

Balanites aegyptiaca Oil Synthesized Iron Oxide Nanoparticles: Characterization and Antibacterial Activity

Hind Baballa Gasmalla^{1,2}, Ahmed Mahmoud Idris¹, Mahgoub Ibrahim Shinger^{1,3}, Dongdong Qin¹, Duoliang Shan¹, Xiaoquan Lu^{1*}

¹Key Laboratory of Bioelectrochemistry & Environmental Analysis of Gansu Province, College of Chemistry & Chemical Engineering, Northwest Normal University, Lanzhou, China

²Forest Products and Industries Department, Faculty of Forestry, University of Khartoum, Khartoum, Sudan

³Chemistry Department, Faculty of Science, International University of Africa, Khartoum, Sudan

Email: hindfnc@hotmail.com, luxq@nwnu.edu.cn

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Abstract

Antibacterial activity of iron oxide nanoparticles, an employing *B. aegyptiaca* oil (L.) Del., was used as natural stabilizer by modifying a co-precipitation method. In this work, we chose *B. aegyptiaca* oil as the new surfactant coating agent, and synthesized *B. aegyptiaca* oil coating with iron oxide nanoparticles which were characterized with a variety of methods, including Gas Chromatography (GC) to determine the fatty acids composition of the seeds oil, Fourier Transform-Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM) equipped with Energy Dispersive Spectroscopy (EDS), X-ray Powder Diffractometer (XRD) and Vibrating Sample Magnetometer (VSM). In antibacterial studies, disk diffusion susceptibility test was used to measure efficacy of iron oxide nanoparticles against Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and Gram-negative bacteria *Escherichia coli* (*E. coli*) in terms of zone inhibition. The *B. aegyptiaca* coated on the surface of iron oxide nanoparticles; its particle size was found to be nanoscale below 50 nm, and the magnetization (δ_s) was 16.975 emu g⁻¹. Antibacterial activity was measured. Efficacy of iron oxide nanoparticles against bacterial strains was found in *Escherichia coli* (*E. coli*). All these findings suggest that the nanoparticles synthesized from *B. aegyptiaca* oil may be a promising reagent for a wide variety of applications in biological fields as well as in nanomedicine.

Keywords

Iron Oxide Nanoparticles, *Balanites aegyptiaca* Oil, Antibacterial Activity

*Corresponding author.

1. Introduction

Nanotechnology had an enormous impact on medical technology, significantly improving the activity, specificity, bioavailability and therapeutic index of various natural products [1]. Therefore, by using nanoscale carriers, the therapeutic value of natural products can be drastically improved [2]. Lately, plant oil extracts are being used as a process for the synthesis of iron oxide nanoparticles that may find very important place in antibacterial activity. With the prevalence and increase of microorganisms resistant to multiple antibiotics and the continuing emphasis on healthcare costs, many researchers have tried to develop new, effective antimicrobial reagents. In the past two decades, some studies have shown that antimicrobial formulations in the form of nanoparticles could be used as effective bactericidal materials because of their high surface-to-volume ratio and novel physical and chemical properties on the nanoscale level [3]. Over the past decade, iron oxide nanoparticles have been extensively studied in biomedical applications because of their unique properties, such as easy handling, low cytotoxicity, good biocompatibility, relatively low cost and eco-friendly performance [4].

Recently, magnetic antimicrobial nanocomposites have received special attention due to the recyclable and localizing properties of magnetic nanoparticles. Magnetic silver nanocomposites are the most studied matrix as antimicrobial agents. Bifunctional $\text{Fe}_3\text{O}_4@Ag$ nanoparticles with both superparamagnetic and antibacterial properties were prepared, and the nanocomposites showed antibacterial performance against *E. coli*, *Staphylococcus epidermidis* (*S. epidermidis*) and *B. subtilis* [5]. A stable aqueous suspension of $\text{Fe}_3\text{O}_4@Ag$ was developed by Chudasama and co-workers, and the nanocomposites displayed antibacterial activity against both Gram-negative and Gram-positive organisms [6]. Also tested human fibroblasts incubated with magnetic nanoparticles showed that lactoferrin and ceruloplasmin coated nanoparticles adhered to the cell surface whereas plain uncoated particles were found to be phagocytosed by the cells [7].

Balanites aegyptiaca (L.) Del., (Zygophyllaceae), is locally known Hegleig tree and its fruits are called lal'loub; it is also known as Desert date in English. The tree is found in stretching from arid and semi-arid regions to sub-humid of tropical savannas of African countries, all over the Sahel and on many sites of the Sudan savanna, extending from the Atlantic coastline of Senegal to the Red Sea and Indian Ocean and the Arabian Peninsula [8]. It is widely used, in traditional medicine, to treat infectious diseases, psychoses, epilepsy, jaundice and rheumatism [9]. In addition, it is an important component of many popular preparations due to its antidiabetic, antiseptic, antimalarial, antisiphilitic and antiviral (Herpes zoster) activity [10] [11]. The fixed oil from the fruits had shown anticancer activity against lung, liver and brain human carcinoma cell lines [12]. The same study reported remarkable antimicrobial activity for the fixed oil against selected strains of Gram-positive and Gram-negative bacteria as well as antiviral activity against Herpes simplex virus [12]. *Balanitis aegyptiaca* oil is a fatty acid which is used for controlling the tendency of precipitation and agglomeration of the hydroxide precursors on the morphology of the iron oxide nanoparticles; also the purpose of natural *B. aegyptiaca* oil is to enhance the biocompatibility. The stabilization of magnetic nanoparticle dispersions can be obtained by adsorbing the surfactants on the surface of the particles [13]. Stable aqueous magnetic nanoparticles' dispersions are produced with various saturated and unsaturated fatty acids as primary and secondary surfactants [14] [15].

