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Eco-friendly biosynthesis of magnetic nanoparticles using water extract of Calotropis procera leaves: preparation and characterization

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Abstract

Nanoparticles have become a promising material for water treatment and the production of more active adsorbents that combine carbon-based adsorbent and magnetite properties. The water extract from Calotropis procera leaves is used as a capping and reducing factor to optimize nanoparticle production and stabilize the produced magnetite nanoparticles. For this reason, the preparation and characters of magnetite nanoparticles were studied. The prepared nanoparticles were described by XRD, FTIR spectroscopy, imaging by SEM and TEM, EDX analysis, surface area, and pH_{pzc} . The FTIR result illustrates the existence of magnetite and goethite, OH and COOH groups worked as an agent for reduction and capping for Fe₃O₄ inside nanoparticles. The results were confirmed by XRD examination, which illustrates the inverse spinal shape with a lattice structure that is face-centered cubic of magnetite and orthorhombic structure of goethite and the calculated crystalline index CI = 37.78%. The EDX analysis of nanoparticles showed O (23.53%) and Fe (66.94%). The particle size ranges from 10 to 50 nm where sizes ranged from 5-25 nm was represented 70% and the calculated average diameter of nanoparticles is 30 nm, with pH_{pzc} is 8.58, and the surface area is 93.338 m²/g. The results were successful to produce nanoparticles of magnetite prepared by eco-friendly biosynthesis method using Calotropis procera leaves extract.

Keywords: magnetic nanoparticles, Calotropis procera, biosynthesis, Nanoparticles, Green synthesis

1. Introduction

Nanoparticles, ranging from 1-100 nm, possess unique properties in physics, chemistry, electricity, optics, and thermodynamics, making them valuable in communications, energy, chemistry, medicine, and environmental science [1]. Traditionally, nanoparticles were synthesized by means of physical and chemical approaches, but recent research highlights the advantages of biological (green) synthesis. Green synthesis is faster, cheaper, and more environmentally friendly, avoiding harmful chemicals. It utilizes microor-

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ganisms, plant biomaterials, and green reagents, with plant extracts offering a simple, scalable, and cost-effective approach [2]. Biomolecules such as sugars, polyphenols, and terpenoids influence the size and shape of nanoparticles, making green synthesis an eco-friendly alternative for nanoparticle production [3].

Nanoparticles have unique properties such as enhanced catalytic and antibacterial functions. Although they are useful, they can cause invisible pollution and pose health risks such as respiratory and cardiovascular problems [4]. Metal ions go through a process known as bio-reduction during synthesis. The researchers found that while watersoluble heterocyclic components stabilize the generated nanoparticles, the polyol components in the plant extract are in charge of reducing iron ions. Ferric chloride or other appropriate precursors can be used to obtain reduced plant extracts [5].

Iron nanoparticles (INPs) are extensively used in numerous scientific and technological fields due to their distinctive physicochemical and biological properties. They are particularly valuable in environmental remediation, biomedicine, and pharmaceuticals. However, traditional chemical and physical methods for synthesizing INPs have limitations, prompting the search for more sustainable alternatives [6, 7]. Green synthesis, which employs natural compounds from plants or microorganisms, has emerged as a promising method, offering several benefits over conventional techniques. Although using microorganisms presents some challenges, plant-mediated green synthesis provides an easy, cost-effective, and environmentally friendly way to produce non-toxic and biosafe nanoparticles. Plant extracts, abundant in bioactive compounds such as flavonoids, polyphenols, and amino acids, perform as reducing and capping agents, aiding the conversion of metal ions into stable nanoparticles. Plants like green tea and Eucalyptus are among those used for this purpose [8].

Calotropis procera (Aiton) W.T. Aiton (CP) is the famous plant in family Asclepiadaceae (Milkweed), which grows in Egypt and in tropical regions. It has a vast range of natural products distributed among the different portions of the plant. The main chemical constituents of CP, as in most plants, are cellulose, hemicellulose, and lignin [9].

