RESEARCH ARTICLE

Green Synthesis of Iron Oxide Nanoparticles by Extract of Eriobotrya japonica Leaves: Comparing the Characteristics by PEGylated method and Conventional Chemical Method

Soudabeh Etemadi[1,2], Edris Yousefi Delcheh[1], Ahmad Mehravaran[3*], Leili Mohammadi[3], Hadi Mirahmadi[1], Aram Khezri[4]

[1] Department of Medical Parasitology and Mycology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

[2] Department of Medical Laboratory Science, Sirjan School of Medical Sciences, Sirjan, Iran

[3] Infectious Diseases and Tropical Medicine Research Center, Research Institute of Cellular and Molecular Science in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran

[4] Department of Medical Parasitology and Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Objective(s): Magnetic iron oxide nanoparticles are remarkably potent drug nanocarriers due to their intrinsic capacity for elevated drug loading, biocompatibility, stability. This study focused on the development of iron oxide (Fe_3O_4) nanoparticles by the green synthesis method and PEGylated method, utilizing *Eriobotrya japonica* leaf extract.

Methods: The physicochemical parameters were determined using Dynamic Light Scattering (DLS), Field Emission Scanning Electron Microscopy (FESEM), Transmission Electron Microscopy (TEM), Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), and cytotoxicity test methods. Eventually, polyethylene glycol (PEG) was utilized to coat the surface and ensure the stability of the final nanomedicine formulation.

Results: The DLS evaluation of iron oxide nanoparticles revealed an average hydrodynamic size of 155 nm. FESEM showed that the network of spherical with small building blocks was formed by the green synthesis method and coated with polyethylene glycol. The X-ray Energy Dispersive Spectroscopy analysis reveals a significant percentage of carbon (C), oxygen (O), as well as some sulfur (S), chlorine (Cl), and iron (Fe). The FTIR analysis verified the existence of acidic O-H and alkene, C-H, C-N, and C-O functional groups compared to the bare nanoparticle. The cytotoxicity test investigation indicated that the inhibitory concentrations (IC50) toxicity of iron oxide nanoparticles, obtained by the green synthesis method on the MCF-7 cell line, was 1763 µg/ml.

Conclusions: These nanoparticles exhibited a favorable dispersion index, efficient incorporation of plant extract fractions into the nanoparticle network, and minimal toxicity in the final product. Consequently, these nanoparticles are well-suited for biomedical research and drug delivery applications.

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INTRODUCTION

In recent years, nanotechnology has been skillfully manipulated in various fields, including medicine. Nanoparticles are commonly utilized as drug carriers, playing an essential role in the fields of diagnostics and treatment[1]. Under this class of particles, prominent instances involve polymer nanoparticles, nanoemulsions, liposomes, immunostimulating complex (Iscom) and solid nanoparticles, all of which possess promising medicinal applications[2-5]. Conversely, selective

* Corresponding Author Email: *ahmadmehravaran55@gmail.com*

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drug delivery of medical substances has been a critical research topic in medicine and biology for many years. Iron oxide (Fe_3O_4) nanoparticles have specific features compared to other nanoparticles, primarily characterized by their exceptional drug-carrying capacity and targeting capabilities[6]. Furthermore, the exceptional magnetic properties of these ions, combined with their biocompatibility, stability, and compatibility with the environment, make them highly suitable for biomedical applications[7].

Iron oxide nanoparticles (IONPs) are extensively utilized in clinical trials due to their controlled physicochemical, electromagnetic, and paramagnetic properties, as well as their chemical stability as drug carriers, low toxicity, numerous production methods, and biological properties. IONPs are valuable in enhancing imaging contrast, Magnetic resonance imaging, cellular monitoring, immunological tests, tissue regeneration, and drug delivery and have been employed in both live organisms and laboratory settings[8-10]. Despite the promising features, the clinical use of these nanoparticles in medical settings relies primarily on their physicochemical properties, toxicity, and functional capabilities[11]. The production of nanoparticles utilizing physical and chemical methods is prohibitively costly, environmentally detrimental, and necessitates specific facilities and elevated temperatures, pressures, and energy consumption. Furthermore, these methods utilize toxic and non-biodegradable reducing agents and, in certain instances, carcinogenic agents, rendering them unsuitable for biological applications. In recent years, the production of nanoparticles using green synthesis methods has been approved as an efficient and environmentally compatible approach. The product has received approval for being non-toxic, cost-effective, and efficient[12]. Plant extracts and microorganisms are viable options for conducting green synthesis. The research findings demonstrate that plant extracts exhibit a significantly higher and more rapid efficacy in reducing metal ions while preserving their stability than microorganisms[13]. Green synthesis involves the utilization of plant extracts to transform iron oxide (Fe3O4) into non-toxic nanoparticles of metal elements (bases). Iron nanoparticles can function as drug carriers to reduce drug resistance, enhance local concentration, and serve as cell-tissue targeting agents to enhance selectivity[8].

Eriobotrya japonica is an evergreen tree native to subtropical areas cultivated for over two millennia. The plant's leaves and fruits are high in active medicinal compounds and have traditionally been employed for their therapeutic benefits, including treating diabetes, kidney stone expulsion, and liver ailments[14, 15]. The plant's leaves include abundant phenols and triterpenes, which have anti-inflammatory effects on alveolar macrophages, reducing blood glucose levels and enhancing insulin production. Moreover, these compounds exhibit anti-radical and anti-cancer capabilities[16-18]. The extract of *Eriobotrya japonica* leaves, which contains bioactive compounds such as phenols, flavonoids, and triterpene acids, can serve as an effective biological stimulus to synthesize iron nanoparticles. These nanoparticles can be utilized to decrease iron salts in green synthesis[19, 20]. Polyethylene glycol is utilized in medical nanoparticle research to facilitate drug delivery and to stabilize and mitigate nanoparticle toxicity through surface coating[21]. The use of polyethylene glycol in synthesizing iron oxide nanoparticles modifies their surface charge, stability, size reduction, and toxicity. This study employed polyethylene glycol for this specific purpose[22].

This study utilizes *Eriobotrya japonica* leaf extract as a reducing agent to produce iron oxide nanoparticles by the green synthesis method. The objective is to examine the physicochemical characteristics of iron oxide green synthesized nanoparticles utilizing *Eriobotrya japonica* leaf extract and compare them to those obtained through the chemical method and PEGylated method.

