Preparation of Fe_2O_3 Nanoparticles from Mixing Henna Extract With Ferric Chloride to Cytotoxic Assay on Cancer Cell Line

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Abstract

Green synthesis of cytotoxic assay of iron oxide nanoparticles (Fe_2O_3) NPs was on cancer cell line prepared from mixing (*henna*) extract with iron chloride (III) salts at 200 ^oC for 2 hours by simple chemical method. (Fe_2O_3) NPs were identified using X-ray diffraction, filed Emission-Scanning Electron Microscopy, Ultravolite visble, and Photoluminescence. XRD measurements explained crystalline size (30) nm with (hexagonal) structure for (Fe_2O_3) NPs using henna extract. FE-SEM showed average grain size of (Fe_2O_3) NPs were (18.61) nm. (Fe_2O_3) NPs were tested for their cytotoxic effect against human cancer cells and inhibition rate % results were very high. Results of inhibition rate % for (Fe_2O_3) NPs using henna extract for human cancer cells were (78.9%).

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1. Introduction

Sustainable development in the process of developing the land, cities and societies, as well as businesses, provided that it meets the needs of the present without compromising the ability of future generations to meet their needs. The world faces the danger of environmental degradation, which must be overcome while not giving up the needs of economic development, as well as equality and social justice [1-2]. The green synthesis was developed ten years ago. Green synthesis is characterized as an easy, simple, clean, and eco-friendly method. Subsequently, green synthesis is classified into biological systems, inclusive of plants. Plant extracts (henna) are highly attractive so they are selected from biological systems because they are safe, fast and produce large quantities of (IONPs) [3-5].

Nanoparticles (NPs) could be defined as small particles with a diameter less than 100 nm in dimensions' synthetic of inorganic or organic materials, having new properties as contrasted to the bulk materials. In addition, NPs very important due to their applications in different fields (medicine fields, sustainable development, and environment remediation) [6-7]. Among the NPs, Iron oxide NPs advantages compared to other materials [8] special physicochemical properties, such as low toxicity, and high catalytic activity [9-10], small sizes, and high surface area to volume ratio, physical and magnetic properties [11-14]. Iron oxide NPs can be categorized to main namely [(Fe_2O_3) triple iron oxide, (α- Fe_2O_3) hematite, (Fe_3O_4) magnetite, and (FeO) wustite] [15-17]. Iron oxide NPs provide a large range of applications such as magnetic targeting, hyperthermia, gene therapy, environmental remediation, antimicrobial agent, and anticancer in vivo and vitro [18-21]. Interest in Fe NPs due to their characteristics and novel properties Such as easy surface engineering for targeted therapy, drug delivery and selective treatment making them a better substituent against traditional therapeutic agents [22]. Cancer is uncontrolled growth or division of cells that transform into cancerous cells [23]. Cancer is treated by First, surgery, as the cancerous mass or tissue is removed, Second, radiation therapy, by shedding rays on the cancerous tissue to kill its cells. Third, chemotherapy, by giving the patient chemotherapy drugs to destroy the cancer cells. Other options for treating cancer including: First, Biological therapy, as the patient is given substances that help his immune system, such as interleukin and interferon [24]. In addition, hormonal therapy. Third, stem cell transplantation. So the aim of the study was to prepare the iron oxide prepared from plant extracts (henna, beta vulgarize and Punica granatum) in a sustainable development method as an alternative to the use of toxic chemical drugs that reduce human life

instead of life. Friendly prepared FeNPs proved their ability to treat cancer with less cost and less side effects [25-26]. In 2020, R. C. Popescu et al., the prepared of iron oxide NPs and application in cancer treatments [27]. In 2019, Jayakumar Sandhya et al., created iron oxide NPs using borassus flabellifer seed coat and application in cancer treatments [28]. In 2018, Helale Kaboli Farshchi, et al., Synthesized iron oxide NPs using Rosemary extract and application in cancer treatments [29]. In 2018, Zahra Izadiyan, et al., prepared iron oxide NPs from using juglans regia green husk extract and application in anticancer [30].

