

Screening of Medicinal Plant Methanol Extracts for the Synthesis of Gold Nanoparticles by Their Reducing Potential

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Development of nontoxic, clean techniques for the synthesis of metal nanoparticles such as gold has attracted increasing attention in recent years. Although many reports have been published about the biogenesis of gold nanoparticles using several plant extracts such as Neem leaf broth (*Azadirachta indica*), the capacity of a large number of such extracts to form gold nanoparticles has yet to be elucidated. In this research a titrimetric assay was employed for preliminary evaluation of the reducing potential of different medicinal plant extracts. All the extracts were used separately for the synthesis of gold nanoparticles through the reduction of aqueous AuCl_4^- . After the screening step, the methanol extracts of *Eucalyptus camaldulensis* and *Pelargonium roseum* were selected for further studies. The reducing ability of these extracts was significantly enhanced as compared to Neem leaf broth (*Azadirachta indica*) which was used as control sample. Transmission electron microscopy, energy-dispersive spectroscopy and visible absorption spectroscopy confirmed the reduction of gold ions to gold nanoparticles. The *E. camaldulensis* and *P. roseum* extracts produced gold nanoparticles in the size ranges of 1.25–17.5 and 2.5–27.5 nm with an average size of 5.5 and 7.5 nm, respectively.

Key words: *Eucalyptus camaldulensis*, *Pelargonium roseum*, Neem Leaf, Gold Nanoparticles, Synthesis, Plant Extracts, Chloroauric Acid, Reduction, Spectrum

Introduction

The development of green processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology [1, 2]. Today, nano-metal particles, especially gold, have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of chemistry, electronics, medicine, and biotechnology at the nanoscale [2–5]. Gold nanoparticles could also have many new applications in biology; for example, they are used for the development of biosensors and DNA labeling [6, 7]. In medicine, gold nanoparticles are used for different purposes. For example, after cellular uptake, they can act as tiny, precise and pow-

erful heaters (thermal scalpels) to kill cancer [8–9], and they are capable of inducing apoptosis in B-chronic lymphocytic leukemia [10]. Many reports have been published in the literature on the biogenesis of gold nanoparticles using several plant extracts, particularly Neem leaf broth (*Azadirachta indica*), alfalfa (*Medicago sativa*) and geranium leaves (*Pelargonium graveolens*) [11–17]. However, the synthesis of gold nanoparticles using total extracts has not yet been studied for a large number of plants. Generally, the reducing property of the plant extracts and their constituents, such as flavonones and terpenoids, plays a critical role in the reduction of Au^{+3} to gold nanoparticles [11]. In this comparative study, the reducing ability of common medicinal plant extracts has been investigated using

Table 1. The reducing ability of some medicinal plant extracts and UV/Vis characteristics of the chloroauric acid solution treated with these extracts for 15 min.

Plant name (Voucher number)	Spectrum characteristics		Amount of KMnO_4 (mg) used for oxidation of 1 mg of dried plant extracts
	λ_{max} (nm)	Optical density	
<i>Artemisia annua</i> L. (83011)	538.9	0.766	2.10
<i>Eucalyptus camaldulensis</i> (84160)	534.4	1.02	2.60
<i>Artemisia absinthium</i> L. (83004)	539.5	0.331	1.41
<i>Lippa citriodora</i> (83411)	542.5	0.749	2.60
<i>Mentha piperita</i> (83453)	538.9	0.911	2.33
<i>Lavandula angustifolia</i> (84410)	535.9	0.794	1.89
<i>Pelargonium roseum</i> L. (84570)	533.2	0.995	3.29
<i>Ocimum basilicum</i> L. (84530)	530.2	0.708	2.30
<i>Ruta graveolens</i> (83638)	–	–	2.4
<i>Hyssopus officinalis</i> (84261)	535.0	0.648	2.5
<i>Rosmarinus officinalis</i> (83636)	540.4	0.930	2.25
<i>Azadirachta indica</i> (positive control)	532.6	0.487	1.2

a wet-analytical technique (titrimetric method). Separately, all plant extracts were used for the synthesis of gold nanoparticles by the reduction of aqueous AuCl_4^- . In this screening process involving a number of medicinal plants, we observed that *Eucalyptus camaldulensis* and *Pelargonium roseum* extracts are potential candidates for rapid synthesis of gold nanoparticles.

