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ORIGINAL ARTICLE

Green synthesis and study of structural properties of Copper nanocrystallites from hawthorn plant extract and study of its antibacterial activities

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Abstract

Recently, the synthesis of metal nanocrystallites using plant extracts has received much attention due to its low cost and compatibility with the environment. In this research, copper nanocrystallites were synthesized using hawthorn extract. The formation of copper nanocrystallites was observed by changing the color of the solution. Synthesized copper nanocrystallites were investigated by techniques such as: Ultraviolet-Visible Spectroscopy (UV-Vis), Scanning Electron Microscope (SEM), Fourier Transform-Infrared Spectroscopy (FTIR), Energy Dispersion Spectroscopy (EDX) and X-ray Diffraction Spectroscopy (XRD). In the UV-Vis spectrum, a dominant absorption peak at 552 nm wavelength was observed, which was related to copper nanocrystallites. In the images taken from SEM, it was found that the nanocrystallites are nearly spherical in shape and their average size on the surface of the sample was about 61-85 nm. Parallerly, in the investigation of antibacterial properties, synthesized nanocrystallites showed better antibacterial activity against Gram-negative bacteria (*Escherichia coli*) respect to other bacteria in the present work. The obtained results show that hawthorn extract can be used for the synthesis of copper nanocrystallites.

Keywords: *Antibacterial; Copper Nanocrystallites; Green Synthesis; Plant Extract; X-ray Diffraction Spectroscopy (XRD).*

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INTRODUCTION

Nanotechnology has been one of the important research areas during the last few decades. This technology has been used in all fields such as pharmaceuticals, textiles, petrochemical industries, health care, food, biomedical sciences, mirror and glass industries, agriculture and in medicine to treat infections and incurable diseases [1].

Among metal nanoparticles such as copper, gold, silver, etc., copper nanoparticles are more popular due to their lower price than gold and silver, low dimension, high surface area, good conductivity and the unique antibacterial it has attracted the attention of researchers [2-7].

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For the synthesis of metal nanoparticles, there are various physical and chemical methods such as: chemical reduction, sol-gel, cello thermal, hydrothermal, frictional erosion, laser abrasion and other such cases, due to the use of energy, temperature and high pressure, the products They are very expensive, toxic and dangerous, which lead to environmental problems. Therefore, researchers are looking for an alternative method to prepare metal nanoparticles [8-11].

Since, plants have attracted the attention of many researchers due to their abundance and availability and due to their unique healing and antioxidant properties. They can be used as a huge source and a suitable alternative to toxic chemicals and dangerous in different areas of

nanotechnology [12-16]. So far, the biological synthesis of nanoparticles has been done using different biological materials such as bacteria, viruses, fungi, and yeasts [17].

In recent years, the synthesis of nanoparticles using plant extracts has attracted a lot of attention under the name of biosynthesis (green). Green synthesis is a simple, cost-effective, non-toxic and environmentally friendly method due to the use of plant extracts. For the synthesis of nanoparticles, different plant parts such as stem, root, flower, fruit and leaf have been used [18].

Low cost, abundance and easy access to plant resources, especially fruits and plants that grow in forests and pastures, can be considered as a good source for green synthesis. Therefore, it is good to know these fruits and plants and use them for the synthesis of metal nanoparticles.

Hawthorn is the name of a plant of the genus *Crataegus* and family *Rosaceae*, which is distributed in Asia, Europe and North America, and is abundantly found in different regions of Iran, especially in the north of the country, with the local name of Velik. Hawthorn is a plant whose maximum height reaches about 3 meters and the diameter of its stem rarely exceeds 15 cm. Due to the presence of chemical compounds such as: flavonoids, proanthocyanins and organic acids, this plant is very useful in the treatment of various diseases such as: cancer, cardiovascular diseases, Alzheimer's and diabetes [19-21].

In recent years, several researches have been presented on the green synthesis of silver, gold and copper nanoparticles from the extracts of different plants and their antibacterial effect [22-29]. In this work, for the first time, the synthesis of copper nanocrystals from hawthorn extract of *Crataegus pseudoheterophylla*, which is an abundant, available and affordable plant, has been used, and UV-Vis, FTIR, EDX and SEM techniques confirm the presence of these nanocrystals. Also, the antibacterial effects of copper nanocrystals synthesized from hawthorn extract were investigated.

MATERIALS AND METHODS

Collection of hawthorn

Hawthorn fruit was collected from the forests of Noor city in northern Iran (Mazandaran province) in November 1400. This hawthorn belongs to the species *Crataegus pseudoheterophylla*.

