



The utility of *Hibiscus sabdariffa* L. to prepare metal oxides NPs for clinical application on osteoporosis supported by theoretical study

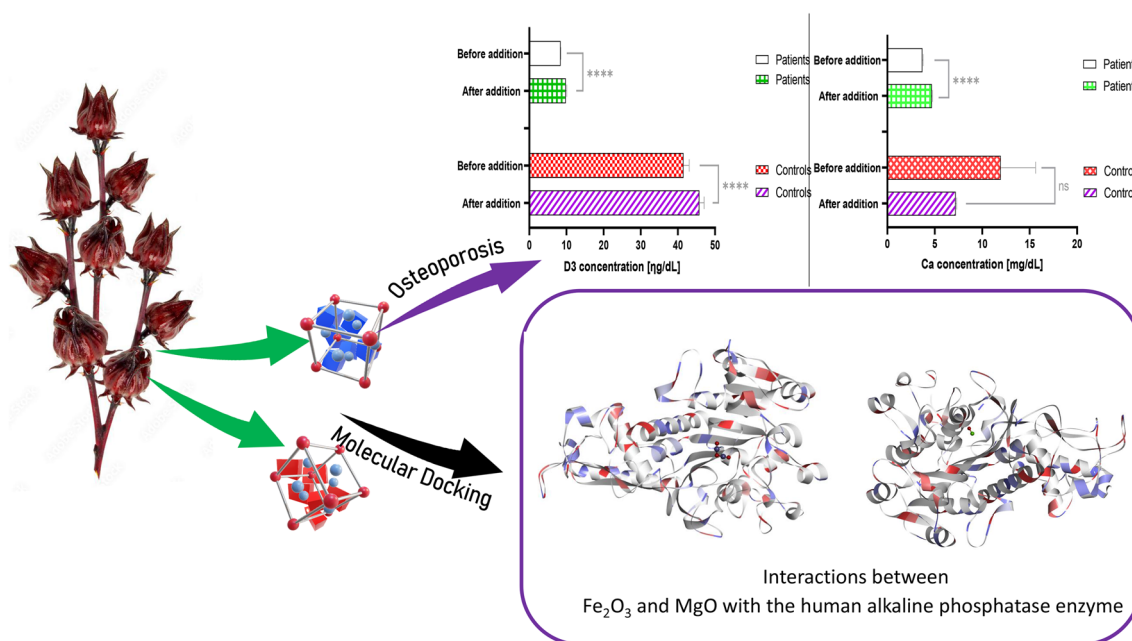
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Abstract

Green synthesis of metal oxides as a treatment for bone diseases is still exploring. Herein, MgO and Fe₂O₃ NPs were prepared from the extract of *Hibiscus sabdariffa* L. to study their effect on vit D³, Ca⁺², and alkaline phosphatase ALP associated with osteoporosis. Computational chemistry was utilized to gain insight into the possible interactions. These oxides were characterized by X-ray diffraction, SEM, FTIR, and AFM. Results revealed that green synthesis of MgO and Fe₂O₃ NPs was successful with abundant. MgO NPs were in vitro applied on osteoporosis patients ($n = 35$) and showed a significant elevation of vit D³ and Ca⁺² ($0.0001 > p < 0.001$) levels, compared to healthy volunteers ($n = 25$). Thus, *Hibiscus sabdariffa* L. is a good candidate to prepare MgO NPs, with a promising enhancing effect on vit D³ and Ca⁺² in osteoporosis. In addition, interactions of Fe₂O₃ and MgO NPs with ALP were determined by molecular docking study.

Graphical abstract



Keywords Fe₂O₃ NPs · Molecular docking · Green synthesis · MgO NPs · Osteoporosis · *Hibiscus sabdariffa* L.

Introduction

Using chemical methods to synthesize nanoparticles has increased the risks of environmental pollution due to toxic and hazardous chemicals [1, 2]. Hence, the current focus is on using biogenic sources for the synthesis of nanoparticles, such as peptides [3–5] saccharides [6, 7], microorganisms, algae, and plants [8–11]. Its efficiency, cost-effectiveness, and environmentally safe nature have opened more spaces for safe nanobiotechnology [12, 13]. Metal oxide nanoparticles (MO NPs) have attracted lots of interest, recently, due to their exceptional physical and chemical properties useful for biosensing, as diagnosing tools, catalysts, and anticancer and antimicrobial agents [14–17]. Among these MO NPs, magnesium oxide (MgO) NPs and iron oxide NPs have been used for several purposes, such as treating various ailments, adsorbent, catalytic support, agricultural products, paints, superconductor products, antimicrobial materials, antifungal activity [18], anticancer [19], antioxidants [20], tissue engineering [21], photonic devices, refractory materials, and sensors [22–25]. They have a biocompatible nature and remarkable stability under harsh conditions [26, 27]. MgO NPs showed high reactivity due to the comportment of a great number of highly reactive edges, structural defects on the surface, unusual lattice planes, and a high surface-to-volume ratio [28]. Moreover, MgO exhibits exceptional optical, electrical, thermal, mechanical, and chemical capabilities in addition to a strong ionic character [29]. Due to its extremely high melting point and low heat capacity, MgO is a good material to use as insulation. The characteristics of iron oxide NPs led to some widespread in vivo applications, including contrast agents in magnetic resonance imaging (MRI), cell separation, and drug delivery fields [11, 30]. Furthermore, the capacity to create heat in the presence of an alternating (AC) magnetic field makes iron oxide NPs a potential material for a variety of applications, including biomedicine, bioremediation, diagnostics, materials engineering, and solid tumor therapy [31].

Hibiscus sabdariffa L. (*H. sabdariffa*), also known as Roselle, is a member of the Malvaceae plant family and is utilized extensively as a diuretic, for treating gastrointestinal disorders, liver diseases, fever, hypercholesterolemia, and hypertension as well as acting as a blood pressure suppressant, chemo-protective antioxidant, and anti-cancerous agent [32–36]. It has been selected here for several reasons. It is often used in alternative medicine because it is rich in phytochemicals like polyphenols especially anthocyanins, polysaccharides, and organic acids, thus having enormous prospective in modern therapeutic uses [37]. Citric acid, hydroxycitric acid, and hibiscus acid (ALLO-2-hydroxycitric acid) are a few examples

of the organic and phenolic acids included in this plant's phenolic components. There are other flavonoids such as gossypetin, luteolin, and quercetin, along with their corresponding glycosides. The presence of anthocyanins in high amounts in the calyces is responsible for their bright red color [38–43], see Table [A]. In addition, *Hibiscus sabdariffa* L. has been used for green synthesis of some metal ions nanoparticles [44–46]. Also, it showed a potent antibacterial agent and is beneficial for those with diabetes mellitus [47]. Strongly basic dye from *H. sabdariffa* helps diagnose different diseases. As the most prevalent bone disease, osteoporosis affects more than 50% of women and 20% of men in the US population aged 50 years and above, it is considered a significant and public health concern [48]. Osteoporosis has been undiagnosed yet due to a lack of epidemiological data and insufficient methods for investigation, a considerable under-recognition of osteoporosis has been studied in clinical practice, in the past century [35].

