Green Synthesis of Gold Nanoparticles Using Three Medicinal Plant Extracts as Efficient Reducing Agents

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ABSTRACT: *The aim of this work is green synthesis of gold nanoparticles using the aqueous extract of Anthriscus sylvestris, Ferula gummosa and Achillea eriohora leaf and stems as the reducing agents. The effects of reducing agent concentration in the reaction mixture and temperature on the size of the gold nanotriangles were studied. The nanoparticles were characterized using UV–Vis spectroscopy, XRD, and SEM. XRD studies show that the particles are crystalline in the cubic phase. However, reduction of gold ions by plant extracts resulted in formation of spherical gold nanoparticles with diameters from 18 to 56 nm.*

KEYWORDS: *Anthriscus sylvestris*; *Ferula gummosa*; *Achillea eriohora; Biosynthesis; Gold nanoparticles.*

INTRODUCTION

Nanoscience is a relatively new and efficient branch of science dedicated to the improvement and utilization of devices and structures ranging from 1 to 100 nm in size, in which new chemical, physical, and biological properties, are not necessarily similar to those observed in bulk materials [1]. Nanoparticles, due to their specific electrical, optical, magnetic, chemical, and mechanical properties are currently used in many high technology fields, such as the medical sector for diagnosis, antimicrobial, drug delivery [2] as well as in the electronic and optoelectronic industry or in the chemical sector for catalysis, for environmental protection, and energy conversion [3,4].

Synthesis of nanoparticles is usually carried out by various physical and chemical methods, such as evaporation–condensation, liquid-phase synthesis, photoreduction, electrolysis and pyrolysis. Most of these methods are expensive and requiring the use of toxic solvents [5]. Nowadays there is a growing need to expand environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis method. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration [6]. Nanoparticles of noble metals, such as gold, silver, and platinum, are widely used in products that come into direct contact with the human body. Nanoparticles are used, or being evaluated for use, in many fields such as medical and pharmaceutical applications, manufacturing and materials, energy and electronics [7]. For instance, a great deal of effort has been put into the biosynthesis of inorganic materials, especially noble metal nanoparticles, using microorganisms and plants or fruit extracts [8-10].

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1021-9986/2019/1/1-10 *10/$/6.00*

Biological methods or greener protocols for synthesis of nanoparticles using microorganisms, enzymes, and plants or plant extracts have been suggested as potential eco-friendly alternatives to chemical and physical methods. The reducing agents involved include the various water soluble plant metabolites (e.g. alkaloids, phenolic compounds, and terpenoids) and co-enzymes [11-13]. Due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization [4,14]. Also, these green methods are low cost, fast, efficient, and generally lead to the formation wide variety of shapes including spheres, rods, cubes, needles, prisms, with sizes between 1 to 100 nm. These features mainly depend on the process parameters, such as the type of plant extract and its concentration and metal salt(s) reacting, pH, temperature, and time of reaction, as well as the rate of mixing of plant extract and metal salt(s) [15]. A leaf extract of *Cassia auriculata* has been used to synthesize spherical and triangular gold nanoparticles (15–25 nm) within 10 min at room temperature [16]. *Parida* reported the synthesis of gold nanoparticles mediated by an extract of *Allium cepa*. The particles had an average size of 100 nm and could be internalized by MCF-7 breast cancer cells via endocytosis [17]. Gold nanoparticles produced using a leaf extract of *Coriandrum sativum* (coriander). The particles ranged in the size from about 7 to 58 nm and had diverse shapes (spherical, triangular, decahedral)[18]. An aqueous extract of *Terminalia chebula* were also used to produce gold nanoparticles with sizes ranging from 6 to 60 nm. These nanoparticles were active against both Gram-positive *S. aureus* and Gram-negative *E. coli* [19].

Hence in this context, the present work describes the synthesis of gold nanoparticles using several medicinal plant extracts. This approach appears to be a cost-efficient alternative to conventional methods for the synthesis of gold nanoparticles in plant extract. The novelty of this investigation is developing a new biocompactible herbal reducing agent for biosynthesis of gold nanoparticles as a very cost effective biocatalyst.