This study aims to explore the efficacy of magnetic iron oxide nanoparticles (Fe_3O_4) synthesized with *B. aegyptiaca* oil (L.) Del., as novel reagent to detect the bacteria strains. Three representative bacteria typically recommended for use in antimicrobial assays, *i.e.*, *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and *Escherichia coli* (*E. coli*) were used. The antibacterial effect was quantized based on the inhibition zone measured in the disk diffusion tests conducted in plates.

2. Experimental

2.1. Plant Material Preparation and Characterization

The fruits of *B. aegyptiaca* were harvested from Central of Sudan-Eldamazen. The kernels of the fruits were separated and powdered using a mortar and pestle. 200 gm of *B. aegyptiaca* seeds weighed and packed in Soxhlet apparatus. The powdered seeds were extracted with petroleum ether 40°C - 60°C. The solvent was then collected and evaporated under reduced pressure using rotary evaporator apparatus. The petroleum ether extract yielded pale yellow colour oil which was then stored in dark bottles at room temperature till use [16].

2.2. Fatty Acid Composition of Seeds Oil of *B. aegyptiaca*

The triglycerides of the *B. aegyptiaca* oil trans-esterified using the following procedures [17] [18]. Typically, the

oil (1 ml) was diluted by 1 ml of hexane. Then 6 ml of NaOH (0.5 M) in methanol were added. The mixture was refluxed for 2 - 3 min. Next, 6 ml of H₂SO₄ 1% were added and shook well-over night, followed by the addition of 2 ml of hexane and shook after that saturated NaCl was added. Then to the upper layer (hexane) a few amount of Na₂SO₄ was added. After decantation the fatty acids composition was determined using gas chromatography (GC-2010, SHIMADZU-Japan).

2.3. Co-Precipitation Synthesis of *B. aegyptiaca* Oil/Fe₃O₄ Nanoparticles

Iron oxide nanoparticles were prepared by a co-precipitation method with the addition of *B. aegyptiaca* oil as a surfactant. Typically, 50 ml of 1 M solution FeCl₃·6H₂O and 50 ml of 2 M FeSO₄·7H₂O were mixed and dissolved in deionized water. Then 3 M of Sodium hydroxide were added into the above solution and the pH value was maintained between 10 - 11 with continuous stirring for 30 minutes and a dark precipitate was formed. 2 ml of *B. aegyptiaca* oil were heated to 70°C under nitrogen atmosphere and added in the above solution with continuous stirring for 3 h at 80°C. After that, the magnetic precipitate was isolated from the solvent by a permanent magnet, which then washed several times with distilled water and ethanol in sequence. Finally it was dried at 50°C for 24 h and grinded to fine powder.

2.4. Physicochemical Characterization

2.4.1. Gas Chromatography (GC) Conditions

The fatty acids in the oil of *B. aegyptiaca* were analyzed on a Shimadzu gas chromatography 2010 equipped with flame ionization detector (FID). Fatty acids were separated using an INNOWax capillary column (0.25 mm i.d. 30 m in length, 0.25 µm film thicknesses). The carrier gas was hydrogen at flow rate of 40 ml min⁻¹, with air 400 ml min⁻¹ and makeup gas of Helium at 30 ml min⁻¹. The column temperature was programmed at 3°C min⁻¹ to 250°C with initial temperature of 100°C. The injector was set at 230°C with split ratio of 50:1 and the detector was set at 250°C (Table 1).

2.4.2. Transmission Electron Microscopy (TEM)

Transmission electron microscopy was used to determine the morphology, size and structure of the magnetite particles (FEI TECNAI G2 operating at 300 kV). The sample dispersions were drop-cast onto the copper grids separately. X-ray energy-dispersive spectroscopy (EDS) was used to determine the elemental composition and purity of the sample by atom percentage of metal, elemental analysis on single particles was carried out using EDS attachment equipped with TEM.

2.4.3. Vibrating Sample Magnetometer

Magnetization curves for the coated cells were obtained with a vibrating sample magnetometer (VSM, Lanzhou University, Lakeshore 730, America).

2.4.4. Fourier Transform Infra Red Spectroscopy (FTIR)

FTIR spectra of the dried powder iron oxide nanoparticles were obtained using a Bruker Vertex 70 FT-IR spectrophotometer and KBr method.

2.4.5. X-Ray Diffraction (XRD)

X-ray diffraction (XRD) measurements were carried out at room temperature using a BRUKER D8 ADVANCE

Table 1. Gas chromatograph of *B. aegyptiaca* oil. The table shows the quantitative composition of the oil.

Peak	CMPD Name	Height %
1	Palmatic acid M.E	2.1756
2	Cis-10-Heptadecenoic acid M.E	22.5294
3	Oleic acid M.E	8.3397
4	Stearic acid M.E	66.9553
Total		100.0000

X-ray powder diffractometer with Cu-K radiation ($\lambda = 1.5406 \text{ \AA}$) in the 2θ range of 10°C to 80°C .