Thus, this study was designed to introduce the green synthesized MO NPS to this field of application. In addition, according to the well-known properties of MO NPs, the study has hypothesized different scopes of application to be confirmed. Theoretical calculations were designed to investigate the possible interactions between the prepared MO NPs and the human alkaline phosphatase enzyme. Molecular docking is one of the most applied computational chemistry methods that becomes increasingly useful based on the 3D structures generated by data of X-ray diffraction and their improved resolution, which is reported in the Protein Data Bank (PDB).

The significance of this research is to prepare MO NPs in an environmentally friendly way utilizing *H. sabdariffa* L. extract and apply those NPs as treatment of low Vit D³ and Ca⁺² in some osteoporosis cases. These oxides were prepared and well characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray tomography (EDX), Fourier transform infrared (FTIR), atomic force microscopy (AFM), and zeta potential. Molecular docking studies indicate that Fe₂O₃ and MgO NPs interact with the human alkaline phosphatase enzyme, which is compatible with our experimental data.

Experimental

Materials

The leaves of the *Hibiscus sabdariffa* L. plant were purchased from a herb shop in Baghdad, and Mg(NO₃)₂ and Fe (NO₃)₂ salts were purchased from BDH Chemical Ltd., England.

Preparation of the plant extract

Fresh flower *Hibiscus sabdariffa* L. was washed thoroughly twice with deionized water to remove any remained material, dried for 2 days with constant stirring to prevent the rotting of the leaves, and then ground well. The plant was extracted by adding 3 g of the ground *Hibiscus sabdariffa* L. to 50 mL of deionized water, heating to 60 °C for 15 min, and then the extract was collected by filtration.

Preparation of iron oxide NPs

The iron nanoparticles were prepared using the extract of *Hibiscus sabdariffa* L. A weight of 0.404 g $\text{Fe}(\text{NO}_3)_2$ was dissolved in 50 mL of deionized water and then added to the extract until changing color to a cloudy black. A volume of 50 mL NaOH was added to the mixture and left to settle and the precipitate was separated. The precipitate was washed five times with deionized water and the precipitate was calcined at 400 °C for 4 h.

Preparation of magnesium oxide NPs

A weight of 0.256 g $\text{Mg}(\text{NO}_3)_2$ was dissolved in 50 mL of deionized water and then added to the extract of *Hibiscus sabdariffa* L. until the color of the mixture changed to red. A volume of 50 mL NaOH was added and left to settle and the precipitate was separated. The precipitate was washed five times with deionized water and the precipitate was calcined at 600 °C for 4 h.

Clinical application of magnesium oxide

Sample collection

This study included 60 participants who visited Yarmouk Teaching Hospital in Baghdad. Thirty-five of them suffered from osteoporosis and twenty-five participants compatible with age (35–55 years) were healthy volunteers. Research ethics of human rights were followed according to the standard ethics of Yarmouk Teaching Hospital. All participants were diagnosed by specialists in the hospital. The study excluded participants who suffered any complications that could interfere with the results, such as rickets or arthritis, or any other bone diseases.

Blood samples

An intravenous blood sample (5 mL) was collected from all participants. The blood was transferred directly to a gel tube, left for 15 min until the blood clot, and centrifuged at (1800×g) for 3–5 min. The serum was stored at −20 °C until the time of analysis.

Methods

To investigate the effect of magnesium oxide NPs on the serum levels of vitamin D3 level, calcium, and the activity of the enzyme alkaline phosphatase (ALP) for patients suffering osteoporosis disease, standard protocols were followed to determine the bio parameters for all participants before and after in vitro adding of MgO, in vitro.

Statistical analysis

The statistical analysis and the significance of differences between groups were done using an unpaired Student *t* test (with *Welch's correction*-two-tailed) at a significant *p* level <0.05. GraphPad PRISM 9 software for Mac @2021 was assisted with all the statistical analyses.

Molecular docking analysis

It is a resource-saving technique that provides accessibility of computational chemistry to academia and industries. Molecular docking calculations were carried out for metal oxides NPs with human alkaline phosphatase enzyme (PDB Code: 2GLQ [49]) AutoDockTools-1.5.7rc1 [50] and Discovery Studio Visualizer [51] software packages were used for molecular docking calculations. The number of water molecules in the protein pocket was eliminated, and polar hydrogens and Kollman charges were added. The root of each ligand molecule was automatically recognized, and torsions were selected. The torsions of the ligand were rotated, and the chosen residues were examined. To indicate where the ligands would bind preferably, they all were docked blindly.

Results and discussion

A mechanism for synthesis of metal oxides using *Hibiscus sabdariffa* L.

The general mechanism of fabricating MO NPs *Hibiscus sabdariffa* L. is shown in Scheme 1. The plant extract contains lots of functional groups such as carbonyl, hydroxyl, and amine (Table 1) able to reduce metal ions to nanoparticles with different shapes and sizes. In addition, the plant has some bioactive substances such as flavonoids, protein, and sugar that possibly contributed to the reduction of metal ions [52, 53].

FTIR spectroscopy

H. sabdariffa L. extract and MO NPs

Figure 1 shows the FTIR spectrum of the crude extract of *Hibiscus sabdariffa* L. and the prepared MO NPs. The FTIR

Scheme 1 Mechanism of preparing the metal oxide NPs from *Hibiscus sabdariffa* L.

