Natural Resources and
Sustainable Development

Volume 8, Issue 1, 2018
DOI: 10.31924/nrsd.v8i1.008

GREEN BIOSYNTHESIS SILVER NANOPARTICLES USING LEAF EXTRACT

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Abstract

Using plant extract to synthesize silver nanoparticles is particularly worth of attention due to the simple, quick, affordable and friendly protocol with the environment. In our experiment, we have proved both the ability of common species (Mentha aquatic, Coleus blumei and Tagetes erecta) of achieving the synthesis of nanoparticles with a diameter between 8 and 300 nm, and the anti-bacterian effects of the obtained nano-silver.

Key words: plant extract, nanoparticles, Mentha, Coleus, Tagetes

INTRODUCTION

An important vector of research in nanotechnology is the synthesis of nanoparticles with controlled size, composition and morphology. Ranging in nanometers, nanoparticles have attracted attention due to their special properties, which find applications in fields like medicine, genetic therapy, DNA analysis, antibacterial agents, biosensors, magnetic resonance imaging etc. (Kulkarni, Muddapur, 2014). Nanoparticles can be obtained through physical, chemical, and biological methods, as well as combinations thereof. (Luechinger et al., 2010). Physical and chemical methods ensure good control of shape, size and quantity produced, but suffer from a series of disadvantages, principally linked to their complexity and to the toxic chemical substances employed. Waste resulting from these processes make them dangerous both to human health and to the surrounding environment (Sasidharan, Balakrishnaraja, 2014). Consequently, there is a growing need to discover technologies for nanoparticle synthesis which do not rely on toxic chemicals. Plant extract, due to its complex chemical composition, offers the biosynthesis process both the reductive, and the stabilizing compounds required (polyphenols, flavonoids, vitamins, protein, amino acids, enzymes, lignin, hemicellulose, pectin etc.) (Zhang et al., 2013; Tippayawat et al., 2016). According to Makarov et al, 2014, the general mechanism of green synthesis of metal nanoparticles consists in the bonding of metallic ions to reductive metabolites and to stabilizing agents, and their reduction to metallic atoms. The resulting complex between the metallic ion and the metabolite interacts with similar complexes, forming a very small nanoparticle, which continues to grow via coalescence and coagulation, until the particles become stable in terms of shape and size. Extracts from certain plant species (Piper longum) work as covering agents for the formation of silver nanoparticles, and can boost cytotoxic effects on tumor cells (Jacob et al., 2012). Both the Mentha aquatica and Coleus blumei species contain flavonoids and polyphenols in their compositions (Sytara et al., 2016). Flavonoids contain various functional groupings capable of forming nanoparticles. It has been postulated that, through tautomeric transformations of flavonoids from enol shape to ceto shape, a reactive hydrogen atom can be released, which can reduce metal (Makarov et al., 2014). Tagetes sp. essential oil is rich in terpenic hydrocarbons (Prakash et al., 2012). Terpenoids represent a different class of organic polymers, synthesized in plants from units of isoprene with five atoms of carbon, which exhibit strong anti-oxidant behavior (Makarov et al., 2014). According to Shankar and collaborators, terpenoids play a key role in the transformation of silver ions to nanoparticles.

MATERIAL AND METHOD

Leaves were harvested from plants belonging to the 3 species of interest (Coleus blumei, Tagetes erecta, Mentha aquatic – Fig. 1), and identified in the Botanical laboratory. They were washed twice in tap water, then cleared with distilled water, to remove epiphytal microflora and impurities, after which they were dried.

Coleus blumei Tagetes erecta Mentha aquatica

Fig. 1. Species used in the experiment

After being desiccated, they were ground, and 10 g of biomass were mixed with 100 ml deionized pater at 80^0 C for 5 minutes. The resulting infusion was decanted and filtered through Whatman paper until perfectly clear. A 1 mM silver nitrate ($AgNO_3$) was prepared, and 90 ml of it were mixed with 10 ml of the clear leaf extract, after which it was kept in the dark, at room temperature. After 12 hours, its color changed, indicating the formation of silver nanoparticles (Fig. 2).

Fig. 2. AgNPs solutions after 12 hours

RESULTS AND DISCUSSION

1. *Characterization of nanosilver using UV-visible spectroscopy*

Visible UV spectroscopy is a frequently used technique (Fig. 3). Wavelengths between 300 and 800 nm are used to characterize various metallic nanoparticles ranging in size from 2 to 100 nm (Feldheim, Foss, 2002). For Ag nanoparticles, the measuring interval is between 400 and 500 nm (Sriram, Pandidurai, 2014).