Magnetite nanoparticles have been successfully prepared by using Calotropis procera with different methods. One of these studies uses aqueous leaves extract as a reducing agent and studies the influence of the entire extract and some of its phytochemicals on the distribution of particle size. phenolic, flavonoid, and saponin extracts gave nanoparticles with mean particle sizes of 62.83 nm, 68.02 nm, and 134 nm, respectively. While leaves extract produces a mean particle size of 11.1 nm. The prepared magnetite nanoparticles have inverse cubic spinal structure and exhibit meaningful anti-microbial activity against some microorganisms [10]. In another investigation, using the extract of Calotropis procera as a reducing and capping factor in the eco-synthesis of iron oxide nanoparticles was carried out by using a Fe^{3+} / Fe^{2+} ratio of 2:1 mol at pH 9. The nanoparticles presented a mean diameter size of 54 nm, and the nanoparticles of ferric oxide with a mean crystallite size of 19.6 nm and inverse cubic spinal structure were formed and confirmed by XRD. The findings are dependable for the green synthesis route's ability to produce nanomaterials with potential use as drilling fluid additives [11].

Using the leaves extract in a single-step preparation method to produce iron phyto-nanoparticles. The extract of CP leaves was used as a stabilizing and reducing agent, and the preparation method was optimized for iron nanoparticle production. The particles take the orthorhombic form and exhibit a size (~32 nm) [12]. Also, the flower extract of CP used to prepare a-Fe₂O₃ yielded an octahedral shape with double-layer edge and edge length of about 4.0 nm, which corresponding to a surface area of approximately 55.42 nm². The synthesized α -Fe₂O₃ nanoparticles exhibit high-rate antibacterial activity against Escherichia coli [13]. Another green method is used for the production of nanoparticles of iron oxide. The iron (II) oxide nanoparticles were produced by means of Calotropis procera leaves extract exhibit particles size 32 nm; the Fe₂O₃ concentration of 1.0 mg/mL can inhibit the development of fungal mycelia and reduce the occurrence of cherry fruit rot [14]. Superparamagnetic behavior refers to the fact that magnetite nanoparticles are attracted to the magnetic field but lose their magnetism when the magnetic effect is removed. Using an external magnetic field, these suspended superparamagnetic particles can be quickly and easily separated from the water sample. Furthermore, the reusability of the magnetite adsorbent allows it to be used for further adsorption runs once the adsorbed dye molecules have been desorbed from the surface of the magnetic adsorbent. Therefore, for simple, effective, economical, and massive adsorption of dye molecules from aqueous samples, magnetite nanoparticles are considered as an affordable, readily available, and magnetically separable

adsorbent.

All previous studies agreed on the preparation method, although there were some minor differences between them, for example, the difference in the raw materials used. These studies also focused on conducting nanoparticle identification using FTIR, XRD, DLS, and UV to characterize the nanoparticles for the main purpose of studying their effect on microbes and fungi, and none of the studies explained the mechanism of the reaction that took place to obtain the nanoparticles. So, the main objective of the present work is to prepare magnetite nanoparticles through a green synthesis technique consuming the water extract of Calotropis procera leaves grown in the Egyptian environment, identify the properties of the nanoparticles, and predict the formation mechanism of the nanoparticles.

2. Experimental

2.1. Materials

All of the chemicals used in this analysis were purchased from the Sigma-Aldrich company in accordance with the specified procedures and were of analytical grade purity.: Potassium hydroxide 99%; Sodium hydroxide ph grade: 99%; Sodium carbonate: 99%; Sodium bicarbonate ACS: 99.7%; and chemicals from Fisher Scientific, as follows: traceable to NIST buffer solutions of pH 4, 7, and 10; Ferric nitrate nonahydrate 98%; Ferrous sulfate heptahydrate CDH ACS grade 99% India; Hydrochloric acid ACROS Organic fuming 37% Belgium; Nitric acid Merck ACS grade 69 % Germany; and Sodim nitrate ACS grade from Reachim 99.8%. The plant grown in El-Mokattam region, Cairo, Egypt, was identified and characterized by examining its botanical properties on an herbarium sample [15-17], the leaves were gathered from the plant in September 2023. High-quality deionized water was adopted for the synthesis of magnetite nanoparticles.

2.2. Dry leaves biomass preparation

The fresh leaves were gathered, cleaned with tap water, then deionized water to get rid of the dirt, dried in a not-so-sunny place for fifteen days, and then dried in an oven at 105 $^{\circ}$ C for eight hours. The dried leaves were crushed by hand and stored in a plastic bag labeled by CPDL until use.

