RESEARCH ARTICLE

Synthesis and evaluation of antibacterial properties of green copper oxide nanoparticles from Hypericum perforatum plant extract and Marrubium Vulgare

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ABSTRACT

In the present study, copper oxide nanoparticles were synthesized using Hypericum perforatum plant extract from the Malpican family and Marrubium Vulgare plant from the mint family. Since it is believed that the antioxidants in the plant reduce the reduction of metal ions to nanoparticles as reducing agents, these two plants were tested for their antioxidant properties by the free radical scavenging method, and the IC_{50} quantity was measured. Hypericum perforatum plant with IC_{50} equivalent to 0.413 had more antioxidant content than Marrubium Vulgare plant with IC_{50} equivalent to 1.562, so it was superior in the process of green synthesis. The properties of the synthesized nanoparticles were analyzed using X-ray diffraction (XRD), Scanning electron microscope (SEM), and Ultraviolet-visible (UV-Visible). The presence of a metal-oxygen bond was confirmed by Fourier-transform infrared spectroscopy (FT-IR). X-ray energy diffraction spectra showed the purity of the synthesized nanoparticles. The synthesized nanoparticles were observed with spherical morphology and size distribution of 30 to 40 nm and with uniform size distribution. The results of the XRD spectrum showed that pH adjustment did not affect the synthesis of copper oxide nanoparticles. The nanoparticles synthesized against the two bacteria used in present study did not show significant antibacterial properties compared to industrial antibiotics.

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INTRODUCTION

Undoubtedly, the use of medicinal plants is the oldest human approach to treating diseases and there has always been a close relationship between man and plant [1–3]. Therefore, plants can be considered as a source of potentially useful chemicals, which can be considered not only as medicine but also as a unique model for the production of natural alternative chemicals [4,5]. In recent decades, the preparation and study of nanoparticles have attracted the attention of scientists in various fields of basic and applied sciences [6,7]. Nanotechnology means the study and development of materials at the atomic, molecular, and macromolecular scales, which leads to the manipulation of building blocks of materials and their conversion to a scale of 1-100 nm [8–13]. According to this definition, it is clear that the effects of quantum mechanics are of particular importance at this scale [14–18]. In recent years, the use of metal nanoparticles has found many applications in new technology [19]. Metal nanoparticles such as gold, silver, and copper nanoparticles are designed nanoparticles that have suitable electronic, catalytic, and optical properties, and because of these desirable properties of such nanoparticles in areas such as sensor fabrication and fabrication, these catalysts are widely used

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[20–22]. Metal nanoparticles, such as gold, silver, and copper nanoparticles, are designed nanoparticles that have appropriate electronic, catalytic, and optical properties, and because of these desirable properties of such nanoparticles in areas such as sensor construction [23]. Also, metal nanoparticles have been highly regarded due to their properties such as surface Plasmon resonance, optical properties, good catalytic performance, good antimicrobial activity, as well as high surface-to-volume ratio, and controlled porosity [24,25]. There are various methods for the preparation of metal nanoparticles such as chemical reduction, hydrothermal, microemulsion, and the use of lasers, among which the synthesis of metal nanoparticles by the chemical method is widely used [26–30]. These methods, known as chemical methods, are very expensive, and in the process of preparing nanoparticles are harmful, toxic, and very dangerous chemicals, which lead to environmental problems [31]. Therefore, materialists and nanochemists are looking for an alternative and environmentally friendly method for the preparation of metal nanoparticles. In recent years, the method of biosynthesis using plant extracts has received more attention than physical and chemical methods. Synthesis is green, simple, low cost, non-toxic, environmentally friendly, and efficient to operate. The use of plant extracts for the synthesis of nanoparticles through a biological process is very beneficial from an environmental point of view. Vishveshvar et al. [32] synthesized copper oxide nanoparticles with a size distribution of 80 to 110 nm using Ixiro coccinea leaves in an environmentally friendly manner. Yugandhar et al. [33] synthesized copper oxide nanoparticles using the fruit extract of Syzygium alternifolium in a spherical shape at a size of 69 nm and then examined its antiviral properties against Newcastle virus. Awwad and Amer [34] synthesized copper oxide nanoparticles using the aqueous extract of Ailanthus altissima leaves in a spherical shape with an average size of 20 nm and then investigated its antibacterial properties. Singh et al. [35] synthesized spherical oxide nanoparticles using Psidium guajava leaf extract in a spherical shape with a size distribution of 2 to 6 nm. Sarkar et al. [36] synthesized copper oxide nanoparticles using the extract of Adiantum lunulatum in a spherical, pure, and very stable form with a diameter of approximately 6.5 nm. Copper oxide nanoparticles (CuO) are one of the most important intermediate metal oxides that have unique properties and they are used in various technologies such as technologies related to superconductors, gas sensors, etc [37]. CuO has recently been used as an antimicrobial agent against several bacterial species [38]. Copper nano oxide plays an important role in today's industrial world, this material has a variety of applications in the electronics and electrical industries due to its conductive and semiconductor capabilities [39]. This product is widely used as a catalyst in the oil and gas and petrochemical industries and the industries of glass, glaze, tiles, and ceramics as well as other chemical industries [40,41]. In this paper, for the first time, the use of tea grass extract and white fraction for the synthesis of copper nanoparticles has been reported, and also the effect of pH regulation on the synthesis process was investigated. The effects of synthesized nanoparticles on two Gram-positive and Gram-negative bacteria are shown.