Chemicals

For the synthesis of nanocrystals, Copper sulphate ($CuSO_4.5H_2O$) from Merck German company was used with a high degree of purity. PH adjustment was done with sodium hydroxide (NaOH), and also, the solvent used to make all the solutions were deionized water.

Preparation of hawthorn aqueous extract

First, the hawthorn was washed with tap water and deionized water, dried in the shade and away from sunlight, and powdered with a home grinder. 10 g of hawthorn powder was added to 100 ml of deionized water and placed on a magnetic stirrer at 60 °C for 40 minutes. Then the extract was cooled for 5 minutes at room temperature and passed through a filter paper, and the filtered extract was stored at 4 °C for later use.

Synthesis of Copper Nanocrystallites

To prepare copper nano crystallites, different concentrations of copper sulfate solution are used, which will have different results. In this research, 0.25 g of copper sulfate of 0.01 M was mixed with 100 ml of deionized water. Then 20 ml of hawthorn extract was added drop by drop and the pH of the reaction mixture was adjusted to 11 using some sodium hydroxide and with continuous stirring, a change in the color of the solution was observed, which indicated the formation of copper nano crystallites. Finally, the formed sediments were centrifuged and washed 3 times with deionized water. Finally, the sediments were dried in an oven at 60 °C for 24 hours.

Characterization Techniques

Cu nanocrystallites synthesized with hawthorn extract were characterized by using some relevant techniques: spectroscopic UV-Vis (Thermo Company, Biomate5 model) methods for finding the metallic name (here, it reveals it is a Cu nano crystallite, see follow section for more details). Furthermore, size and shape of nanocrystallites were examined using scanning electron microscopy (SEM) (TESCAN Company, MIRA III model). Also, in order to distinguish the percentage of element in the present sample, EDX machine model DXP-X10P was used. The functional groups attached to the nanocrystallites surface were detected using an FTIR spectroscope (Thermo Company, AVATAR model), where the

spectra were recorded from 4000 to 400 cm^{-1}. The structure of Cu nanocrystallites was determined by x-ray diffraction (XRD) analysis (PHILIPS Company, PW1730 model).

Antibacterial activity of synthesized

Antibacterial activity of copper nanocrystals was investigated using agar disk diffusion method. Nanocrystals were tested against two bacteria *Staphylococcus aureus* (*Staphylococcus aureus*, Gram positive bacteria), *Escherichia coli* (*E. Coli*, Gram negative bacteria). The cultured bacteria were uniformly spread on the surface of the plate and the desired samples (copper nanocrystals) with concentrations of (12.5, 25 and 50 µg/µL) were poured onto sterilized 6 mm discs. It immediately after that was incubated at 37 °C for 24 hours. Then incubation the inhibition zone was measured. For more accuracy, the experiment was repeated several times (3 times) and the best results were recorded.

RESULTS AND DISCUSSION

UV-Visible spectroscopic analysis

The surface plasmon resonance property of nanocrystallites was monitored by an UV-Vis spectroscopy. Copper nanocrystals have a plasmonic resonance band in the wavelength range of 450-650 nm. Fig. 1 shows the results of ultraviolet-visible spectroscopy of nano crystallites synthesized from hawthorn extract. As shown in Fig. 1, nano crystallites synthesized from hawthorn extract have a dominant absorption peak at the wavelength of 552 nm. The presence of a dominant absorption peak indicates that the geometric structure of copper nano crystallites synthesized from hawthorn extract is nearly spherical, and surface plasmon have a main role in this regard (for more details about crystallite's shape, see our pervious works in Refs [111-222].

Scanning electron microscopy

Scanning Electron Microscope (SEM) is an advanced tool for studying and checking the morphology structure and determining the size of nano crystals. Fig. 2 shows the FESEM image of the nanocrystal synthesized with hawthorn extract at a scale of 500 nm. As shown in Fig·2a, copper nanocrystals synthesized with hawthorn extract are nearly spherical and the size of nanocrystals is about 61-85 nm. Also, according to Fig. 2b, the average size of nanocrystals is approximately 226.54 nm.