MATERIALS AND METHODS

Extraction of the plant

The leaves of the *Eriobotrya japonica* plant were obtained from trees in the northern region of Iran during spring. The leaves were cleansed, dried in the sunlight, and then powdered. The hydroalcoholic extract was prepared by mixing 100 g of plant leaf powder with a ratio of 20% water and 80% alcohol (Ethanol 96%), resulting in a total volume of 600 ml. The mixture was subsequently stirred for 24 hours on a rotator at 325 rpm. Afterward, a sterile cloth was used to remove coarse sediments, and the remaining liquid extract was filtered through the Whatman 4 filter paper. Next, utilizing a vacuum distillation device (evaporator), the liquid extract solvent was evaporated at a temperature

of 40 degrees Celsius for 6 hours, resulting in the precipitation of the final extract.

Chemical method

The preparation of iron oxide nanoparticles was carried out using a simple co-precipitation method. Iron nitrate Nona hydrate (99.99%) and iron sulfate heptahydrate (99.5%) were purchased from Ghatran Chimi Compani, Iran. Iron nitrate Nona hydrate and iron sulfate heptahydrate were dissolved in water at 60 degrees Celsius for 30 minutes using a heater stirrer with 200 rpm. Afterward, HCL 30% and ammonium 25% were added to raise the pH level over 11. After 5 minutes of stirring (200 rpm), a solution undergoes a chemical reaction, forming a dark brown precipitate. The solution was placed into a vacuum distillation device, and the resultant powdery precipitate was retained[23].

Simple and PEGylated green synthesis method

Iron nitrate nonahydrate and iron sulfate were dissolved in liquid herbal extract (before evaporation) and then subjected to a heater stirrer (200rpm) at 60 degrees Celsius for 30 minutes. Afterward, HCL 30% and ammonium 25% were used to achieve a pH above 11. Following 5 minutes of stirring with 200 rpm, a solution manifested a dark brown precipitate. Within this method, the liquid plant extract performs as both a solvent and a reductant in synthesizing nanoparticles from metal salts. Polyethylene glycol 600 (PEG-600) was provided from Merck Company. In order to obtain PEGylated iron oxide nanoparticles, the pH was raised, and then polyethylene glycol 600 (PEG-600) was added into the solution, producing a more intense precipitate. An evaporator was used to desiccate the nanoparticles obtained by this method[22].

Dynamic light scattering (DLS)

Dynamic Light Scattering (DLS) is a contemporary method for measuring particle sizes and electrical charge within the nanometer to micron scale. The suspension system scatters light in a manner that exhibits oscillations. In order to measure the particle diameter, a detector is positioned at a specific angle to record the intensity of the scattered light over a defined period. This method provides a rapid means of measurement[24]. The dynamic light scattering approach was utilized to quantify the diameter, polydispersity index, and electrical charge of iron oxide nanoparticles obtained by the chemical, simple, and PEGylated green synthesis methods.

Field Emission Scanning Electron Microscopy (FESEM)

The surface morphology, chemical element composition, and texture of iron oxide nanoparticles obtained by simple green synthesis and PEGylate green synthesis were investigated using a field emission scanning electron microscope (model: KYKY EM8000F FESEM-China). Some of the diluted powder suspension was applied onto a glass surface to achieve this objective. Subsequently, a thin layer of gold coating was deposited onto the surface at 25 kV under vacuum conditions. The X-ray energy diffraction spectroscopy (EDS) method was utilized to determine the elemental composition of solid powder samples.

Transmission electron microscopy (TEM)

A small amount of PEGylated green synthesized iron oxide nanoparticle powder was introduced into a glass filled with distilled water to investigate nanoparticle constituents' morphology, size, and interconnectedness utilizing a transmission electron microscope. Subsequently, the glass containing nanoparticles underwent dispersion in an ultrasonic device for 20 minutes. Subsequently, a few drops of the sample were taken from the glass using a micropipette and transferred onto the carbon-coated grid Cu Mesh 300 (EMS-USA) Formvar. This grid serves as a platform for holding nanoparticles in the TEM device. After a few minutes, the dispersant on the grid evaporated. Subsequently, the grid was subjected to analysis using the Transmission Electron Microscope for observation.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR method was employed to ascertain the bonds and functional groups in the iron oxide nanoparticle obtained by the simple and PEGylated green synthesis methods. A comparison was made with the iron oxide nanoparticle obtained by the chemical method, and the quantity of extract loaded into the nanoparticle composition was assessed. In this analysis, the nanoparticle powder obtained by the chemical method, simple green synthesis, and PEGylated green synthesis was applied onto the ATR crystal as a thin layer (approximately 2 mm) and compressed using a rotary press to ensure the optimal contact between the sample and crystal.

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X-ray diffraction (XRD)

The undiluted nanoparticle solution was deposited onto a glass slide and allowed to dry at 25°C to obtain the X-ray diffraction pattern of iron oxide nanoparticles synthesized by either of the methods. The procedure above was conducted to create a layer on the glass slide. A Rigaku X-ray diffractometer (model: Advance D8, BRUKER, Germany) was utilized to evaluate the dried samples. The instrument was operated at 40 kV, and 40 mA current with CuKα radiation (l = 1.54056 Å) was employed. The diffraction angle ranged from 20 to 90 degrees, while the scanning frequency was 0.02/s.

Cytotoxicity test (MTT Assay)

The test was conducted utilizing a breast cancer epithelial cell line (MCF7). The PEGylated green synthesized iron oxide nanoparticles were evaluated using the colorimetric method involving tetrazolium 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl bromide (MTT)[25]. This method is based on the enzymatic activity of mitochondrial succinate dehydrogenase in living cells. This enzyme converts the yellow MTT solution into purple formazan crystals, which are then dissolved in DMSO and evaluated with the Elisa Reader device. Each well of a 96-well plate was filled with 180 microliters of cell suspension, resulting in a concentration of 3 x 10^4 cells per milliliter of culture media. Subsequently, 20 µl of various doses of medication were added to each well. Doxorubicin was utilized as the positive control, while the negative control consisted of a culture medium containing 0.5% DMSO without any medication. Following a 48-hour incubation period, 20 µl of MTT solution (5 mg/mL) was added to each well. Then, after a 2-hour incubation period,100 µl of DMSO was replaced with the prior solution to dissolve the formazan crystals. The optical absorption of all three plates was then measured at a wavelength of 560 nm using an Elisa reader device. Three repetitions were determined for various dilutions of each substance. The cell survival percentage was obtained using the following formula:

$$\text{Cellsurvival percentage} = \left(\frac{\text{optimal absorption in treated cells} - \text{Optical absorption in the negative control group}}{\text{Optical absorption in the positive control group} - \text{Optical absorption in the negative control group}}\right) \times 100$$

Statistical analysis

The statistical analysis was conducted using GraphPad Prism software, and the results were evaluated using a one-way ANOVA. The observed differences reached statistical significance at a significance level of $P<0.05$.