In this work, sustainability development for IONPs [Fe_2O_3 (triple iron oxide)] was prepared using (*henna* leaf) at 200 ^oC for 2 hours. After that, IONPs [Fe_2O_3 (triple iron oxide)] were diagnostic via X-ray diffraction", Filed Emission Scanning Electron Microscopy, and Photoluminescence spectroscopy. A clinical trial of IONPs has been conducted to treat cancer in harmful human cells due to of the effectiveness, safety and ease of IONPs because they are prepared from plant extracts, they are considered a strong antibiotic in the body of the organism against harmful cells. The aim of the current study is to determine the cytotoxic assay of these synthesized IONPs on breast cancer cell line (MCF-7).

2. The Experimental Work

2.1 The Sample Collection

Five of the different plants listed below were represented using leaf (henna). These lants are rich sources of (flavonoids, phenols, alkaloids, vitamins, amino acids, quinones, minerals, sulfur, proteins, compounds (allicin)). There are very rich in flavonoids, fructose that can reduce ions to be NPs because of the presence of (vitamin C) in these plant extracts. The henna extract has been collected from the local market in (Baghdad & Basrah/Iraq), as preliminary work. Table 1 shows the bionomical, family, and plants type which used in the bio-synthesis of NPs.

Table 1: A List of plant Bionomical name that used for the sustainability development of IONPs:

Plant Bionomical name	Common Name	Family	Type
Henna	*Lawsonia inermis*	*Lythraceae*	Leaf

2.2 Preparation of plants extract

A mixture of 5 gm of henna extract and 200 ml of distilled deionized water and using the magnetic stirrer at 80 ° C for two hours. The final solution is left at room temperature to cool. The solution is then filtered with Whatman filter paper of pore size(1μm). Finally, the henna extract was placed in sealed glass tubes for a period of 1 to 3 days for future preparations. The same process steps are repeated to prepare the other henna extract [31-33]. Figure (1) shows the steps of transferring henna parts to henna extract.

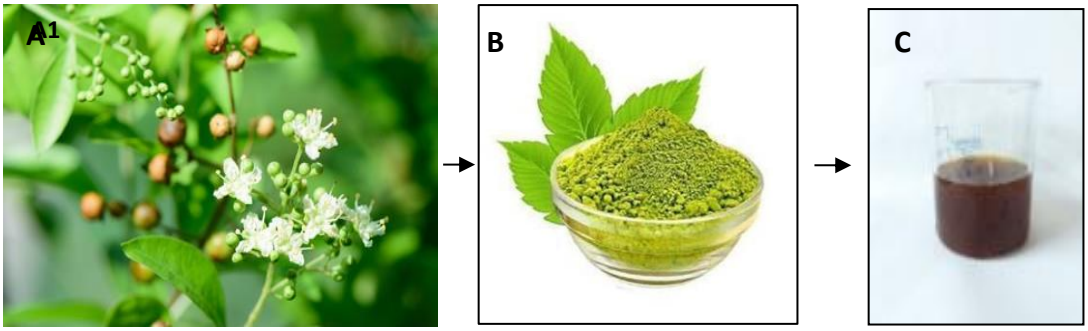

Figure 1: the steps transferring to plant extract, A) *henna* , B) *henna* powder, and C) *henna* extract.

2.3 Synthesis of IONPs using henna extract

IONPs were prepared from mixing (100 ml, 1M) of $FeCl_3$ salts with 100 ml of (*henna*) extract and using the magnetic stirrer at 70 ° C for 30 minutes. The reaction was stopped once a change in colour happened, and this is an indication of the formation of nanomaterials. The solution was then cooled to room temperature and placed in an ice bath to equilibrate the nanoparticles. A nanopowder of IONPs (Fe_2O_3) was prepare by placing 25 ml of the solution in a ceramic tray inside the oven at 200 ° C for 2 hours the powder was kept in sealed glass tubes for future assignments. The same protocol was applied to prepare the remaining IONPs. Figure (2) shows the steps of transferring plant extracts to IONPs.