2.3. Preparation of Calotropis procera leaves extract

CPDL sample about 100 g was added to 1000-mL of deionized water in a clean glass bottle. The mixture was stirred in a shaker @ 25 $^{\circ}$ C/24 hours, then transferred into a clean 1000-mL beaker. The mixture was heated in a water bath @ 70 $^{\circ}$ C/1 hour, then left to cool down, the extract filtered, labeled as CPLWEX, and stored in a dark glass bottle in the refrigerator @ 4 $^{\circ}$ C until use.

2.4. Preparation of magnetite nanoparticles by green method

Fe₃O₄ nanoparticles were prepared using the benefitting methods reported in [10, 18-20] with modifications and using Calotropis procera leaves extract (WEX). The preparation method is shown in Figure 1. In brief, 50 ml of 0.04 M ferric nitrate solution was added to 50 ml of 0.02 M ferrous sulfate solution in a 250 mL three-neck flask. The mixture was degassed with nitrogen gas for 10 minutes and then heated to 80 °C in a water bath under continuous nitrogen gas flow and gentle mechanical stirring (200 rpm). 10 mL of WEX was added to the mixture over 10 minutes with a stirring speed of 1200 rpm (the black dispersed residue appeared upon addition), and finally, 1.0 M KOH was added dropwise until the pH value of 11. Under the same conditions, the mixture continued to heat for one hour, and the black dispersion was enhanced. At the end of the reaction, the solution was cooled down to 25 °C, and the produced black mud was separated by a strong magnetic field, cleaned four times with deionized water, and recollected with a magnet. The prepared magnetite nanoparticles were dried over night at 70 °C, weighed, labeled as MGNP, and stored in a polypropylene Falcon tube until use.

2.5. Calotropis procera leaves extract lyophilization

The freeze-drying technique was used to eliminate the water from WEX and convert it to powder for comparison analysis. 100 mL of WEX was distributed on four 25 mL sterile glass bottles and put in a freeze dryer (Thermo Heto Powerdry LL1500: Czech Republic) with heating conditions (freeze at -68 °C under vacuum for 48 hours, then heating through two steps (step 1 at 25 °C for 24 hours and step 2 at 35 °C for one hour). The produced powder was collected, labeled as EXL, and stored in a clean, capped falcon tube at 4 °C until use.

2.6. Characterization techniques

The characterization was performed for the samples (MGNP, CPDL, and EXL) by using many techniques to determine the properties of the prepared green synthesis magnetite nanoparticle and the lyophilized sample of leaves water extract. The characterization of function groups was achieved at regular temperature for all samples by the FT-IR spectrometer in the range of wavenumber from 4000 to 400 cm⁻¹ a scan resolution of 1 cm⁻¹ (Thermo Scientific, Nicolet IS50 FTIR Spectrometer, USA). The sample surface morphology was accomplished by scanning electron microscopy (SEM) equipped by an energy dispersive X-ray (EDX) analyzer with a TESCAN Vega TS 5136LM, typically at a working distance of 10 mm, an accelerator voltage of 25 kV, and with various magnification values (FEI Company, Netherlands). Particle morphology and transmittance electron microscopy (TEM) from Thermo Fisher Talos F200i: USA used to examine particle diameter size, and the images of MGNP were operated at 200 kV. The structure of MGNP was analyzed by X-ray reflectometer diffractometer from Panalytical X'Pert Pro : UK equipped with Cu $K\alpha$ source using and Ni-filtered at 6/min scanning speed rate, 2q ranging from 5 to 90 and l = 1.54 Å. The crystallinity index was calculated using Eq. 1, and the crystallite size (Ds) was evaluated using Scherrer's equation Eq. 2.

$$CI(\%) = \frac{S_{cp} - S_{ap}}{S_{cp}} \times 100$$
(1)

where S_{ap} : the area of the total peaks and S_{cp} : represents the area of the crystalline peaks.

$$D_s = K \times \frac{\lambda}{\beta \, Cos\theta} \tag{2}$$

where q: the Bragg's peak, b: the width (FWHM) of the diffraction peak, K: a constant that depends on

crystallite morphology, usually assumed as unity @ 0.94, and (l):the x-ray wavelength, assumed as 0.1540598 nm.