RESULTS

Dynamic Light Scattering (DLS)

Dynamic light scattering was employed to perform hydrodynamic distillation of iron oxide nanoparticles synthesized using chemical methods and simple and PEGylated green synthesis methods. Graph 1 illustrates the determined frequency-based size distribution of nanotubes. The average hydrodynamic size for Fe3O4 obtained by chemical and simple and PEGylated green synthesis is 478, 290, and 155 nm, respectively. All three samples exhibited a single peak in the particle-size distribution verticals, with a relatively low polydispersity index (PDI) indicating a low accumulation of nanoparticles in the solution. All three nanoparticles exhibited a negative zeta potential (Table 1).

Field Emission Scanning Electron Microscopy (FESEM)

Surface morphology, composition of chemical elements, and texture of iron oxide nanoparticles obtained by simple green and PEGylate synthesis methods were observed using a field emission scanning electron microscope. Figure 1 depicts the formation of spherical iron oxide nanoparticles among irregular clusters of plant components. Figure 2 depicts the application of polyethylene glycol as a surface coating on spherical iron oxide nanoparticles, crystals, and plant clusters. The X-ray energy diffraction spectroscopy analysis of the particular area of the iron oxide nanoparticle, obtained by the simple and PEGylated green synthesis method (depicted in Figure 3 and 4), demonstrates a significant percentage of carbon (C) and oxygen (O) elements, along with traces of sulfur (S), chlorine (Cl), and iron (Fe).

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was utilized to investigate the morphology and size of iron oxide nanoparticles obtained by the PEGylated green synthesis method (Figure 5). Particle aggregation occurred as expected due to the significant surface-to-volume ratio, resulting in enhanced surface energy and a more indistinct appearance. The results reveal that most nanoparticles have a quasi-spherical form, with an

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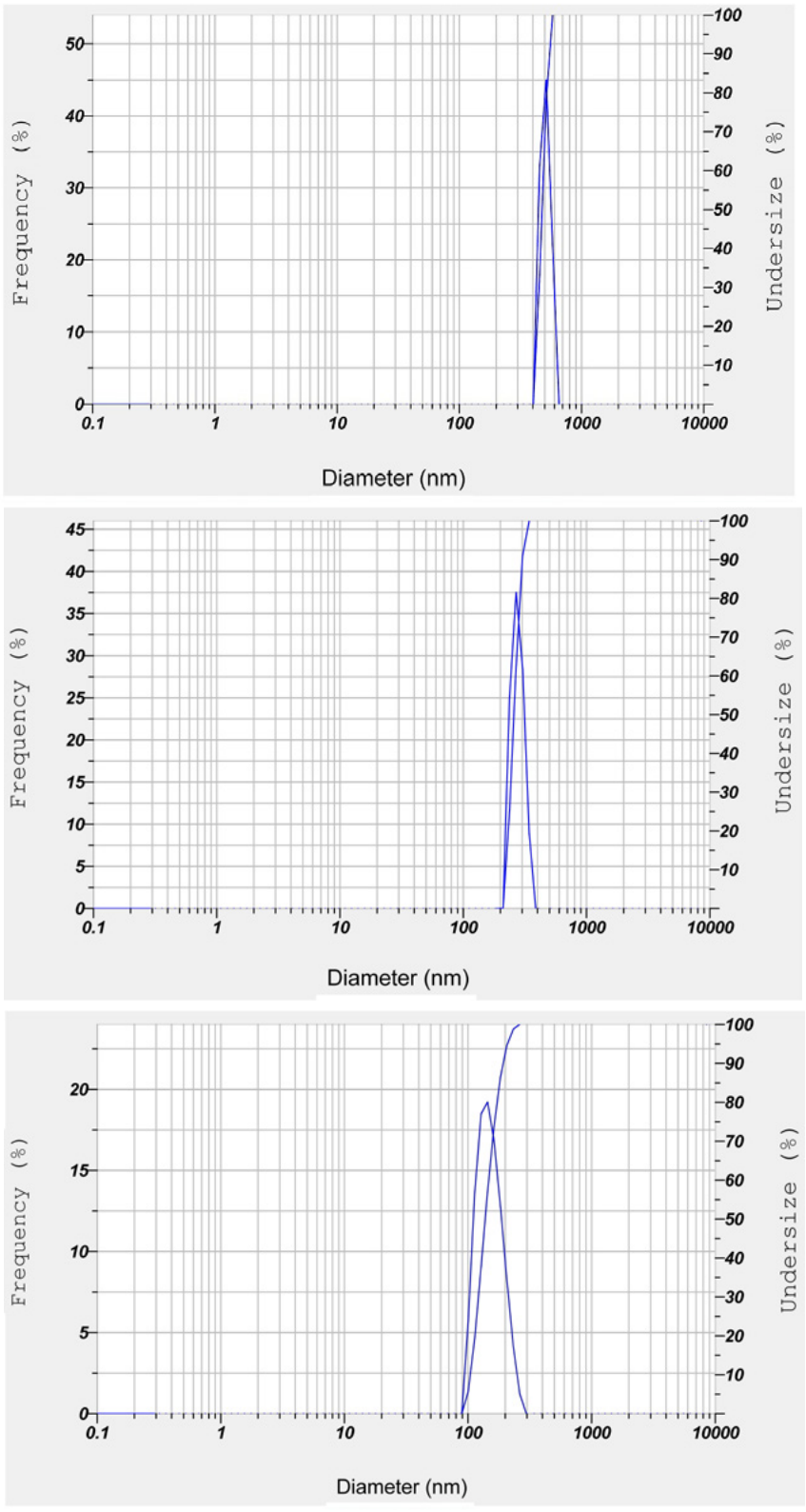

Graph 1. The mean, median, and range were derived from the frequency-based size distribution analysis conducted using Dynamic Light Scattering (DLS). (a): bare Fe_3O_4 nanoparticles (b): Fe_3O_4 obtained by simple green synthesis (c): Fe_3O_4 obtained by PEGylated green synthesis.