2.5. Antibacterial Activity

In antibacterial studies, agar well disk diffusion susceptibility test was used to measure efficacy of iron oxide nanoparticles against Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and Gram-negative bacteria *Escherichia coli* (*E. coli*) in bacterial sensitivity to antibiotics is commonly tested using a disk diffusion test, employing antibiotic impregnated disks [19]. A similar test with nanoparticles laden disks was used in this study. Briefly, A 5 ml suspension of nanoparticles (5 mg ml^{-1}) was sonicated and subsequently filtered through a membrane filter ($0.2 \mu\text{m}$, 47 mm diameter). The nanoparticles laden filter paper was dried in an oven for 1 h and small disks of uniform size (6 mm diameter) containing $100 \pm 15 \mu\text{g}$ nanoparticles were punched out and stored in a desiccators at room temperature. The bacterial suspension ($100 \mu\text{l}$ of $10^4 - 10^5 \text{ CFU ml}^{-1}$) was applied uniformly on the surface of a beef agar plate before placing the disks on the plate (3 per plate). The plates were incubated at 35°C for 24 h, after which the average diameter of the inhibition zone surrounding the disk was measured with a ruler with up to 1 mm resolution terms of zone inhibition [20].

3. Result and Discussion

Synthesis and characterization of magnetite nanoparticles (MNP) of *B. aegyptiaca* oil.

3.1. Chemical Composition of *B. aegyptiaca* Oil

In order to determine the fatty acids composition of *B. aegyptiaca* seed oil gas chromatography analysis of the petroleum ether extract of the seed oil revealed the presence of stearic acid M. (66.9%), Cis-10-Heptadecenoic acid (22.5%), oleic acid (8.3%) and palmitic acid (2.1%). The fatty acids composition of *B. aegyptiaca* seed oil is presented in Figure 1. The chromatograms of *B. aegyptiaca* oil are composed of essentially four carboxylates, corresponding to four fatty acid. Cis-10-Heptadecenoic acid (17:1) has long been recognized as minor constituent of ruminant fats and its isomeric definition remains undefined in most reports on ruminant milk and intramuscular fat [21] [22]. Recently it was proposed, along with other odd-chain fatty acids, as a potential marker of microbial biomass [23].

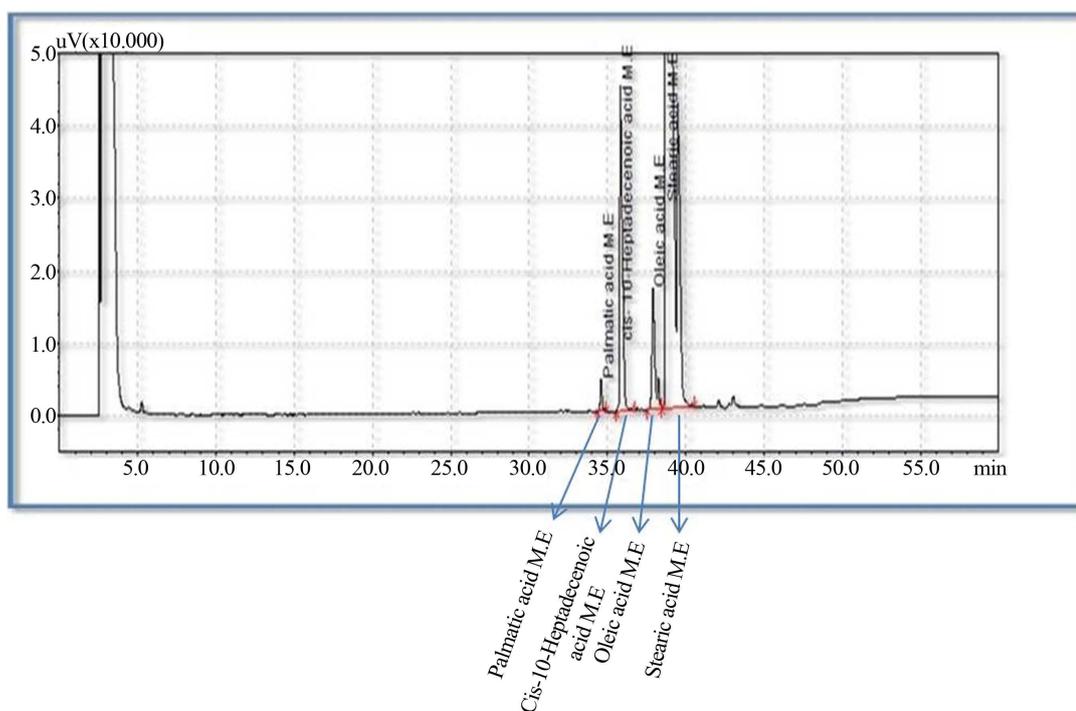


Figure 1. Chromatogram of *B. aegyptiaca* oil analyzed by gas chromatography.

