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Aqueous Phase Lavender Leaf Mediated Green Synthesis of Gold Nanoparticles and Evaluation of its Antioxidant Activity

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Abstract

In this paper, green synthesis of gold nanoparticles (AuNPs) using leaf extract of Lavender (*Lavandula angustifolia*) and their antioxidant activity was evaluated under the ambient aqueous phase condition. As-synthesized AuNPs were characterized by visual, ultraviolet–visible–near infrared (UV–vis–NIR) spectroscopy, transmission electron microscopy (TEM), and dynamic light scattering (DLS) technique. Formation of AuNPs produces an intense absorbance peak at 530 and 1055 nm in UV–visible spectroscopy. TEM and DLS results confirmed that the synthesized AuNPs were crystalline, polydisperse, quasi-spherical and triangular shape with an average size ranging from 34–300 nm. UV-vis-NIR, TEM, and DLS study showed that the phytochemicals from the Lavender leaf extract have dual properties of reducing and stabilizing agents. Further, AuNPs (21.53%, 0.2 mL) showed higher antioxidant activity than Lavender leaf extract (4.73%, 0.2 mL) against 2,2-diphenyl-1-picrylhydrazyl. On looking these advantages, green synthesis of AuNPs without inert atmosphere is highly recommended for future medicinal and industrial application.

Keywords: Antioxidant activity; Nanobiotechnology

Introduction

In recent years, nanobiotechnology is one the most popular branch of nanotechnology, which received the most attention from the researchers, due to its economic and eco-friendly procedures to generate particles with a dimension smaller than 100 nm, with specific functions [1]. Nanoparticles are synthesized by various methods such as chemical, physical, mechanical and biological [2]. The gold nanoparticles (AuNPs) is of particular interest because of its valuable application in catalysis, sensor, electronics, medicine, drug delivery, biomedical diagnostics, biolabeling, tissue/tumor imaging, photothermal therapy and immunodiagnostic [3,4].

Green synthesis of AuNPs using plant extract is recommended as an eco-friendly alternative to chemical methods that reduces the maintenance of septic environment and eliminates the generation of toxic byproducts [5]. The plant materials like *Azadirachta indica* leaf [6], *Cinnamomum camphora* leaf [7], *Lantana camara* flower [2], *Genipa americana* fruit [8], *Emblica officinalis* fruit [9], *Capsicum baccatum* fruit [10], *Abelmoschus esculentus* seed [11] and *Plukenetia volubilis* oil [12] have been reported for the extracellular synthesis of AuNPs.

Lavender (*Lavandula angustifolia*) is a widely distributed ornamental plant belongs to the family Lamiaceae and cultivated extensively in temperate climates of South America, Europe, and Asia. It has been traditionally considered for its very pleasant smell and a bitter taste. Its purple flowers and essential oil are used in toiletry, cosmetics, perfume, pharmaceutical, food and flavor industries. Many compounds have been detected in lavender aerial parts and flowers extract, including geraniol, linalool, linalyl acetate, ursolic acid, luteolin, umbelliferone, coumarin etc. The plant is used in traditional and folk medicines in the different parts of the world for the treatment of several skin sores, insect bites, gastrointestinal, nervous and rheumatic disorders. It also showed carminative, diuretic, anti-epileptic, anti-rheumatic, pain reliever, relaxant, sedative, antioxidant, burn healing, antibacterial and anti-inflammatory properties [13,14].

So, green synthesis of nanoparticles using plant materials is of great interest. Hence, the main goal of the present study was the facile green synthesis of AuNPs using the aqueous leaf extract of Lavender. The synthesized AuNPs were characterized using ultraviolet–visible–near infrared (UV–vis–NIR) spectroscopy, transmission electron microscope (TEM), and dynamic light scattering (DLS) technique. Further, the antioxidant efficacy of AuNPs was assessed *in vitro* against 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and compared with the leaf extract.

Materials and Methods

Materials

Gold chloride ($AuCl_4^-$, 99.0%) was purchased from Spectrum, USA and DPPH$^{\bullet}$ (>99.5%) was purchased from Sigma-Aldrich, USA. Lavender leaves were collected from the local garden of Universidad de las Fuerzas Armadas ESPE, Sangolqui, Ecuador. The thoroughly washed Lavender leaf (2 gm) was chopped and ultrasonicated in 25 mL of water for 3 mins. Ultrasonication was performed with ultrasonic processors (DAIGGER GE 505, 500 W, 20 kHz) immersed directly into the reaction solution. The operating condition was at 30 sec pulse on/30 sec pulse off time with an amplitude of 72% at 25°C for 3 minutes. After ultrasonication, yellow color Lavender leaf extract (LLE) was filtered using Whatman paper no. 1 and stored at 4°C for further use.

