Efficient Green Synthesis of (Fe$_3$O$_4$) and (NiFe$_2$O$_4$) Nanoparticles Using Star Anise (*Illicium verum*) Extract and Their Biomedical Activity against Some Cancer Cells

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Abstract: Magnetite Fe$_3$O$_4$ and spinel (2:1) and (4:1) NiFe$_2$O$_4$ magnetic nanoparticles (MNPs) were prepared by simple and affordable co-precipitation methods using an extract of star anise (*Illicium verum*) as a green reducing agent. The morphology and chemical composition of these MNPs were confirmed by field-emission scanning electron microscopy, energy-dispersive X-ray spectroscopy, UV–visible spectroscopy, and X-ray diffraction (XRD). The synthesized magnetite Fe$_3$O$_4$ and spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs were in the size range of 0.1–1 µm. The MNPs had irregular clustered platelets (magnetite Fe$_3$O$_4$) and pyramidal structures (spinel (2:1) and (4:1) NiFe$_2$O$_4$ NPs). The average sizes of the synthesized magnetite Fe$_3$O$_4$, and spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs calculated using XRD analysis were 66.8, 72.5, and 72.9 nm, respectively. In addition to the characteristic absorption peaks of magnetite Fe$_3$O$_4$, those of spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs were detected at ~300–350 nm and ~700 nm, respectively. Overall, the results of this study indicate that the synthesized magnetite Fe$_3$O$_4$, and spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs showed high biomedical activities against liver carcinoma cells and non-small lung adenocarcinoma cells.

Keywords: green synthesis; magnetite; spinel NiFe$_2$O$_4$; star anise; co-precipitation

1. Introduction

Magnetic nanoparticles (MNPs) have been extensively investigated owing to their interesting properties, such as excellent magnetic activity, chemical and thermal stability, high surface-area-to-volume ratio, good adsorption behavior, and photocatalytic activity [1–4]. However, ferrite in the form of magnetite (Fe$_3$O$_4$), maghemite (γ-Fe$_2$O$_3$), and doped-ferrite have particularly received a large amount of attention [5–7]. Generally, based on the crystal structure, ferrite nanoparticles are classified as hexagonal (MFe$_5$O$_8$), garnet (M$_3$Fe$_5$O$_{12}$), or spinel (MFe$_2$O$_4$) structures, where M is a transition metal cation such as Ni, Mg, Co, Cu, or Zn [8,9]. MNPs have potential uses in a wide variety of applications based on their electrochemical and antimicrobial activity, such as organic catalysis, photocatalysis, fuel cells, electronic devices, water remediation, drug delivery, and cell therapy [10–13]. The conventional physical and chemical methods that are used to synthesize MNPs are co-precipitation, thermal decomposition, sonochemical, sol–gel, hydrothermal, and chemical combustion methods [14–17]. In recent decades, the limitations of these methods have been clarified, including long processing times, high cost, use of hazardous chemical compounds, and the release of toxic reagents into the environment [18,19].

Currently, green nanotechnology is attracting significant scientific attention owing to its high potential for addressing environmental challenges as it is inexpensive, safe, and eco-friendly [20,21]. Therefore, improved protocols for the green synthesis of MNPs...
using natural extracts and microorganisms have been reported. Prokaryotes (e.g., bacteria, algae, and fungi) produce a large number of inorganic materials as by-products because of their huge biodiversity [22]. In addition, the leaves, roots, seeds, and flowers of plants contain diverse phenolic compounds that can reduce metal ions to metal nanoparticles (NPs) simply and rapidly [23,24]. Therefore, the analysis of such compounds for MNP biosynthesis may be of use more broadly for other applications as well. In addition, there is potential for MNPs to have biotechnological and medical applications [25,26].

The main purpose of the current research was to develop a green method for the synthesis of two types of MNPs: magnetite (Fe$_3$O$_4$) and spinel nickel ferrite [(2:1) and (4:1) NiFe$_2$O$_4$], via a simple, rapid, economical, and effective co-precipitation method. Star anise (Illicium verum) extract was used as an eco-friendly reducing agent for the reduction of metal ions to metal nanoparticles. To the best of our knowledge, this is the first time that the preparation of magnetite Fe$_3$O$_4$, and spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs using star-anise extract has been reported. The synthesized MNPs were characterized using field-emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDS), UV–visible spectroscopy (UV–vis), and X-ray diffraction (XRD). Once characterized, the cytotoxicity effect of the MNPs against liver carcinoma cells and non-small lung adenocarcinoma cells was examined as an example of a biomedical application.

2. Experimental Section

2.1. Chemicals

Analytical-grade iron chloride (FeCl$_3$·6H$_2$O, FeCl$_2$·4H$_2$O), and nickel chloride (NiCl$_2$·6H$_2$O) were obtained from Sigma-Aldrich and used without additional purification. All chemicals and plant extracts were prepared in this investigation using double-distilled water as the solvent.