The surface area examination was performed at 77.35 K by Nova Touch Quantachrome: USA for all samples (MGNP and CPDL) by using nitrogen adsorption-desorption measurements to study the surface area of the samples. The samples were degassed under a high vacuum for 6 hours at a temperature of 150 °C before measurements. The samples were cooled by liquid nitrogen, and the volume of gas (N₂) absorbed at specific pressures was measured.

The salt addition technique was applied to measure the pH at the point of zero charge (PZC) [21] for all samples (MGNP and CPDL) using a 0.1 M NaNO₃ solution at 25 °C. In the current study, 0.1 g of each sample was added to 20 mL of 0.1 M NaNO₃ in a distinct 50 mL Falcon. The initial pH was adjusted to (1, 3, 5, 7, 9, and 11) by using 0.1 M HNO₃ and 0.1 M NaOH solutions combined with a calibrated pH meter. The tubes shake for 24 hours at 25 °C. At the end of the ignition time, the residual was centrifuged, and the final pH of each solution was accurately measured. The difference between the final and initial pH was computed using Eq. 3.

$$\Delta pH = pHf - pHi \tag{3}$$

where: pH_i : initial pH and pH_{f} : final pH

3. Results and discussion

The results of the characterization of the prepared nanoparticle depend on several factors, such as the active part of the plant used, the season of the plant collection, and the environment surrounding the place where the plant grows like region, environment, quality of soil, age of plant etc [22].

3.1. Fourier transformation infrared

C.P. leaves extract EXL, CPDL, and magnetite nanoparticles (MGNP) were studied by FTIR spectroscopy, as shown in Figure 2. The CPDL sample spectra in Figure 2 (c) exhibit absorption at the wavenumbers of 1372-1023 cm⁻¹, 1732 cm⁻¹, and 3662-2880 cm⁻¹ which indicated the presence of



Figure 1: Green synthesis of magnetite nanoparticles schema

lignin, hemicellulose, and cellulose, respectively, as reported in previous work [23]. The peaks at 2921, 1622, 1515, 1372, and 1023 cm⁻¹ related to the lignin structure [23]. According to Kalu et al. [10], the leaf extract of C.P. (EXL) contains phenols, flavonoids, alkaloids, tannins, and saponins. The EXL spectra in Figure 2 (b) exhibited characteristic bands at 3391, 2926, 1615, 1420, 1106, 670, and 596 cm⁻¹, illustrated the existence of the OH phenolic function group, the C-H function group of alkanes, the C=O carboxylic group, the O-H carboxylic group, and the C=C bond of aromatic compounds or the C-O stretching of the phenolic group, respectively. By comparing the spectra of CPDL and EXL, the extract contains aromatic, phenolic, and carboxylic compounds.

According to the MGNP FTIR spectral data, as shown in Figure 2 (a), MGNP has a characteristic

band (a broad band ranges from 3640 to 3000 and is centered at 3150 cm^{-1} , a very weak band at 2925, 1620, 1389, 891, 792, and 559 cm⁻¹). By comparing the FTIR spectral data of EXL and MGNP, it was illustrated that the OH phenolic peaks at 3391 and 3259 cm^{-1} in EXL were shifted to 3150 cm^{-1} may be due to the presence of OH groups of phenolic compounds of C.P. extract or the presence of OH groups of FeO(OH) goethite and this designates that the phenolic OH function group may be worked as a reducing factor in the formation of Fe₃O₄ in MGNP. Also, the band of the O-H carboxylic group at band 1420 cm^{-1} in EXL shifted to 1389 cm^{-1} in MGNP, and the bands associated with the C-O phenolic group disappeared at band 1106 cm⁻¹ in MGNP, indicating that carboxyl in leaves extract worked as a capping agent for Fe₃O₄ in MGNP formation. The magnetite nanoparticle (MGNP) spectra showed the main absorption band at 559 cm^{-1} as shown in Figure 2 (a), belonging to the basic stretching vibrations of iron and oxygen at the tetrahedral shape (Fe-O) [24]. Derva et al. [3] reported that the bands at 400 to 570 cm⁻¹ correspond to magnetite (Fe₃O₄), 630-660 cm⁻¹ correspond to maghemite $(g-Fe_2O_3)$, and peaks at 352,470, and 540 cm⁻¹ are corresponded to hematite (a-Fe₂O₃). The peaks at 792 cm^{-1} (-OH) and 891 cm^{-1} (-OH) are wavenumbers of goethite a-FeO(OH) [25-27]. The FTIR spectrum shows that the phytoconstituents found in the aqueous extract of C.P leaves were effectively working as capping and reducing factors in the formulation of Fe₃O₄/NPs. The same results were reported in previous research [10, 28].