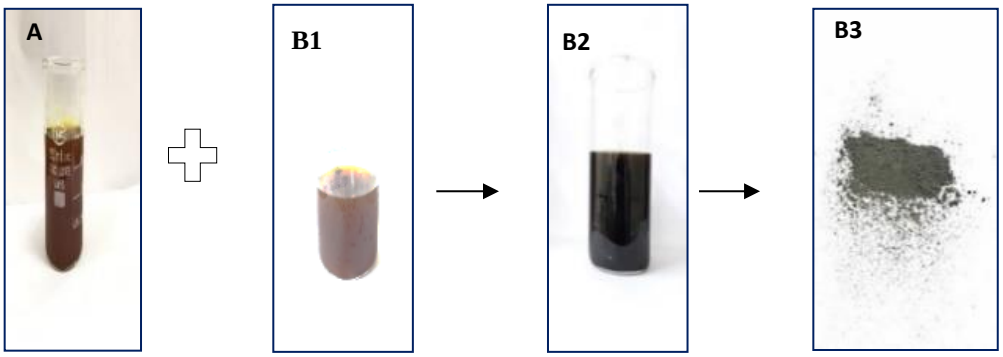

Figure 2: Steps transferring plant extracts to IONPs, (A) $FeCl_3$ salts, (B1) *henna* extract, (B2) Fe_2O_3 NPs, (B3) Fe_2O_3 NPs powder.

2.4 Cytotoxic assay

2.4.1 Cell culture

Human breast adenocarcinoma (mcf-7) is the acronym of Michigan Cancer Foundation-7 cell lines was used in the current study. The cells were maintained in DMEM supplemented with 10 % fetal bovine serum 0.5 % antibiotic solution (penicillin and streptomycin stabilized with glutamine), 0.5 % antimycotic solution (amphotericin "B") at 37 °C supplemented with 5 % CO_2.

2.4.2 Preparation of (Fe_2O_3) NPs to Cytotoxic assay

Stock solution of nanoparticles Fe_2O_3 NPs using (*henna*) extract were Filtered through 0.2µm. The NPs were diluted using serum free media: 1:1. 1:2, 1:3 and 1:4 for each nanoparticles. Cultured cells were seeded in 96 wells plate at density of 80,000 cells per well and incubated. After 24 h, cells were treated with NPs for another 24 h. Then stained with crystal violate for 30 min and read out at absorbance 492 nm using ELISA reader. In the figure (3) blow show of the breast cancer image which it took by mammogram (Digital MicroDose Mammography) from infected cells to make our experimental test on it.

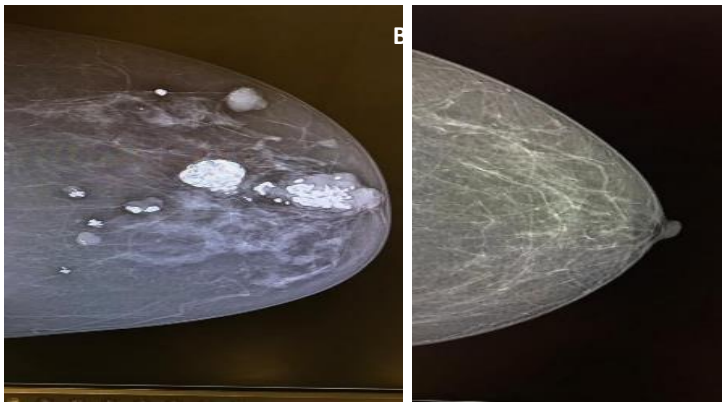

Figure 3: Images of the mammogram for cancer cells (MCF-7), (A) the normal breast tissue and (B) the abnormal breast tissue, and

3. Results and discussions

3.1 XRD patterns of Fe_2O_3 NPs using plant extracts

The results showed that Fe ions have reduced to Fe_2O_3 NPs by (*henna*) extract at 200 ^oC for 2 hours. All diffraction peaks correspond to the distinguishing (hexagonal) according to (JCPDS card no. 00-040-1139).