EXPERIMENTAL

Preparation of Hypericum perforatum and Marrubium Vulgare

Hypericum perforatum plant of the Malpician family and of the species H. perforatum, which is found as a vehicle in wheat and cornfields [42]. This plant also has high antioxidant properties that its role is undeniable in the treatment of depression (Fig. 1a). Marrubium Vulgare is a flowering plant of the mint family (Fig. 1b).

This plant has antioxidant properties that are useful in treating diabetes, cough, chest disorders, regulating heart rate, and reducing menstrual pain. The plants were dried in the shade after collection and disinfection.

Extraction of Hypericum perforatum and Marrubium Vulgare plant

Aerial parts of Hypericum perforatum and Marrubium Vulgare after collected and approved by a botanist, were washed with distilled water, and then dried at room temperature. The dried plant was crushed separately with an electric grinder and prepared as a uniform, fine powder. 20 g of the prepared powder was poured into a clean Erlenmeyer flask and 200 g of deionized water was added to it. The mixture was stirred and then boiled for 15 min and after cooling, strained through filter paper to completely separate the solid particles, the solution under the strainer was maintained for later use in a dark closed container in the refrigerator (4° C).

Fig. 1 (a) Hypericum perforatum (b) Marrubium Vulgare

Comparison of antioxidant properties of Hypericum perforatum and Marrubium Vulgare by DPPH method

For this purpose, free radical DPPH and synthetic antioxidant butylhydroxytoluene (BHT) were used. The percentage of oxidation inhibition of each sample can be calculated by the following equation:

$$AI\% = \frac{(A_{control} - A_{sample})}{A_{sample}} \times 100 \quad (1)$$

Where %AI, Acontrol, and Asample are percentage of inhibition, Adsorption of control solution at 517 nm, and sample Absorption at 517 nm respectively. By plotting the %AI curve against different concentrations of the extract, the value of IC50 for each extract was determined. GraphPad Prism software was used to calculate the IC50 of the extracts. According to the IC50 values obtained from the two studied plants, it was found that the antioxidant property of the Hypericum perforatum is higher than that of Marrubium Vulgare. See Fig. (2) for a specific antioxidant comparison of the extracts of the two plants.

Synthesis of green copper oxide nanoparticles

Pour the anhydrous copper sulfate solution into a separate small glass Erlenmeyer flask, then raise the temperature of the solution to 100-120 °C while the Erlenmeyer was on a magnetic stirrer, then 2 ml of the plant extract was added to the broth drop by drop.

At first, the blue color of the solution turned green, and after 24 hours of continuous stirring, the color of the solution turned Terra Cotta. Aluminum cap was placed on Erlenmeyer for 24 h to ensure good synthesis of nanoparticles and no side reactions in the presence of air.

After synthesizing, the solution containing the nanoparticles was centrifuged at 5500 rpm for 15 min and the resulting powder was placed in an oven at 90 ° C overnight to dry. The obtained nanoparticles were stored in a dark container for identification and spectroscopy. This synthesis method was performed for both Hypericum perforatum extracts and Marrubium Vulgare. But the synthesis results showed that the solution obtained from Hypericum perforatum extract did not contain synthetic nanoparticles. This is probably due to the lower antioxidant properties of the Marrubium Vulgare plant compared to the Hypericum perforatum plant.