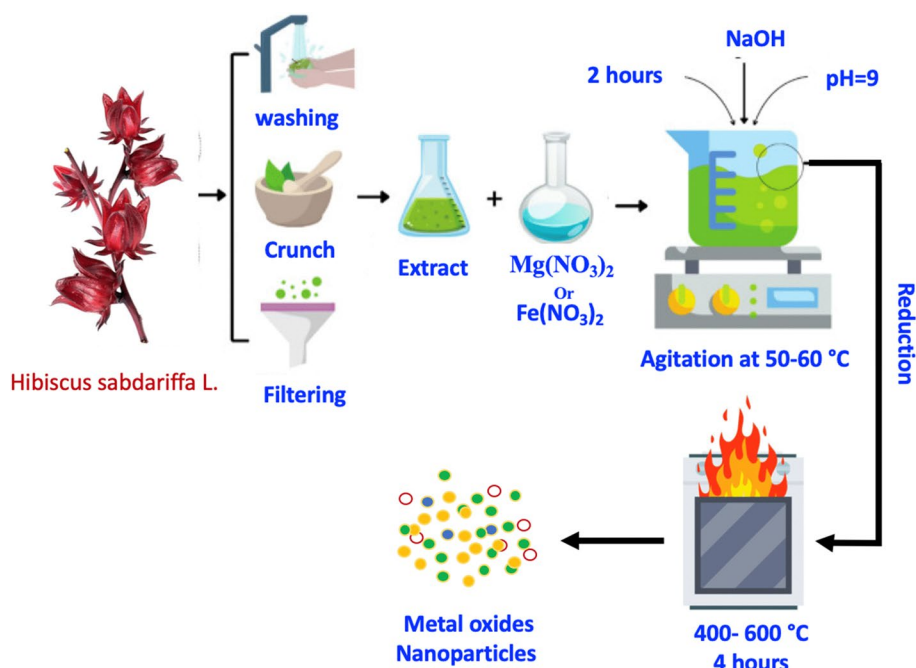


Table 1 Some of the chemical contents of *Hibiscus sabdariffa* L. [21]

Chemicals

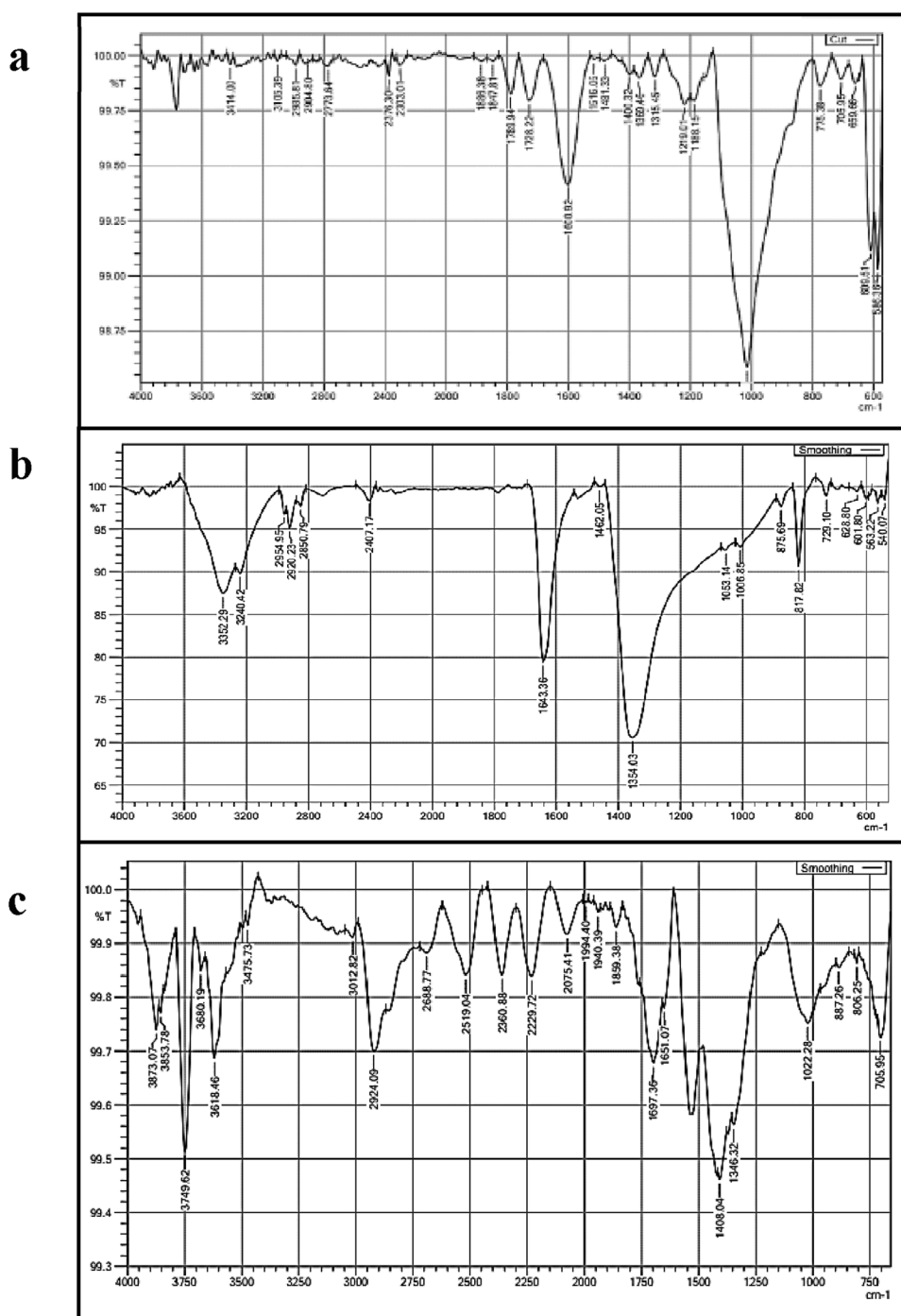
Hydroxycitric acid	Chlorogenic acid isomer I	Protocatechuic acid
Kaempferol-3-O-rutinoside	Chlorogenic acid isomer II	Protocatechuic acid glucoside
Hibiscus acid glucoside	5-Hydroxymethylfurfural Methyl gallate	Coumaroylquinic acid
Hibiscus acid 6-methyl ester	2-O-trans-Caffeoyl-hydroxycitric acid	Quercetin-3-rutinoside
Anthocyanins Delphinidin-3-sambubioside	5-Caffeoylquinic acid	5-O-Caffeoylshikimic acid
Cyanidin-3-sambubioside	Myricetin-3-arabinogalactoside	Leucoside (kaempferol-3-O-sambubioside)
Flavonoids, phenolic acid and gallic acid	3-Caffeoylquinic acid	Quercetin-3-sambioside
Kaempferol-3-p-coumaroyl glucoside	Methyl (AS in methyl-epigallocatechin)	Quercetin-3-glucoside

spectrum of the extract showed a broad and characteristic peak at $\sim 2979\text{ cm}^{-1}$ indicating the presence of lipid-carbohydrate bonding, Fig. 1a, The peaks at 1785 and 1724 cm^{-1} referred to the ($\text{C}=\text{O}$) stretching of esters and the bands between 1399 and 1042 cm^{-1} were due to the presence of CH_2 stretching. A peak at 3343.61 cm^{-1} was assigned for O–H stretching; [54, 55]. In general, the spectra of the extract indicated the presence of the hydroxyl group of phenolic compounds [56], and cellulose-fatty acids $\nu(\text{C}=\text{O})$ stretching of esters [57]. The vibrational mode of angular deformation of CH_3 was observed at 1364 cm^{-1} , the stretching of C–O–C was assigned at 1185 cm^{-1} , and the stretching of C–O was seen at 1031 cm^{-1} . The spectrum at 771 cm^{-1} indicated angular deformation of N–H [58]. The broad peak at $3600\text{--}3400\text{ cm}^{-1}$ was due to alcoholic or phenolic –OH groups. Strong (–OH) stretching vibrations at 3439 cm^{-1} were due to the water molecules, Fig. 1a. Bands between