FT-IR spectroscopic analysis

Fourier transform-infrared spectroscopy is one of the unique techniques in qualitative and quantitative measurement of different molecular

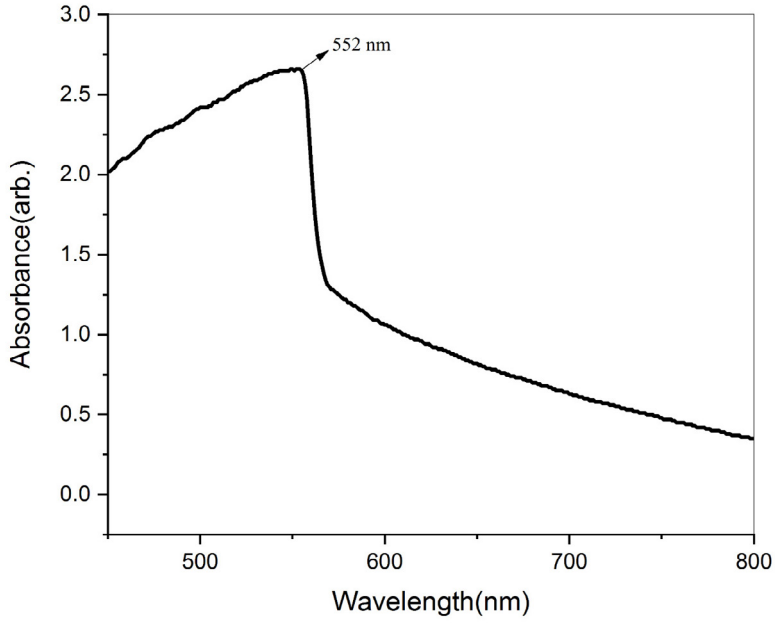

Fig. 1. UV-Visible spectrum of copper nanocrystallites synthesized with hawthorn extract.

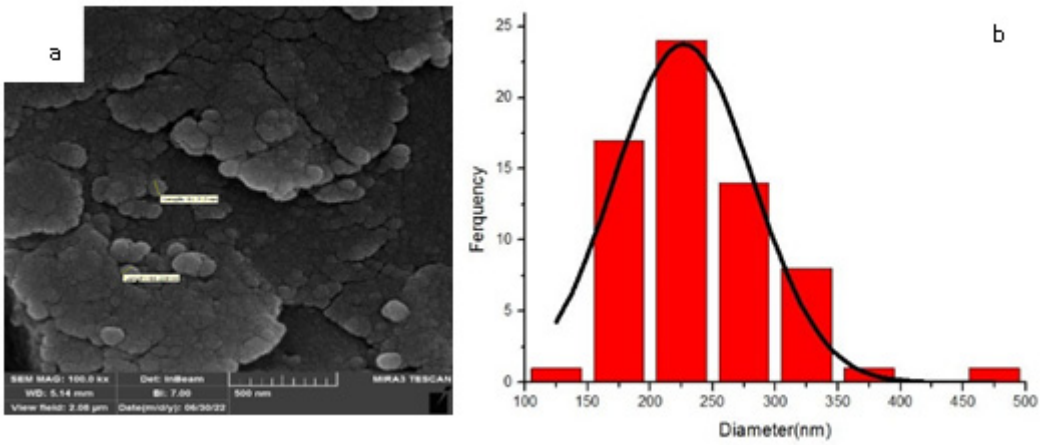

Fig. 2. a) FESEM image of copper nanocrystallites synthesized with hawthorn extract. b) Size distribution of nano crystallites synthesized with hawthorn extract.

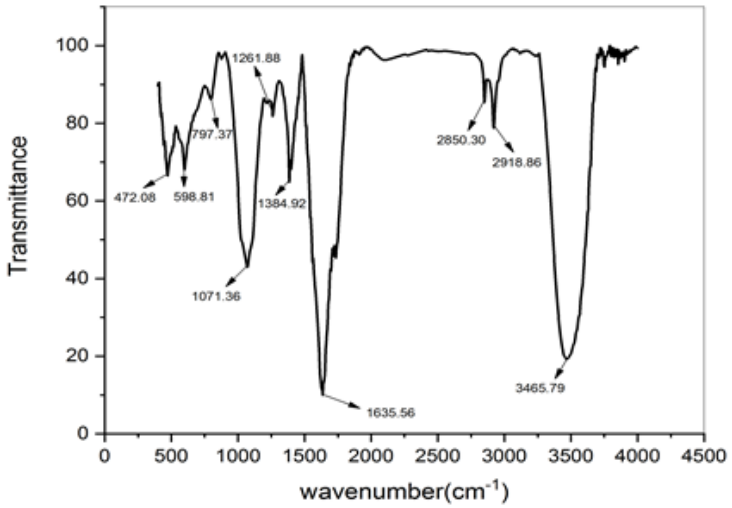

Fig. 3. FTIR spectrum of nanocrystallites synthesized with hawthorn extract.

