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ORIGINAL ARTICLE

Facile preparation of Au nanoparticles mediated by *Foeniculum Vulgare* aqueous extract and investigation of the anti-human breast carcinoma effects

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Green synthesis

Abstract This experiment evaluated antioxidant, anti-human breast cancer activities, and cytotoxicity effects of green synthesis of Au nanoparticles (AuNPs) containing *Foeniculum Vulgare* aqueous extract. Mixing *Foeniculum Vulgare* aqueous with Au chloride solution produced Au nanoparticles. The characteristics of Au nanoparticles determined using Fourier Transformed Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM), UV–Visible Spectroscopy (UV–Vis), and Field Emission Scanning Electron Microscopy (FE-SEM). To check the cytotoxicity and anti-breast cancer effects of Au chloride, *Foeniculum Vulgare* aqueous extract, and AuNPs on common breast cancer cell lines i.e., ZR-75-30, T47D, and HCC1187 was used MTT assay. AuNPs showed no cytotoxicity and the most effective anti-breast cancer features compared to other items that were tested. They had no cytotoxic effects on normal cell line (HUVEC) and had very low cell viability, high anti-breast cancer activities dose-dependently against ZR-75-30, T47D, and HCC1187 cell lines. In the presence of butylated hydroxytoluene as the positive control, the DPPH

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test was used to evaluate the antioxidant features of Au chloride, *Foeniculum Vulgare* aqueous extract, and Au nanoparticles. AuNPs showed the best antioxidant properties compared to other items that were tested. Perhaps remarkable anti-human breast cancer activities of Au nanoparticles synthesized by *Foeniculum Vulgare* aqueous extract due to its antioxidant properties. After clinical trial and confirmation of results, this formulation can be used as an effective drug to treat breast cancer.

1. Introduction

Foeniculum Vulgare is a herb with many medicinal properties that is known by various native names around the world, generally known as *fennel*. It belongs to *Plantae* kingdom, *Apiales* order, *Apiaceae* family, and *Foeniculum* genus. Fennel is a perennial and aromatic herb with light yellow flowers that grow up to 2 m high and general appearance is similar to dill (Kaur and Arora, 2009; Manonmani and Abdul Khadir, 2011; Orhan and B. Ozc ¨ ¸elik, M. Kartal, and Y. Kan, , 2012; Morales et al., 2012; Dua et al., 2013). Some studies reported two sub-species of fennel one of them has bitter seeds is called *Pipertum* and another has sweet seeds is called *Vulgare* (Rather et al., 2016). In ancient times and contemporary in different parts of the world the fruits, stems, leaves, seeds, and the whole plant of *Foeniculum Vulgare* were used to treat the several diseases such as cold, cough, renal diseases, cancer, antiemetic, colic, diarrhea, gastralgia, fever, etc. Also, *Foeniculum Vulgare* has galactagogues properties and it is suitable for breastfeeding mothers. (Badgujar et al., 2014; Carrio and J. Vall ´ es, 2012). *Foeniculum Vulgare* has a protein, fat, minerals, fiber, carbohydrates, and phytochemical compounds. Phytochemical researches have indicated the presence of numerous valuable compounds, such as volatile compounds, phenolic, flavonoids compounds, amino acids, and fatty acid. Some pharmacological compounds isolated from fennel are as follows: Scopoletin, phenolic glycosides, Fenchone, phenols, Bergapten, *para*-Anisaldehyde, Psoralen, (z)-9-Octadecenoic, Dillapional, Beta-myrcene, Imperatorin, Limonene, Dillapiol, Terpineol, Fenchone, 1,8-Cineole, 5-Methoxypsoralen, *para*-Anisaldehyde, Photoanethole, *trans*-Anethole, Dianethole, (E)-Phytol, Neophytadiene, Phenylpropanoid, *o*-Cymene, 1,3-Benzenediol, Methylchavicol, Anethole, Linoleic acid, 1-Methoxycyclohexene, Estragole, Isoquercetin, Rosmarinic acid, Oleic acid, 3-O-Caffeoylquinic acid, Quinic acid, Quercetin-3-O-glucoside, Exo-fenchyl acetate, *cis*-Miyabenol C, Dillapional, Limonene-10-ol, Eriodictyol-7-rutinoside, Isorhamnetin-3-O-glucoside. Researches have shown that *Foeniculum Vulgare* has antithrombotic, antioxidant, anti-inflammatory, antibacterial, antifungal, hepatoprotective, oestrogenic, antidiabetic activities, memory enhancer, acaricidal, anti hirsutism, and in vitro cytoprotection and antitumor activity (Badgujar et al., 2014; Pradhan et al., 2008; Koppula and Kumar, 2013; Rahimi and Ardekani, 2013; Nassar et al., 2010; Albert-Puleo, 1980; Tripathi et al., 2013). The remedial potentials of the *Foeniculum Vulgare* because of its phytochemicals role were mentioned above.