EXPERIMENTAL SECTION

The aerial parts of the three plant species were collected from Ilam, the Darrehshahr area all in July 2015, during the flowering stage. Voucher specimens were deposited at the Herbarium of the Research Institute of Forest and Rangelands (TARI). Tehran, Iran. Chloroauric acid ($HAuCl_4$) was obtained from Sigma-Aldrich Chemicals and used as received.

Plant Extract Preparation

A 5 g portion of thoroughly washed plant leaves were finely cut and boiled in 100 mL of sterile distilled water and filtered to get the extract. The resulting extracts were used for further experiments.

Synthesis of Gold Nanoparticles using Plant extracts

Different volumes (0.5-3 mL) of the plant extract were added to 20 mL solutions of 10^{-3} M aqueous $HAuCl_4$ separately. The effect of the amount of extract on the biosynthesis of gold nanotriangles was investigated by evaluating the nanoparticles formed using UV-Vis measurements after allowing the reaction mixture to stand for 48 h. The bioreduction of the Au^+ ions in solutions was monitored by periodic sampling of reaction mixture aliquots (1 mL) and measuring the UV-Vis spectra of the aliquots as a function of time of reaction. The effect of temperature was also studied by performing the reaction at 30 and 40 °C.

UV-Vis Absorbance Spectroscopy Studies

UV-Vis spectroscopic measurements of the nanoparticles synthesized were carried out on a PerkinElmer precisely-Lambda 25, dual beam spectrophotometer operated at a resolution of 1 nm.

XRD measurement

The gold nanoparticle solutions were separated by centrifuge at 10000 rpm for 15 min followed by redispersion of the nanoparticles into 1mL of deionized water. After freeze drying of the purified gold particles, the structure and composition were analyzed by XRD. The dried mixture of gold nanoparticles was collected for the determination of the formation of Au nanoparticles by an STOE-STADV X-ray diffractometer operated at a voltage of 40 kV and a current of 40 mA with Cu Kα radiation in a θ-2θ configurations. The crystallite domain size was calculated from the width of the XRD peaks, by considering this fact that they are free from non-uniform strains, using the Scherrer formula.

$$D = 0.94 \lambda / \beta \cos \theta$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray

wavelength, β is the Full Width of peak at Half Maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample.

β corrected = (FWHM2sample- FWHM2si)1/2

SEM analysis of gold Nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using VEGA TESCAN-XMU SEM machine. After freeze drying of the purified gold particles, the structure and composition were analyzed by SEM.

RESULTS AND DISCUSSION

UV-Vis spectral studies

Addition of plant extract to 10^{-3} M aqueous $HAuCl_4$ solution led to the appearance of a purplish-pink color in solution after about 1 h of incubation, indicating the formation of gold nanoparticles. The UV–Vis spectra of the reaction mixtures are also shown in Fig. 1-4. In each case, a peak was observed in the range of 500–600nm confirming the synthesis of gold nanoparticles as also reported in literature for other biological systems [20].

A. sylvestris

Both leaf and stem extract of this plant were examined for bioreduction of Au^+ ions at two temperatures (30 and 40 °C). A change in color was also associated with well-defined peaks characterized by maxima centered around 530 nm (Fig. 1a-e). Such peaks are known to be due to the surface plasmon resonance (SPR) displayed by gold nanoparticles. Control solutions (without plant extracts) neither developed the purplish-pink colors nor displayed the characteristic peaks. The appearance of purplish-pink color was due to the excitation of surface plasmon vibrations which is absent in bulk material [21]. These results indicated that reduction of Au^+ ions did not occur under the reaction conditions that were used. A variety of biomolecules are postulated to be involved in biological nanoparticle synthesis [22]. The optical absorption spectrum of metal nanoparticles is sensitive to several factors like particle size, shape, particle–particle interaction with the medium and local refractive index [23].

From Fig. 1a,b; it can be concluded that SPR wavelength has a small shift to shorter wavelength (from 536 to 526 nm) showing decrease in particle size. Higher than 10% v/v of the extract, no SPR band was observed.

The UV–vis spectra of Au nanoparticles synthesized by stem extract at 30 °C are shown in Fig. 1c-e, respectively. For 5, 10 and 15 v/v% ratios, individual bands are observed at around 555, 533 and 529 nm respectively; the SPR bands become broader by increasing the plant extract.