Green synthesis of AuNPs

For the green synthesis of AuNPs, 1.0 mL of LLE was mixed with 10 mL of 0.5 mM $AuCl_4^-$ solution at room temperature (22-25°C). Reduction of Au^{3+} to Au and the formation of AuNPs indicated by the appearance of a pink color after 4 hours of incubation period.

Antioxidant activity of LLE and AuNPs

The antioxidant activity of the LLE and was measured by using the DPPH• method adapted from Kumar et al., [5,12] with slight modifications. The LLE/ AuNPs (1000-200 µL) or control and (1000-1800 µL) of H_2O was mixed with 2.0 mL of 0.2 mM (DPPH•) in 95% ethanol. The mixture was vigorously vortexed and allowed to reach a steady state in dark incubation at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm, and the free radical scavenging activity was calculated using Equation (1)

$$Scavenging\ activity\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) x\ 100 \qquad (1)$$

where $A_0$is the absorbance of the control, (blank, without extract, or AgNPs) and A_1 is the absorbance in the presence of the LLE or AuNPs. The final result was expressed as % of DPPH• free radical scavenging activity (mL).

Characterization of AuNPs

The synthesized AuNPs were primarily characterized with the help of a UV–vis–NIR single beam spectrophotometer (Thermo Spectronic, GENESYS^{TM} 8, England). TEM and selected area electron diffraction (SAED) were performed in support film of 2% polyvinyl formal solution stabilized with carbon and recorded digitally (Tecnai G2 Spirit TWIN, FEI, Holland). The hydrodynamic size distributions and polydispersity index (PDI) of nanoparticles were analyzed by using DLS instrumentation (LB-550, HORIBA, Japan).

Results and Discussion

Visual and UV–vis–NIR study

The visual color change from light yellow to magenta pink after treatment of Au^{3+} with LLE clearly indicates the synthesis of AuNPs via reduction of Au^{3+} to Au (Figure 1, Inset). This change of solution color may be attributed to the surface plasmon resonance (SPR), a specific phenomenon which arises due to the collective oscillations of electrons in the conduction band with that of electromagnetic radiation owing to which it gives absorption in the UV–vis–NIR region [15]. In Figure 1, the time-dependent appearance of two new absorption peaks at λ_{max}=530 and 1055 nm corresponding to transverse and longitudinal SPR component of spherical and triangular AuNPs. The alteration in the position of these bands gives information about the particle size, dielectric constant morphology, and adsorbed species on the surface [16,17]. Whereas LLE shows only one absorption peak at 270 nm, it may be due to the presence of flavonoids and coumarins in LLE extract.

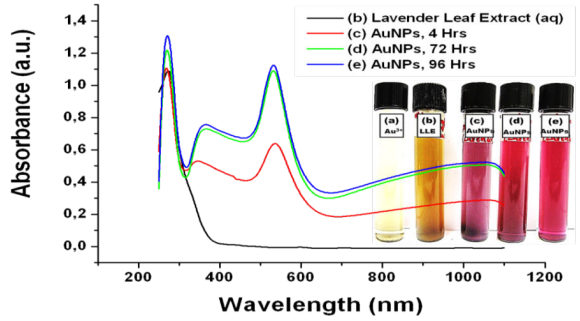

Figure 1: Visual picture and UV–vis–NIR absorbance spectra of as-synthesized AuNPs at different time interval.

TEM study

The morphology, size and crystallinity of the as-synthesized AuNPs were investigated by TEM and SAED measurements. Figure 2 shows TEM images of AuNPs synthesized by using LLE. This study clearly indicates the formation of quasi-spherical and triangular particles of diameter in the range of 30-300 nm. It could be also seen that the triangular AuNPs are coexisting with quasi spherical AuNPs, indicating that the absorption in the NIR region (1055 nm) and this result that was constant with UV–vis–NIR analysis. The AuNPs are considerable anisotropy, well separated from each other indicating good capping and absence of aggregation. The bright hexagonal spot in the SAED pattern (Figure 2c) from the triangular AuNPs reveals that the AuNPs are single crystalline and highly (111) oriented with the top normal to the electron beam [18,19].

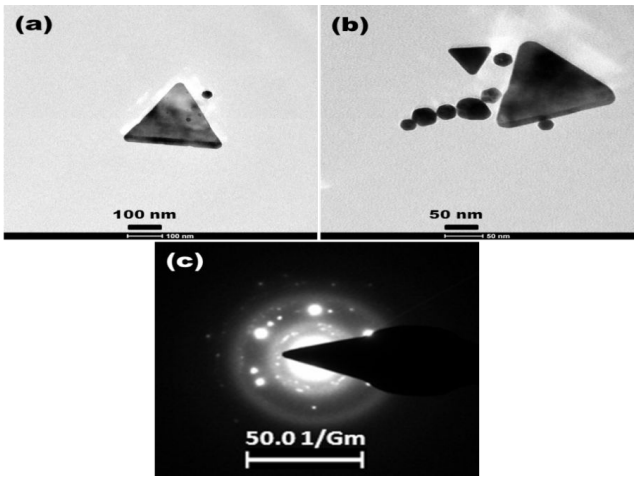

Figure 2: TEM (a-b) and SAED (c) images of AuNPs synthesized by using LLE.