2.2. Preparation of the Star Anise Extract

Star anise (Illicium verum) was purchased from a local market. To prepare the extract of star anise, we used the same procedure as reported previously [27]. The extract was filtered and centrifuged at 15,000 rpm before being used for the preparation of MNPs.

2.3. Synthesis of the Magnetic Nanomaterials

Magnetite Fe$_3$O$_4$, and spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs were synthesized by green co-precipitation methods. First, FeCl$_3$·6H$_2$O and FeCl$_2$·4H$_2$O with 1:2 molar proportions were dissolved in 100 mL of double-distilled water. The reaction mixture was then boiled for 10 min at 60 °C on a hot plate with vigorous stirring. Next, 10 mL of the star-anise extract was added to the reaction mixture. The color changed from light to darkish brown, indicating the production of Fe$_3$O$_4$ MNPs. Then, the solution was dried in an oven for 24 h at 80 °C. Subsequently, the obtained powder was calcined at 700 °C for 2 h [28]. Second, the spinel NiFe$_2$O$_4$ MNPs with (2:1) and (4:1) ratio of (NiCl$_2$·6H$_2$O: FeCl$_3$·6H$_2$O) were synthesized following the same above procedure.

2.4. Instrumentation and Characterization

The morphology and elemental composition of the MNPs were studied using FESEM and EDS measurements (JEOL JSM-7600 F Technologies Ltd., Raleigh, NC, USA). A Scanting XDS 2000 diffractometer equipped with a Cu Kα radiation source was used to obtain the XRD patterns of the MNPs. The spectroscopic analysis of the MNPs was performed using a UV/VIS/NIR spectrometer (Lambda 750, Parkin Elmer).

2.5. In Vitro Cytotoxicity Evaluation

In vitro cytotoxicity evaluation of the three different types of MNPs was performed against liver carcinoma cells (Hep G2, ATCC number HB-8065) and non-small lung adenocarcinoma cells (A549, ATCC number CCL-185). All cell products were purchased from the American Type Culture Collection (ATCC). Living cellular models were used between
passages 12–27. Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), streptomycin (100 µg/mL), and penicillin (100 U/mL) were used to maintain the two cell lines.

The cellular viability of Hep G2 and A549 cells following the application of the MNPs was assessed by measuring the cellular metabolic activity using MTS assay kit (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA). Following the cell confluency, cells were detached from the flask using trypsin, then counted with the trypan blue exclusion test, and seeded at a seeding density of $1.5 \times 10^4$ cells/well into 96-well plates. The samples were then incubated overnight in a humidified 5% CO$_2$ cell culture incubator at 37 $^\circ$C. The next day, 100 µL samples of increasing concentrations of the tested MNPs (15.62–1000 µg/mL) were incubated with the two human cancerous cell types for 24 h. The cells were incubated with only DMEM, or with Triton X-100, as the positive and negative controls, respectively. The investigated nanoparticles were aspirated from the wells, 100 µL of DMEM was added, and then 20 µL of the MTS reagent was added to each well. Thereafter, cells were covered with aluminum foil and incubated for 2–3 h at 37 $^\circ$C. A Cytation 3 absorbance microplate reader (BioTek Instruments Inc., Winooski, VT, USA) was used to measure MTS absorbance at 490 nm. The percentage of viable cells was calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{(S - T)}{(H - T)} \times 100$$

where S is the absorbance of the cells treated with the MNPs, H is the absorbance of the cells treated with DMEM (positive control), and T is the absorbance of the cells treated with Triton X-100 (negative control).

3. Results and Discussion
3.1. Morphology

Figure 1a–c, Figures 2a–c and 3a–c display the FESEM images of the MNPs synthesized using the star-anise extract. Figure 1a–c shows that the magnetite Fe$_3$O$_4$ MNPs comprised irregular clusters of overlapping platelets. However, Figures 2a–c and 3a–c clearly depict the distinct pyramidal shapes of both spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs. Therefore, the precursor metallic salts did not have a significant effect on the morphology of the spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs. It is possible that the highly agglomerated shapes might be due to the magnetic properties of the MNPs [29]. The size of the MNPs was determined to be in the range of 0.1–1 µm.

The EDS spectra of the MNPs fabricated using star-anise extract are shown in Figures 1d, 2d and 3d. In the case of magnetite Fe$_3$O$_4$ (Figure 1d), strong signal peaks at 6.2 keV and 0.6 keV for iron and at 0.5 keV for oxygen were observed. The presence of iron and oxygen verified the formation of magnetite Fe$_3$O$_4$ MNPs [30]. Typical peaks of nickel, iron, and oxygen were noticed in the EDS spectra for the (2:1) and (4:1) spinel MNPs (Figures 2d and 3d). However, it is clear that the use of star-anise extract via simple co-precipitation forms highly crystalline Fe$_3$O$_4$, and spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs.
Figure 1. Representative FESEM images (a–c) at different magnifications of the synthesized Fe$_3$O$_4$ NPs and (d) EDS.