Reductions occurred at 30 and 40 °C was compared for phytosynthesis of Au nanoparticles. At 40 °C the bioreduction rate was increased and stable colloid with SPR bands at 516 and 529 nm for leaf extract was obtained. Au nanoparticles produced by stem extract at 40 °C had SPR bands at 536, 525 and 525 nm in 5, 10 and 15% v/v respectively (Fig 2a-e). Thus, reduction occurs at higher temperatures leading to large-sized nanoparticles.

F. gummosa

The optical absorption spectrum of metal nanoparticles is sensitive to several factors like particle size, shape, particle–particle interaction with the medium and local refractive index [23]. The digital photograph and UV–Vis spectra of Au NPs are shown in Fig. 3a-f. For 5, 10 and 15% of leaf and stem extracts, SPR bands are observed for gold around 540-579 nm. With an increase in the leaf extract quantity a red shift to higher wavelength and the peaks broadening is observed for leaf extract (5%, 540 nm; 10%, 545 nm; 15%, 579 nm).

From Fig. 3d-f, it is seen that as the quantity of the *Ferula* stem extract was increased, the SPR bands become broader and shift to higher wavelengths signifying an increase in particle size [24,25].

A.eriohora

By using stem extract of *A. eriohora*, there was neither a change in color nor a characteristic peak. Also the leaf extract (5% v/v) did not display the well characteristic peaks. A change in color for bioreduction by leaf extract at 10 and 15 % v/v was also associated with well-defined peaks characterized by maxima centered at 550 and 526 nm which indicated the smaller nanoparticles by using higher amount of leaf extract (Fig. 4a-c).

