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A novel green one-step synthesis of gold nanoparticles using crocin and their anti-cancer activities

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ABSTRACT

Functionalized nanoparticles are specifically designed to deliver drugs at tumor cells and can potentially enhance anticancer activity of drugs such as crocin. In the present study, we have applied antioxidant crocin as a reducing agent for one pot green synthesis of controlled size gold nanoparticles (AuNPs). Spherical, stable and uniform AuNPs were synthesized using crocin. These AuNPs are characterized by UV–Vis, TEM and XRD techniques. The prepared AuNPs showed surface plasm on resonance centered at 520 nm with the average particle size of about 4–10 nm. The anti-cancer effect of AuNPs was determined using MTT and LDH tests. The cellular data showed that these AuNPs significantly decreased cancerous cells' growth after 24 and 48 hours in a time- and dose-dependent manner ($P < 0.05$). The results suggest that such AuNPs can be synthesized simply and quickly with invaluable clinical as well as pharmaceutical activities which can help to treat human breast cancer.

1. Introduction

Recent advances in nanotechnology have caused a wide growth in synthesis of nanoparticles in various fields of science including medicine, agriculture, electronics, etc. [1–3]. Because of unique characteristics of gold nanoparticles (AuNPs), they have been drawn into important biological pathways as well as cancer diagnosis and treatment [4–6]. AuNPs hold great promise to solve several limitations such as nonspecific bio-distribution, lack of targeting, lack of aqueous solubility, poor oral bioavailability, and low therapeutic indices [7]. It has been highly demanded to develop non-toxic synthesis methods, green chemistry and renewable biological resources that have played a pivotal role in preparation of AuNPs [8].

Some typical AuNPs produced by microorganisms such as Pseudomonas aeruginosa and Alkalotolerant actinomycete bacteria in the range of 15–30 nm and 5–15 nm, respectively [9,10]. Among bacteria, microbes [11–13], and plants which are used in biosynthesis process of AuNPs, plants are more suitable due to optimal environmental preservation, fast, available, simple production, and high stability of nanoparticles. Recently, synthesis of metal nanoparticles has been reported by extract of many plants including olive and *Aloe Vera* [14,15]. Their antioxidant components are known to charge nanoparticles and prevent their aggregation by stabilizing the particles. In this context, researchers have synthesized, stabilized, and optimized the AuNPs using biochemical constituents of herbs [16].

Saffron stigma contains several active phytocompounds for instance carotenoids (crocins and crocetin), monoterpen aldehydes, and a- and b-carotenes. This herb has enough medicinal properties such as moderating Alzheimer, relieving premenstrual syndrome, reducing depression, and suppressing many cancers [17–21]. Crocin ($C_{44}H_{64}O_{24}$), the main ingredient of saffron stigma is one of the water soluble carotenoids in the nature. As an antioxidant, it protects cells against oxidative stress via reducing free radicals [22].

Since AuNPs have not been yet synthesized by crocin, the present study proposed a novel method to produce AuNPs using an antioxidant component. In addition to, their inhibitory effects on proliferation of human breast cancer cells were investigated.

2. Experimental

2.1. Preparation of Crocin–AuNPs

The reagents included $HAuCl_4$ salt (>99.8% purity, Merck) and NaOH (99.8% purity, Merck). According to Bolhasani et al. [23], crocin was separated and purified from the Iranian saffron stigma. In brief, saffron ethanolic extract was applied on a glass column (2 × 80 cm) packed with neutral aluminum oxide 90-active (Merck, Darmstadt, Germany) and eluted with 50% ethanol, followed by 50% ethanol containing acetic acid (4:1 v = v). Crocin fractions of 4 mL were collected, and their absorbency at 440 nm was monitored. Purity of the products was confirmed by high performance liquid chromatography (HPLC), by thin

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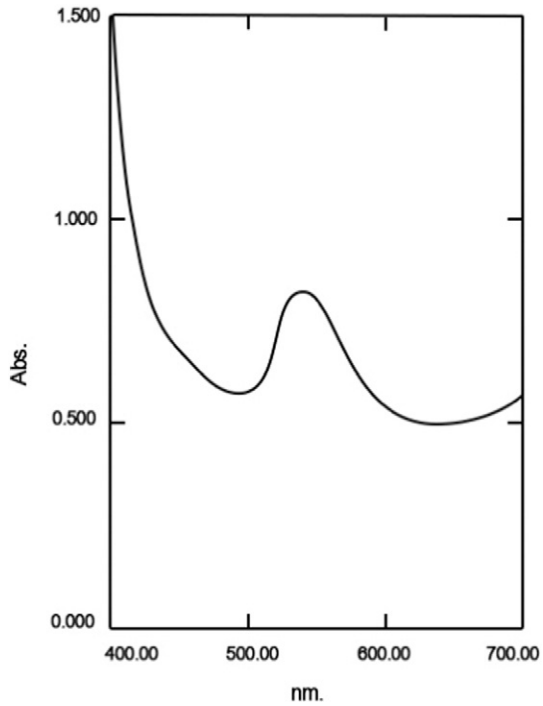

Fig. 1. UV–vis spectrum recorded after 24 h of reaction of mixture of 5 ml of $HAuCl_4$ 1 mM and 6 ml of crocin 682.38 mM at 50 °C.

layer chromatography (TLC), and by determining their melting temperature. In addition, infrared (IR) spectroscopy was applied for characterization of the components [23].