The previous studies have been indicating when metallic nanoparticles are green-synthesized by ethnomedicinal plants rich in antioxidant molecules, their therapeutic properties such as anti-human cancer effects significantly increase (GBD, 2015; GBD, 2015; Ebell, 2016; Grossman, 2018; Gibson, 2016; Jalalvand et al., 2019). One of the simplest nanostructures that is widely used in industry today is metallic nanoparticles. Metallic nanoparticles can bind non-destructively to single-stranded DNA, which are important in medical diagnostics. Nanoparticles also can pass through the vessel and position the target organ in the body, which is used in biomedicine, imaging and therapy (Jalalvand et al., 2019). Biomedical applications of nanoparticles include drug carriers, tracking or labeling materials, carriers for gene therapy, hyperthermia, and materials for magnetic resonance imaging. To use nanoparticles to deliver a drug molecule or DNA or a gene in gene therapy, chemical changes at the nanoparticle surface are always required for specific interactions with the desired biomolecule. Nanoparticles are used for imaging for medical purposes or in vitro and *in vivo* chemical processes. Metallic nanoparticles have received a lot of attention due to their properties such as antifungal, photocatalytic and UV absorbing properties (Jalalvand et al., 2019). Due to the antibacterial properties of these metal oxide nanoparticles, they can be used in the food industry and active food packaging. Also, metallic nanoparticles are potentially used in hyperthermia, magnetic resonance imaging (MRI), diagnosis and treatment of tumors or cancer, biomarkers, biodegradation, biotechnology and the removal of important organic, inorganic and radioactive contaminants due to their high biocompatibility (Gibson, 2016; Jalalvand et al., 2019). Metallic nanoparticles have many applications in various fields such as fuel cells (hydrogen, methanol), glucose detection, drug delivery, toxicology, and biological interactions (Jalalvand et al., 2019). Metallic nanoparticles as a strong antioxidant resource are much less toxic than metals and also these nanoparticles have high power in scavenging free radicals (FR), so, it can be used as a natural antioxidant. Studies show that these nanoparticles detoxify hydroperoxidases and lipohydroperoxidases at the cytoplasmic and mitochondrial matrix levels. Metallic nanoparticles such as copper, silver and titanium have very high antimicrobial properties that can be used in various industrial and biomedical sectors (Zangeneh et al., 2019). Nanoparticles can also be used as coatings on molecules to bind or interact with biological targets, to ensure the presence of these nanoparticles in the target part of the body, carriers are used to accurately deliver these nanoparticles, in which peptides have been introduced as one of the best carriers (Gibson, 2016; Shaneza, 2018).

The last decade has seen the promising emergence of nanoparticles in cancer treatment systems such as drug delivery and recombinant proteins with anti-tumor properties. Special features of the microenvironment around the tumor allow nanoscale systems to accumulate at the tumor site

(Jalalvand et al., 2019). Therefore, some nanoparticles with adjuvant properties, when they carry peptides or proteins, can increase the activity of cells in the reticuloendocardial system and activate macrophages and dendritic cells (Zangeneh et al., 2019). Activated macrophages and dendritic cells swallow and process the complex, and immune responses are formed more efficiently. These nanoparticles can increase the response of the immune system to the target antigen, as well as to direct and direct this system to create a specific type of response (Shaneza, 2018). By using these nanoparticles as antigen carriers, the amount of recombinant protein used as well as antigen toxicity is reduced and the destructive effects of proteases on protein antigen are reduced (Dua et al., 2013; Rather et al., 2016; Badgujar et al., 2014; Ghorbani, 2005). This strategy enhances the efficiency of the target protein in inducing immune responses against the tumor, which is important in advancing functional goals such as protein and effective drug delivery (Zangeneh et al., 2019).

Metallic nanoparticles containing medicinal plants have so significant anti-cancer effects. In recent years, these metal nanoparticles containing herbs have been used to treat various cancers of the ovaries, prostate, esophagus, stomach, lungs and various leukemias. One of the most important cancers in recent years is breast cancer (Gibson, 2016; Jalalvand et al., 2019; Zangeneh et al., 2019; Shaneza, 2018).

In this study, the effects of Au nanoparticles formulated by *Foeniculum Vulgare* seed aqueous extract against common human breast cancer cell lines i.e., ZR-75-30, T47D, and HCC1187 were evaluated.

2. Material and methods

*2.1. **Synthesis of Foeniculum Vulgare** seed **green-synthesized AuNPs***

Extraction from *Foeniculum Vulgare* is the first step to produce a green synthesis of Au nanoparticles. For this purpose, we used the method of extracted with distilled water in the microwave. In this research used seeds of the plant. Generally, the method of synthesis AuNPs are as fallows 100 mL of $HAuCl_4{\cdot}H_2O$ (1 mM) and 1.5 g of NaOH pellets were dissolved in 50 mL of distilled water following by a mixture of solution added 20 mL of *Foeniculum Vulgare* seeds extract and then stirring at 25 °C for 1 h. After the reaction time, the color of the solution changed to the dark yellow color that indicates the formation of Au nanoparticles (AuNPs). In the next step, the solution was allowed to precipitate, then the precipitate was filtered and washed with distilled water, acetone, and ethanol. Finally, the resulting precipitate was dried at 90 °C for 14 h to obtain the best Au nanoparticles powder (Shaneza, 2018).