Results and Discussion

Screening and synthesis of gold nanoparticles

The chemical reduction of aqueous solution of chloroauric acid (HAuCl_4) is one of the most widely used methods for the synthesis of gold colloids. In this study, the reducing properties of different medicinal plant extracts were independently investigated using an oxidation-reduction titrimetric assay involving KMnO_4 (Table 1). The extracts from *E. camaldulensis* and *P. roseum* showed the highest reducing ability among the tested extracts including the control sample (Neem leaves). In a series of parallel experiments, the formation of gold nanoparticles by these methanol extracts was also investigated. The appearance of a purple color in the reaction vessels suggested the formation of gold nanoparticles with size < 20 nm [18]. Fig. 1 shows the test tubes containing the plant extracts of *E. camaldulensis* and *P. roseum* before (tube A) and after reaction with Au^{3+} for 15 min (tubes B and C). The gold containing solution (tube A) that was transparent yellow at first turned into purple on completion of the reaction (tubes B and C). Also, the methanol extracts of

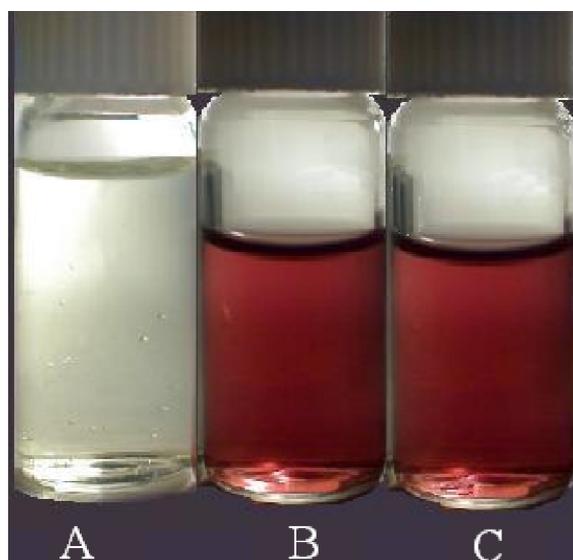


Fig. 1. Solutions of chloroauric acid (1 mM) before (A) and after exposure to the methanol extracts of *Eucalyptus camaldulensis* (B) and *Pelargonium roseum* (C) plant extracts.

other plants changed the color of solutions from clear to other colors such as green, violet, purple and grayish after 15 min of reaction with Au^{3+} (pictures not shown).

These reaction mixtures were further characterized by UV/Vis spectroscopy. The technique outlined above proved to be very useful for the analysis of nanoparticles [18–21]. As illustrated in Fig. 2, a strong, broad absorption band with maxima located between 530 and 542 nm was observed due to formation of gold nanoparticles produced by the extracts and the control sample (*Azadirachta indica*). This peak is assigned to a surface plasmon, a phenomenon that is well-documented for various metal nanoparticles with sizes ranging from 2 to 100 nm [18–21]. The strong surface plasmon resonance maxima of *E. camaldulensis* and *P. roseum* were centered at *ca.* 534.4 and 533.2 nm, respectively. The most intense plasmon resonance bands were observed for *E. camaldulensis* and *P. roseum* extracts; therefore, these plant extracts were selected for further experiments and characterizations. As mentioned above, these samples showed the greatest reducing ability compared to the other plant extracts in the oxidation-reduction titrimetric assay using KMnO_4 solution (Table 1). In contrast, the *Ruta graveolens* extract showed considerable reducing ability, but the surface plasmon resonance band was not observed for this extract (Table 1). Also, this extract did not change the

