each case and the silver nanoparticles which were precipitated at the bottom of the tube, mixed with deionized water and centrifuged again. This procedure was repeated for three times to increase the purity of nanoparticles. Then, the best samples were selected based on their appearance properties, such as intensity of darkness and applied for further experiments.

Characterization of silver nanoparticles

The revival of pure Ag^{+} ions was evaluated through UV-Vis spectroscopy. The UV-Vis spectrum analyses were performed using Array Photonix Ar 2015 spectrophotometer in the range of 300-350 nm wavelengths. In order to determine the size distribution of synthesized nanoparticles, Particle Size Analyzer (Vasco, France) was applied. To do this, samples were diluted (10 folds) with distilled water prior to analyses. Also, for more precise determination of particle sizes and morphological study of nanoparticles, transmission electron microscopy (Leo 912AB) was carried out at a voltage of 120 kV. TEM samples were prepared on carbon coated copper grids by dropping AgNPs colloidal solutions and the following drying steps under vacuum.

Results and Discussion

At first, plant extracts were pale yellow or green but following the addition of silver nitrate solution, their color changed to dark brown in 24 hours. As demonstrated in figure 1, this color changes were apparently visible (Figure 1).

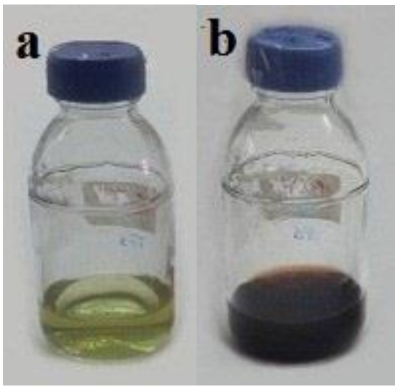

Figure 1. Color differences between (a) Marigold extract solutions and (b) Marigold extracts in the presence of silver nitrate solutions.

Plant extracts, which were kept in the absence of silver nitrate solution and didn't show any color changes, were considered as controls. The establishment of dark brown color after mixing the extracts with silver ions was a clear indicator of the metal ions reduction and the formation of silver nanoparticles in the environment. The addition of plant extracts to silver nitrate solution at a ratio of 10 to 100 was the best and produced relatively darker nanoparticle solutions.

In general, if color changes are associated with the formation of sediments, so they will show the formation of large particles; but, when color changes happened without any sedimentation, it could be concluded that synthesized nanoparticles have a very small particle size distribution. During our experiments no sedimentation was observed in nanoparticle solutions of marigold extracts.

Formation and stability of silver nanoparticles

To confirm the formation and proper stability of silver nanoparticles, their absorption spectrum was read after 24 hours in the range of 300-350 nm using a spectrophotometer (Unico Gene Company, USA). Results of UV-Vis analyses (Figure 2) showed that the aqueous extracts of various plant organs of Marigold, were produced different concentrations of silver nanoparticles. The highest absorbance at 430 nm was 1.6 a.u (Absorbance unit) and recorded for the nanoparticles which were synthesized using hairy root extracts, while, the lowest was obtained for nanoparticles synthesized by seed extracts (0.8 a.u). Absorptions of 1.5 a.u and 1.17 a.u were respectively observed at 430 nm for nanoparticles synthesized by petal and root extracts. Marchiol et al, (2014) used extracts of different organs (leaves, stems and roots) of various plants including *Brassica juncea*, *Festuca rubra* and *Medicago sativa* for synthesis of silver nanoparticles. Extracts of different plant organs indicated different performances during synthesis of nanoparticles. In *Brassica juncea* and *Festuca rubra*, root extracts showed a better performance, while, in *Medicago sativa*, leaf extracts were the best. In the present study the potential of various organ extracts of marigold (leaves, petals, seeds, roots and hairy roots) for production of silver nanoparticles were assessed comparatively, and the priority of hairy root extracts were confirmed.

The results of particle size analyses were shown in Figure 3. Results indicated that nanoparticles which were synthesized using hairy root and root extracts had the smallest size, respectively. The size of smallest and largest nanoparticles synthesized were equal to 5.4 and 22.4 nm in hairy root extracts, 14.8 and 61.7 nm in root extracts, 16.2 and 67.6 nm in petal extracts, and 29.5 and 67.6 nm for seed extracts. As demonstrated in Figure 3 (C, D), nanoparticles synthesized by hairy root and root extracts of marigold had the smallest size.