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Table 1. Average hydrodynamic size, zeta potential, and Polydispersity index (PDI) of iron oxide nanoparticles obtained by chemical methods (Bare NPs), simple green synthesis, and PEGylated green synthesis.

feature / nanoparticles	Size (nm)	Zeta potential (mV)	PI
Bare NPs	478	-5.4	0.751
Green synthesized NPs	290	-33.6	0.711
PEG-Green synthesized NPs	155	-37.9	0.669

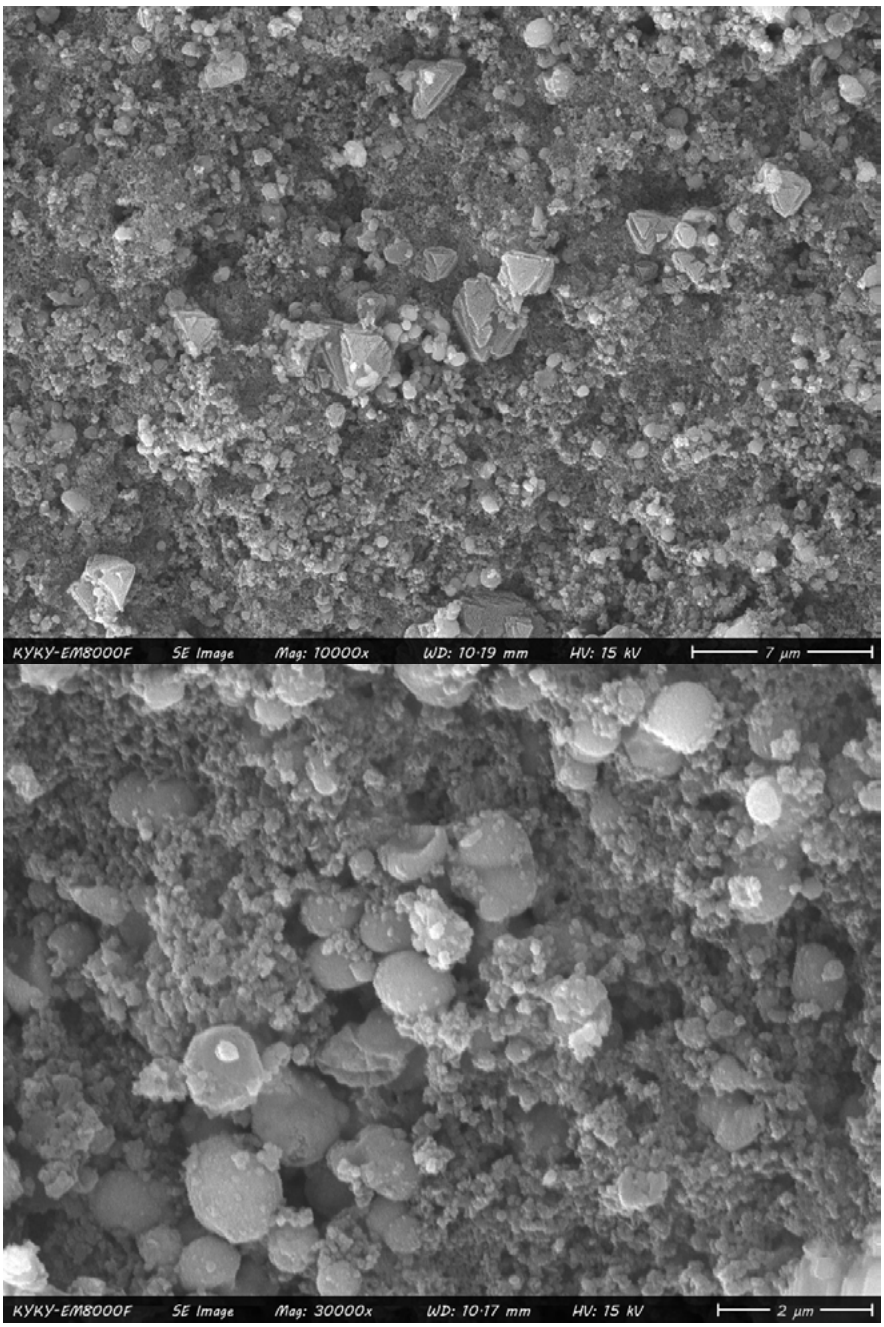

Fig 1. FESEM micrographs of iron oxide nanoparticles obtained by the simple green synthesis method

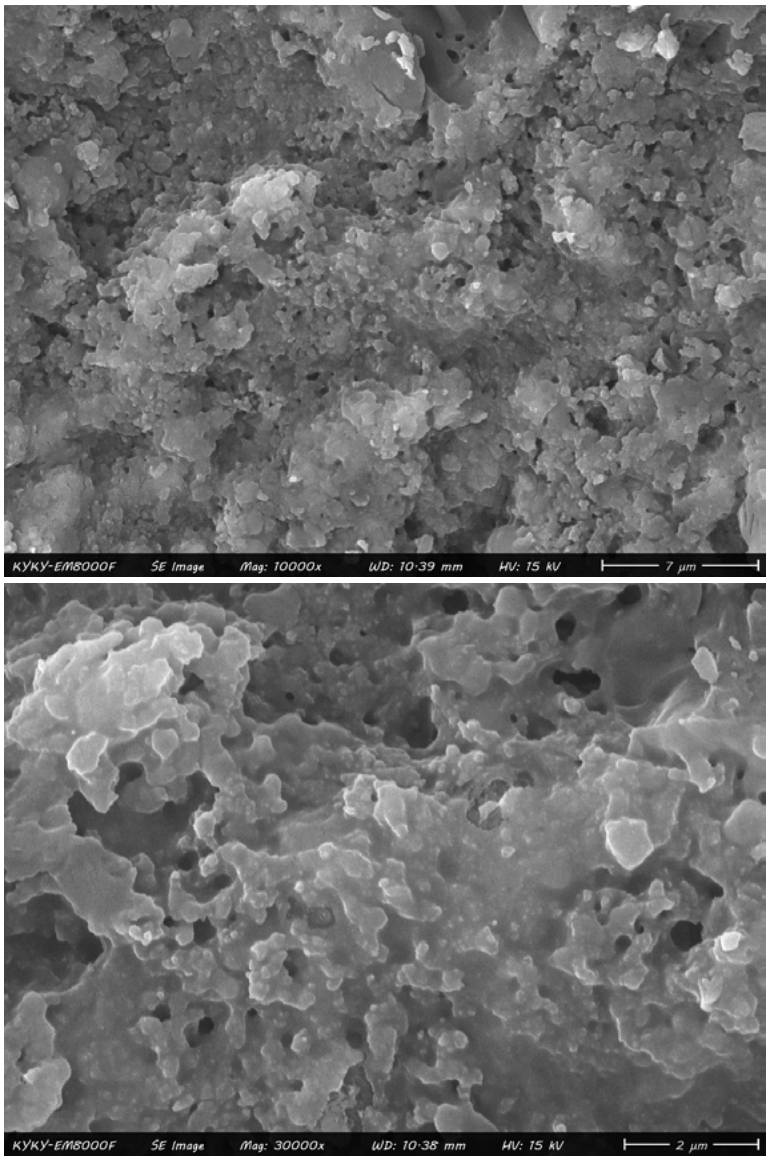

Fig 2. FESEM micrographs of iron oxide nanoparticles obtained by the PEGylated green synthesis method.

average diameter of about 100 nm. Furthermore, the plant extract, nanoparticles, and polyethylene glycol have interlinked to create a network.