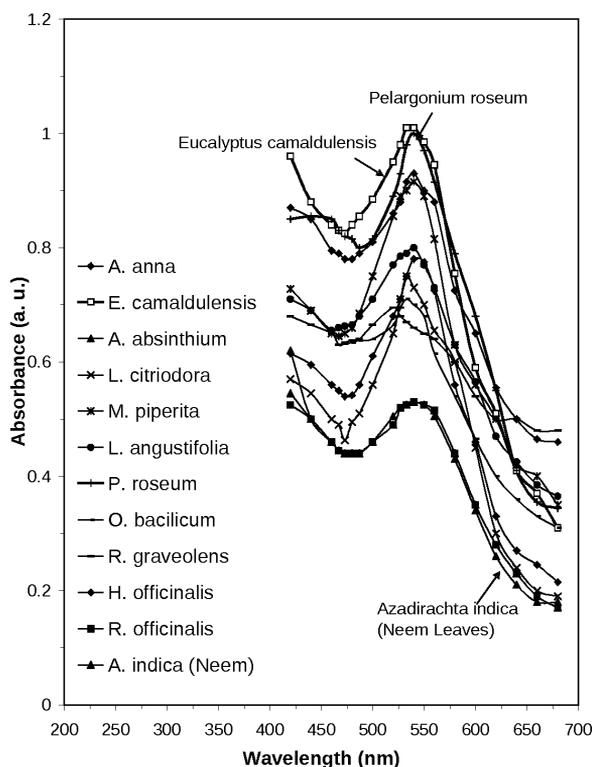


Fig. 2. UV/Vis spectra of gold colloids: Spectra recorded after adding different medicinal plant extracts (10 mL) to 90 mL of chloroauric acid solution (1 mM). The curves are recorded after a period of 15 min. The largest plasmon resonance band was observed for *Eucalyptus camaldulensis* and *Pelargonium roseum* methanol extracts.

solution color to purple after 15 min of reaction with Au^{3+} . This indicates that gold ions cannot be reduced by the type of reducing agents that are present in the *R. graveolens* methanol extract.

Fig. 3 shows the UV/Vis spectra recorded for the reactions of aqueous chloroauric acid solutions with *E. camaldulensis* (top) and *P. roseum* extracts (bottom) as a function of time. The strong resonance bands centered at about 433 nm increases with time.

Particle size and its chemical composition

Fig. 4 shows representative TEM images recorded from the drop-coated film of the gold nanoparticles synthesized by treating the chloroauric acid solution with the plant extracts of *E. camaldulensis* and *P. roseum*. The size histogram of gold particles produced by *E. camaldulensis* extract (upper right illustration in Fig. 4) shows that the particles range in size from 1.25 to 17.5 nm, and possess an average size of 5.5 nm. Fur-

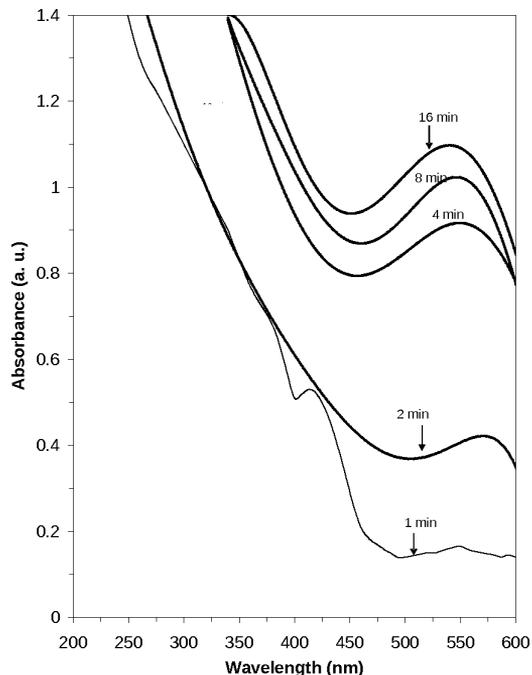
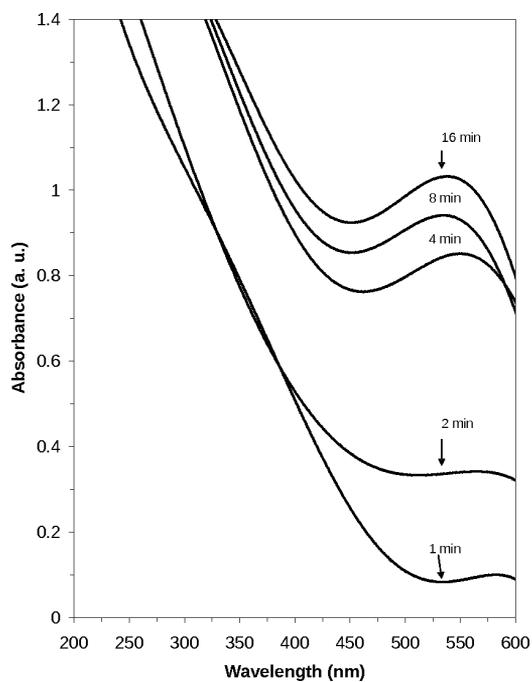


Fig. 3. UV/Vis Spectra recorded as a function of time for 1 mM aqueous solutions of chloroauric acid reacted with *Eucalyptus camaldulensis* (top) and *Pelargonium roseum* (bottom) plant extracts.

thermore, the *P. roseum* extract reduces Au^{3+} to gold nanoparticles (particle size from 2.5 to 27.5 nm) with