species. Fig. 3 shows the FTIR spectrum of copper nanocrystallites synthesized with hawthorn extract. The peak at 3465.79 cm^{-1} is related to the vibrations of the O-H functional group (alcohols and phenols), the peaks at 2918.86 cm^{-1}, 2850.30 cm^{-1} and 1384.92 cm^{-1} are related to the vibrations of the functional group C-H, 1635.56 cm^{-1} and 797.37 cm^{-1} peaks correspond to C=C functional group vibrations, 1261.88 cm^{-1} and 1071.36 cm^{-1} peaks correspond to C-O functional group vibrations. Also, the peaks at 598/181 cm^{-1} and 472/08 cm^{-1} are related to copper nanocrystallites and vibrations between copper and oxygen.

Energy dispersive X-ray spectrometry

This analysis is used to determine the percentage of elements in the sample. Figure 4 shows the EDX energy dispersive spectroscopy for copper nanocrystallites synthesized with hawthorn extract. Copper nanocrystallites have an absorption peak at 1 Kev, which is an indicator for the presence of copper nanocrystallites, according to Fig. 4, the presence of copper nanocrystallites with a weight percentage of 20% has been confirmed. Also, other peaks can be observed in the spectrum, which can be caused by physical absorption during sample preparation or by contamination of the net of the device.

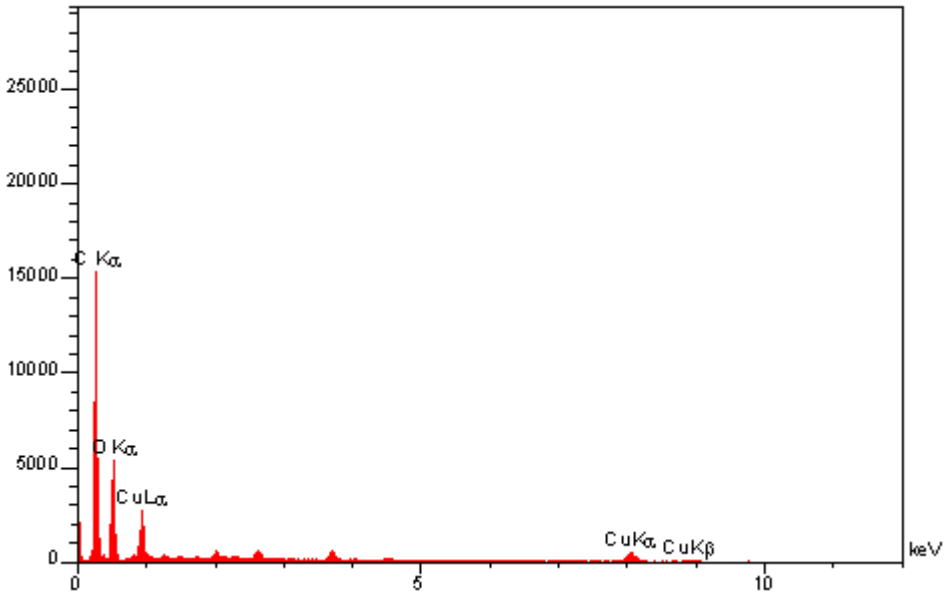

Fig. 4. EDX spectrum of copper nanocrystallites synthesized with hawthorn extract.

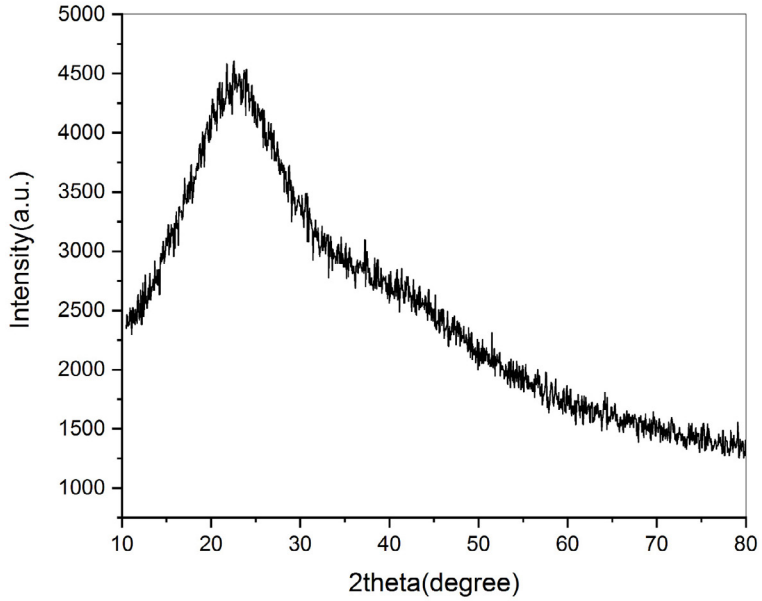

Fig. 5. XRD pattern of copper nanocrystallites synthesized with of hawthorn extract.