3.2. Transmission Electron Microscopy (TEM)

Transmission electron microscopy allows obtaining information about the shapes and sizes of the particles. **Figure 2** shows the TEM images and histogram of the size distribution of the magnetite particles with and without *B. aegyptiaca* oil coating. **Figure 2(a)** denotes presence of agglomeration of the particles in pure Fe_3O_4 , while **Figure 2(b)** shows each particle separated from the neighbors because of the organic chain absorbed on its surface of iron oxide nanoparticles. Surfactant molecules (also called stabilizing agents) are attached to the nanoparticle surface, preventing clotting and providing the desired dispersion and surface properties. It is well known that magnetic Fe_3O_4 prepared by the co-precipitation method has a large number of hydroxyl groups on its surface in contact with the aqueous phase. The OH groups on the surface of Fe_3O_4 particles react readily with carboxylic acid head groups of oil fatty acids molecules. Excess fatty acids on oil of *B. aegyptiaca* and then adsorbed the fatty acids to form as hydrophobic shell [24]. The size and shape of magnetite nanoparticles may vary depending on the nature and concentration of the stabilizing agent [25]. The size of bare Fe_3O_4 nanoparticles is 29 nm with a dominant population at 5 - 10 nm and the size of nanoparticles coated *B. aegyptiaca* oil is 35 nm with a dominant population at 11 - 12 nm. The nanoparticles coated *B. aegyptiaca* oil has a size slightly higher than the nanoparticles without *B. aegyptiaca*. A mean size of samples was calculated by programme nano measurer 1.2.

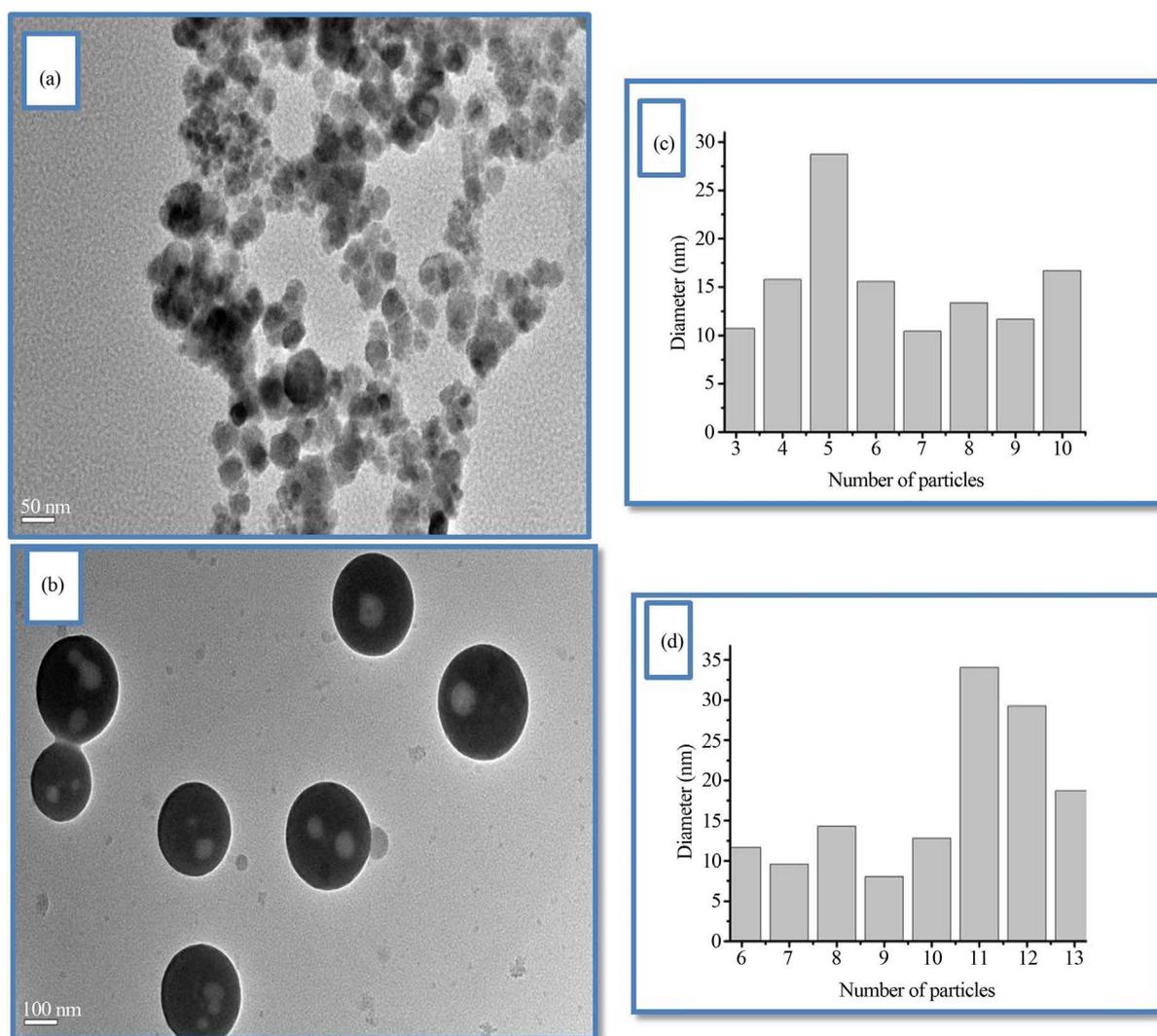


Figure 2. TEM micrographs of (a) the magnetite Fe_3O_4 and (b) *B. aegyptiaca* oil coated magnetite and the corresponding histograms (c) and (d).

3.3. X-Ray Energy-Dispersive Spectroscopy Analysis (EDS)

Figure 3 shows the composition of the Fe_3O_4 with *B. aegyptiaca* was confirmed by X-ray EDS microanalysis of the analyzed point. The presence of Fe and O can be observed, with iron abundance higher than oxygen signals that indicate oil coating and surrounding iron oxide nanoparticles. Moreover, together with Fe and O ones, the copper signal is present because of the copper grids used for sample preparation, in which traces of Cu are present, as reported by the manufacturer. The C signals are attributed mainly to organic molecules (fatty acid) in *B. aegyptiaca* oil.