2100 and 2500 cm^{-1} showed alkynes stretching, and 1876 , 1604 cm^{-1} , and that at $1460\text{--}1350\text{ cm}^{-1}$ corresponded to C=O stretching (amide linkages), C=C stretching (alkenes), and –CH stretching (alkanes), respectively [9]. Figure 1b for MgO NPs shows bands at 3308 , 2139 , 1635 , 1346 , 419 , and 407 cm^{-1} . The strong infrared band near 3308 cm^{-1} was assigned for the O–H bond vibrations of the OH group, band at 1635 cm^{-1} indicated $\nu\text{C}=\text{O}$, typical for the structure of flavonoids which can also be found in the extract [59]. The peaks between $\lambda 450\text{--}560\text{ cm}^{-1}$ were assigned to MgO stretching vibrations. The absence of a peak at 694 cm^{-1} confirmed that there was no $\text{Mg}(\text{OH})_2$ [60, 61].

Figure 1c shows the FTIR spectrum of Fe_2O_3 NPs. The bands at 2923 and 2853 cm^{-1} were for aromatic and aliphatic C–H stretching, bands in the region $400\text{--}850\text{ cm}^{-1}$ (metal–oxygen bond formation), bands at 618 and 467 cm^{-1} (stretching vibration of Fe–O [10, 62], the band

Fig. 1 The FTIR spectra of the extracts and metal oxides. **a** *Hibiscus sabdariffa* L. extract, **b** the synthesized MgO NPs, **c** the synthesized Fe₂O₃ NPs



at λ 618 cm⁻¹ (Fe–O–H stretching vibration), the bands at 3422 and 1630 cm⁻¹ (O–H stretching vibration of surface-adsorbed water molecules) [63]. the peaks at 3448 and 1635 cm⁻¹ (O–H of H bond or carboxylic acid and C=C ring stretching on the surface) [64], bands at 1668 and 1720 cm⁻¹ (asymmetric and symmetric stretching of the –CO–) [65], bands between 1000 and 1250 cm⁻¹ (vibration and stretching of various methyl groups (–CH₃, –C(CH₃)₂) [66], absorption bands at 3500 and 3270 cm⁻¹, (strong OH

and NH stretching of phenol and amine groups), 2700, 2610, 1755, and 1170 cm⁻¹ (=C–H aldehyde stretch, O–H from acidic groups, C=O stretch and C–O groups, respectively) [66]. The appearance of two absorption bands at 474 and 575 cm⁻¹ was due to the formation of Fe–O bonds [67].

Interestingly, incubation of both MgO NPs and Fe₂O₃ NPs with organic solvents (DMSO, toluene, ethanol) for up to 3 weeks showed similar stabilities over time. The FTIR spectra of MgO NPs and Fe₂O₃ NPs in DMSO solvent

showed a reduction in the intensity of peaks compared to that of the pure extract (see Table 2). This may result from the dissolving of organic components of the NPs in the organic DMSO solvent. Upon dissolving both oxides NPs with toluene, the intensity of peaks reduced more than that of DMSO. Over the third week of incubation, all functional groups disappeared, which perhaps means complete dissolution. In ethanol, the FTIR spectra showed a mild reduction in the intensities of peaks after 2 weeks of incubation for both MO NPs which could indicate good stability in ethanol. The stability in DMSO was moderate over time while the stability in toluene was very poor, Table 2.

X-ray diffraction

Magnesium oxide NPs

The powder sample of MgO NPs was analyzed by a Cu K α X-ray diffractometer to confirm the structure of MgO NPs. Figure 2a shows peaks at 2θ values mainly of 42.87° and 62.35° which were identified in (2 0 0) and (2 2 0) planes and matched the crystalline plane of MgO NPs. The intensities and positions of diffraction peaks confirmed a cubic lattice. The major peak appeared at 42.9 corresponding to (200) planes, related to the MgO particle phase with a cubic structure in agreement with a previous study [68]. The broad peak from 10° to 20° 2θ was due to the presence of organic materials from leaf extract which are responsible for the surfactant from small nanoparticles. The major peak of 42.87° , reflected the plane (2 0 0), was chosen to calculate the average crystalline size, which found to be 20.62 nm [69] calculated by Debye–Scherrer Eq. (1) [70]:

$$D = (K \times \lambda) / \beta \cos \theta \quad (1)$$

where K value = 0.94 (shape factor for the spherical crystallites with cubic symmetry), $\lambda = 1.584 \text{ \AA}$, β = full-width half maximum in peak radians, and θ = Bragg's angle.

Iron oxide NPs

The crystal structure and phases of the Fe₂O₃ NPs were analyzed in the XRD pattern shown in Fig. 2b. The crystalline structure was characterized using XRD (Rigaku t III) with Cu-K α radiation (1.500 $\text{\AA}$, 40 kV, 30 mA). The particle size distributions were measured by SZ-100 nanoparticles (Hiroba USA). The XRD pattern of Fe₂O₃ NPs showed distinctive diffraction peaks corresponding to the amorphous nature of those particles [71]. The XRD pattern was at 2θ values of 18.97° (100), 29.81° (111), 35.24° (200), 39.53° (210), and 48.30° (211). The peaks confirmed the crystalline inherent. The planes at (111), (200), and (220) corresponded to the face-centered cubic nature of Fe₂O₃ [62]. Based on

that, XRD patterns indicated that the Fe₂O₃ was formed by the reduction of metal ions found in the extract. The calculated particle size of the Fe₂O₃ NPs was 45.09 nm, using Scherrer's formula in Eq. (2):

$$\text{Crystal size} = K\lambda / \beta \cos \theta \quad (2)$$

where K : shape factor (0.94), λ : the incident wavelength of Cu–K α radiation $n = 0.154252 \text{ nm}$, β : full-width half maximum (radian), Bragg's angle (radian).

Scanning electron microscopy (SEM) and atomic force microscopy (AFM)

The SEM images in Fig. 3 indicate aggregation of spherical structures resulting in moderately dispersed MgO NPs with smooth surfaces and non-uniform shapes. The MgO NPs were observed at different sizes between 30 and 100 nm. These results agreed with other previous bio-prepared MgO NPs from different sources [68, 69, 72], Fig. 3a. SEM image of Fe₂O₃ NPs in Fig. 3b shows a mix of fine and cluster spherical structures, Fig. 3b. Agglomeration of the particles could have resulted from heat exposure during the preparation process.