2.2. Chemical characterization of Foeniculum Vulgare seed green-synthesized AuNPs

Various techniques were used to determine the characterization of Au nanoparticles such as FT-IR, UV–Vis, and TEM.

FT-IR (Shimadzu IR affinity.1) was used to identify the biomolecules that participate in the reduction of Au nanoparticles.

Characteristic absorption bands of Au metal were evaluated with the method of UV–Vis spectroscopy analysis.

The sizes and shape of nanoparticles were evaluated by the TEM method.

2.3. Evaluation of the antioxidant activities of Foeniculum Vulgare seed green- synthesized AuNPs

Free radicals are unstable atoms that have one or more unpaired electrons. These active species are very harmful due to their high reactivity. They are most often formed when oxygen molecules in the body split into separate unstable atoms. This process can turn into a chain reaction. Free radicals excessive production in the body causes cell damage and oxidative stress. Genetics and the environment affect the extent of free radical damage in individuals. These active molecules are produced as part of the body's natural biological processes. One of the most important free radicals is DPPH. DPPH is widely used to study the antioxidant activities of natural compounds and nanoparticles (Jalalvand et al., 2019; Zangeneh et al., 2019).

Used 2,2-diphenyl-1-picrylhydrazyl (DPPH) for evaluation of the antioxidant features of Au nanoparticles, *Foeniculum Vulgare* aqueous extract, and Au salt (Arunachalam, 2003). Simultaneously different samples of Au nanoparticles, *Foeniculum Vulgare* seed extract, Au salt of variable concentrations (0–1000 μg/mL), and 39.4% DPPH solution (w/V) in 1:1 aqueous MeOH were prepared. Then the DPPH solution was added to the samples that were mention above and incubated at 37 °C. The absorbances of the mixture after 30 mint of incubation were measured at 517 nm. In this study respectively butylated hydroxytoluene (BHT) and MeOH (50 %) were considered as positive and negative controls. The antioxidant features of the samples were expressed as a percentage of inhibition and were calculated based on the following formula:

$$\text{Inhibition}(\%) = \frac{\text{SampleA.}}{\text{Control} - \text{A.}} x100$$

2.4. Evaluation of anti-human breast cancer features of Foeniculum Vulgare seed green- synthesized AuNPs

With the advancement of life sciences, measuring the rate of proliferation, survival and cell mortality under different conditions has become very important. In this regard, MTT analysis has greatly contributed to the study of biocompatibility of various materials by providing a highly safe non-radioactive colorimetric system. Cytotoxicity tests are tests that examine the side effects of various compounds on the cell. These processes take place in the environment outside the human body or the so-called extraterrestrial. Most of these processes also use cell culture. In MTT analysis according to ISO 10993-5 international standard, different equipments are tested for cytotoxicity, if they do not have toxic effects, they will obtain the necessary standards and licenses and enter the buying and selling market. The MTT set is the best-known test for cell viability. The main purpose of this test is to evaluate the toxicity of compounds, drugs or other supplements on the cell. Of course, it may also be mentioned in articles as a process for examining cell proliferation or counting (Jalalvand et al., 2019; Zangeneh et al., 2019). MTT analysis can differentiate between living and dead cells by affecting intracellular organs. In this method; the cells, after being cultured in the laboratory, are "treated" with the desired substances to evaluate their toxicity. At the end of this test, for each concentration of the substance, the cell viability is determined. Although this method is primarily for water-soluble solutions and compounds, it is currently used for other compounds soluble in organic solvents and nanoparticles. The behavior and rate of cell proliferation may increase or not change at all under the influence of hormones, growth factors, cytokines and mitogens. Also, some drugs and cytotoxic (toxic) substances, such as anticancer drugs, may cause necrosis or apoptosis (death) of cells or slow down the rate of proliferation and growth or even loss of cell structure (Zangeneh et al., 2019). Proper analysis of the MTT test can evaluate many of these behaviors. The MTT analysis basis is based on mitochondrial activity. This activity is usually stable in living cells. Hence; any change in several active and living cells is linked to mitochondrial property. This examination is a colorimetric way based on the breakdown and reduction of yellow tetrazolium crystals by the succinate dehydrogenase, and the formation of insoluble purple crystals performs the final analysis. Unlike other methods, MTT analysis eliminates the cells washing and shrinking steps, which usually causes the loss of cells part and increases the work error. That is, all the steps of the experiment, from the cell culture beginning to reading and analyzing the findings with a photometer, are done in a completely compact way and in a "micro plate". Hence the sensitivity, accuracy, and repeatability of the test is high (Shaneza, 2018).