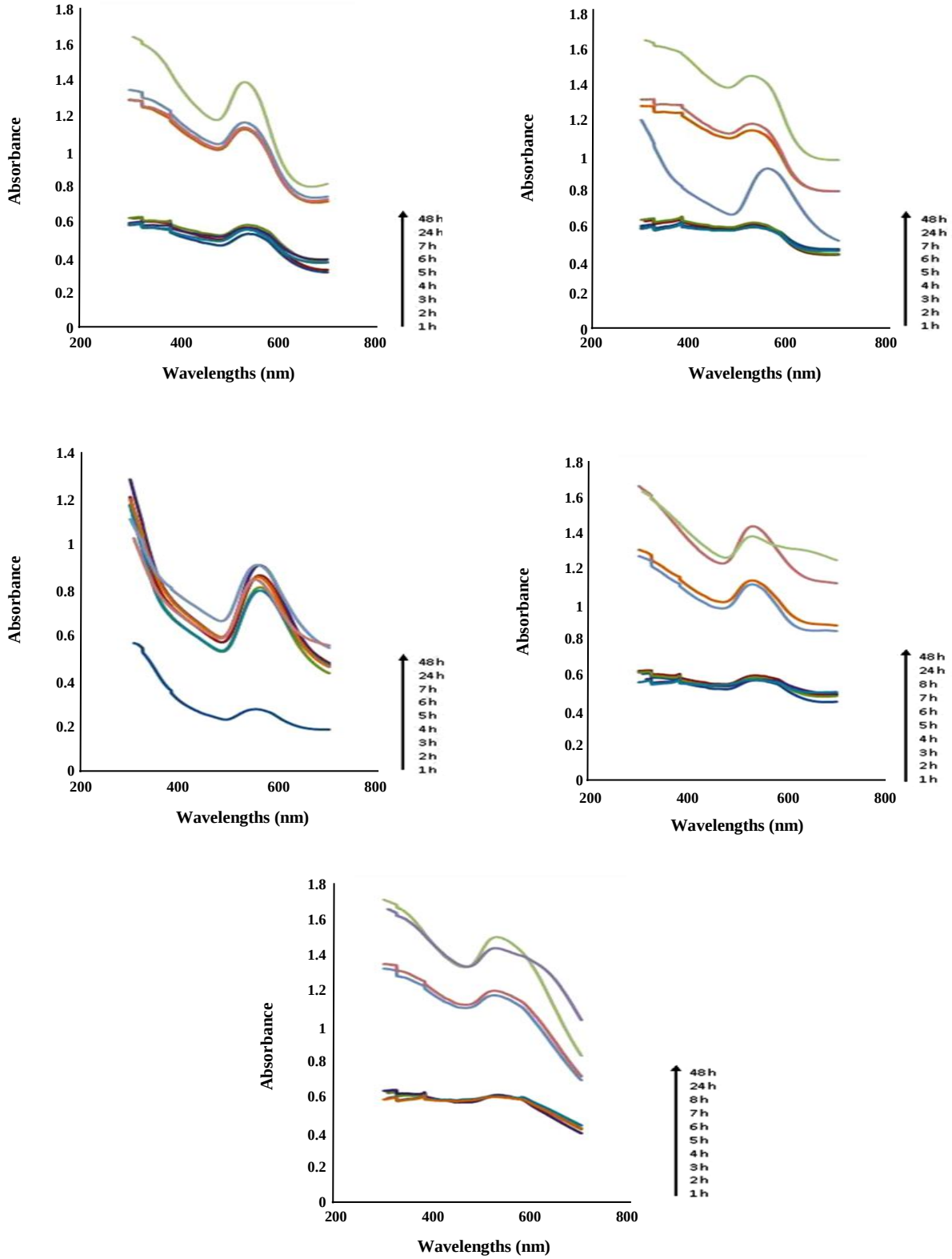

Fig. 1: UV-Vis absorption spectra of reaction mixtures of Anthriscus sylvestris with varying concentration at 30 °C [(a) leaf extract (2.5 % v/v), (b) leaf extract (5 % v/v), (c) stem extract (5 % v/v), (d) stem extract (10 % v/v), (e) stem extract (15 % v/v)] after 48 h of reaction.