Fourier-Transform Infrared (FTIR) Spectroscopy Analysis

Graph 2 depicts the results of FTIR spectroscopy, which investigated the bonds and functional groups present in nanoparticles obtained by the chemical method, simple green synthesis, and PEGylated green synthesis. A sharp peak, indicative of Fe-O bond absorption, is observed in the 540 to 620 w/cm spectral range[26, 27]. Furthermore, sharp peaks are observed at 1033.45, 1067.69.18, 1395.55, 1397.22, and 1401.59 waves/cm in all three synthesized nanoparticles. These peaks indicate a higher absorption level in the green synthesized nanoparticles, especially in the PEGylated green synthesis. The absorption in this spectrum is caused by the bending bonds of C-H and O-H, as well as the stretching bonds of S=O, C-N, and C-O. Within the spectral range of 1613.13 and 1687.53 w/cm, two sharp peaks are observed in the profiles of simple and PEGylated

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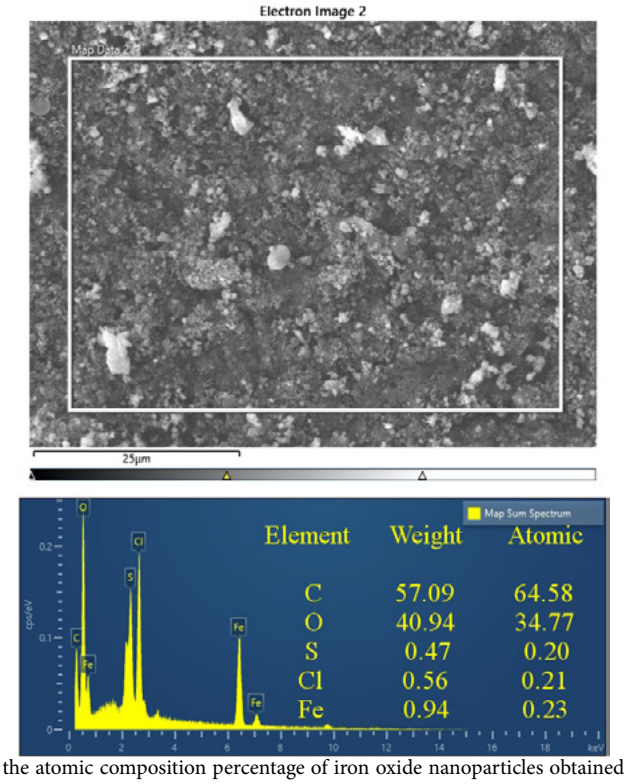

Fig 3. EDS imaging area and the atomic composition percentage of iron oxide nanoparticles obtained by the simple green synthesis method.

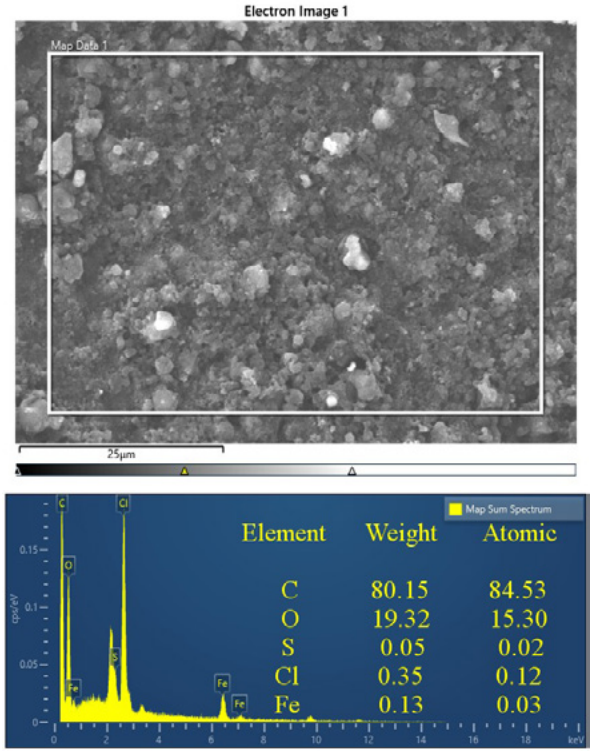

Fig 4. EDS imaging area and the atomic composition percentage of iron oxide nanoparticles obtained by the PEGylated green synthesis method.