Cell lines have been used in this research for evaluating anti-human breast cancer and cytotoxicity effects of *Foeniculum Vulgare* seed extract, AuNPs, and Au salt are as follows:

a) Human breast cancer cell lines: ZR-75-30, T47D, and HCC1187.

b) Normal cell line: HUVEC.

For evaluating anti-human breast cancer and cytotoxicity effects MTT assay was used.

The cell lines that were mentioned above were plated in the DMEM media using 96 well plate with 10% FBS, penicillin, streptomycin, and antimycotic solution, the distribution of cells within each plate well was 10,000 and incubated at 37 °C for 24 h, then all cells were treated with various dilation of Au nanoparticles, *Foeniculum Vulgare* seed extract, Au salt (0–1000 μg/mL) and incubated for 24 h. Then added 5 mg/mL of MTT to all wells and finally incubated at 37 °C for 4 h. After the determined of absorbance at 531 nm, the percentage of cell viability calculated based on the following formula (Tahvilian et al., 2019):

$$\text{Cell} \quad \text{viability}(\%) = \frac{\text{Sample} \quad \text{A.}}{Control \quad A.} x100$$

2.5. Qualitative measurement

To compare the results, in addition to the formula mentioned above, which was calculated as an average of 5 repetitions of experiments. The results were analyzed using SPSS software version 22 and the statistical differences between the treatments were examined by *t*-test and $P < 0.05$ was considered significant.

3. Results and discussion

Cancer is a genetic disease that includes 277 types of diseases. There are also more than 100,000 types of chemicals in our environment, of which only 35,000 have been analyzed and about 300 of them produce cancer. The remaining 65,000 chemicals in nature have not yet been tested. Cancer occurs due to uncontrolled cell division, which is the result of environmental factors and genetic disorders (Gibson, 2016). The four key genes involved in cancer cell conduction include DNA repair genes, tumor suppressor genes, oncogenes, and programmed death genes (Jalalvand et al., 2019). If a genetic mutation is produced in a cell, normal cells go out of their way and are affected by new commands that progress to cancer cells. In addition to chemicals, sunlight, shortwave, viruses and bacteria also have a special role in causing cancer (Zangeneh et al., 2019). Cancers have existed since the beginning of mankind. In recent decades, advances in computer molecular medicine have been able to not only study the causes and mechanisms of this deadly disease but also to perform better in its early diagnosis and treatment (Jalalvand et al., 2019; Zangeneh et al., 2019). More than 50% of cancers are currently being treated, especially if diagnosed early. Cancer can be treated in several ways: surgery, chemotherapy, radiation therapy, immunotherapy, gene therapy, or a combination of these. Due to the relative inefficiency and very severe side effects of chemotherapy drugs, researchers and scientists have sought a new formulation of various compounds, especially metallic nanoparticles (Gibson, 2016).

In this experiment, we formulated AuNPs using *Foeniculum Vulgare* aqueous seed extract. Moreover, in vitro condition, we evaluated the anti-human breast cancer features of AuNPs against common human breast cancer.

3.1. UV–visible spectroscopy of Foeniculum Vulgare seed green-synthesized AuNPs

Fig. 1 shows the UV–Vis spectra analysis from AuNPs containing *Foeniculum Vulgare* seed extract. UV–Vis spectroscopic analysis revealed in this research that an absorption peak at 531 nm, in this peak the formation of AuNPs is confirmed, due to some researches that have been done the wavelength range for AuNPs formation from pharmaceutical plants have been determined between 510 and 550 nm (Shaneza, 2018). Fig. 1 reveals the instability of this spectrum, which indicates a complete reduction of the Au cation.

3.2. TEM analysis of Foeniculum Vulgare seed green-synthesized AuNPs

TEM is a technique by which the morphology and structure of materials such as size, shapes, and distribution were determined. As you can see in Fig. 2, the AuNPs containing *Foeniculum Vulgare* seed extract are spherical and about 17–20 nm in sizes which are well distributed.