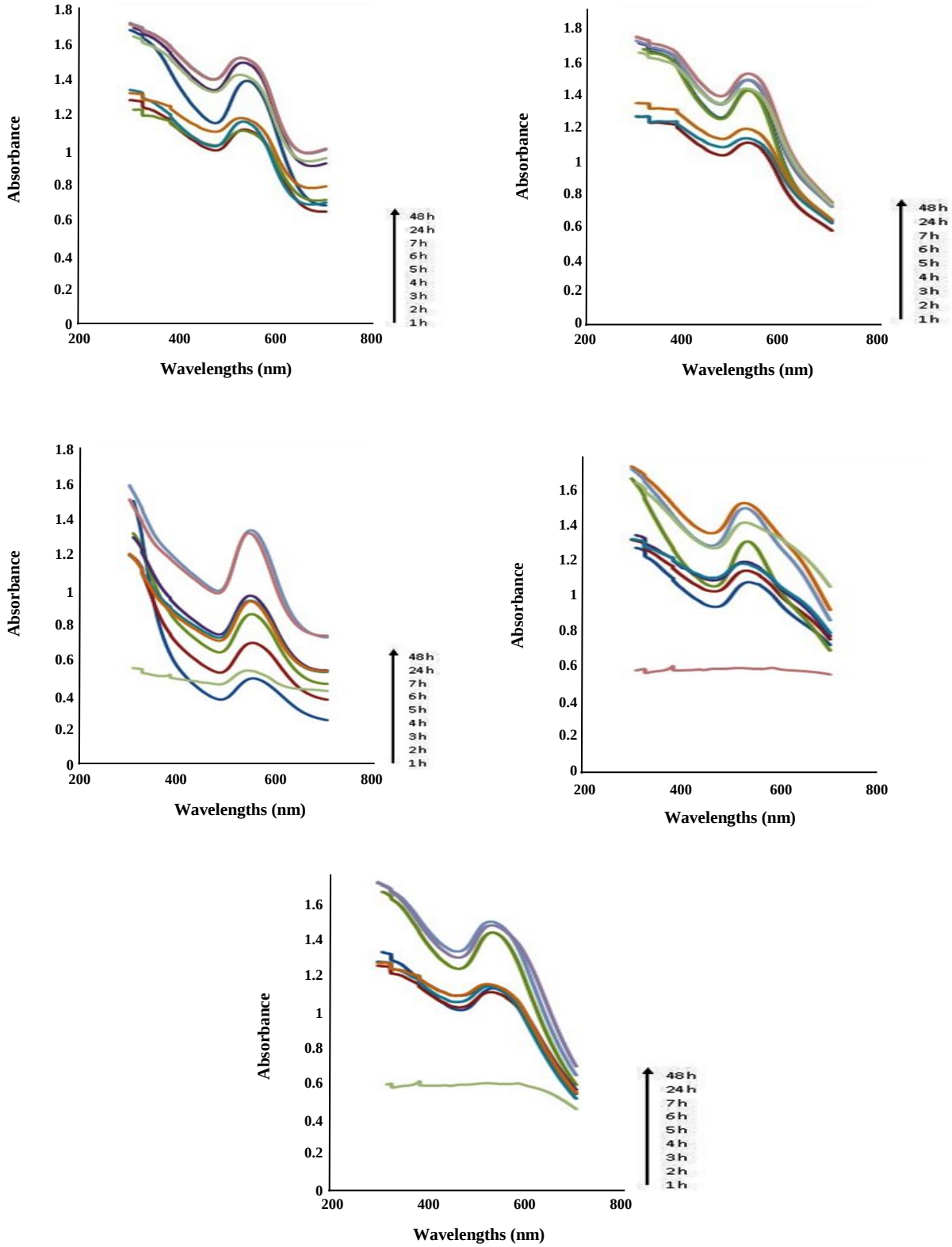

Fig. 2: UV-Vis absorption spectra of reaction mixtures of Anthriscus sylvestris with varying concentration at 40 °C [(a) leaf extract (2.5 % v/v), (b) leaf extract (5 % v/v), (c) stem extract (5 % v/v), (d) stem extract (10 % v/v), (e) stem extract (15 % v/v)] after 48 h of reaction.