The peaks intensities of (Fe_2O_3) NPs are increasing when using the henna extract as shown in Figure 4. The position, height, and width of the diffraction peaks depend on the nanocrystalline nature of the (Fe_2O_3) NPs. The peaks (*104*), and (*017*) are the preferred orientation of (Fe_2O_3) NPs using (*henna*) extract, followed by the (104), (*102*), (017), (110), and (112) [34-36]. Table 2 explains XRD results of (Fe_2O_3). More parameters determined the structural properties of the materials such as crystallite size.

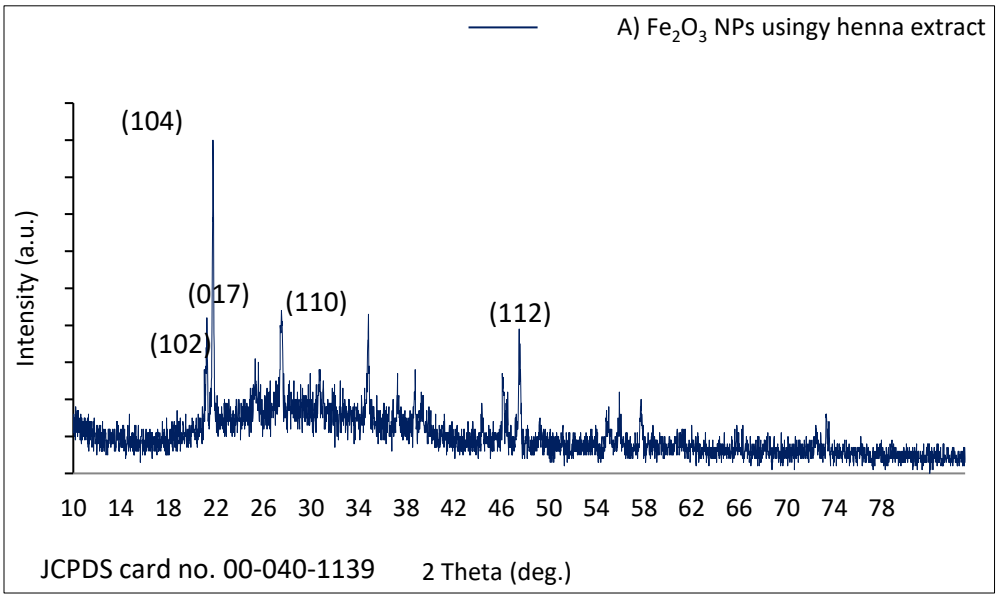

Figure 4: XRD pattern of IONPs using plant extracts at 200 ^{o}C for 2 hours, Fe_2O_3 NPs using *henna* extract.

Table 2: XRD explains results of (Fe_2O_3), (α-Fe_2O_3) and (Fe_3O_4) NPs using plant extracts.

Plant Extract	Material	(hkl)	Crystallite size D (nm)
Henna	Fe_2O_3	(104)	30
		(017)	35

3.2 FE-SEM analysis of IONPs using plant extracts

FE-SEM analysis measurements have been performed to determine the surface morphology and average grain size of Fe_2O_3 NPs using (*henna*) extract by a simple chemical method at 200 ^{o}C for 2 hours. The morphology and average grain size of Fe_2O_3 NPs were analysed using FE-SEM images of the synthesized Fe_2O_3 NPs using a (*henna*) extract at 200 ^{o}C for 2 hours, which was deposited on a glass substrate. Figure 5 (A-B) shows a micrograph of the notable nanoparticle structures observed with average grain sizes of 18.61 to 27.91nm [37].