The formation of Nano Silver was confirmed by UV-visible spectroscopy. The results shown in Figure 3 have maximum absorbance between 400-500 nm region. This result is similar with other findings reported in literature. The UV-vis absorption spectrum of these solutions has a typical value of 420 nm, which corresponds to the characteristic resonance of the surface plasmon of silver nanoparticles. Also, the plasmon bands are symmetrical, which indicates that the solution does not contain multiple aggregated particles.

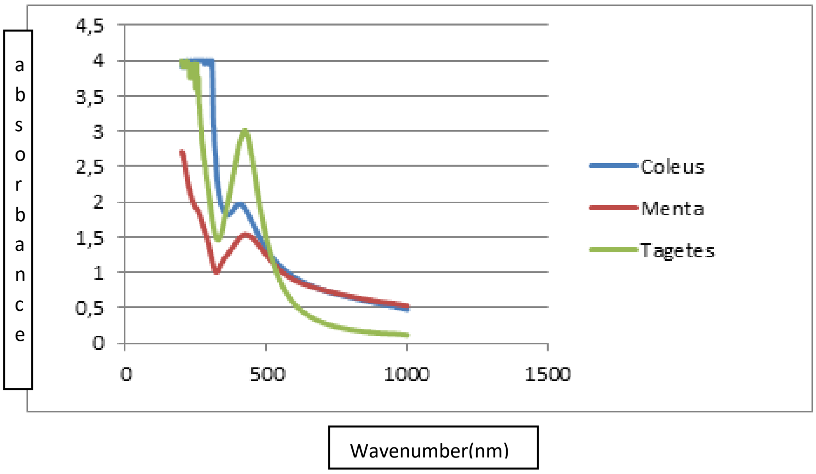


Fig. 3. UV-vis spectrum

2. *Dynamic Light Scattering*

DLS was applied to colloidal solution, using ZEN 3690 (Malvern Instruments), in order to determine the average particle size and size distribution (Jiang et al., 2009). The green method we employed ensured synthesis of silver nanoparticles with an average diameter of 53.08 nm in the case of Tagetes erecta, and 58.42 nm in the case of Coleus blumei (Fig. 4, 5, 6). The average sizes on the smallest peaks are of 15.27 nm in Tagetes and 12.45 nm in Coleus, and on the highest peaks, they were 105.8 and 113.6 nm respectively.