Energy dispersive X-ray analysis (EDX)

The EDX analysis was utilized to indicate the elemental composition of the biosynthesized MgO NPs [73]. The quality of the synthesized NPs is associated with the existence of significant peaks in the EDX spectrum, specifically related to the prepared compounds. MgO and Fe₂O₃ NPs were pure samples, consisting of Mg, Fe, and O and no other elements. The ratio of Mg/O and Fe/O elements was measured as shown in Table 3.

Figure 3 shows AFM images, granularity distribution, and SEM images of MgO, Fe₂O₃ NPs, and *Hibiscus sabdariffa* L. The topographical images of the prepared MgO showed aggregation of nano spherical particles Fe₂O₃ revealing a folded mountain-like structure. The average length of the rock structure was 400 to 630 nm. It may be due to the metal bindings or the deposition of oxide layers. SEM images of MO NPs (a and b) indicated aggregation of spherical-like nanoparticles compared to the unshape surface of *Hibiscus sabdariffa* L. in (c).

Zeta potential (ζ -potential)

Z-potential measurement is based on the movement of nanoparticles under the influence of an electric field, according to the charge and environment. NPs with high negative or positive ζ -potential values would never aggregate due to the electrostatic force of repulsion, while particles with

Table 2 Analysis of FTIR spectra for the functional groups in the plant extract, Fe₂O₃ NPs, and MgO NPs after incubation with organic solvent for 3 weeks of course study

Metal oxides (time)	Solvent	Band cm ⁻¹	Functional groups
Plant extract		3271	Amide I (N–H stretching in resonance with amide II overtone)
		2125	Terminal alkyne (C≡C stretch)
		1635	Amide (C=O stretch)
		968	Choline asymmetric stretch
		659	Amide IV (mainly O=C–N deformation)
		605	Cyanidin-3-sophoroside [203]
		578	
Fe ₂ O ₃ and MgO NPs (1st week)	DMSO	3300	Amide I (N–H stretching)
		1650	Amide (C=O stretch)
		900–1050	Choline asymmetric stretch
		500, 600	Cyanidin-3-sophoroside
Fe ₂ O ₃ and MgO NPs (2nd week)	DMSO	330	Amide I (N–H stretching)
		1650	Amide (C=O stretch)
		1000	Choline asymmetric stretch
		500	Cyanidin-3-sophoroside
Fe ₂ O ₃ and MgO NPs (3rd week)	DMSO	3300	Amide I (N–H stretching in resonance with amide II overtone)
		3050	Amide II
		2900	Asymmetric CH ₂ stretching
		1650	Amide (C=O stretch)
		1400, 1500	Amide II (N–H bend in plane and C–N stretch)
		1050	Choline asymmetric stretch
		650	Amide IV (mainly O=C–N deformation)
Fe ₂ O ₃ and MgO NPs (1st week)	Toluene	3300	Amide I (N–H stretching)
		1650	Amide (C=O stretch)
		500	Cyanidin-3-sophoroside
Fe ₂ O ₃ and MgO NPs (2nd week)	Toluene	3300	Amide I (N–H stretching)
		1700	Amide (C=O stretch)
		1400, 1500	Amide II (N–H bend in plane and C–N stretch)
		900	Choline asymmetric stretch
Fe ₂ O ₃ and MgO NPs (3rd week)	Toluene	–	–
Fe ₂ O ₃ and MgO NPs (1st week)	Ethanol	3300	Amide I (N–H stretching)
		2100	Terminal alkyne (C≡C stretch)
		1650	Amide (C=O stretch)
		500	Cyanidin-3-sophoroside
Fe ₂ O ₃ and MgO NPs (2nd week)	Ethanol	3300	Amide I (N–H stretching)
		3000	Asymmetric CH ₂ stretching
		1650	Amide (C=O stretch)
		1450	Amide II (N–H bend in plane and C–N stretch)
		1100	C–O stretch
		950	Choline asymmetric stretch
		500	Cyanidin-3-sophoroside
Fe ₂ O ₃ and MgO NPs (3rd week)	Ethanol	3300	Amide I (N–H stretching)
		2950	Asymmetric CH ₂ stretching
		1650	Amide (C=O stretch)
		1450	Amide II (N–H bend in plane and C–N stretch)
		1100	C–O stretch
		950	Choline asymmetric stretch
		500	Cyanidin-3-sophoroside

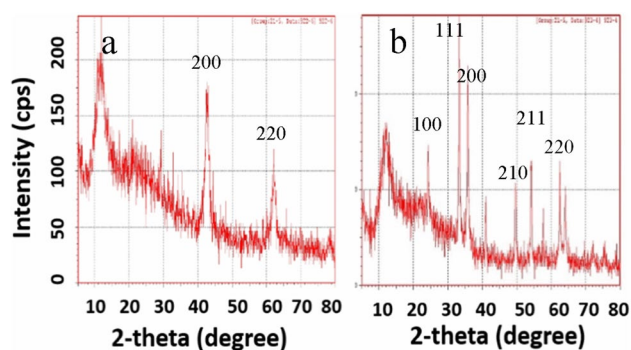


Fig. 2 The XRD patterns of MO NPs. **a** MgO NPs, **b** Fe₂O₃ NPs

low ζ -potential values tend to flocculate. Herein, MgO NPs possessed a high negative ζ -potential of -19.8 mv (results not shown), which indicated that the synthesized MgO

NPs were highly stable and prevented agglomeration due to strong electrostatic repulsive forces between them [61].