The current result agrees with the results of the presence of Fe-O bound that characterized for magnetite as reported in [10–12] but surpasses them in the FTIR result exhibit the presence of another face of magnetic material at peak 792 and 891 cm⁻¹ are characterized for goethite a-FeO(OH).

3.2. The Morphology and particle size

The TEM photos shown in Figure 3 show the surface morphology and particle size distribution of MGNP nanoparticles. The sample probably comprises homogeneous nanoparticles with spherical shapes and a mean diameter of about 10-50 nm, where the particle sizes of 5-25 nm represented 70% of the total particle sizes in the sample, as shown in Figure 4. The sample is shown in the form of dense aggregations of nanoparticles. The particle size is consistent with the size computed from the Scherrer equation, which exhibits a mean diameter of about 30 nm. The good relationship between the particle size determined by using the Scherrer equation Eq. 2 and TEM images confirms the crystalline shape of the Fe₃O₄ nanoparticles, with a calculated crystalline index CI = 37.78% Eq. 1. Thus, the magnetite NP was successfully prepared by the green method by consuming the water extract of C.P. leaves as a reducing and stabilizer agent for nanoparticles.

SEM/EDX analysis was applied to study the surface morphology and the elemental analysis of MGNP. The SEM image in Figure 5 shows irregularshaped to semi-spherical agglomerated particles; this aggregation may be due to the reaction of magnetite with the phytocompounds from C.P. water extract during the green synthesis process. This is supported by the existence of the carbon element in the EDX analysis in Figure 5 , where the analysis shows carbon (9.53%), oxygen (23.53%), and iron (66.94%).

In the current study, the using of the TEM technique give a unique clear image as shown in Figure 6 for the formation of core shell around Fe_3O_4 nanoparticle which provide more information about the characterization of the magnetite nanoparticle reported in the previous studies in [10–12]. The results confirm the concept that the particle size of the nanoparticle changes with change the raw materials as reported by Dominikus et al. [29].

3.3. X-Ray Diffraction

From the X-ray analysis of MGNP in Figure 7, the peaks of diffraction can be noticed for MGNP at 2q values of 30.15° , 35.71° , 43.29° , 53.39° , 57.29° , and 62.90° , corresponding to the crystalline planes (220), (311), (400), (422), (511), and (440), respectively. The peak values show perfect coherence with the XRD pattern of magnetite, which corresponds with standard JCPDS values (file no. 01-19-0629). Similar results were reported in various studies and imply that the magnetite (Fe₃O₄) has



Figure 2: FTIR spectra of CPDL, EXL and MGNP



Figure 3: EM images of MGNP

an inverse spinal with a face centered cubic lattice structure [10, 26, 30]. The 2q values of 11.69° correspond to the phytoconstituent of C.P. extract; also, the 2q values of 17.75°, 21.18°, 33.22°, 36.63°, 39.9°, 41.18°, 50.5°, 58.9°, and 61.25° correspond to (200), (101), (301), (111), (211), (401), (112), (511), and (020), respectively. The peak values show good consistency with those from the standard XRD pat-



Figure 4: EM images particle size distribution of MGNP



Figure 5: EM/EDX images of MGNP



Figure 6: EM image of MGNP to illustrate the core shell

tern of goethite, which is an orthorhombic structure a-FeO(OH), which corresponds with standard JCPDS values (file no. 96-100-8767), the same result reported by Hessien M. [25]. This result confirms the result of FTIR. The XRD results support the results of FTIR and confirm the formation of magnetite nanoparticles using the green synthesis method in sample MGNP.





The current result agrees with the results of the presence of magnetite pattern with inverse cubic spinal shape as reported in [10, 11] but also confirmed the result of FTIR in the current study for the presence of orthorhombic structure of goethite a-FeO(OH) which is a new phase of magnetic material found in MGNP prepared in the current study.