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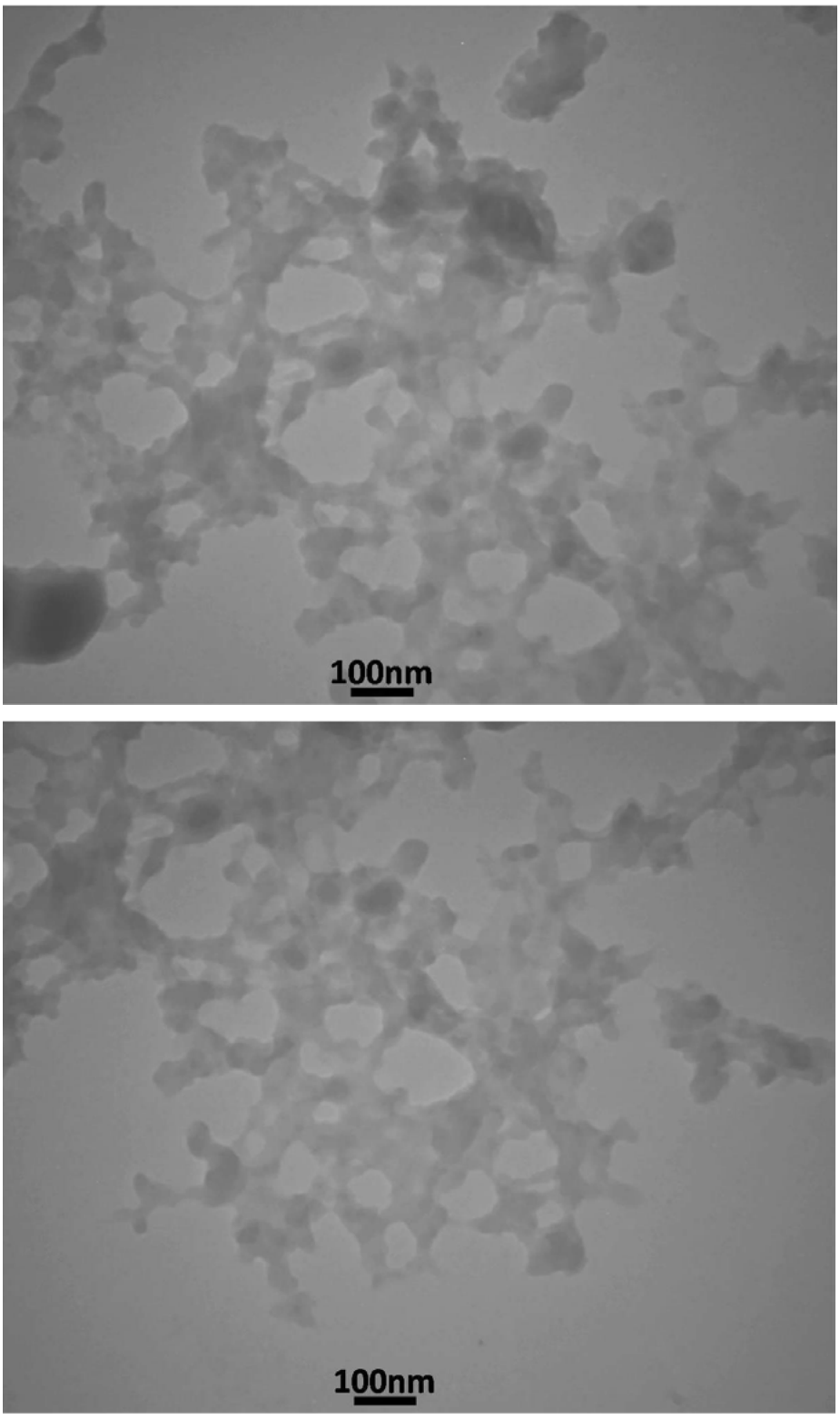

Fig 5. TEM micrographs of iron oxide nanoparticles obtained by the PEGylated green synthesis method.

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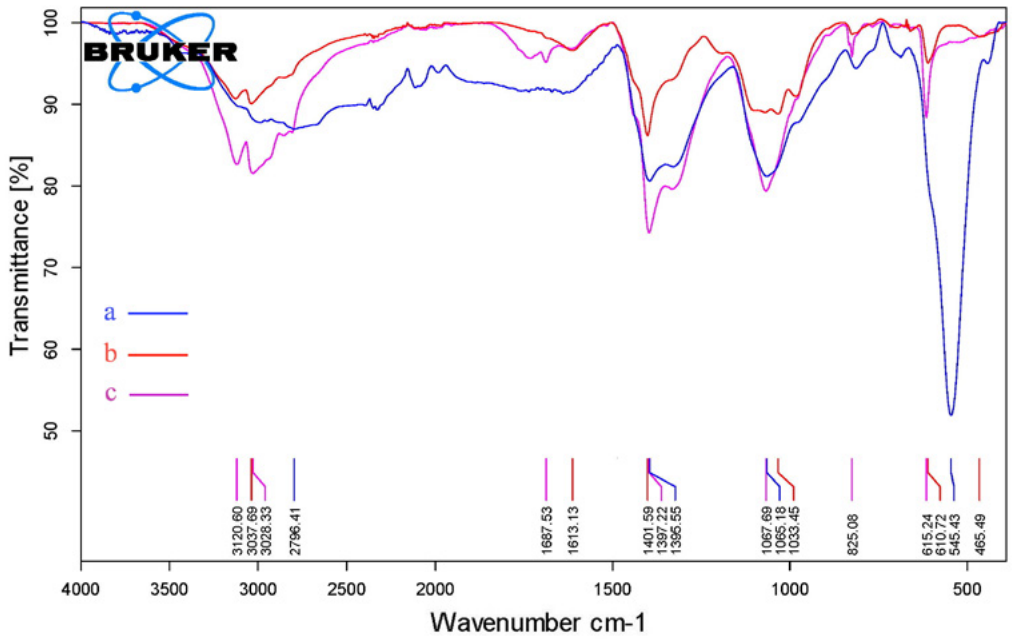


Graph 2. FTIR analysis: a: bare Fe_3O_4 nanoparticles b: iron nanoparticles obtained by simple green synthesis c: iron nanoparticles obtained by PEGylated green Synthesis.

green synthesis iron oxide nanoparticles. These peaks represent the absorption of C=O stretch bonds in this specific wavenumber range. Within the spectral range of 1613.13 and 1687.53 w/cm, two sharp peaks are observed in the profiles of simple and PEGylated green synthesis iron oxide nanoparticles. These peaks represent the absorption of C=O stretch bonds in this specific wavenumber range. The sharp peaks within the spectrum of 2400 to 3400 w/cm can be attributed to the absorption characteristics of the acidic O-H bonds.

X-ray diffraction (XRD) study

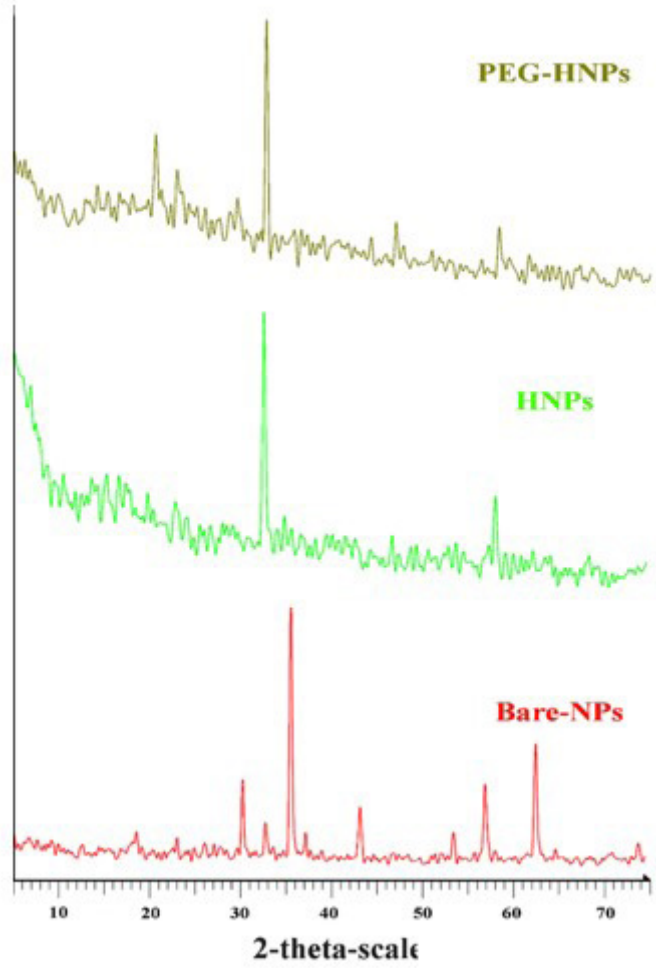
XRD diffraction determined the phase purity and crystallinity of the biosynthesized nanoparticles (Graph 3). The X-ray diffraction pattern of bare iron oxide nanoparticles, exhibited sharp 2θ peak values at 30.8°, 36.1°, 43.3°, 57.4°, and 63°. The X-ray diffraction pattern of iron oxide nanoparticles, obtained by the simple green synthesis method, exhibited sharp 2θ peak values at 15.3°, 16.9°, 32.9°, 34.9°, and 58.5°. The X-ray diffraction pattern of iron oxide nanoparticles, obtained by the PEGylated green synthesis method, exhibited sharp 2θ peak values at 20.9°, 23.1°, 29.8°, 32.7°, 47.4° and 58.6°. The crystallite sizes of Fe_3O_4 nanoparticles were determined from the X-ray diffraction data using the Debye–Scherrer formula. Iron oxide nanoparticles with an average size of 454,262 and 124 nm were estimated by chemical methods, simple green synthesis and PEGylated green synthesis, respectively.