Synthesis of copper oxide nanoparticles by adjusting pH

In this method, the above procedure was repeated with the difference that this time before adding the extract, the pH content of copper sulfate was increased to seven and then added to the extract.

RESULTS AND DISCUSSION

Visible-ultraviolet light spectroscopy (UV-Vis)

The synthesized nanoparticles were first investigated by visible-ultraviolet light spectroscopy at a wavelength of 200-700 nm using

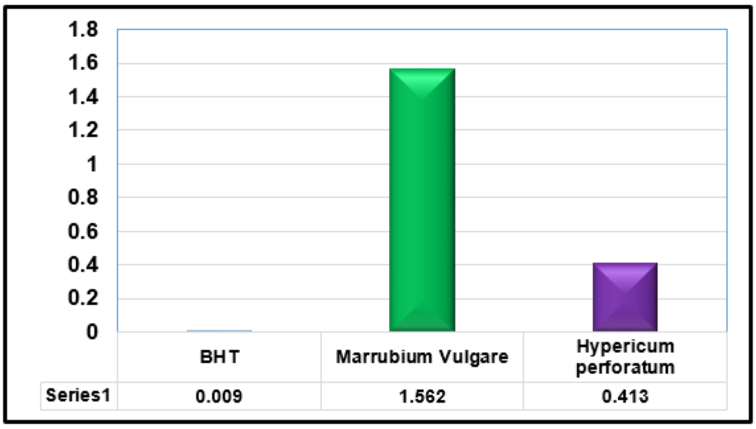

Fig. 2. Comparison of IC50 for BHT and Marrubium Vulgare and Hypericum perforatum extract.

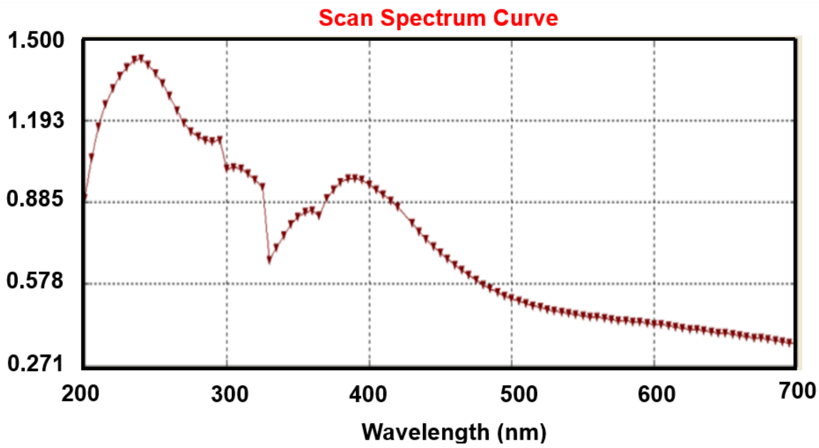

Fig. 5. (UV-Vis) spectrum of colloidal composition containing copper oxide nanoparticles

quartz coats and plant extracts as controls. Visible-ultraviolet spectroscopy is one of the methods used in experimental sciences to study scientific and practical information, using the interaction of light and spectroscopic material. In metal nanoparticles, surface Plasmon resonance is due to their unique optical properties, which undergo factors such as the size of the nanoparticles, their distance from each other, and the refractive index of the surrounding environment. The displacement of the peaks and the thinning of their intensity and the formation of thinning in the observed colors are among the factors that depend on the size of the nanoparticles. Therefore, the optical properties of nanoparticles depend on the diameter of the nanoparticles. Larger nanoparticles show more dispersion and have wider peaks and change to longer wavelengths. 4 µl of the extract containing nanoparticles was poured into 16 cells so that not to bubble and placed in the embedded position of the device. The peak in the range of 200-400 nm indicated the presence of copper oxide nanoparticles (Fig. 5).