3.4. Zeta potential

The results in Figure 1S show that nanoparticles are more stable than CPDL at all pH values due to the value of the zeta potential of nanoparticles being more negatively charged than CPDL. In the case of MGNP, at pH values between 6 and 8, the zeta potential shows a minimum value close to -60 mV; beyond this range, particularly at lower or higher pH values, the zeta potential becomes less negative. The most negative zeta potential observed between pH 6 and 8, where the particles tend to disperse better, suggests high stability, but outside this pH range, the nanoparticles may agglomerate or become less stable. These results give more information about nanoparticle added to the results reported in the other studies in [10–12].

3.5. The pH of point of zero charge

Figure 2S shows the relationship between the initial pH and the change in pH with regard to the final pH. The crossing between the curve and the initial pH (x-axis) at a zero change in pH is the pH of the point of zero charge (PZC), which represents the pH at which the surface charge of the material is zero. The pH point zero charges were 5.85 and 8.58 for CPDL and MGNP, respectively. Therefore, the surface charge of MGNP is positive below pH 8.56 and negative above this pH point. These results provide extra information about magnetite nanoparticle added to the results reported in the other studies in [10–12].

3.6. Surface area - Brunauer-Emmett-Teller (BET

The surface area of MGNP has been investigated through N_2 adsorption and desorption measurements at 77 K, and the results show that the surface area was 93.338 m²/g. These results provide extra information about magnetite nanoparticle added to the results reported in the other studies in [10–12].

3.7. Ferric oxide-nanoparticle formation mechanism

The formation of magnetite nanoparticles was confirmed by the visual method, where during preparation the color of the reaction mixture transformed from a yellow to black dispersion, which is the main observation for the formation of Fe_3O_4 nanoparticles [31]. This color change was a result of the interaction between the phytochemical compounds in EXL and the iron salt mixture. The second method is that the nanoparticles are collected by a magnet during preparation. The predicted chemical reaction mechanism for the synthesis ferric oxide nanoparticles can be hypothesized as shown in Figure 8 as follows:

The FTIR spectra of C.P. extract (EXL) suggested the presence of COOH and OH groups, the -COO⁻ groups can interact with Fe^{2+} and Fe^{3+} in the aqueous medium and undergo chelation reactions. The elevation of the aqueous reaction medium temperature to 80 °C in the presence of KOH leads to the formation of bonds between OH⁻ ions and Fe ions, resulting in the development of Fe(OH)₃ and Fe(OH)₂ core shells. With the progress of the reaction, the water dehydrated from the Fe(OH)₂ and Fe(OH)₃ and ended with the production of stabilized nanoparticles surrounded by the phytocompounds present in the extract. The same mechanism was proposed by Awwad and Salem [32].



Figure 8: Suggested mechanism of Fe₃O₄ nanoparticles

In conclusion, based on the characterization findings of MGNP in the current study and compared with other studies reported in [10-12], the results suggested that the formation of magnetite nanoparticles by the green method changes with the change of active ingredients in the water extract of Calotropis procera leaves, which is effected by several conditions, mainly the collection season of the plant and the environmental conditions as reported in [22, 33]. In many studies, it was reported that polyphenols play an important role in the formation of stable magnetite nanoparticles, where they act as reducing agents and capping agents. [34]. The ratio of the leaf extracts to salt and the type of iron salt used are important factors in the determination of nanoparticle properties, as reported by Hoag et al. [34], where in this study, 0.1 M FeCl₃ was added to 20 g/L of green tea extract in a ratio 2:1 and produced nanoparticles with a diameter range of 5-10 nm. In another study by Markova et al. [35] use ferric nitrate in ratio of 1:5 with the green tea extract under nitrogen and this produce nanoparticles 70 nm. Nadagouda et al. [36] reported that the production of nano particles with different sizes and properties is due to changes in synthesis procedures,