In order to identify the crystalline phase of synthesized copper nanocrystals, X-ray diffraction analysis (XRD) was performed (Fig. 5). The results show that the formed nanocrystals have an amorphous structure.

Antibacterial Activity

The antibacterial activity of copper nanocrystals synthesized with hawthorn extract was investigated against various pathogenic organisms such as Staphylococcus aureus (+) and Escherichia coli (-). The diameter of the inhibitory zones (mm) around each disk with the solution of copper nanocrystals with different amounts is shown in Table 1. Copper nanocrystals synthesized from hawthorn extract with a concentration of 50

Table 1. Antibacterial Activity of Synthesized Cu nanocrystallites with hawthorn extract.

Concentration of Nanocrystallites (µg/µl)	Zones of inhibition in millimeters (mm)	
	E. coli (-)	*S. aureus* (+)
50	3	-
25	1	-
12.5	-	-

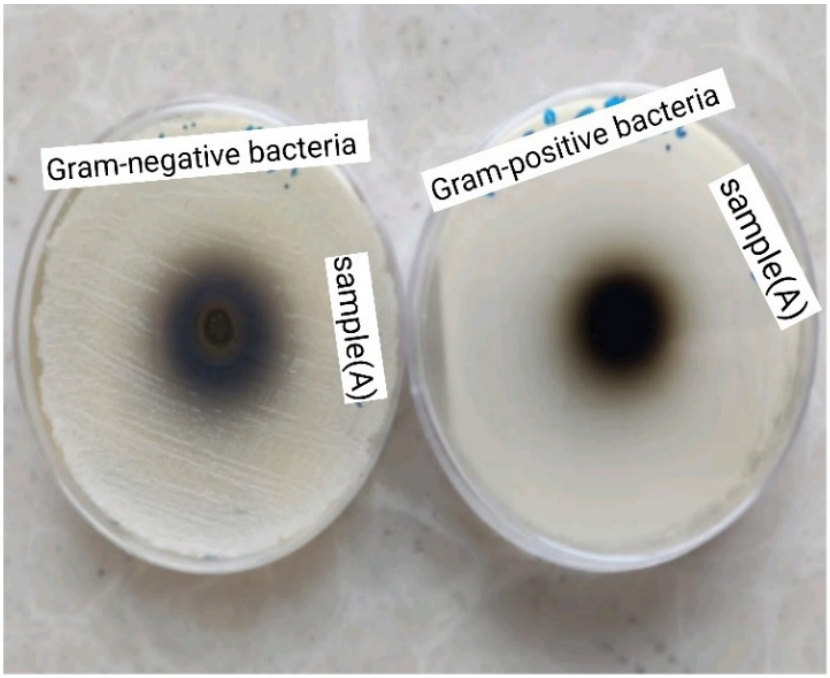

Fig. 6. Antibacterial activity of copper nanocrystals synthesized from hawthorn extract with a concentration of 50 µg/µL.

µg/µL showed good inhibition against Escherichia coli (Fig. 6). Its antibacterial activity is due to the fact that copper nanocrystals synthesized with hawthorn extract can penetrate the bacterial cell membrane and disrupt the structure of the cell membrane and prevent their proliferation and growth.

CONCLUSION

The present study demonstrates advantageous and green method for the synthesized of copper nanocrystallites. The UV-visible spectra, XRD pattern and FTIR spectra substantiated the formation of cu nanocrystallites in the presence of biomolecules (tannins, flavonoids and proanthocyanins) of hawthorn extract. The absorbance maxima λ_{max} of 552 nm was confirmed by the formation of Cu nanocrystallites. SEM analysis showed the size of the nanocrystals to be around 61-85 nm. Nanocrystals synthesized from hawthorn extract showed better antibacterial activity against Escherichia coli bacteria, and it can be concluded that copper nanocrystal synthesized from plant extracts have the ability to kill the disease-causing bacterial strains.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest.

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