Cytotoxicity test (MTT Assay)

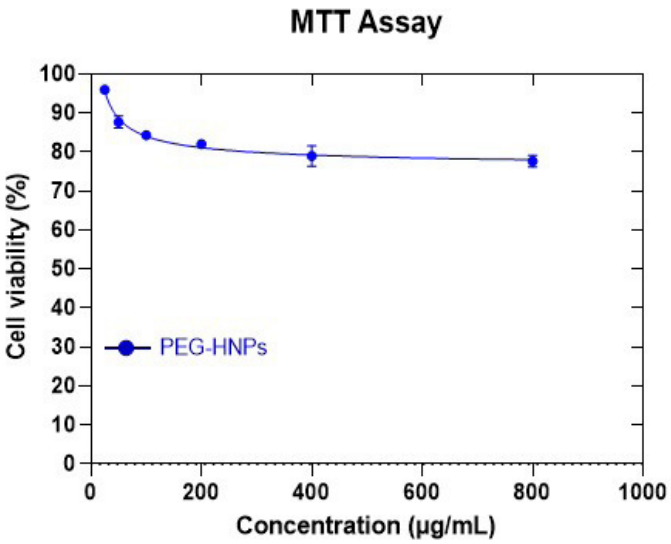
The cytotoxicity effect of PEGylated iron oxide nanoparticles obtained by green synthesis, at doses of 25, 50, 100, 200, 400, and 800 µg/ml was assessed on the MCF-7 cell line (Graph 4). Moreover, their IC50 value has been calculated (Table 2). The results indicated that the negative control (0.5%) exhibited no cytotoxicity. The results obtained from the impact of PEGylated iron oxide nanoparticles on MCF-7 cells revealed that the percentage of cell survival reduced with an increase in concentration. The toxicity test revealed the lowest cell survival at the highest nanoparticle concentration (800 µg/ml) (Graph 4). The IC50 value of the PEGylated green synthesized iron oxide nanoparticles is 1763 µg/ml on MCF-7 cell line (Table 2).

DISCUSSION

The production of nanoparticles utilizing physical and chemical methods is prohibitively costly, environmentally detrimental, and necessitates specific facilities and elevated temperatures, pressures, and energy consumption. Furthermore, these methods utilize toxic and non-biodegradable



Graph 3. XRD pattern of bare iron oxide nanoparticles, synthesized by simple and PEGylated green synthesis.



Graph 4. The toxicity determination of green synthesized PEGylated iron oxide nanoparticles on MCF-7 cell line according to concentration. Error bar represents mean±SD (n = 3).

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Table 2. IC50 values of PEGylated green synthesized iron oxide nanoparticles according to their effects on the MCF-7 cell line.

Medications	PEG -HNPs
IC50 (µg/ml)	1763

reducing agents and, in certain instances, carcinogenic agents, rendering them unsuitable for biological applications. In recent years, the production of nanoparticles using green synthesis methods has been approved as an efficient and environmentally compatible approach [28,29].

Iron oxide nanoparticles (Fe_3O_4) were selected as the drug carrier and plant extract stabilizer in this study. Utilizing the PEGylated green synthesis method in producing these nanoparticles provides several advantages compared to simple green synthesis and chemical methods. These advantages include smaller size and accumulation, increased zeta potential, and enhanced stability. These differences are substantiated by the findings obtained from utilizing dynamic light scattering and transmission electron microscopy. Iron oxide nanoparticles as carriers and effective medicines are suitable antimicrobial and anti-parasite agents, as Gudkov et al. and Kannan et al. revealed[30, 31]. However, their magnetic features, limited stability, and high toxicity at elevated concentrations challenge their application in in-vitro studies. As mentioned in the studies by Soenen et al. and Yang et al., the impact of iron oxide nanoparticles on cellular environments and the role and sensitivity of iron released from molecular disintegrations are significant[32, 33]. The method of PEGylation, as demonstrated by Arslani et al., is an efficient and non-toxic method for producing magnetic nanoparticles with enhanced stability and reduced size[34]. The present study replicates the findings of Abakumov et al. that using PEG in producing iron oxide nanoparticles reduces their cytotoxicity on cells and increases their stability[35].

The study's findings indicate that using simple green synthesis and green synthesis with polyethylene glycol is a viable, cost-effective, and environmentally benign approach for producing iron oxide nanoparticles. The results of Piro et al. study indicate that using the simple green synthesis method yields iron oxide nanoparticles with reduced sizes, improved dispersion, and enhanced stability compared to those produced using the chemical method[36]. Consistent with the research conducted by Yew et al., plant extract materials have been utilized as reducing agents and stabilizers in producing metallic iron oxide nanoparticles[37]. Furthermore, the preset study also demonstrated the advantages of the PEGylated green synthesis method for producing iron oxide nanoparticles compared to the simple green synthesis method. Joshy et al. provided a clear demonstration of the efficacy of PEGylated green synthesis in the manufacturing of iron oxide nanoparticles. Compared to alternative approaches, this method yields nanoparticles characterized by their small size, durable particles, and reduced cytotoxicity[22]. These findings are consistent with previous studies, such as the study conducted by Antarnusa et al.[38].