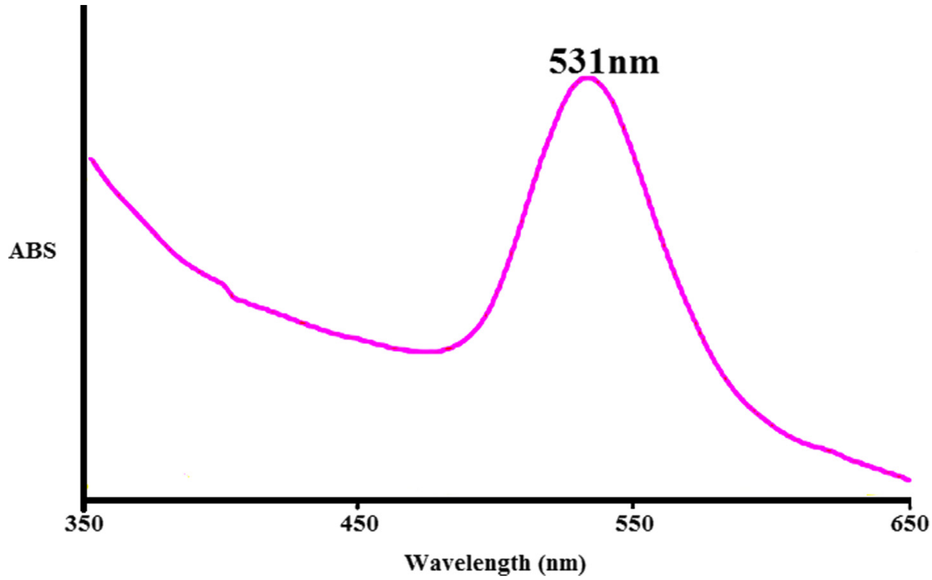

Fig. 1 UV–Vis spectra of Au nanoparticles green-synthesized by *Foeniculum vulgare* seeds.

3.3. FE-SEM analysis of Foeniculum Vulgare seed green-synthesized AuNPs

FE-SEM analysis is a technique for evaluation of size, morphology, and topography of materials such as AuNPs and is widely used in physics, chemistry, and biology researches. In this experiment, the FE-SEM image of AuNPs containing *Foeniculum Vulgare* seed aqueous extract shown in Fig. 3. In this figure, the AuNPs are spherical, 17–20 nm in size that arranged regularly and closes together. A small number of AuNPs appear to form the agglomerated structure, which can be caused by functional groups such as hydroxyl.

3.4. FT-IR analysis of s seed green- synthesized AuNPs

One of the important uses of the FE-SEM technique is to determine the functional groups of materials. Fig. 4 shows the FT-IR spectrum of AuNPs containing *Foeniculum Vulgare* seed aqueous extract in the wavenumber of 400–4000 cm^{-1}. In Fig. 4, the absorption peak at wavenumber 3382 is due to alcoholic and phenolic hydroxyl groups, the band at 2873 cm^{-1} is referred to as the asymmetric CH_2 stretching. The bands at 1376–1605, 1009, and 562 cm^{-1} are referred to as aromatic rings, C-O stretching, and Au-O respectively.

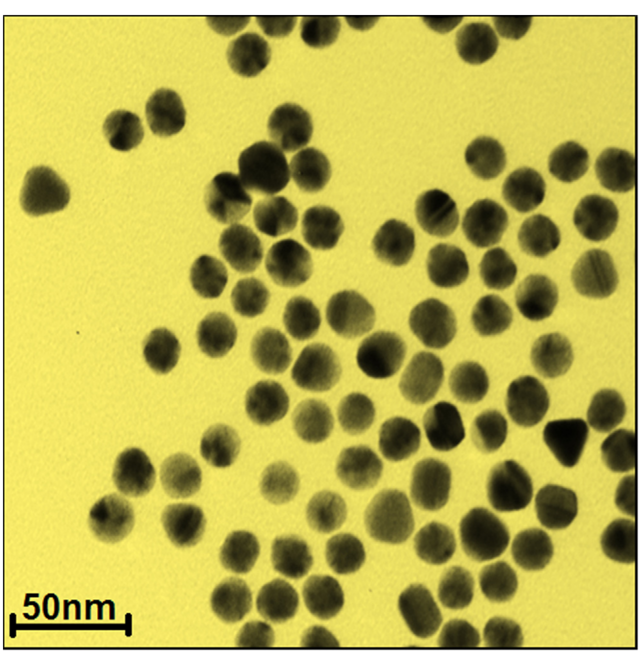

Fig. 2 TEM image of Au nanoparticles green-synthesized by *Foeniculum vulgare* seeds.

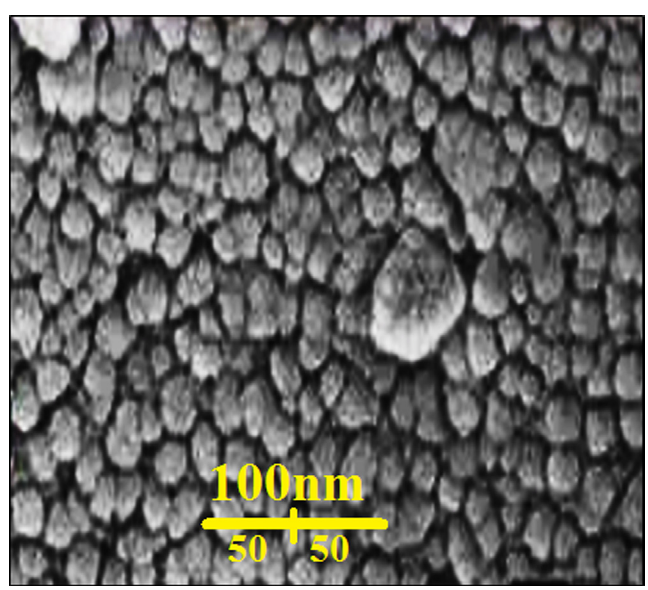

Fig. 3 FE-SEM image of Au nanoparticles green-synthesized by *Foeniculum vulgare* seeds.