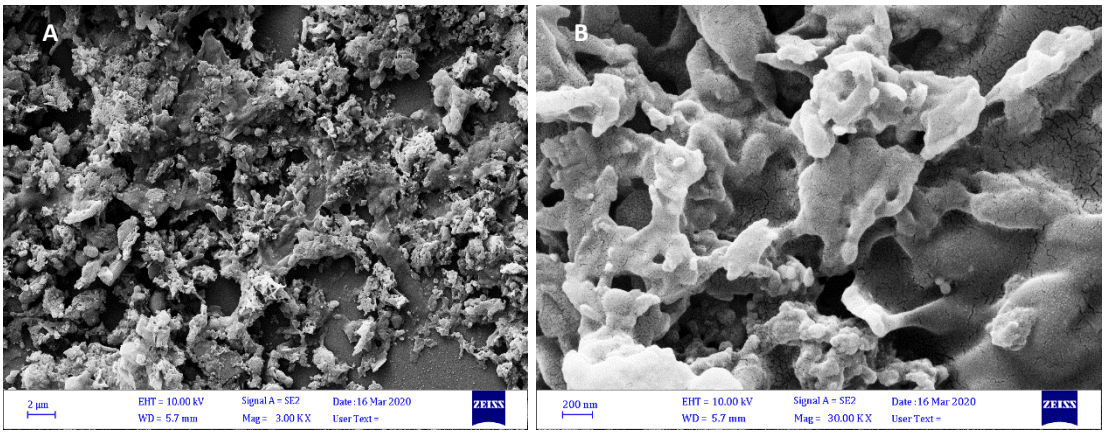

Figure 5: FE-SEM analysis of Fe_2O_3 NPs using (*henna)* at 200 ^oC for 2 hours A-B) using (*henna)*.

3.3 Cytotoxicity of Fe_2O_3 NPs using henna extract

The result of crystal violate assay was used to determine of percentage cell death with respect to control (untreated cells), inhibition rate of cancer cell line was determined in figure 6 [39-40]. Figure (8) reveals breast cancer cell images for infected cell before and after treatment by IONPs within 24 hours [41-44]. The results of inhibition rate % of cancer cell line using three different types and dilutions of IONPs as shown in table (3).

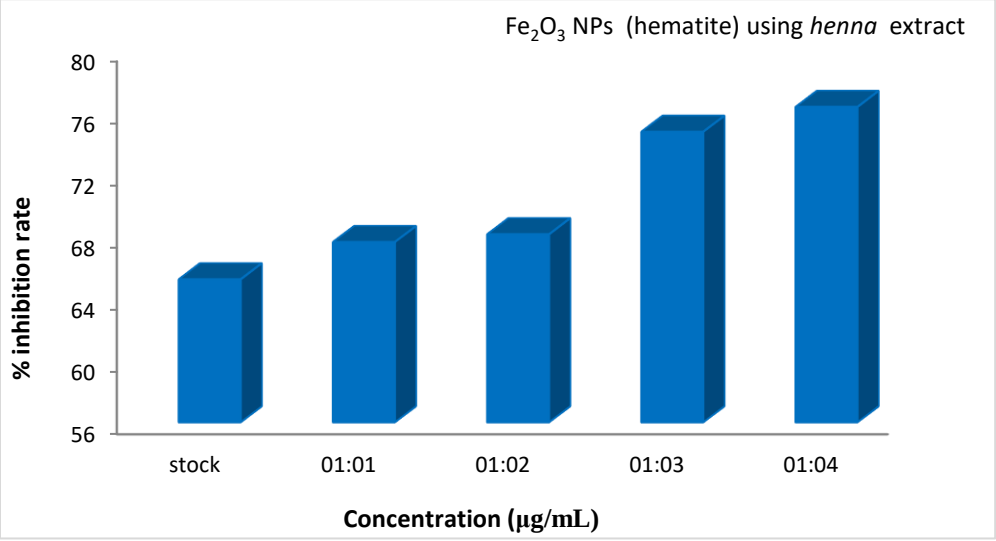

Figure 6: The inhibition rate of cancer cell line dilutions of Fe_2O_3 NPs (hematite) using *henna extract*.