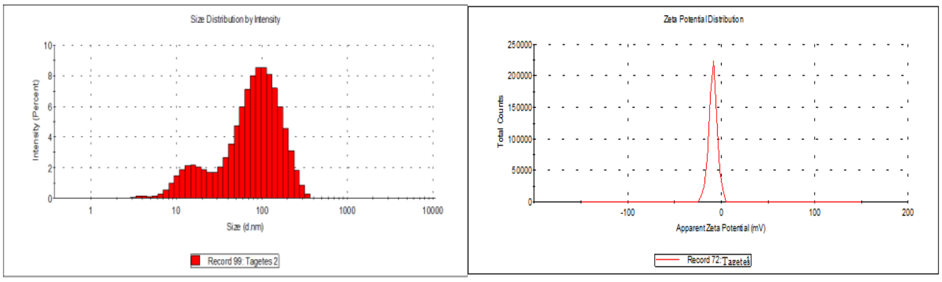


Fig. 4. DLH Tagetes erecta

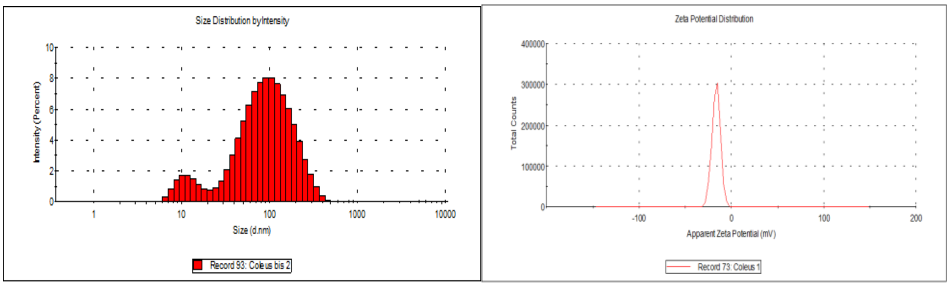


Fig. 5. DLH Coleus blumei

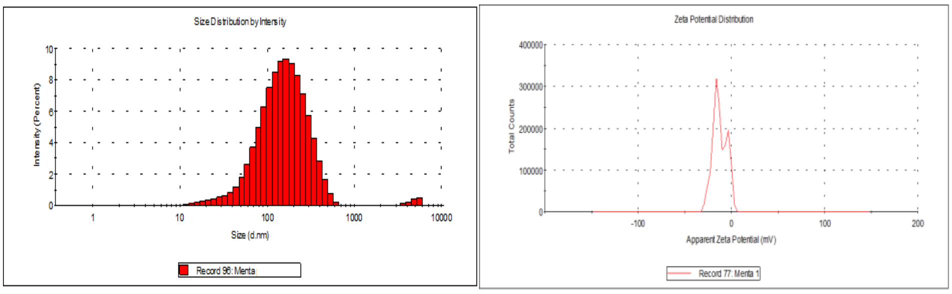


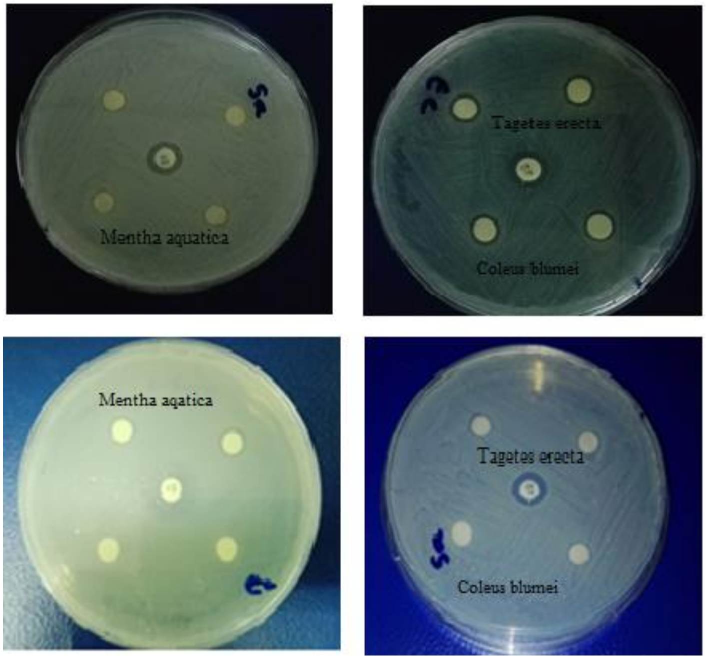
Fig. 6. DLH Mentha aquatica

The Zeta potential supplies data referring to the surface charge, the stability of nanoparticles, evaluating the purity of the system etc. Zeta potential is established by determining the electrophoretic mobility, combined with measuring the speed of the particles. The value of the Zeta potential offers information on the stability of the colloidal system. The general value which provides the distinction between a stable and an unstable system is placed at + 30 mV, or – 30 mV. Particles with potentials exceeding +30mV, or smaller than – 30 mV are normally considered stable. The Zeta potential values determined by us in our nanosilver solution show a good stability thereof. An important factor which influences Zeta potential is the pH value. The pH values of the nanosilver solutions of the 3 species tested by us was 5. Ag nanoparticles generally have low Zeta potential in strongly acid pH, and high Zeta potential in an alkaline pH (Dubey et al., 2010).

3. Antibacterial action

Silver nanoparticles exhibit bactericide action both against Gram-positive and Gram-negative bacteria (Valodkar et al., 2011). The antimicrobial activity of AgNPs takes places based on multiple mechanisms. According to Pal et al. (2007), the antimicrobial effect is due to the formation of pores in cellular walls, which facilitate the loss of cellular contents. Another suggested mechanism is the penetration of ionic channels by the silver ion, and the degradation of the ribosome, as well as the inhibition of the expression of enzymes and proteins which contain essential thiol for the production of ATP and DNA, thus inducing cell death. The interactions between bacteria and silver nanoparticles were also linked to their coupling to the active site of the cellular membrane, and the inhibition of the functions of the cellular cycle (Kim et al., 2007).

We tested the antimicrobial effects of AgNPs (Kirby-Bauer method) by using 2 strains of E.coli bacteria and Staphylococcus aureus. We used an unselective medium for bacterial development (Nutritional Agar) and, as reference, cellulose disks supplied by Liofilchem, impregnated with 10 µg of Gentamicin. In order to test the antimicrobial action, ø6 mm blank cellulose disks (BioMaxima S.A.) were impregnated with 10 µl AgNPs solution (Fig. 7).



Staphylococcus aureus Escherichia coli

Escherichia coli Staphylococcus aureus

Fig. 7. Antibacterial effect of silver nanoparticles

The antibacterial effect at tested concentrations is obvious in Tagetes erecta, Coleus blumei and Mentha aquatica, on E.coli and Staphylococcus aureus, and less evident in the case of Coleus blumei on Staphylococcus aureus.

CONCLUSIONS

AgNPS biosynthesis using plant extracts is a simple, cost-effective, and environmentally friendly method. In this study, we have highlighted the possibility of obtaining Ag nanoparticles, and their antibacterial effects, using leaves from the plant species Tagetes erecta, Coleus blumei and Mentha aquatica.

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Received: May 3, 2018
Revised: May 31, 2018