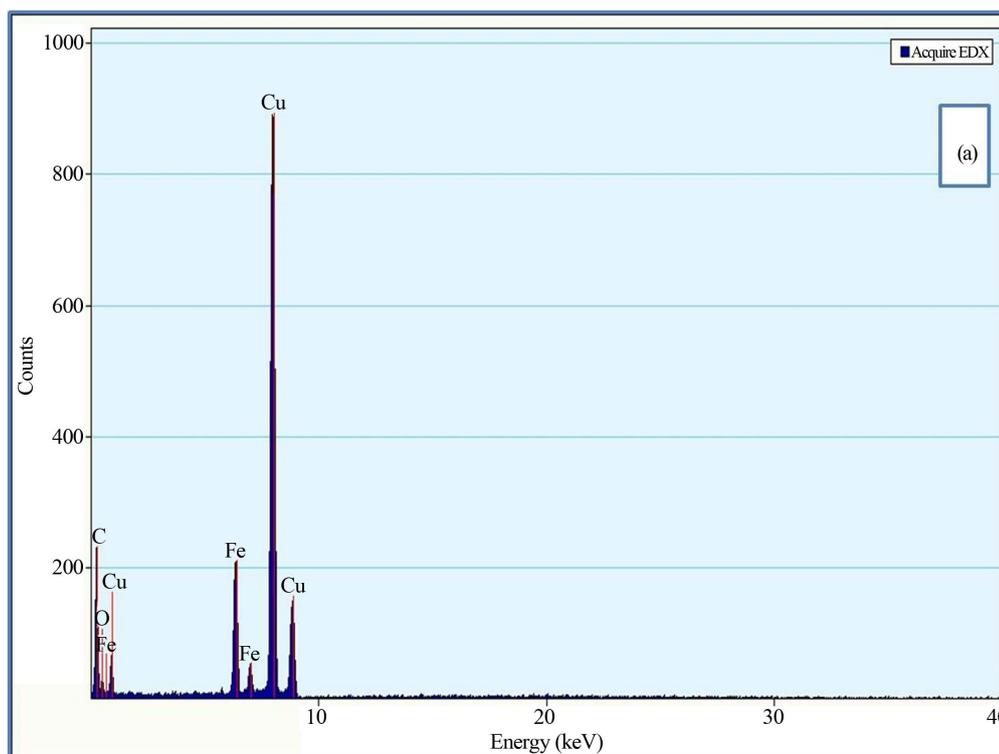
3.4. Vibrating Sample Magnetometer

The magnetic properties of the *B. aegyptiaca* coated Fe_3O_4 nanoparticles were investigated with a vibrating sample magnetometer at 300 k. **Figure 4** is shown the magnetic hysteresis loop at 300 k. The saturation magnetization (M_s), remanence (M_r), coercivity (H_c) and loop squareness ratio (M_r/M_s) of *B. aegyptiaca* coated iron oxide nanoparticles is shown in **Table 2**. The coated sample had a higher M_s but a lower M_r , H_c and M_r/M_s . The increase in magnetization of the coated sample is probably due to the nanoparticles, spinal structure, confirmed that the good paramagnetic property of the resulting sample at room temperature [26].

3.5. Infrared Spectrum of *B. aegyptiaca* Oil

To understand the adsorption spectrum of the *B. aegyptiaca* oil on the surface of Fe_3O_4 nanoparticles, infrared measurements were carried out on the *B. aegyptiaca* oil, pure Fe_3O_4 and the composite Fe_3O_4 nanoparticles coated with *B. aegyptiaca* oil. **Figure 5** shows the typical IR spectrum of the *B. aegyptiaca* oil. The functional groups present in the ester *B. aegyptiaca* oil are explained as follows: The vibration caused by C-H stretching could be found at wavelength around 3000 cm^{-1} (**Figure 5(c)**). This indicates the presence of aliphatic hydrocarbons. Between 1600 and 1800 cm^{-1} another distinctive peak could be noticed. This is C=O, *i.e.* carbonyl group, which could account for the carboxylic acid of *B. aegyptiaca* oil. Lastly, C-O ether groups can be seen between 1000 and 1100 cm^{-1} .

In **Figure 5(c)** two sharp bands at 2955 and 2852 cm^{-1} were attributed to the asymmetric CH_2 stretch and the symmetric CH_2 stretch, respectively which were not found in the pure Fe_3O_4 . The intense peak at 1745 cm^{-1} was