Nanoparticle X-ray diffraction (XRD) spectroscopy

X-ray diffraction is an old and widely used technique in studying the properties of crystals. In this method, X-ray diffraction by the sample is used to investigate the characteristics of the sample. XRD can be used to determine the general quantities of crystalline hardness such as lattice constant, lattice geometry, quality determination of unknown materials, crystal phase determination, crystal size determination, single-crystal orientation, etc. The X-ray diffraction pattern of the synthesized copper oxide nanoparticles is shown in (Fig. 6). XRD analysis was performed to prove copper oxide metal nanocrystals. Based on the findings,

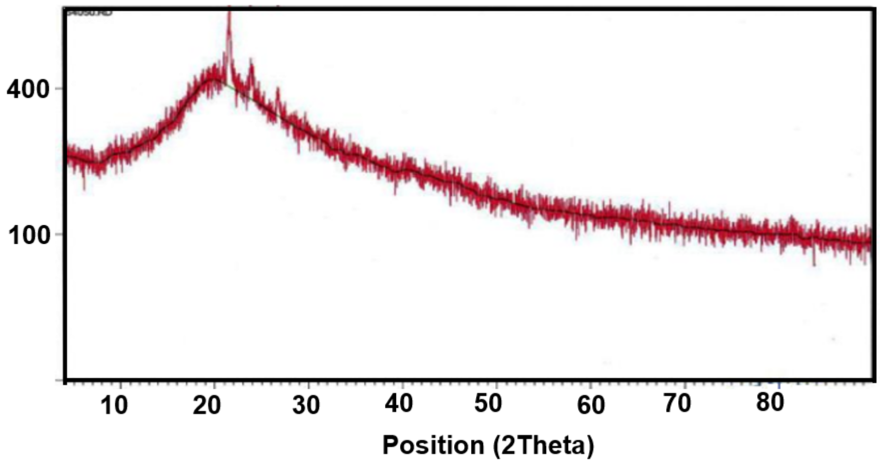

Fig. 6. XRD spectrum of copper oxide nanoparticles synthesized from Hypericum perforatum extract

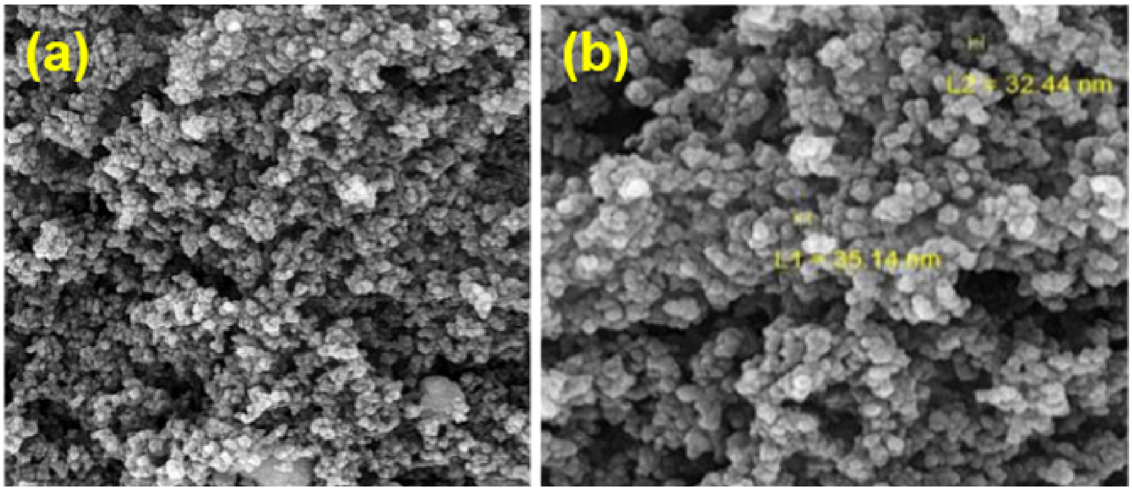

Fig. 7. SEM spectrum of copper oxide nanoparticles synthesized from Hypericum perforatum extract (a) 50.0 KX (b) 100KX

synthetic copper oxide metal nanocrystals are given at the level of 20-30 peaks, which is completely consistent with the standard sample of copper oxide nanocrystals.

Scanning electron microscope (SEM) imaging

The shape and size distributions of the synthesized nanoparticles were examined by scanning electron microscopy image analysis; a vacuum medium was required to work with the electron microscope. SEM analysis was used to determine the size of nanoparticles and study their morphology. To prepare the samples, the extracted powder from the initial solution was glued to the sample holder. Then a very thin layer of gold with a thickness of 10 nm was applied to that layer by the sputtering method. After preparing the samples, the images were taken by scanning electron microscopy (FE-SEM). According to Fig. 7 copper oxide nanostructures were observed as nanospheres with a diameter of approximately 32-36 nm.