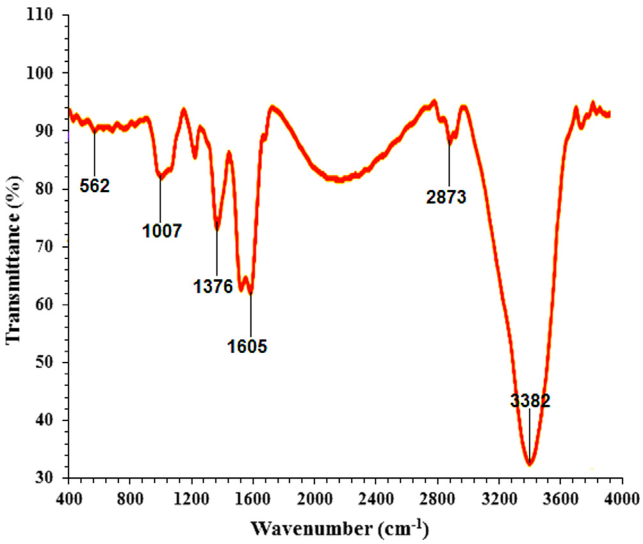

Fig. 4 FT-IR spectra of Au nanoparticles green-synthesized by *Foeniculum vulgare* seeds.

3.5. Cytotoxicity and anti-breast cancer features of Foeniculum Vulgare seed green- synthesized AuNPs

In this study, the treated cells with various concentrations of the present $HAuCl_4$, *Foeniculum Vulgare* seed aqueous extract, and AuNPs were tested by MTT assay for 48 h about the cytotoxicity features on normal (HUVEC) and common breast cancer cell lines i.e. ZR-75-30, T47D, and HCC1187. The absorbance rate was determined at 570 nm, which indicated extraordinary viability on normal cell line (HUVEC) even up to 1000 µg/mL for $HAuCl_4$, *Foeniculum Vulgare* seed aqueous extract, and AuNPs (Fig. 5; Table 1). In the case of a malignant breast cell line, the viability of them reduced dose-dependently in the presence of $HAuCl_4$, *Foeniculum Vulgare* seed aqueous extract, and AuNPs. The IC50 of AuNPs were 321, 269, and 234 µg/mL against ZR-75-30, T47D, and HCC1187 cell lines, respectively (Fig. 5; Table 1). Metallic nanoparticles have different parameters such as texture, size, shape, etc. Sizes have a very important feature in the therapeutic effects of nanoparticles, according to some researches, small metallic nanoparticles have better penetration into cells and have better anti-cancer effects. It has been evaluated that particle size lower than 50 nm showed excellent therapeutic activity in the corresponding cancer cell lines (You et al., 2012;