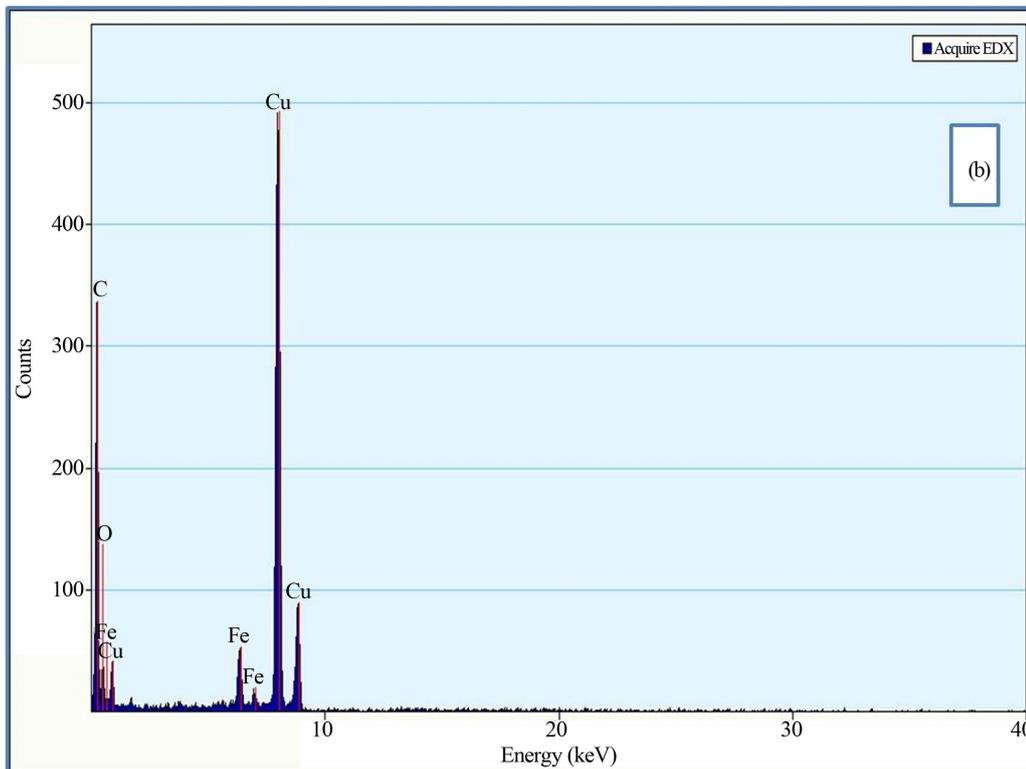


Figure 3. X-ray energy-dispersive spectroscopy analysis (EDS) (a) pure Fe₃O₄ (b) Fe₃O₄ *B. aegyptiaca* oil. EDS spectra showing elemental composition analysis of Fe₃O₄ nanoparticles.

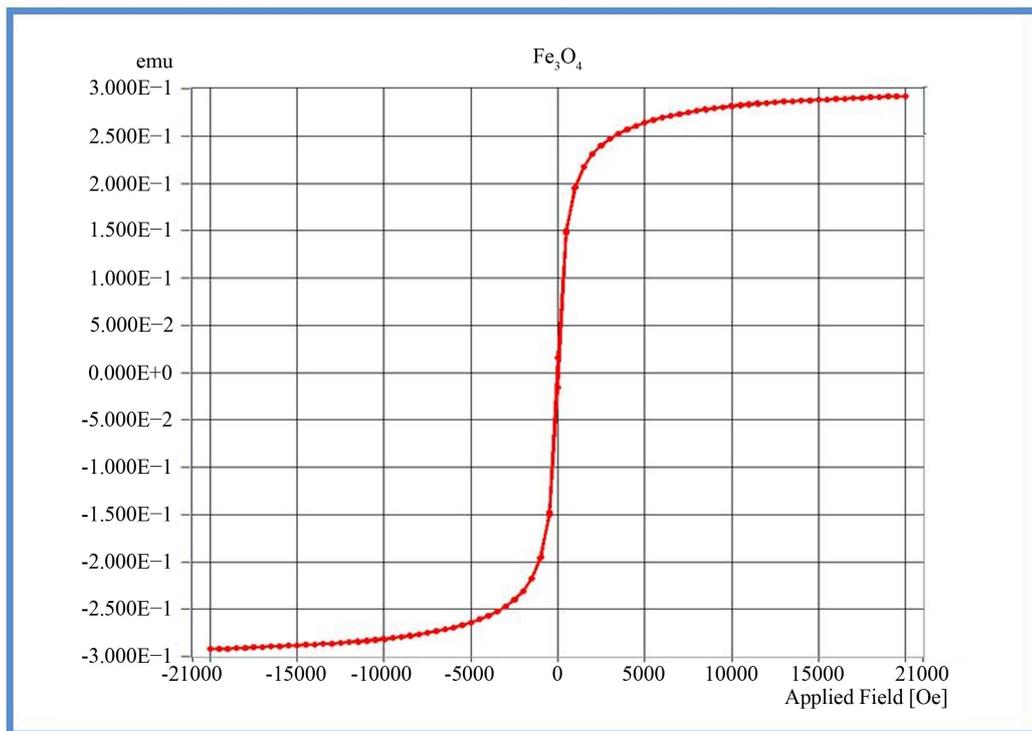


Figure 4. Magnetization curves for the *B. aegyptiaca* oil coated of Fe₃O₄ determined with a vibrating sample magnetometer. σ_s, saturation magnetization; emu, electromagnetic unit; Oe, Oersted.