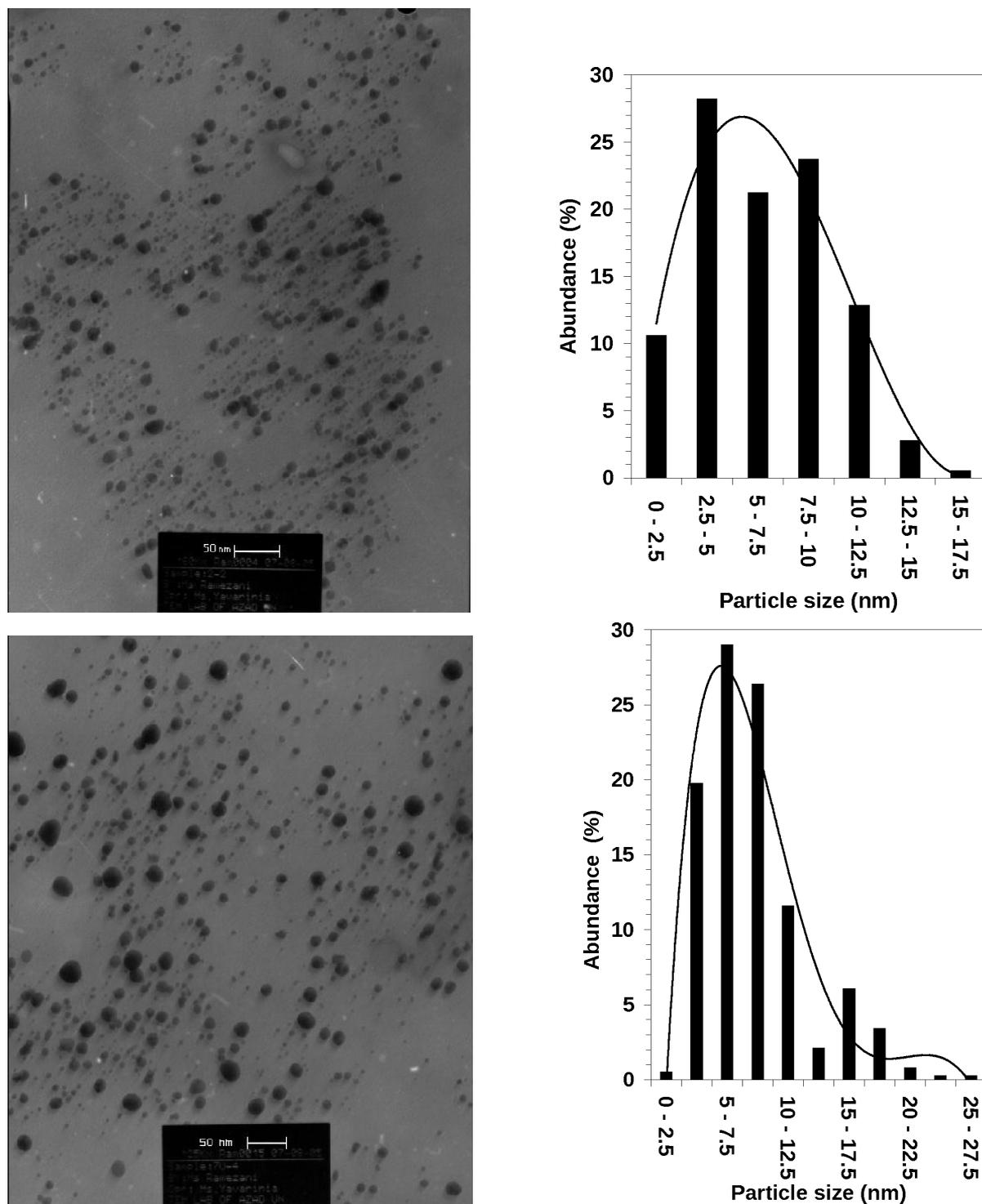


Fig. 4. Transmission electron micrographs recorded from a small region of a drop-coated film of chloroauric acid solution treated with the methanol extracts of *Eucalyptus camaldulensis* (upper left picture) and *Pelargonium roseum* (lower left picture) for 15 min (scale bars correspond to 50 nm). The related particle size distribution histograms obtained after counting 350 individual particles for each sample, are also shown (right side pictures).