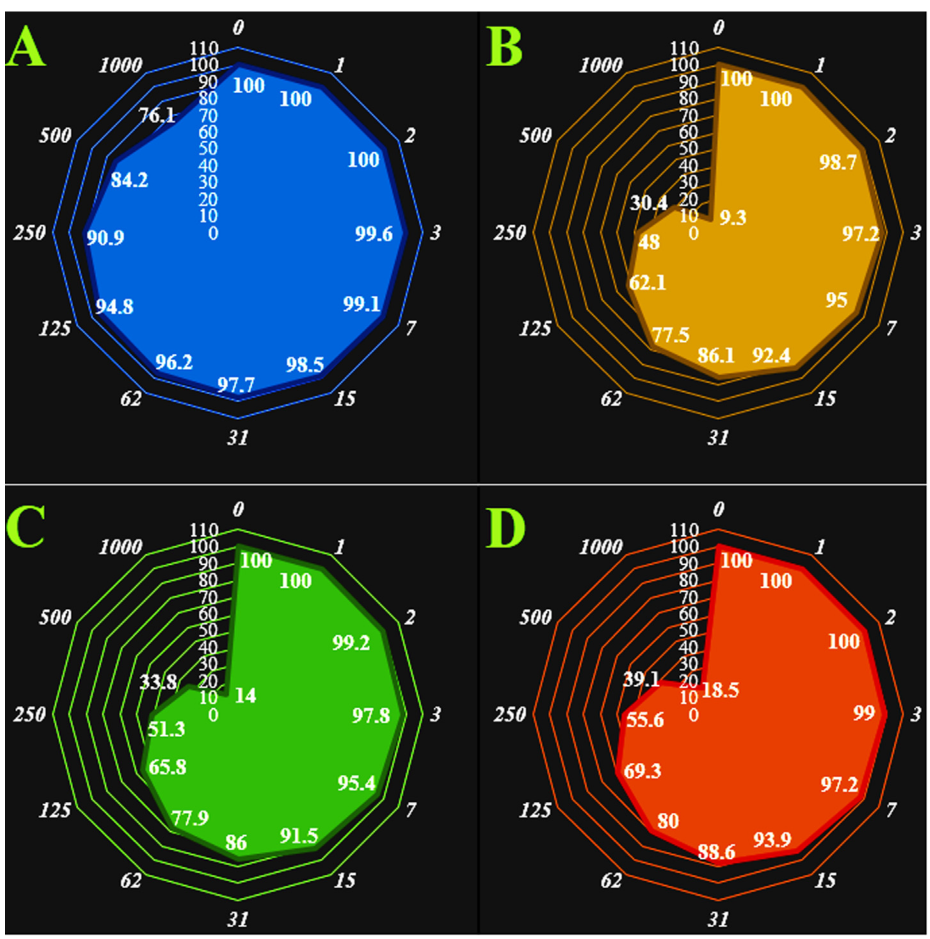

Fig. 5 The anti-human breast cancer properties (Cell viability (%)) of gold nanoparticles (Concentrations of 0–1000 µg/mL) against normal (HUVEC: A) and human breast cancer (ZR-75–30 (A), T47D (B), and ZR-75–30 (C)) cell lines. The numbers indicate the percents of cell viability in the concentrations of 0–1000 µg/mL of gold nanoparticles against several human breast cancer cell lines.

Table 1 The IC50 of gold nanoparticles in the anti-human breast cancer test.

	ZR-75-30	T47D	ZR-75-30
IC50 (μg/mL)	234	269	321

Mao, 2016; Namvar et al., 2014; Sankar et al., 2014; Katata-Seru et al., 2018). As you see in Figs. 3 and 4 of our research; the average size of AuNPs synthesized by *Foeniculum Vulgare* seed aqueous extract is 18.5 nm. Au nanoparticles have been used to treat several cancers, including human glioma, human lung cancer, uterus cancer, mammary carcinoma, lung epithelial cancer, Lewis lung carcinoma, and colon cancer.

3.6. Antioxidant features of Foeniculum Vulgare seed green-synthesized AuNPs

Oxidative stress is caused by an imbalance between the production of free radicals and metabolic reactions, which leads to damage to lipids, proteins and nucleic acids. These damages may be due to low levels of antioxidants or an excessive increase in the production of free radicals in the body (Gibson, 2016; Jalalvand et al., 2019). In humans, oxidative stress is associated with chronic diseases such as diabetes and cancer. Therefore, the production of synthetic and natural antioxidants is necessary to prevent oxidative stress and its destructive effects. Antioxidants effectively and in various ways reduce the harmful effects of free radicals in the biological and food systems and cause detoxification (Shaneza, 2018). In this regard, green nanoparticles can be used (using plant substrates to prepare nanomaterials that are environmentally friendly and do not contain any harmful chemicals) that show antioxidant properties. At present, the use of non-toxic substances in synthesizing nanoparticles to prevent biological hazards, especially in medical and pharmaceutical applications is considered (Jalalvand et al., 2019; Zangeneh et al., 2019). Many researchers have focused on bioactive substances derived from plants or other sources such as bacteria, fungi and yeast for synthesizing nanoparticles. The green synthesis method is thought to increase the biocompatibility and performance of metal nanoparticles for biological applications due to removing harmful chemicals (Shaneza, 2018). During the bio-production stages of nanoparticles, their extracellular production using plants or their extracts is more beneficial and their production can be adjusted in a controlled way based on size, distribution and shape for different purposes (Zangeneh et al., 2019).

In this research, we evaluated the antioxidant features of *Foeniculum Vulgare* seed aqueous extract green-synthesized Au nanoparticles against common free radicals (Fig. 6).