MgO NPs and osteoporosis

The known MO NPs which were synthesized by physico-chemical strategies, such as ZnO, CuO, CoO, NiO, and Cr₂O₃, were reported with potential carcinogenicity in addition to their environmentally toxic effect due to the hazardous reducing agents, such as organic solvents that used for synthesis [75, 76]. Therefore, the clinical and bioapplications of MO NPs are limited. This study has rationally designed MO NPs fabricated from green technology as an alternative method to reduce the harmful effects. For biosynthesis of functional MO NPs, medicinal plant components were highly recommended due to enriched stored phytochemicals. Probably, the therapeutic potency comes from those phytochemicals, which eliminate the toxic effects of the MO NPs

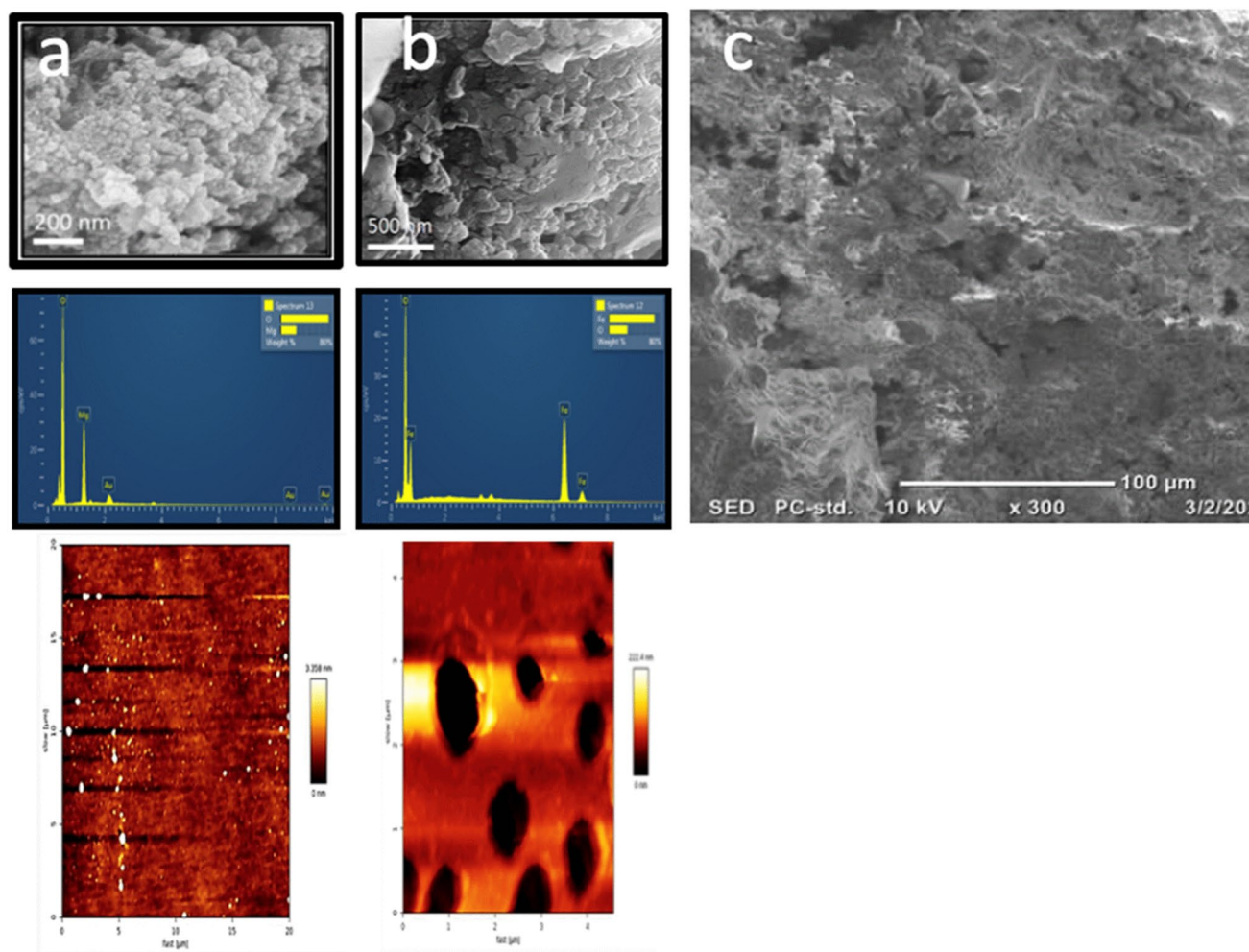


Fig. 3 SEM, topography images, and EDX elemental distribution of the synthesized metal oxides. **a** MgO NPs, **b** Fe₂O₃ NPs. **c** SEM image of *Hibiscus sabdariffa* L., which was reproduced from Ref. [74]

Table 3 EDX data of MgO and Fe₂O₃ NPs

Element	Apparent concentration	k ratio	Wt%	Wt% sigma	Atomic %
O in MgO sample	21.74	0.07314	75.40	0.16	82.32
Mg	3.57	0.02364	24.60	0.16	17.68
O in Fe ₂ O ₃ sample	13.92	0.04683	28.83	0.19	58.58
Fe	18.80	0.18804	71.17	0.19	41.42

through several unique properties, such as magnetic, physical, chemical, thermal, electrical, and optical properties [77]. Thus, many medicinal plants have been used for biosyntheses, such as *Amarantus blitum*, *Lawsonia inermis*, *Lime* peel extract, *Artemisia anua* hairy root, *Piper nigrum*, *Tragacanth gum*, *Opuntia dileni haw*, *Boswellia carteri resin*, *Limonia acidissima*, *Moringa oleifera*, and sugar cane juice. *Hibiscus sabdariffa* L. extract has been extensively utilized in traditional medicine to improve lifestyle and treat infectious diseases, however, is not validated. On the other hand, MgO

NPs were extensively studied due to their antimicrobial activity. Herein, MgO NPs were synthesized using *Hibiscus sabdariffa* L. extract and evaluated the in vitro effects on the main parameters disturbed in osteoporosis model systems (vitamin D3, Ca²⁺, and ALP). The mean age of the selected osteoporosis patients (37.05 years) was compatible with that of the control group (41.32 years). The BMI values of patients (29.74 kg/m²) were non-significantly different from that of the control group (40.44 kg/m²), ($p > 0.01$). Before in vitro adding the MgO NPs, osteoporosis patients suffered lower serum levels of D³ and Ca²⁺ compared to the control, ($p < 0.0001$), Fig. 4. After adding the MgO NPs (2:1, serum:MgO), a significant elevation of D³ and Ca²⁺ was observed for both patients and controls ($p < 0.0001$ and $0 < 0.001$, respectively), Fig. 4a and b and Table 4. D³ of osteoporosis patients significantly increased after treatment by MgO NPs up to 9.82 ± 1.49 ng/dL compared to that before treatment 8.41 ± 0.48 ng/dL, ($p < 0.0001$) which agreed with another work [78], Fig. 4a and Table 4. Furthermore, the level of Ca²⁺ significantly increased for a patient up to 4.69 ± 0.40 mg/dL compared to that before treatment 3.71 ± 0.41 mg/dL, ($p < 0.0001$), which agreed with another