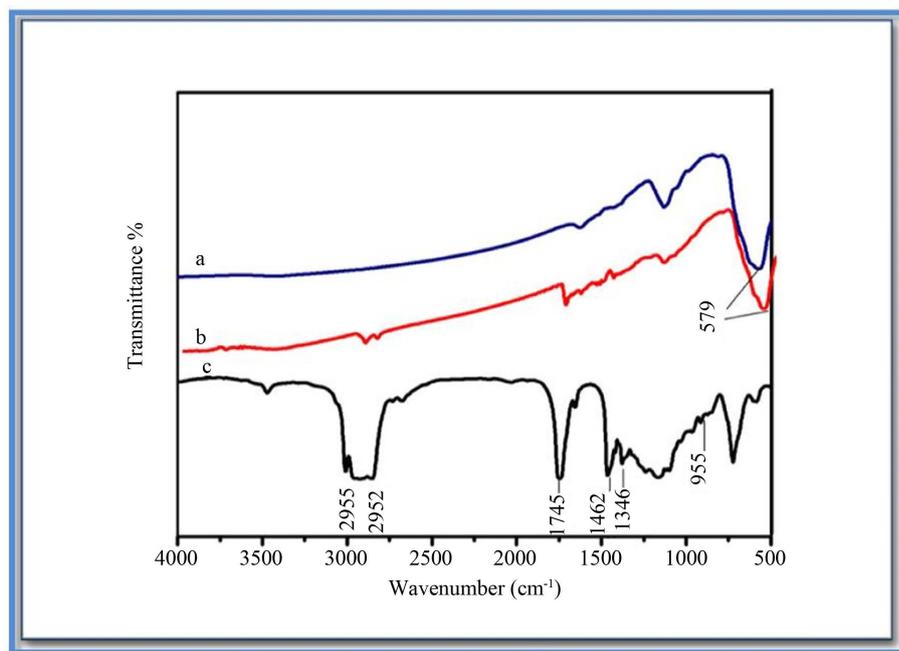


Figure 5. FTIR spectra of (a) pure Fe_3O_4 ; (b) Fe_3O_4 coated with *B. aegyptiaca* oil and (c) *B. aegyptiaca* oil.

Table 2. Magnetic properties of the *B. aegyptiaca* oil coated of Fe_3O_4 .

Parameter	Upward part	Downward part	Average	Hysteresis parameter
Mr (emu/g)	-18.006E^{-3}	15.944E^{-3}	16.975E^{-3}	Remanent magnetization: $M_H = 0$
S	0.063	0.056	0.059	Squareness: Mr/Ms
S*	0.070	0.064	0.067	$1-(M_r/H_c)$ (1/slop at H_c)
Ms (emu/g)	286.832E^{-3}	-286.651E^{-3}	286.741E^{-3}	Saturation magnetization: maximum M measure
Hc Oe	40.26	-39.65	39.96	Coercive field: field at which M/H changes sign

derived from the existence of the C=O stretch and the band at 1346 cm^{-1} exhibited the presence of the C-O stretch. The O-H in-plane and out-of-plane bands appeared at 1462 and 955 cm^{-1} , respectively. In the **Figure 5(b)**, the asymmetric CH_2 stretch and the symmetric CH_2 shifted to 2916 and 2850 cm^{-1} , respectively. The surfactant molecules in the adsorbed state were subjected to the field of the solid surface. As a result, the characteristic bands shifted to a lower frequency region which indicated that the hydrocarbon chains in the monolayer surrounding the nanoparticles were in a closed-packed, crystalline state [27]. Which were characteristic of the asymmetric in as (COO^-) and the symmetric in s (COO^-) stretch, instead. This result can be explained that the bonding pattern of the carboxylic acids on the surface of the nanoparticles was a combination of molecules bonded symmetrically and molecules bonded at an angle to the surface [28]. The characteristic band for pure Fe_3O_4 usually appears at 570 cm^{-1} , whereas the present sample was shifted to 579 cm^{-1} . This can be explained as the carboxyl groups of *B. aegyptiaca* oil combined with the Fe atoms on the surface of the Fe_3O_4 nanoparticles and render a partial single bond character of the C=O bond to weaken the bond, and then shift the stretching frequency to a lower value [29].

3.6. X-Ray Diffraction (XRD)

The crystallinity of the magnetic nanoparticles was investigated by powder XRD (**Figure 6**). Both Fe_3O_4 and Fe_3O_4 *B. aegyptiaca* oil nanoparticles exhibit six characteristic peaks at $2\theta = 30.1, 35.3, 43.6, 53.6, 57.3$ and

62.8, indexed as (220), (311), (400), (422), (511) and (440), respectively. The peak positions and relative intensities of the nanoparticles match well with those from JCPDS card 19-0629 for magnetite [30], All the reflections in the pattern can be indexed from those of a standard sample of the Fe_3O_4 spinel structure [JC-PDS card No: 26-1136], indicating that the pure Fe_3O_4 has been obtained [31].

3.7. Antibacterial Activity Study

Antimicrobial activity of *B. aegyptiaca* synthesized iron oxide nanoparticles suspension was tested against different gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative *Escherichia coli* (*E. coli*). It was shown in **Figure 7** moderate activities against most of the tested microorganisms. The antimicrobial activity test of the fixed oil against selected strains of Gram-positive and negative was shown the highest activity on the gram positive bacteria. While *B. aegyptiaca* oil didn't shown any result for gram negative bacteria, in contrasted it was shown highest antimicrobial activity against gram negative bacteria when used Fe_3O_4 with *B. aegyptiaca* oil and no result in gram positive bacteria these results are consistent with recent reports the bacitracin-conjugated Fe_3O_4 @PAA nanoparticles also shown antibacterial ability towards Gram-negative *E. coli* bacteria [30]. The antimicrobial activity of this nanoparticles synthesized by oil Fe_3O_4 is less than standard *B. aegyptiaca* oil that as reflected by lower size of the inhibition zone. The inhibition zones were calculated and taken as the measure of the inhibitory power of the oil [32]. The picture was shown that the oil at the tested dose of 10 μl /disk had antimicrobial activity with maximum inhibition zone against *Staphylococcus aureus* (*S. aureus*) compared with Fe_3O_4 -*B. aegyptiaca* with maximum inhibition against *Escherichia coli* (*E. coli*).