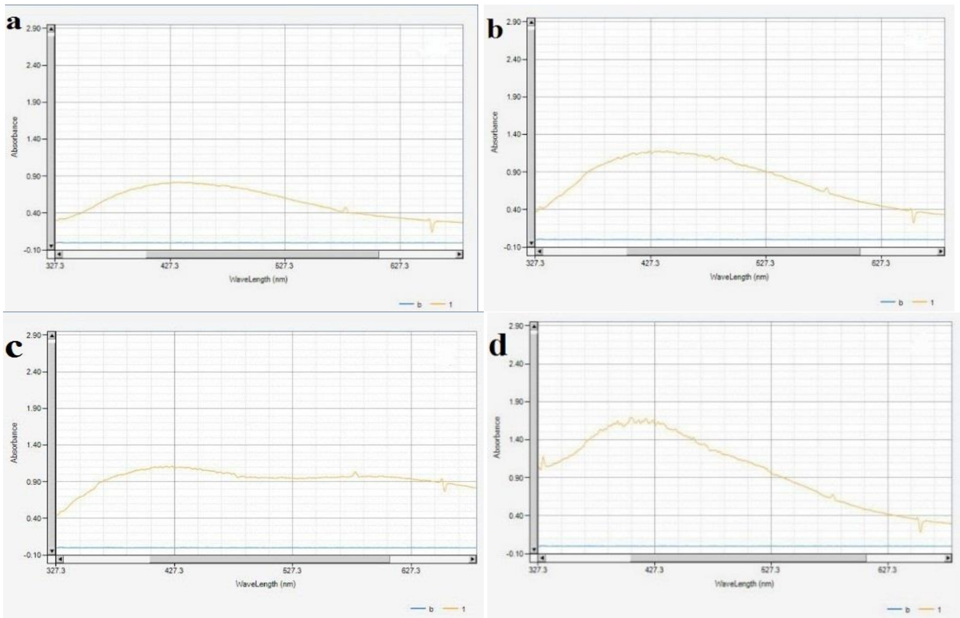

Figure 2. The results of UV-Vis analyses of silver nanoparticles synthesized by seed (a), petal (b), root and (c), hairy root (d) extracts of Marigold.

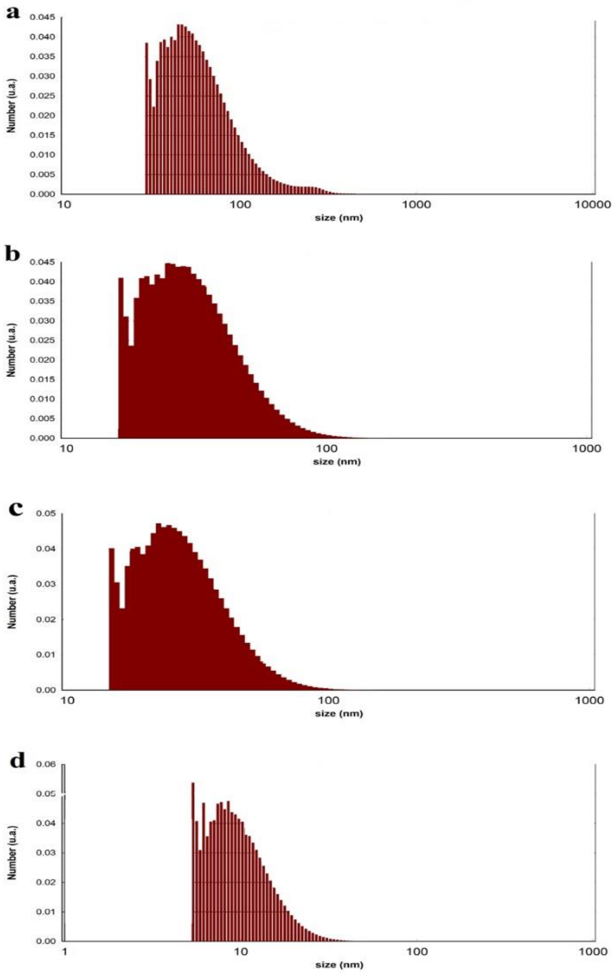

Figure 3. The results of nanoparticle size analyses obtained based on dynamic light scattering experiments (Cumulants mode) for silver nanoparticles synthesized by seed (a) petal (b) root (c) and hairy root (d) extracts of *Calendula officinalis.*

For more certainty and also to determine the morphological features of silver nanoparticles, they were investigated via transmission electron microscope.