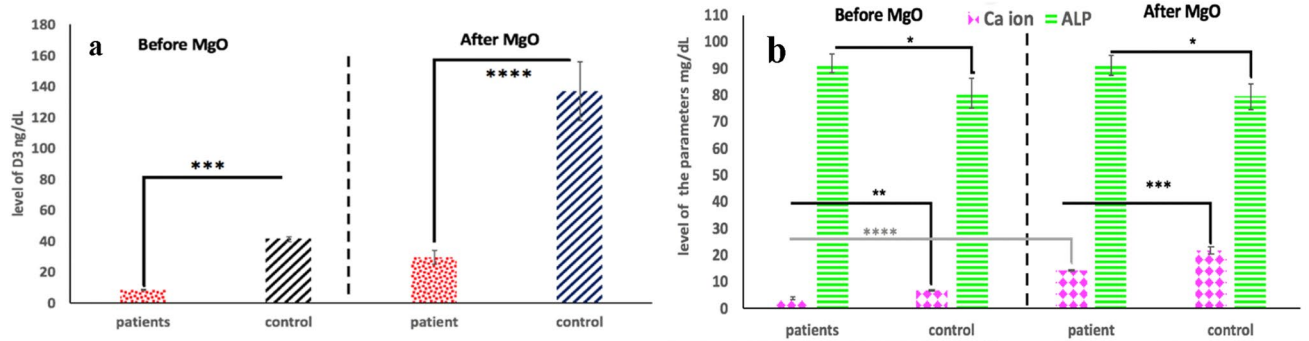

Fig. 4 Levels of Ca and ALP in sera of osteoporosis patients and control before and after adding MgO NPs. **a** Levels of vitamin D3, **b** levels of Ca²⁺ and ALP. Error bars represent standard deviation at a confidence level of 95%

Table 4 Effect of MgO NPs on serum levels of vitamin D3, ALP, and Ca for patients and healthy controls

Parameters	Before adding MgO Mean $\pm$ SD		After adding MgO Mean $\pm$ SD	
	Patients	Control	Patients	Control
D3 (ng/dL)	$8.49 \pm 0.47 \alpha \xi$	$41.52 \pm 1.52 \xi^{***}$	$29.46 \pm 4.47 \alpha^{****}$	$137 \pm 19.11^{****}$
Ca ²⁺ (mg/dL)	$3.70 \pm 0.44 \alpha \xi$	$6.64 \pm 0.48 \xi^{**}$	$14.07 \pm 0.43 \alpha^{***}$	$21.63 \pm 1.38^{****}$
ALP (mg/dL)	91.89 ± 3.68	$80.56 \pm 5.42 \alpha^{ns}$	$91.00 \pm 3.54 \xi^*$	$79.36 \pm 4.72 \alpha^{ns \xi}$

Values were calculated before and after treatment with MgO NPs under standard conditions. The standard deviation (SD) of each mean value was included

**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$, ns non-significant. Ξ = comparison between patients and control before addition, α = comparison between patients and control after addition, $\alpha \xi$ = comparisons between patients before and after addition

work [79]. The fundamental function of the ALP enzyme is to transfer the metabolites across the cell membrane, and therefore a high level of this enzyme suggests liver failure and bone homeostasis [80]. Interestingly, MgO NPs did normalize the studied biochemical parameters, perhaps through free-radical-quenching potential as previously suggested [81, 82], Fig. 4b and Table 4. Above all, the biosynthesized MgO NPs did not previously show any toxic, hemorrhage, or inflammatory effects on lab animal models, in vivo [68, 81], which supported this work by the nontoxic nature imparted from the phytochemicals of *medical plant* extracts.

Figure 5 reveals a single significant correlation between vitamin D3 and ALP after adding MgO NPs (0.43, $p < 0.02$). This probably means that increasing the level of vitamin D3 after adding MgO NPs has improved the activity of ALP, Fig. 5.

Molecular docking results of MgO and Fe₂O₃ with the human alkaline phosphatase target

Molecular docking calculations have been used extensively in drug design in recent years. It is done to predict the interaction between the determined target region and the functional groups in drug content. Many interactions and binding energies such as π - π hydrogen bonds, π -alkyl, π -sulfur, T-shaped, π -lone pair, van der Waals were obtained from this analysis. The presence of hydrogen bonds and the lowest negative binding energy (score) reveal important drug-protein interactions. The binding energies (ΔG) of Fe₂O₃ and MgO with the human alkaline phosphatase enzyme are -4.2, and -2.4 kcal/mol, respectively. According to these results obtained, Fe₂O₃ has a higher binding affinity. MgO interacts with the active site of the human alkaline phosphatase

Fig. 5 Pearson correlations between the studied parameters before and after adding MgO NPs. **a** Heat map of all correlations before adding MgO NPs, **b** heat map of all correlations after adding MgO NPs. **c** Correlation matrix between the parameters (ALP vs D³, and ALP vs Ca²⁺) before adding MgO NPs. The correlations were non-significant at $p < 0.05$, confidence intervals 95%, **d** Correlation matrix between parameters after adding MgO NPs (ALP vs D³, and ALP vs Ca²⁺), * $p < 0.02$