Compared to the chemical method, the reduction in the diameter of Fe3O4 nanoparticles obtained by simple green synthesis is potentially attributed to the surface charge of herbal ingredients molecules surrounding the Fe3O4 nanoparticles during the green synthesis method. Moreover, the increased potential difference between the primary fluid and the fluid layer corresponds to the opposite charge on the nanoparticle's surface, as demonstrated by Piro et al.[36]. Additionally, this reduction in diameter of Fe3O4 nanoparticles was observed in the PEGylated method in comparison with simple green synthesis and chemical method, which could be attributed to the surface charge of herbal ingredients and PEG-600 molecules surrounding the Fe3O4 nanoparticles during the PEGylated green synthesis method, and its zeta potential. The alteration in surface charge resulted in a decrease in the aggregation of nanoparticles inside the aqueous solution. The hydrodynamic size determined using the dynamic light scattering approach for magnetic nanoparticles was greater than the measurements obtained using the transmission electron microscope. The hydrodynamic diameter observed in an aqueous suspension encompasses a solvation layer of water molecules and ions. This discrepancy in data collected from dynamic light scattering and transmission electron microscopy might be attributed to this solvation layer[39].

The FESEM photographs revealed the presence of a network consisting of spherical iron oxide nanoparticles, crystals, and plant extract compounds. The polymer coating made of polyethylene glycol was observed in the sample prepared by the PEGylated green synthesis method. The bioactive reducing agents and polyethylene

glycol in the iron oxide nanoparticles, obtained by the simple and PEGylated green synthesis method, are positioned between magnetic nanoparticles. This placement potentially decreases the accumulation of the nanoparticles by increasing their zeta potential, which agrees with the findings of Bhuiyan et al[40].

The X-ray energy diffraction spectroscopy investigation results can indicate the presence of carbon, which may be attributed to the functional groups of plant compounds and polyethylene glycol, which is especially visible in the final nanoparticles of PEGylated synthesis. The studies conducted by Kannan et al. and Santos et al. have yielded comparable findings[29, 39]. The presence of sulfur and chlorine in the final products can be attributed to the utilization of iron sulfate and hydrochloric acid in the synthesis of nanoparticles. The TEM and SEM investigation revealed the spherical form of the iron nanoparticles. At the same time, EDS verified the presence of an iron oxide core and the coating of plant components with polyethylene glycol. The XRD pattern provided clear evidence for the crystalline structure of iron oxide nanoparticles in the final product obtained by the chemical method. However, the XRD pattern did not indicate a crystalline structure for the iron oxide nanoparticles obtained by the simple and PEGylated green synthesis method. The XRD test results were probably affected by the low atomic percentage and weight percentage of the Fe element, which was less than 5%. The peaks of iron oxide nanoparticles obtained by the simple and PEGylated green synthesis method do not exhibit a complete correspondence with the primary diffraction pattern of iron oxide nanoparticles. Conversely, the diffraction pattern of iron oxide nanoparticles obtained by the chemical method demonstrates a strong alignment[42].

The presence of a sharp peak in the absorption spectrum of the Fe-O bond in the results of FTIR analysis, particularly in the chemical nanoparticle and iron oxide nanoparticle obtained by the PEGylated green synthesis method, confirms the existence of the magnetic core. These findings are consistent with the discoveries made in the study conducted by Silva et al.[26]. The presence of a magnetic core is more pronounced in iron oxide nanoparticles obtained by the chemical method due to the high purity level of the nanoparticles. The utilization of iron sulfate salt in the production of all three nanoparticles potentially contributes to the presence of sharp peaks in the absorption spectrum associated with S=O stretching bonds. In addition, in line with the findings of Qasim et al., the significantly increased intensity of the peak in the absorption spectrum associated with C-H and O-H bending and stretching bonds, as well as S=O and C-N stretching bonds, can be achieved by adequately incorporating aldehyde, alkene, alcohol, and carboxylic acid functional groups of plant extract within the network of iron oxide nanoparticles, particularly those produced by the PEGylated green synthesis method[43]. As demonstrated in the study conducted by Aisida et al., C=O stretch bond absorptions were present in both simple and PEGylated green synthesized iron oxide nanoparticles, which were manufactured using green methods[27]. Furthermore, the detection of absorption peaks corresponding to C-H and O-H bending bonds in the absorption spectrum of 2796.41, 3028.33, 3037.69, and 3120.60 w/cm, as observed in the study conducted by Bhuiyan et al., indicates the incorporation of plant extract, particularly in simple and PEGylated green synthesis iron oxide nanoparticles, which indicate sharp peaks[40].

The cytotoxicity of iron oxide nanoparticles, obtained by the PEGylated green synthesis method on the MCF-7 cell line, was minimal at the administered concentrations in the study. Naqvi et al. conducted a study that obtained comparable results and observed that the cytotoxicity of iron oxide nanoparticles in the presence of reactive oxygen mediators (ROS) and the process of apoptosis was affected by both concentration and treatment time[44]. As indicated by the present study's findings and observed by Santos et al. and Huang et al., incorporating PEG in many drug delivery systems and nanoparticles, in addition to drug delivery coating, provides biocompatibility, enhances drug stability, and reduces toxicity. The FDA has approved its utilization in human applications[41, 45].

CONCLUSION

The results of this research show that green synthesis and PEGylation of iron oxide nanoparticles are efficient methods for enhancing stability, reducing toxicity, reducing size, and facilitating accurate and convenient utilization of these particles in bio-medical studies. The simple and PEGylated green synthesis method produces iron oxide nanoparticles (Fe3O4), resulting in stable, non-toxic, and small-sized particles. In this study, for the first time, a fast, easy, practical and environmentally friendly approach for the synthesis of IONPs and PEG-IONPs using the hydroalcoholic

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extract of *Eriobotrya japonica* Leaves is reported. In general, the comparison of our research results shows that the PEGylation method for producing iron nanoparticles (Fe3O4) is suitable and low-cost and creates non-toxic, stable, and small particles. Since the PEG-IONPs nanoparticles have a smaller size and a modified surface, this of innovation is very helpful to researchers in the fields of targeting of cancer cells, drug delivery and imaging of cells. Further research is needed to thoroughly examine the mechanisms of these methods and explore the biological potential, carrier, and cellular targeting of nanoparticles in treating cancer and other disorders. Utilizing iron oxide nanoparticles due to their size and the lack of a clear and well-defined tissue excretion mechanism in living organisms requires the improvement of the synthesis method and achieving the final medicine with more appropriate characteristics.

CONFLICT OF INTEREST STATEMENT

The authors declare there is no conflict of interest.

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