Figure 2. Representative (a–c) FESEM images at different magnifications and (d) EDS spectrum of the synthesized spinel (2:1) NiFe$_2$O$_4$ MNPs.
3.2. UV Spectra

The electronic transitions of the MNPs during the chemical reaction between the extract of star anise and precursor metallic salts were studied using UV–vis spectrophotometry in the range of 200–800 nm. The spectrum of the Fe$_3$O$_4$ MNPs has a strong peak at approximately 300–350 nm \cite{31} (Figure 4a), while this peak shifted to ~700 nm for spinel (2:1) and (4:1) NiFe$_2$O$_4$ MNPs (Figure 4b) \cite{32}. The clear absorption peaks indicate that the synthesized nanoparticles were stable and well dispersed in the solution.

3.3. Crystal Structure

Figure 5 displays the XRD spectra of the synthesized (a) magnetite Fe$_3$O$_4$ and (b) spinel (2:1) NiFe$_2$O$_4$ and (c) (4:1) NiFe$_2$O$_4$ MNPs. Intense reflection peaks of the synthesized
magnetite Fe₃O₄ MNPs were observed at 2θ = 30.8, 38.5, 43.7, 53.7, 56.5, and 62.5° of magnetite Fe₃O₄ [33], as shown in Figure 3a. Scherrer’s equation D = 0.9λ/β cosθ was used to calculate the average crystallite size (D), where β is the full width at half maximum (FWHM) line broadening of the most intense peak, K is the Scherrer constant, θ is Bragg’s angle, and λ is the X-ray wavelength. This analysis gave values of 66.8 nm.

![Image](image.png)

**Figure 5.** XRD spectra of the synthesized Fe₃O₄, and spinel (2:1) and (4:1) NiFe₂O₄ MNPs.

The diffraction patterns of spinel (2:1) and (4:1) NiFe₂O₄ MNPs are shown in Figure 5 (line a,b,c), respectively. The peaks are intense and sharp, indicating the excellent crystallinity of the synthesized MNPs. Peaks were observed at 2θ values of 29.7, 31.5, 35.5, 45.3, 56.7, and 63.2° of the spinel NiFe₂O₄ structure [34]. No additional peaks were observed, indicating that the synthesized MNPs were of high purity. In addition, the crystallite size was determined using Scherrer’s formula as 72.5 and 72.9 nm.

### 3.4. In Vitro Cytotoxicity

The in vitro cytotoxicity assessment of the applied MNPs is an essential step toward biomedical application. In this experiment, increased concentrations of the MNPs were tested against Hep G2 and A549 cell lines to define the optimal concentrations that do not cause cytotoxic effects to living tissue and to identify the safety of MNPs for further studies.

Liver carcinoma cells were used because it is known that the liver is the main site for the metabolism for chemicals and food. Whether the medication is taken orally and then crosses the digestive system to the blood circulation system or whether it is taken by intravenous administration, it should pass through the liver. Furthermore, many chemicals should be metabolized through the liver before they are excreted. Either the chemical compound or the metabolite could be toxic to the liver. Therefore, the liver is one of the main organs that needs to be safe during the administration of any chemicals. Cancer in hepatic cells is ranked in fourth place for causing death for cancer-related deaths [35]. Moreover, the liver has a vital role in immunological reaction and inflammation due to its unique structure and function [35].

Figure 6a represents the effect of magnetite Fe₃O₄, spinel (2:1) and (4:1) NiFe₂O₄ NPs with different base concentrations on the cellular metabolic activity of Hep G2 cell line using MTS assay after a 24 h incubation time. The results showed high metabolic activity of all the applied nanoparticles comparable to the positive control with no observable effect of the tested nanoparticles on the cell viability. High viability of the human cancerous cells was achieved at all concentrations applied even at the maximum concentration used (1000 μg/mL). The level of viability of the highest and the lowest concentrations used was almost comparable.
The star anise extract was used as an environmentally friendly reducing agent instead of a highly toxic chemical reagent. According to the findings, the synthesized MNPs were found to be extremely crystalline and pure. FESEM measurements revealed that the particle sizes of the MNPs were in the range of 0.1–1 µm. Furthermore, according to XRD analysis, the sizes of the prepared MNPs were 66.8, 72.5, and 72.9, respectively. The MNPs fabricated with the star-anise extract could be used as cancer treatments due to excellent biological activity against liver cancer and non-small lung adenocarcinoma cells.

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