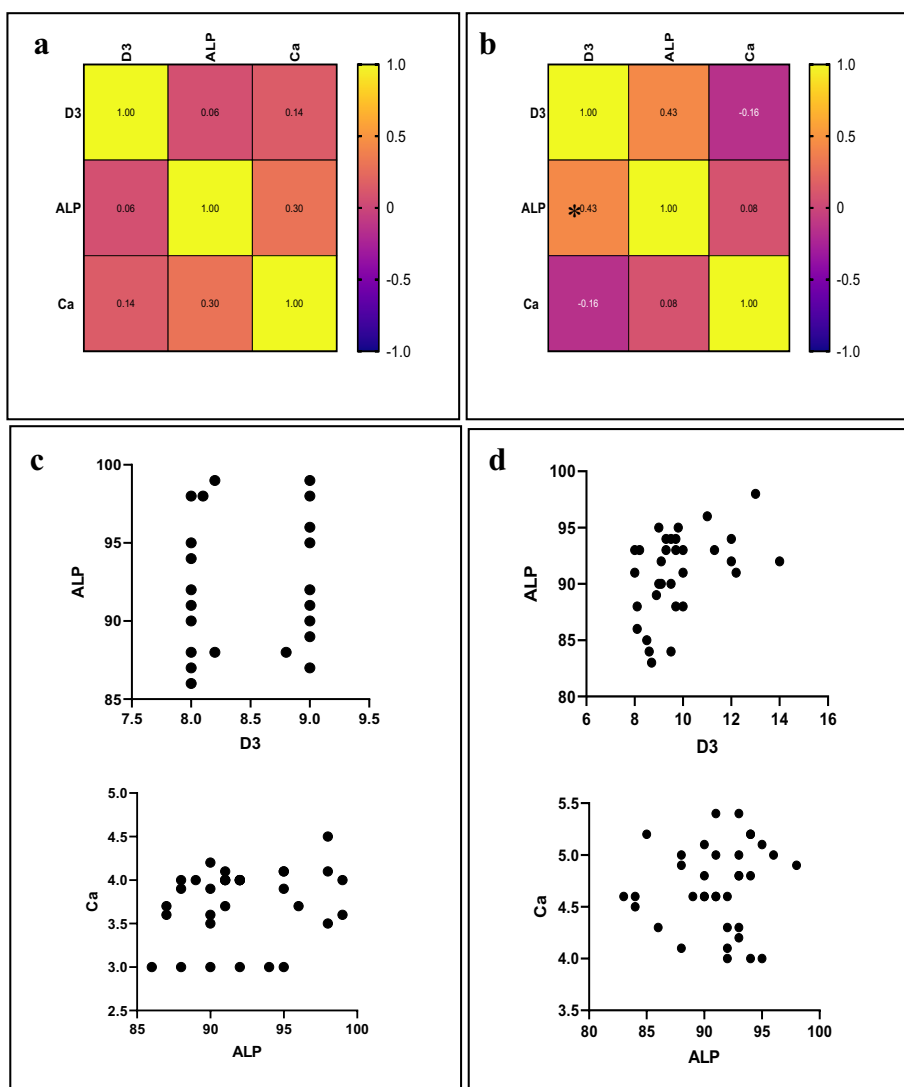


Fig. 6 Two-dimensional view of the molecular docking of MgO at the active site of the human alkaline phosphatase enzyme (a), and hydrogen bond interactions between MgO and the active site of the human alkaline phosphatase enzyme (b)

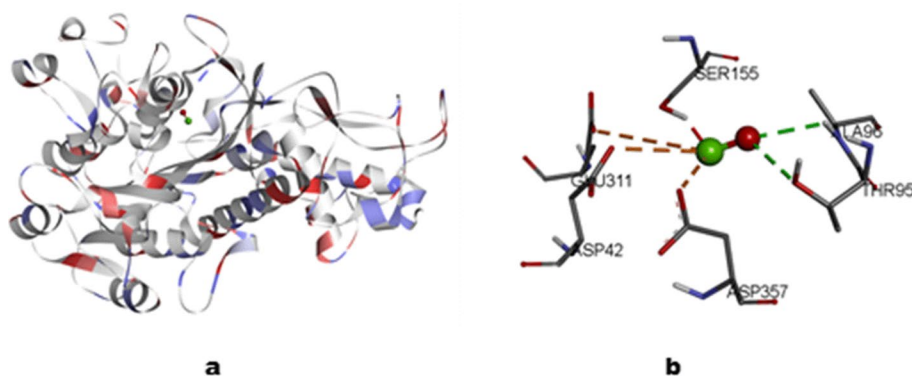
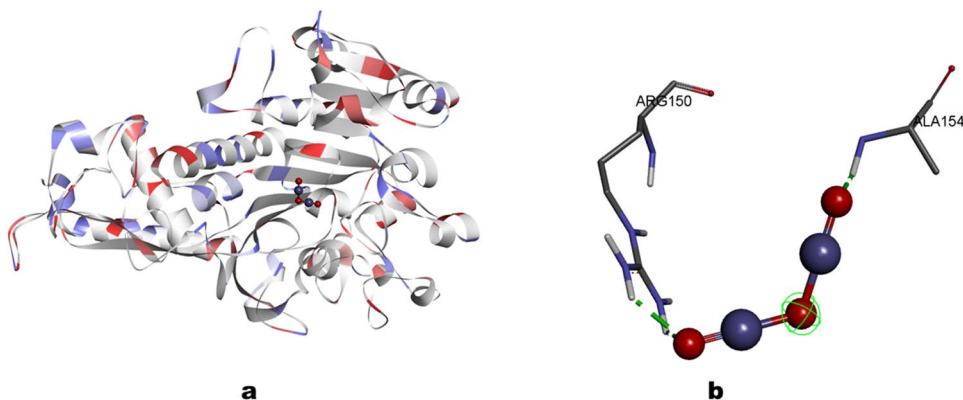


Fig. 7 Two-dimensional view of the molecular docking of Fe_2O_3 at the active site of the human alkaline phosphatase enzyme (a), and hydrogen bond interactions between Fe_2O_3 and the active site of the human alkaline phosphatase enzyme (b)



enzyme via the attractive charge interactions with asp42 and glu311 amino acid residues, attractive charge; metal receptor interactions with asp357 amino acid residue, and conventional hydrogen bond with thr95 (bond distance = 2.88 Å) and ala96 (bond distance = 2.81 Å) (Fig. 6). Fe_2O_3 binds with the human alkaline phosphatase enzyme through the conventional hydrogen bond with agr150 amino acid residue (bond distance = 2.34 Å) and ala154 amino acid residue (bond distance = 2.13 Å) (Fig. 7). Although the interaction type between MgO and the human alkaline phosphatase enzyme is high, the interactions between Fe_2O_3 and the human alkaline phosphatase enzyme are thought to be stronger when the binding affinity and hydrogen bond distances are taken into account.

Conclusion

From the results of the study, we could conclude that the local extract of *Hibiscus sabdariffa* L. is a good reducing potent in the preparation of iron oxide and magnesium

oxide at nanoscales by low-cost and eco-friendly methods. The prepared iron oxide NPs were $\alpha\text{-Fe}_2\text{O}_3$ NPs with a rhombohedral structure, while MgO NPs were cubic crystals with an average size of 18 nm. In vitro, MgO NPs have a significant effect on vitamin D3 and Ca^{2+} levels in the serum of osteoporosis patients (35 patients 35–55 years old), compared to the control group. Thus, this study argued that MgO NPs have a therapeutic potentiality for bone defect as the serum levels of vitamin D3 and Ca^{2+} were significantly elevated post-addition. Consistent with our results, molecular docking studies show that these metal oxide NPs interact with the human alkaline phosphatase enzyme. Nevertheless, MgO NPs have shown better results than Fe_2O_3 NPs because it has more extensive H-bonding. However, further investigations in different in vitro and in vivo models are especially recommended to understand the real potential of those nano oxides before they could be suggested for the benefit of humankind.

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Authors' contribution A-G and A-Q have equally contributed to designing the study. A-G has done the clinical and statistical analyses and wrote and edited the manuscript. A-S has performed all the experiments. Ö and N have designed, performed, and wrote the computational chemistry part of the study and revised the manuscript.

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Data availability This published article includes all data produced or analyzed during this study.

Declarations

Conflict of interests The authors declare no conflict of interest.

Ethics approval and consent to participate This study was registered and approved in Mustansiriyah University according to registration no PG 23/01/2021. It has been considered by the scientific and ethical committee at Mustansiriyah University. It meets the requirements of the Helsinki Declaration. All authors gained permission from each participant to do an in vitro application.

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