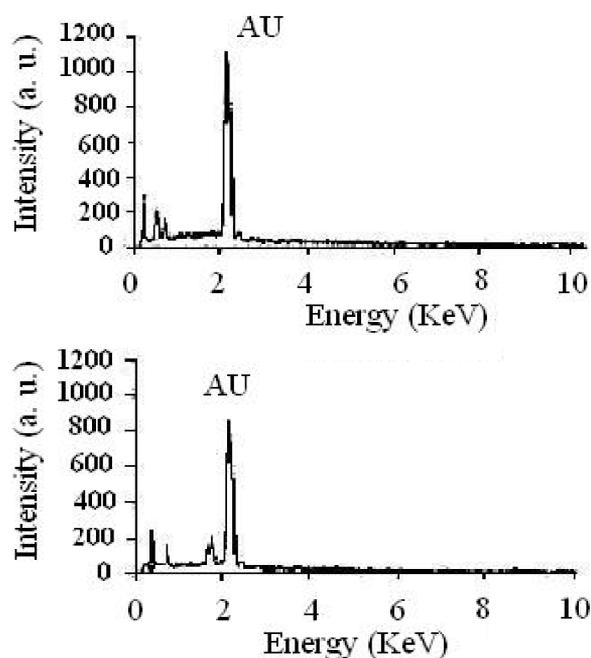


Fig. 5. EDS spectra of gold nanoparticles for Au^{3+} ion solutions treated with two methanol extracts provided from *E. camaldulensis* (upper spectrum) and *P. roseum* (lower spectrum). Gold X-ray emission peaks are labeled. Strong signals from the atoms in the nanoparticles are observed in two spectra and confirm the reduction of gold ions to gold nanoparticles.

an average size of 7.5 nm (lower right illustration in Fig. 4). In the analysis of the gold nanoparticles by energy dispersive spectroscopy (EDS), the presence of the signal of elemental gold was confirmed in two samples (Fig. 5). The Au nanocrystallites display an optical absorption band peaking at 2.15 keV, which is typical of the absorption of metallic Au nanocrystallites [18].

Conclusion

The potential ability of different plant extracts for the reduction of Au^{+3} to gold nanoparticles was investigated. In this screening program, the methanol extracts of *E. camaldulensis* and *P. roseum* were selected for further experiments. Characterization by UV/Vis, TEM, and EDS techniques confirmed the reduction of gold ions to gold nanoparticles. To the best of our knowledge, and based on a thorough literature surveys, this is the first report on the synthesis of gold nanoparticles using total extracts of *E. camaldulensis* and *P. roseum*. Also in this investigation, a wet-analytical technique (titration with KMnO_4) was used to determine

the total reducing ability of plant extracts. This method can have significant potential for finding potent reducing extracts for the synthesis of gold nanoparticles.

Experimental Section

Plant materials and extraction

Whole plants of different medicinal plants (listed in Table 1) and *Azadirachta indica* leaves (Neem) as control sample were harvested in May, 2006, from the botanic garden of the Faculty of Pharmacy, Medical University/ University of Tehran, Tehran, Iran. The plants were air-dried at r. t. and then pulverized (50 g). Each powder was extracted three times with 200 mL of methanol (Merck) by maceration (48 h). The combined solvent extracts were evaporated to yield a brownish or greenish, viscous residue. All experiments were performed on the basis of dry mass of the concentrated extracts. Different stock solutions (10 mg/mL) were prepared in methanol for further experiments.

Titrimetric analysis

The reducing properties of the different stock solutions were quantitatively investigated by the conventional potassium permanganate back-titration method. All samples (0.5 mL) were diluted by distilled water (20 mL) and acidified with 2 mL of sulfuric acid (3 N). Subsequently, all diluted samples were oxidized with an excess of potassium permanganate (0.1 N) for 30 min at 60 °C, and the unreacted permanganate was titrated with 0.2 N oxalic acid solutions. The end-point was determined when the violet solution produced by the excess of potassium permanganate faded into colorless. The concentration of reducing agents in different samples was calculated using the volume and normality of the permanganate solution used for reduction of the samples and reported as mg of KMnO_4 per mg of the extract.

Synthesis and characterization of gold nanoparticles

Aliquots of an aqueous chloroauric acid solution (10^{-3} N) are added to the reaction vessels containing different plant extracts (10% v/v), and the resulting mixtures were allowed to stand for 15 min at r. t. Chloroauric acid was purchased from Merck, Germany. Methanol solution (10% v/v) was used as negative control. The reduction of the Au^{+3} ions by different plant extracts in the solutions was monitored by sampling the aqueous component (2 mL) and measuring the UV/Vis spectrum of the solutions. All samples were diluted three times with distilled water, and the UV/Vis spectra of these samples were measured on a Cecil model 9200 spectrophotometer at a resolution of 1 nm. Gold nanoparticles were characterized by transmission electron microscopy (model EM 208 Philips) and energy-dispersive spectroscopy (EDS).

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