and the most important ratio of extract to iron salt. The phenolic compounds are major compounds in the leave extract of Calotropis procera, as reported by Ashraf et al. [37]. The study found that the extract contains phenolic compounds such as vanillin (46.79%), chlorogenic acid (30.24%), gallic acid (8.06%), and daidzein (3.45%). Also, the phenolic compounds and flavonoids were found in high content in the aqueous extract of Calotropis procera leaves as reported by Armin et al. [38] and the extract has high antioxidant properties. Which also affects the particle size of the nanoparticles, as reported by Machado et al. [7]. Moreover, the type of phytocompounds also affects the particle size, as reported by Kalu et al. [10], where the leaves extract showed the presence of phenols, flavonoids, saponins, alkaloids, and tannins. The magnetite nanoparticles produced average particle sizes of 62.83 nm, 68.02 nm, and 134 nm for pure extracts of phenolic, flavonoid, and saponin, respectively. Sheth [33] reported that the phenol content in mature leaves was 1.52, 0.49, and 1.086 g% in winter, summer, and monsoon, respectively. In conclusion, the type and concentration of active compounds, especially phenolic compounds, in the leaf extract affect the particle size and morphology of the nanoparticle because, as mentioned, the ratio of extract to iron salt is one of the key factors in determining the particle size, and the concentration of phenolic compounds changes from season to season, so the plant collection period plays a role in the composition of the leaf extract.

The formation of the magnetite nanoparticles by coprecipitation method affected by several parameters such as the type of the salt, molar ratio of the reactants, pH level, stirring rate, and reaction temperature as reported by Dominikus et al [29]. Also, the effect of salt type change affect the particle size and magnetization saturation reported by Yazdani and Seddigh [39] as shown in Table 1S.

The result, illustrated by the FTIR spectra of Calotropis procera leaves water extract, which contain a peak value of goethite that was not reported in the other studies, may be due to the change in the side chain function groups of phytocompounds in water extract due to the difference in plant growth region and season of collection. This

is described by the slight shift of characteristic peaks of phytocompounds in the FTIR aspect of the extract. The XRD confirms the result of FTIR in the current study by confirming the confirming the presence of goethite patterns as orthorhombic crystal structures, which can be formed due to the difference in phytocompound composition and concentration, which led to the interaction with iron ions in different manners during the core shell formation and led to the formation of a crystal structure different from the structure of magnetite. Also, the presence of goethite may be due to the high presence of Fe (III) or the increase in the speed of agitation to 1200 rpm, which may induce goethite formation, as reported by Tukan et al. [29]. The goethite also has a magnetic effect, and the adsorption applications reported for using magnetite and goethite separately when preparing a nanocomposite medium of magnetite and goethite with zeolite and used in adsorption of phenolic compounds, where the percent removal recorded 79% and 80% for magnetite/zeolite and goethite/zeolite, respectively, as reported by Fadwa et al. [40]. A recent study by Yen-Hua and Fu-An [41] reported that the goethite nanoparticles used in the removal of copper ions have an adsorption capacity of 149.25 mg/g.

Also, the current study provides new information about the charge of magnetite nanoparticles at different pH values and the stability of the produced nanoparticles. The range of pH for maximum zeta potential (-60 mV) between 6-8 suggests that the magnetite nanoparticles have a strong negative charge at the surface when suspended in a solution within the pH range of 6-8. This level of zeta potential typically has excellent stability [42] and give evidence for the small particle diameter of the nanoparticles, where electrostatic repulsion between particles prevents them from aggregating. As a result, the nanoparticles will remain well-dispersed in the solution. This gives an advantage to the produced nanoparticle for use in biomedical applications such as drug delivery and hyperthermia treatment [43], where the reported zeta potential - 11.49 mV, environmental remediation as an adsorbent [44], and magnetic isolation for ions, cells, DNA, proteins, and various bioactive

Table 1: Comparison of Fe ₃ O ₄ nanoparticle particle size and surface area			
Method	Particle size	Surface area	Ref.
	(nm)	\mathbf{m}^2 /g	
Current study	10-50 by TEM with calculated 30	93.338	Our
	nm		Study
Green synthesis by use	11.1 by DLS62.83 by DLS68.02	-	[12]
Calotropis procera leaves water	DLS 134 DLS		
extractPhenolic			
extractFlavonoid extract			
Saponin extract			
Green synthesis by use	19.6 by DLS	-	[13]
Calotropis procera leaves			
water extract			
Green synthesis by use	32 TEM	_	[14]
Calotropis procera leaves			
water extract			
Green synthesis by use Uncaria	20 TEM	_	[30]
tomentosa leaves aqueous			
extract			
Green synthesis by use	20 TEM	_	[20]
Calotropis procera leaves			
water extract			
Coprecipitation method	15 TEM	_	[22]
Coprecipitation method	10 TEM 30 TEM	_	[25]
Solvothermal method			
Seaweed (Kappaphycus	14.7 TEM	_	[27]
alvarezii) water extract			
Green synthesis by use	10-12 TEM	127 - 318	[29]
MyrtuscommunisL leaf extract			