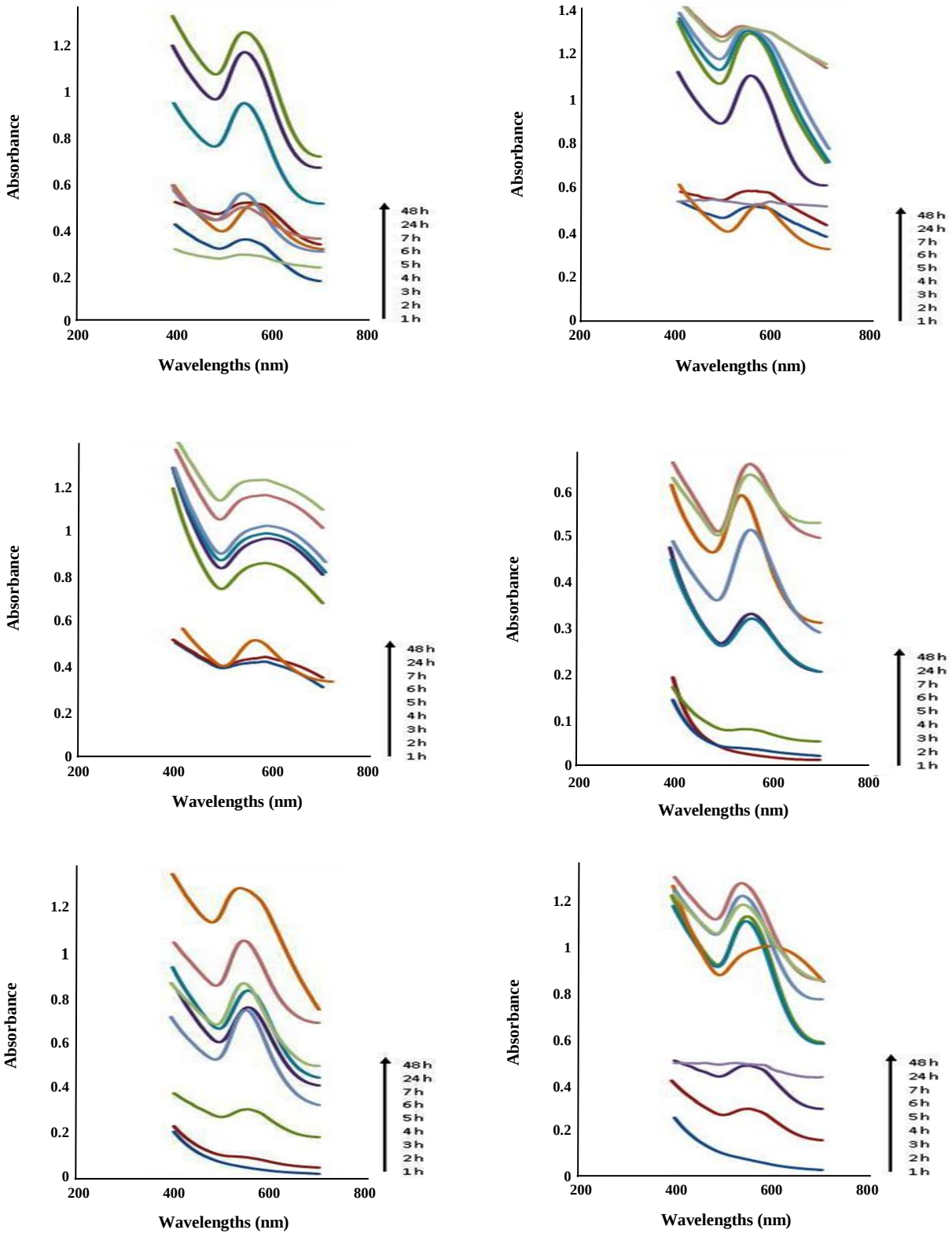

Fig. 3: UV-Vis absorption spectra of reaction mixtures of Ferula gummosa with varying concentration at 30 °C [(a) leaf extract (5 % v/v), (b) leaf extract (10 % v/v), (c) leaf extract (15 % v/v), (d) stem extract (5 % v/v), (e) stem extract (10 % v/v), (f) stem extract (15 % v/v)] after 48 h of reaction.