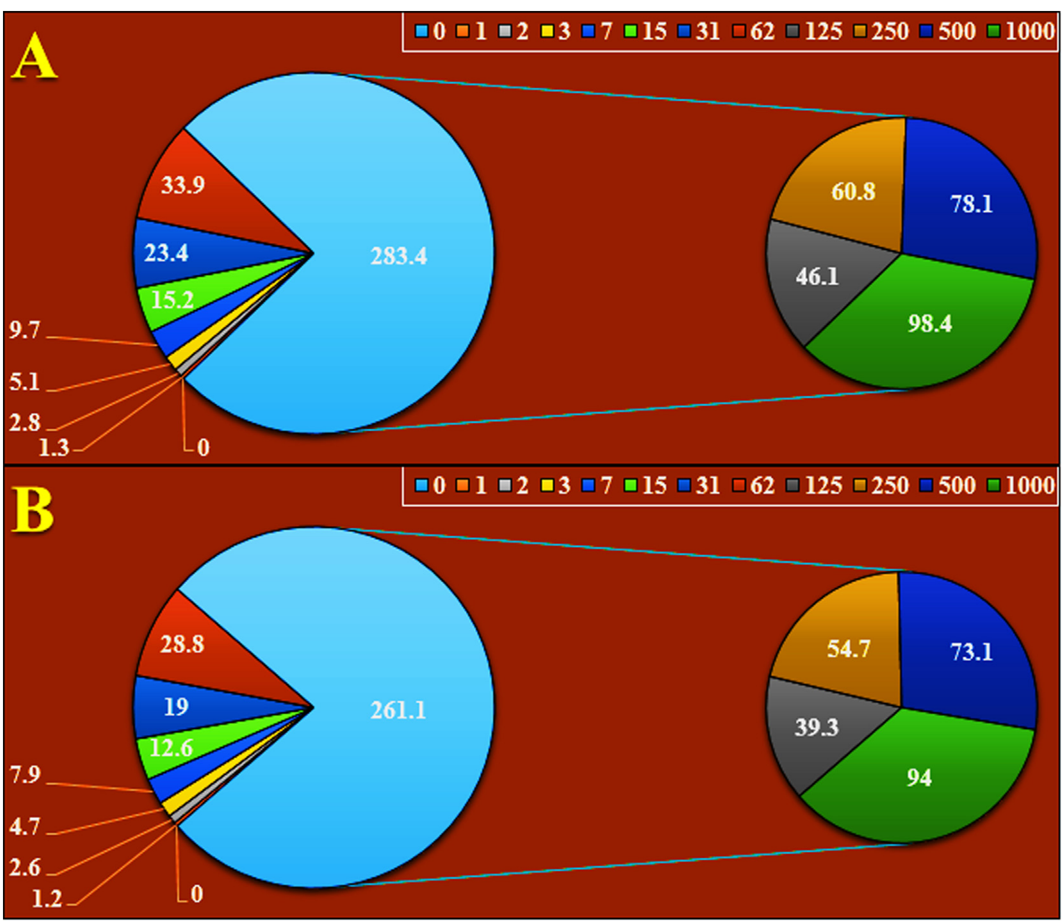

Fig. 6 The antioxidant properties of gold nanoparticles (A) and BHT (B) against DPPH. The numbers indicate the percents of free radical (DPPH) inhibition in the concentrations of 0–1000 μg/mL of gold nanoparticles (A) and BHT (B).

Table 2 The IC50 of gold nanoparticles and BHT in the antioxidant test.

	Gold nanoparticles	BHT
IC50 (μg/mL)	156	201

In the antioxidant test, the IC50 of AuNPs and BHT were 150 and 201 μg/mL, respectively (Table 2).

Investigations have shown that the antioxidant features of AuNPs green- synthesized by pharmaceutical herbs are more significant than other metal nanoparticles. So far considerable antioxidant features of AuNPs green- synthesized by several pharmaceutical herbs such as *Falcaria vulgaris*, *Allium noeanum* Reut. ex Regel, *Gundelia tournefortii* L., *Thymus vulgaris*, and *Camellia sinensis* have been proved (Shaneza, 2018). AuNPs green- synthesized by pharmaceutical herbs show significant antioxidant effects against free radicals formation within the living system (Zangeneh et al., 2019). The AuNPs green- synthesized-formulated have excellent redox features and have a significant role in free radicals deactivating (Shaneza, 2018).

Studies in the recent decade have indicated that phenolic and flavonoids compounds attached to the metallic nanoparticles have important antioxidant features (Shaneza, 2018).

As it was mention previously in the introduction *Foeniculum Vulgare* contains antioxidant compounds.

Perhaps significant anti-human breast cancer potentials of AuNPS synthesized by *Foeniculum Vulgare* seed aqueous extract due to their antioxidant effects. Many researchers around the world reported that AuNPs synthesized by pharmaceutical plants have a considerable role in removing free radicals and are involved in inhibiting the growth of cells in some types of cancer (Katata-Seru et al., 2018; Sangami and Manu, 2017; Beheshtkhoo et al., 2018; Radini et al., 2018; Oganesvan et al., 1991).

4. Conclusion

In this experiment, the AuNPs were obtained from the reaction between $HAuCl_4$ and *Foeniculum Vulgare* seed aqueous extract in vitro condition. TEM, FE-SEM, FT-IR, and UV–Vis methods were used to determine nanoparticle characteristics. According to the FT-IR spectrum the presence of a large amount of antioxidant compounds that created proper conditions for the reduction of Au. In the TEM technique, the average size of Au nanoparticles (AuNPs) were determined to be 18.5 nm, which are desirable. The AuNPs showed excellent antioxidant features against DPPH. AuNPs had suitable anti-breast cancer activities dose-dependently against ZR-75–30, T47D, and HCC1187 cell lines without any cytotoxicity on the normal cell line (HUVEC). After clinical research AuNPs containing *Foeniculum Vulgare* seed aqueous extract can be used as an effective drug in the treatment of breast cancer in humans.

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