4. Conclusion

Magnetite nanoparticles were successfully synthesized and coated with *B. aegyptiaca* oil to obtain stable suspensions in hexane without the need of external stabilizing agents. The *B. aegyptiaca* magnetic nanoparticles displayed antibacterial activity against Gram-negative bacterial strains compared with *B. aegyptiaca* oil itself, and the *B. aegyptiaca* oil had antimicrobial activity against Gram-positive bacteria. The results confirmed that *Balanites aegyptiaca* (L). Del. fixed oil has great potential as medicinal oil beside its use as edible oil. Due to the antibacterial effect and magnetism, the *B. aegyptiaca* synthesized magnetic nanoparticles may have a promising reagent for a wide variety of biological applications as well as in nanomedicine.

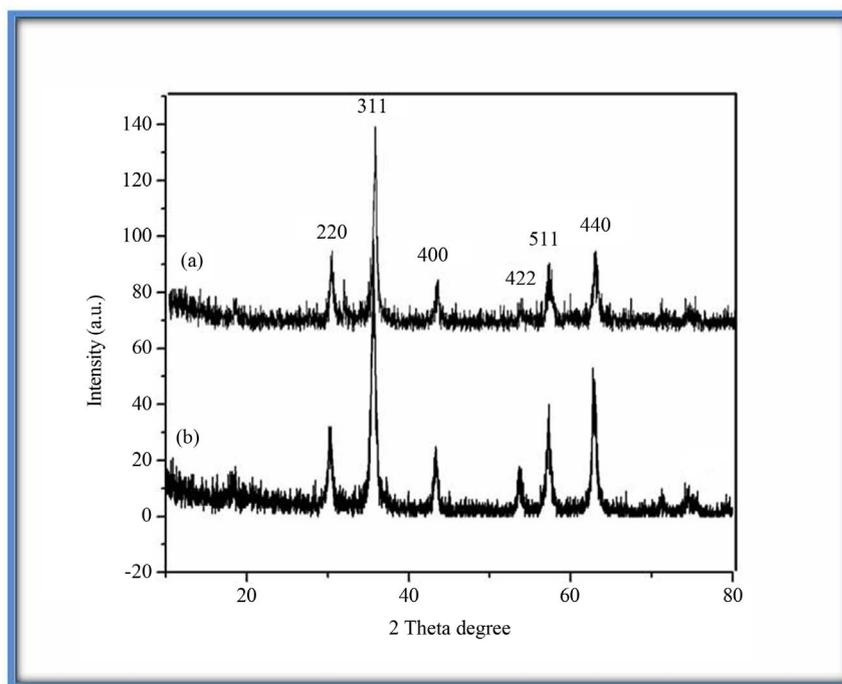


Figure 6. XRD patterns of (a) Fe_3O_4 pure and (b) Fe_3O_4 *B. aegyptiaca* oil nanoparticles.

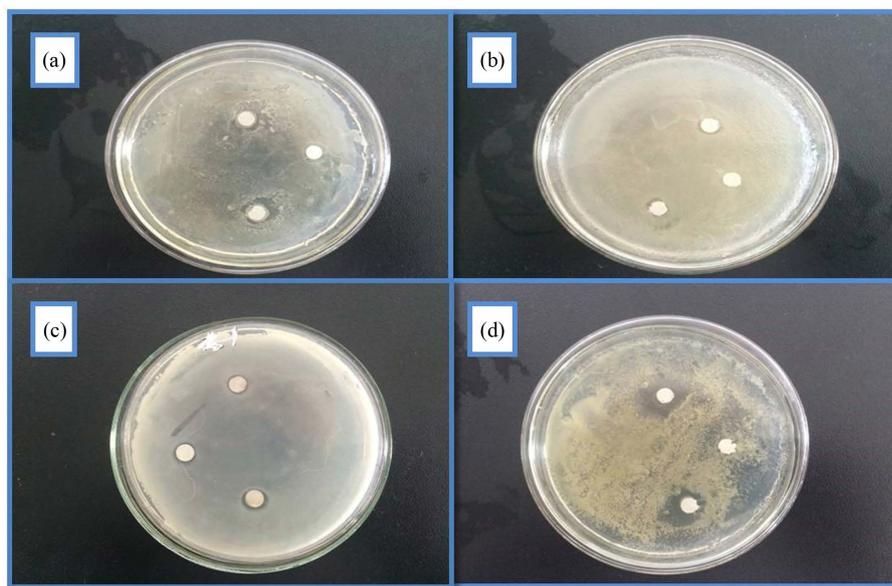


Figure 7. Representative images of agar plates containing *B. aegyptiaca* oil (a) *Staphylococcus aureus*; (b) *Bacillus subtilis*; (c) pure iron oxide nanoparticles and (d) iron oxide nanoparticles with *B. aegyptiaca* oil impregnated disks and diameter of inhibition zone for *Escherichia coli*.

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