The size of silver nanoparticles can be very different. This depends on materials applied to reduce silver ions, silver nitrate concentration, temperature conditions, and so on. In 2011, Darroudi et al, from Putra Malaysia University reported green synthesis and characterization of silver nanoparticles.

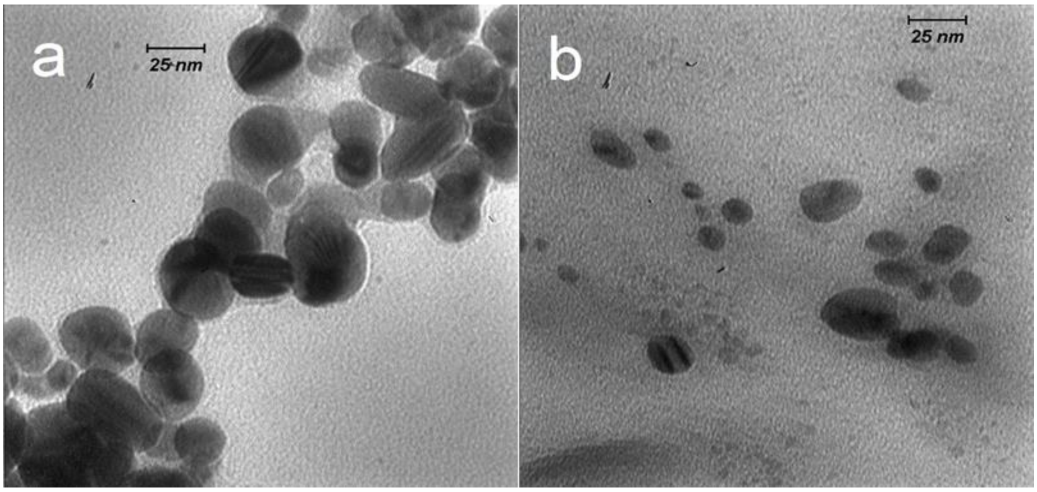

Figure 4. Images obtained via transmission electron microscopy (TEM) for silver nanoparticles with spherical appearance, which were synthesized using root (a) and hairy root (b) extracts of *Calendula officinalis*, scale bar: 25 nm.

They showed that by increasing the temperature, smaller silver nanoparticles are produced. Also, silver nanoparticles which were obtained in gelatin solution were smaller than the ones prepared in sugar containing gelatin solution. In another study, *Catharanthus roseus* was applied for synthesis of nanoparticles and their properties were determined by spectrophotometer UV-VIS, SEM and X-Ray analyses. They reported the preparation of nanoparticles with a diameter between 48 and 67 nm.

This method was introduced as eyewitness to produce nanoparticles with added value for commercial and industrial applications (Mukunthan et al., 2011). Padalia et al. (2015) produced silver nanoparticles by *Calendula officinalis* extract. The size of the synthesized nanoparticles was between 10-90 nm and they had spherical, hexagonal, and irregular shapes. Chidambaram et al. (2014) prepared silver nanoparticles from petal extracts of *Calendula officinalis* with 2-20 nm size ranges. Baghizadeh et al. (2015), reported the synthesis of silver nanoparticles form marigold seed extracts with spherical shape and diameter of 5 to 25 nm. Most of the nanoparticles produced in this study were located between 5 to 10 nm, considering their size distribution. It seems that the size and shape of synthesized silver nanoparticles, vastly, depend on the used materials and synthesis conditions. TEM confirmed the desired spherical appearance for silver nanoparticles which were synthesized in present study (Figure 4).

Conclusion

During last decades, silver nanoparticles have been attracted worldwide attention due to their unique physical and chemical properties. Considering disadvantages of physical and chemical methods of nanoparticle synthesis, green synthesis technologies were suggested as efficient alternatives. Different plant organs could be examined to achieve best results. Results from the present study, for the first time, confirmed that hairy root extracts of marigold had a better performance for production of desirable silver nanoparticles in comparison to other organs extracts.

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Conflict of Interest

The authors report no conflicts of interest.

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