DLS study

The DLS particle size analyzer was used for determining the hydrodynamic size distribution of synthesized AuNPs and the average size was found to be 113.6 ± 56.9 nm (Figure 3). The size distribution of AuNPs ranged from 34 nm to 400 nm and polydisperse in nature (PDI = 0.12566), due to the presence of a small quasi-spherical and big triangular AuNPs. This result supports the UV–vis–NIR and TEM analysis.

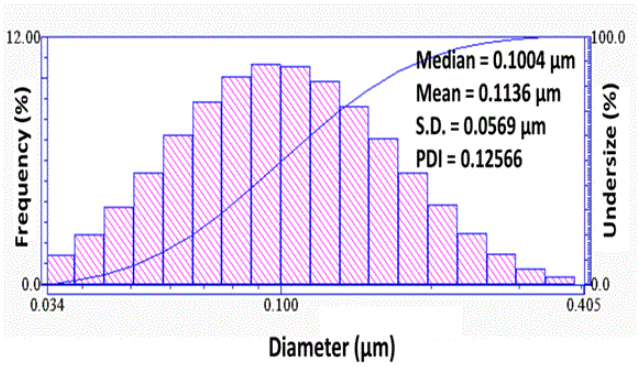

Figure 3: DLS size distribution of AuNPs synthesized by using LLE.

Antioxidant study

In normal metabolism, levels of free radicals ($O_2^{\bullet}$, $O^{\bullet}$, $HO^{\bullet}$, $NO^{\bullet}$) and antioxidants are balanced. However, the overproduction of free radicals results in oxidative damage, leading to a range of chronic diseases, such as cancer, diabetes, and inflammation. The intake of antioxidants provides protection against damage caused by free radicals. However, the use of synthetic antioxidants has been limited because of their toxicities. Therefore, research is now directed toward antioxidants of natural origin [20]. $DPPH^{\bullet}$ is a stable synthetic free radical which is readily reduced by antioxidants, either by accepting or donating electrons, during which the color of $DPPH^{\bullet}$ changes from purple to yellow due to the formation of hydrazine molecules [21]. The antioxidant activity of the LLE and AuNPs was estimated by comparing the % inhibition of $DPPH^{\bullet}$ radicals (Figure 4). It can be observed that AuNPs (21.53%, 0.2 mL) show better $DPPH^{\bullet}$ quenching activity as compared to LLE (4.73%, 0.2 mL) at a lower concentration. The $DPPH^{\bullet}$ scavenging activity of LLE increased with increasing concentrations (4.73%, 0.2 mL; 7.70%, 0.4 mL; 14.09%, 0.6 mL; 22.27%, 0.8 mL and 34.28%, 1mL) whereas, AuNPs showed the opposite trend (21.53%, 0.2 mL; 19.56%, 0.4 mL; 17.18%, 0.6 mL; 10.55%, 0.8 mL and 4.14%, 1mL) due to the less solubility of AuNPs. The antioxidant effects of the AuNPs might be the result of an active physicochemical interaction of Au atoms with the functional groups of the LLE [22].

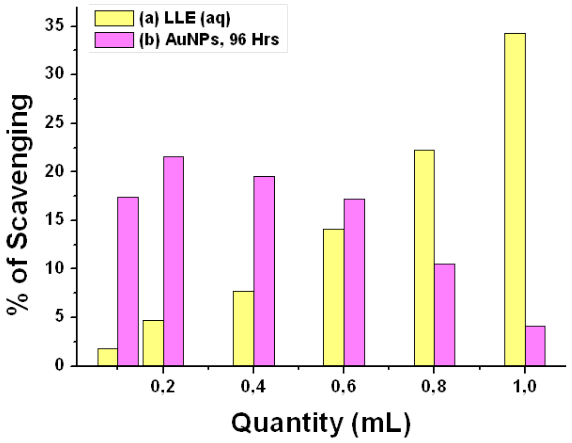

Figure 4: Antioxidant activity of (a) LLE and (b) AuNPs against $DPPH^{\bullet}$.

Conclusion

In conclusion, we have investigated the use of LLE as a reducing and stabilizing agent for the synthesis of AuNPs in an aqueous medium. The UV-vis-NIR, TEM with SAED and DLS results show that as synthesized AuNPs are polydisperse nature, quasi-spherical and triangular shape with an average size ranging from 34–300 nm. It shows enhanced antioxidant activity than LLE alone at low concentration. This study provides an eco-friendly route for the synthesis and application of AuNPs with excellent reproducibility.

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