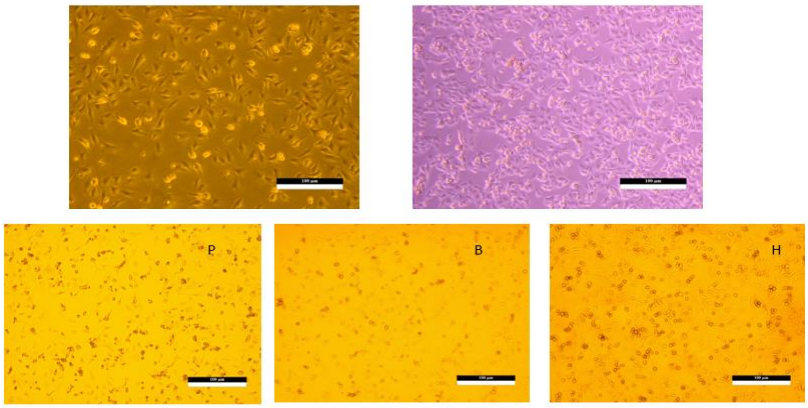

Figure 7: breast cell line before treatment with IONPs and after treatments for 24 hr.

Table 3: The inhibition rate % of cancer cell lines using three different types and dilutions of IONPs.

Inhibition rate %	Dilutions	Fe_2O_3 NPs using *henna*
	stock	68.9
	01:01	75.5
	01:02	76.8
	01:03	78.1
	01:04	78.9

4. Conclusions

Use of henna extract was effective in preparing iron oxide nanoparticles (IONPs) via mixing iron triple chloride ($FeCl_3$) salts with plant extracts (*henna*) and the use of the product in killing cancer cells. The same plant mentioned above contain a wide range of biomolecules that act as a powerful nanoparticles against cancer cells. It also acts as a reducing, stabilizing and anti-caking agent. XRD measurements explained the crystalline size (30) with (hexagonal) structure (hematite) for (Fe_2O_3) NPs using (*henna*) extract. FE-SEM showed the average grain size of IONPs were (18.61) nm, respectively. Fe_2O_3 NPs were applied to human cancer cells and the inhibition rate %

results were very high. The results of inhibition rate % for IONPs using (henna) extract for human cancer cells were (78.9%).

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تحضير جزيئات Fe_2O_3 النانوية من خلط مستخلص الحناء مع كلوريد الحديد الثلاثي للمقايسة السامة للخلايا على خط الخلايا السرطانية

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6. قسم تمريض الصحة النفسية والطب النفسي - كلية التمريض - جامعة بغداد - بغداد – العراق

المستخلص

تم التوليف الأخضر للمقايسة السامة للخلايا لجسيمات أكسيد الحديد النانوية (Fe2O3) NPs على خط الخلايا السرطانية المحضر من خلط مستخلص (الحناء) مع أملاح كلوريد الحديد (III) عند 200 درجة مئوية لمدة ساعتين بطريقة كيميائية بسيطة. (3Fe2O) تم تحديد NPs باستخدام حيود الأشعة السينية ، الفحص المجهري الإلكتروني الماسح للانبعاثات ، ومطياف الاشعة فوق البنفسجية Ultravolite ، والتألق الضوئي. أوضحت قياسات XRD الحجم البلوري (30) نانومتر مع بنية (سداسية) لـ (Fe2O3) NPs باستخدام مستخلص الحناء. أظهر FE-SEM متوسط حجم حبيبي (Fe2O3) NPs (18.61) نانومتر. (3Fe2O) تم اختبار NPs لتأثيرها السام للخلايا ضد الخلايا السرطانية البشرية وكانت النتائج٪ معدل تثبيط عالية جدا. كانت نتائج معدل التثبيط٪ لـ (Fe2O3) NPs باستخدام مستخلص الحناء للخلايا السرطانية البشرية (78.9) ٪.