Table 1: Comparison of Fe₃O₄ nanoparticle particle size and surface area

substances [45]. Also, the current study provides new information about the point of zero charge of the nanoparticles, which is an important factor because it affects the interaction of nanoparticles with their environment, including other particles, solvents, and biological materials. Moreover, the current study gave information about the surface area of the produced magnetite nanoparticle. The surface area of Fe_3O_4 nanoparticles affects their reactivity and magnetic properties. A larger surface area allows for more active sites for reactions or interactions with other molecules, which is beneficial in applications like catalysis [46], also the surface functionalization of magnetite nanoparticle is advantageous for the detection of molecular interactions; the large surface area of magnetite nanoparticle permits efficient functionalization for targeted biomolecule interactions [47, 48]. The higher specific surface area seen in nanoparticles is attributed to the small size of the nanoparticle which give advantage to use in biomedical applications as reported by Wu et al. [48], where the average specific surface area reported 147 m²/g. in another study by Xuan et al. [49], the biocompatible composites possess a large BET surface area (222.3 m²/g); the composite NPs have been used as a bi-functional agent for both MRI and drug carriers. The two main factors that need to be considered in the usage of magnetite nanoparticles in vivo include size and surface modification. Magnetite nanoparticles possess a huge surface area that can be surface functionalized with a huge quantity of functional groups for crosslinking to tumor-targeting ligands, diagnostic imaging, or therapeutic agent delivery [50]. Finally, the current study provides a predicted mechanism for the formation of magnetite nanoparticles (Table 1).

4. Conclusions

In conclusion, this study was performed using the green synthesis technique to produce a magnetite nanoparticle (MGNP) by utilizing a water extract of Calotropis procera leaves as a reducing and capping factor in order to investigate the properties of the produced nanoparticle by using the phytochemical extract from the plant grown in the Egyptian environment and provide more information about the produced nanoparticle. The prepared magnetite nanoparticles (MGNP) were described using several analytical methods. The prepared nanoparticles have a homogeneous spherical shape in aggregation with an average diameter of 10-50 nm (5-25 nm represented 70%) and a calculated particle size of 30 nm, a crystalline index of 37.78%, a surface area of 93.338 m²/g, and pH_{*pzc*} = 8.56. The FTIR spectra show the characteristic wavenumber at 559 cm^{-1} for Fe-O and peaks at 792 and 891 cm⁻¹corresponding to goethite FeO(OH). The results suggested the participation of COOH and OH groups in the production of Fe₃O₄ as reducing and capping factors, and the structure was confirmed by XRD. The EDX confirms the presence of iron (8.82%) and oxygen (40%). MGNP exhibits pH_{pzc} = 8.56 with a buffering effect in the range of pH 5-9. The present study offers an alternative approach to the synthesis of economically viable and well-designed iron nanoparticles using plants from Egyptian nature as biomass and provide extra new information about the nanoparticle properties and predicted mechanism.

5. CRediT authorship contribution statement

Tarek M. Sabry: Conceptualization, Methodology, Software, Data curation, Writing – original draft, Software, Validation, Writing – review & editing. Sabry Abd El-Hamied El-Korashy: Conceptualization, Methodology, Software, Supervision. Hossam Eldin Samir Jahin: Conceptualization, Methodology, Software, Supervision. Gasser Mohamed Khairy: Conceptualization, Methodology, Software, Data curation, Writing – original draft, Supervision, Writing – review & editing. Nadia Fathi Abdel Aal: Conceptualization, Methodology, Software, Visualization, Investigation, Supervision.

6. Declaration of competing interest

The authors declare that there is no conflict of interest.

7. Data availability

Data will be provided upon request.

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