Different concentrations of gold ions (prepared from $HAuCl_4$, 0–5 mM) and crocin as a reducing agent (300–700 mM) were used at various time spans (2 h–8 weeks) and temperatures (25–75 °C) to investigate their effect on size and amount of produced AuNPs. The pH of all solutions was adjusted to 7.5 as physiological pH. The color of solutions was depended on time which determined by a UV–Vis spectrophotometer (2030 CECIL Company, UK) in the range of 520–550 nm. Finally, the solutions were centrifuged three times for 15 min at 10,000 rpm, each time washed using deionized water to separate pure AuNPs. The crystalline structures and their corresponding morphology were investigated by XRD (Philips, X'pert-MPD system using Cu Kα) and TEM (TEMZIESS, TEM PHILPS) techniques, respectively. The line broadening owing to the instrument was computed by Warren's method [24]. The average crystallite size was calculated by Debye Scherer's equation [25]. Also, the histogram of nanoparticle size distribution was calculated from the TEM images by randomly measuring the diameters of at least 650 particles.

2.2. Cell Line and Culture Conditions

Human breast cancer cell line (MCF-7) was provided from the Iranian Biological Resource Center (Iran). The cells were cultured in RPMI 1640 medium (Inoclon, Iran), supplemented by 10% FBS serum, 100 units/mL penicillin and 100 mg/mL streptomycin and grown at 37 °C in humidified atmosphere containing 5% CO2. The cells were then treated with different concentrations of crocin as a control and crocin–AuNPs (0–3 mg/ml) at different time periods (0–48 h).

2.3. Cytotoxicity Assays

2.3.1. MTT Assay

The in vitro cytotoxic effects of crocin–AuNPs on MCF-7 cells were evaluated using 3-(4,-5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT; Sigma-USA) assay as described by Hoshyar et al. [21]. The various concentrations of crocin and crocin–AuNPs (0–3 mg/ml) were added to the 2×10^4 cells that seeded onto the flat-bottomed 96 well plates. The absorbance read at 570 nm by an ELISA plate reader (Biochrom Anthos, USA) after 24 and 48 h of incubation. The absorbance values were converted into percentages of cell viability. In addition, the IC50 values (drug concentration that reduced the absorbance of treated cells by 50% compared with untreated cells) of crocin–AuNPs against the MCF-7 cells were examined at different times (24 and 48 h).

2.3.2. Neutral Red Cytotoxicity Assay

The cell viability was also determined by neutral red test. The cells were treated with different dilutions of crocin and crocin–AuNPs in the fresh media. Following the 24 h of incubation, 100 μL serum free media containing neutral red (100 μg/mL) was added to cells and incubated for 2–3 h. After treatment, 50 μL of dye release agent (a solution of 1% acetic acid: 50% ethanol) was added to each and incubated for 10 more minutes. The plate was placed on a shaker (Vortex Genie) for 30 min after which the optical density of 540 nm was determined by a spectrophotometer.

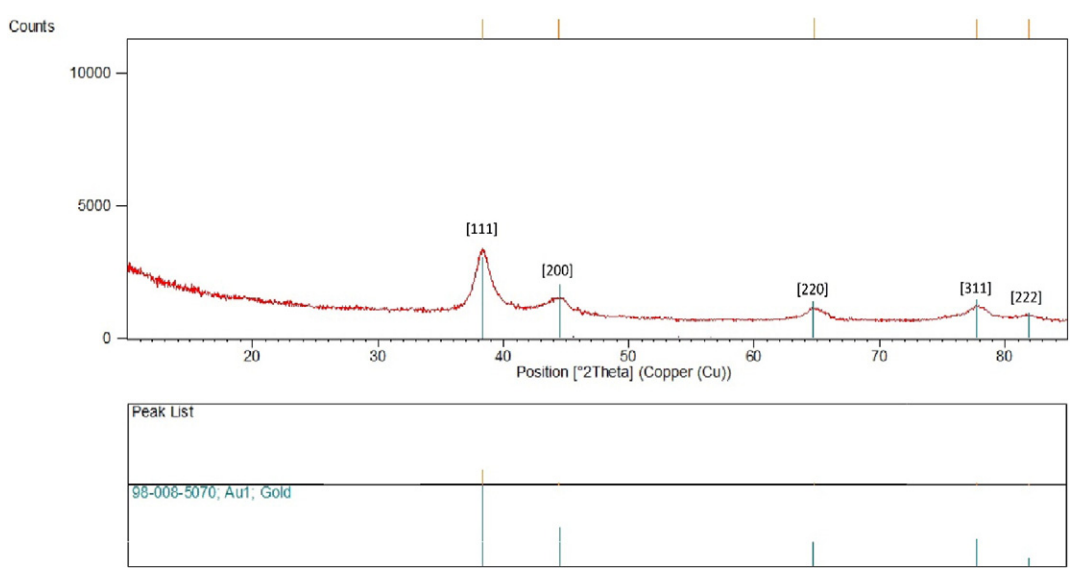

Fig. 2. XRD pattern of AuNPs after 24 h of reaction of mixture of 5 ml of $HAuCl_4$ 1 mM and 6 ml of crocin 682.38 mM at 50 °C.