concentrated and freeze-dried plant extract (equivalent to 100 g of 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. The conversion was fast and completed in 5 h. Results from TEM and DLS analysis demonstrated that by increasing the reaction time, the NPs were aggregated further. Methanol extract produced spherical and more single NPs, whereas dichloromethane produced porous polyhedral and more aggregated NPs. Therefore, it might be concluded that the solvent of extraction may influence the shapes and sizes of the produced silver NPs. Methanol extract of this plant seems to be quite attractive for industrial scale production of silver NPs.

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## 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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