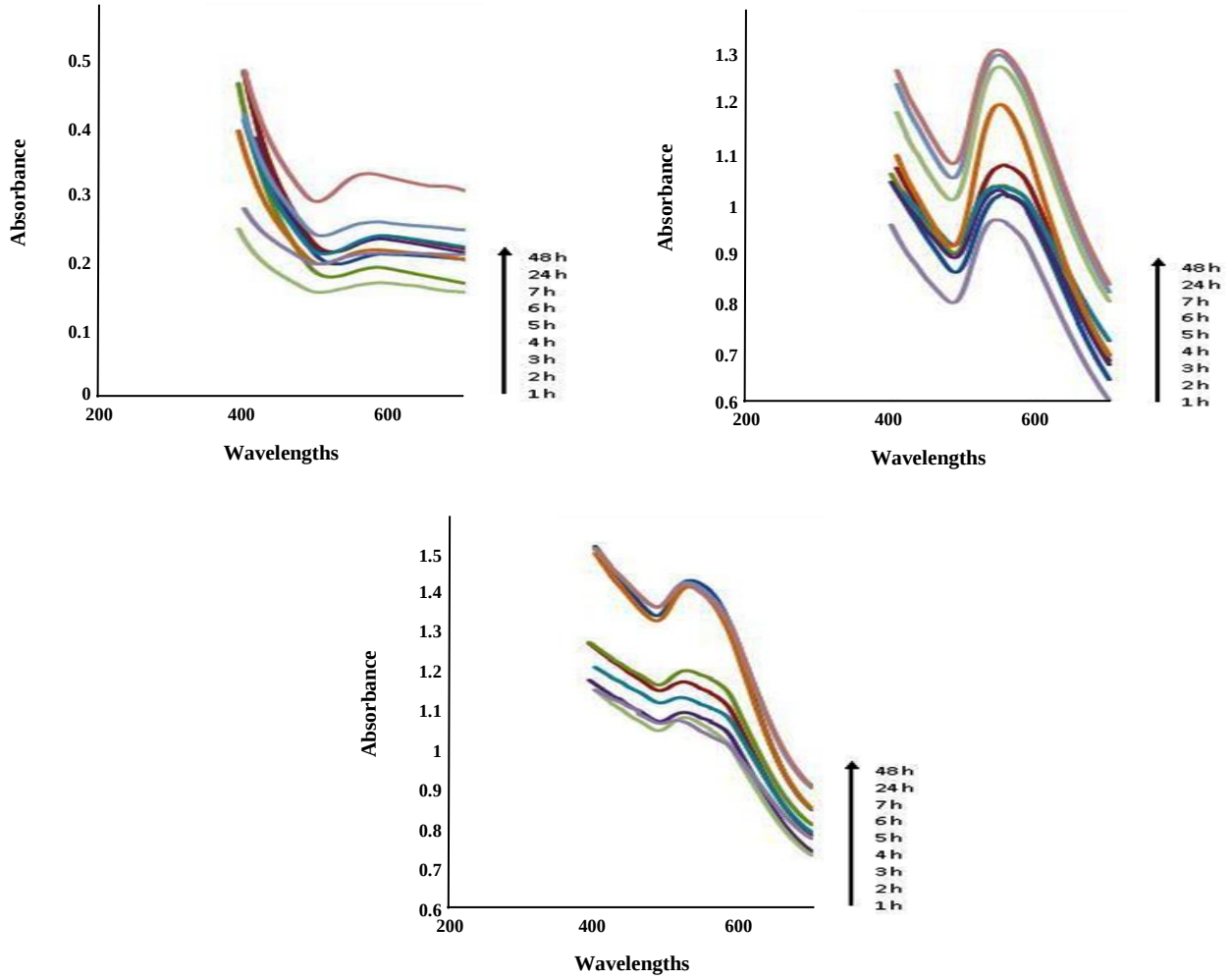

Fig. 4: UV-Vis absorption spectra of reaction mixtures of Achillea eriohora with varying concentration at 30 °C [(a) leaf extract (5 % v/v), (b) leaf extract (10 % v/v), (c) leaf extract (15 % v/v)] after 48 h of reaction.

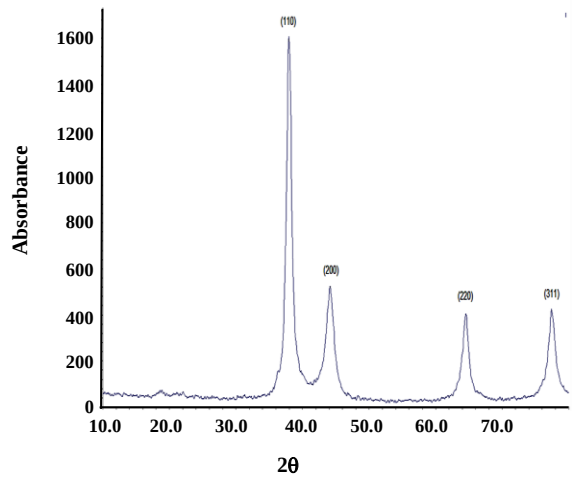

Fig. 5. Representative XRD profile of dried Au NPs.

X-ray diffraction (XRD) analysis

The XRD patterns of the freez-dried gold NPs displaying the structural information and crystallinity are shown in Fig. 5. After reaction, the diffraction peaks at 2θ = 37.9°, 44.1°, 64.53° and 77.42° assigned to the (1 1 1),(2 0 0), (2 2 0) and (3 1 1) planes of a faced centre cubic (fcc) lattice for gold nanoparticles were obtained. The XRD analysis showed predominant peaks at (1 1 1) and (2 0 0) indicative of the presence of microcubes and microwires. These peaks confirm lattice structure. The XRD patterns are consistent with earlier reports on microstructures [26-30].

Scanning electron microscopy analysis

The SEM analysis was used to determine the structure of the nanoparticles that were formed. Representative

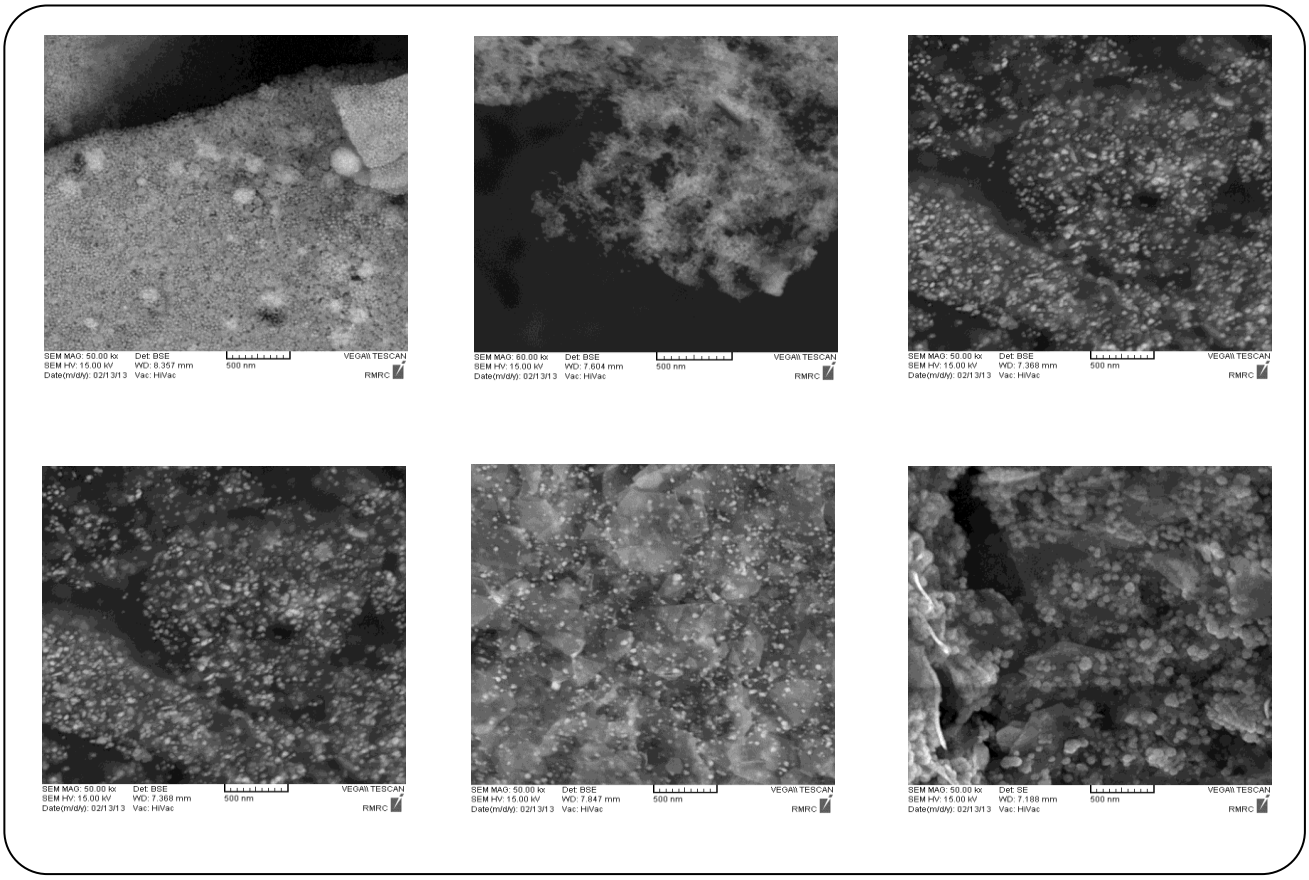

Fig. 6: Scanning electron micrographs of gold nanoparticles synthesized with plant extracts:
(a) Anthriscus sylvestris 5% leaf extract , (b) Anthriscus sylvestris 10% stem extract , (c) Ferula gummosa 10% leaf extract , (d) Ferula gummosa 10% stem extract, (e) Achillea eriohora 10% leaf extract.

SEM micrographs of optimized NPs in each plant extract magnified 50.00 kx and 60.00 kx times are shown in Fig. 6a–f. The average particle size (diameter) of the nanoparticles is 18 nm in case of 5% *A. sylvestris* leaf extract; 18 nm in case of 10% *A. sylvestris* stem extract, 32 nm in case of 5% *F. gummosa* leaf extract; 30 nm in case of 5% *F. gummosa* stem extract and 56 nm in case of 5% *A. eriohora* leaf extract. It is shown that relatively spherical nanoparticles are formed.

CONCLUSIONS

To conclude, we have used a simple and green method to synthesize gold nanoparticles using the aqueous extract of leaf and stems of *A. sylvestris*, *F. gummosa* and *A. eriohora* as reducing agents. The size of the gold nanotriangles can be easily varied from 18 to 56 nm by merely adjusting the amount of extract used in the gold ion reduction. The reaction mixtures displayed typical colors and UV–Visible spectra characteristic of gold nanoparticles. The reduction of the gold ions is believed to occur by an enzymatic process. The nature of the plant extract and its concentration could influence the quantity and size of gold nanoparticles.

Received : Dec. 28, 2016 ; Accepted : Dec. 18, 2017

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