Fourier transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy is one of the most widely used methods in qualitative identification of different molecules, determination of the molecular structure of different species (especially organic species), and identification of functional groups in the structure of a species. This method investigated synthesized copper oxide nanoparticles. FT-IR spectroscopy was performed to identify the molecules in the nanoparticles synthesized from the Hypericum

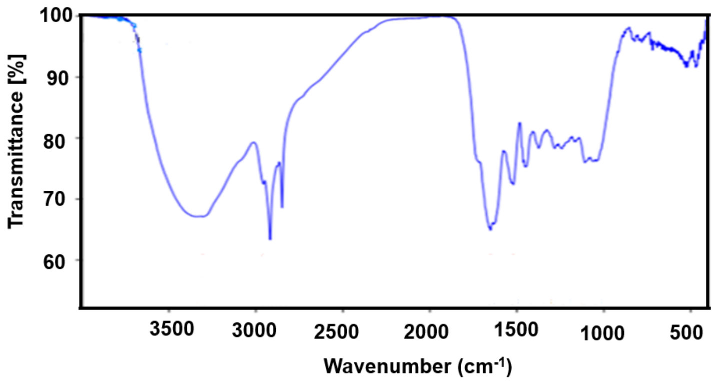

Fig. 8. FT-IR spectrum of copper oxide nanoparticles synthesized from Hypericum perforatum extract

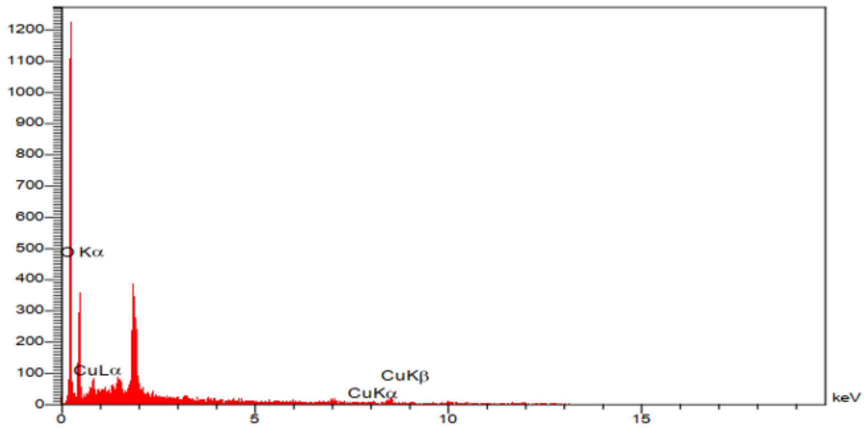

Fig. 9. EDS spectrum of copper oxide nanoparticles synthesized from Hypericum perforatum extract

perforatum extract. To prepare the powder sample for analysis, 20 ml of suspension containing copper oxide nanoparticles and 20 ml of suspension were centrifuged separately for 10 min at 6500 rpm without copper oxide nanoparticles. After pouring out the top phase, the samples were dried in a freeze dryer for 72 hours. Device model Tensor27 made in Germany was used for analysis of powder obtained from FT-IR. The triple peak in the range of 500-700 and the strong peak in the range of 2800-4000 indicated the presence of copper oxide nanoparticles. The wide peak in the 3000-3600 region represented the tensile vibration of the O-H group, in the 2990 tensile region of the C-H group, in the 1651 tensile region C = O and N-H, and the 1042 and 1422 regions related to the tensile movement of the C-O slate. Since the organic compounds of plant extracts were placed around the nanoparticles and caused the stability of the nanoparticles, movements related to these organic groups could be seen in the FT-IR spectrum (Fig. 8).

Investigation of X-ray energy diffraction spectrum (EDS) of copper oxide nanostructures

According to the EDS spectrum of copper oxide nanoparticles shown in Fig. 9, the presence of the element copper is confirmed. The purity of the synthesized nanoparticles is very high. However, a small amount of Cu+2 ions can still be seen in the environment, which can be due to incomplete solid washing by centrifugation.

Investigation of antibacterial properties

The antimicrobial activity of the synthesized nanoparticles was investigated under optimal conditions on two bacterial species including a gram-negative bacterium (Escherichia coli) and a gram-positive bacterium (Staphylococcus aureus). In this investigation the growth inhibition halo of nanoparticles and control antibiotics against (a) Staphylococcus aureus and (b) Escherichia coli. The results obtained from the antibacterial activity of these nanoparticles were compared with the antibiotic species used as control in this experiment. According to the results, the two bacteria did not show much sensitivity to copper oxide nanoparticles, which could be due to changes in the concentration and size of nanoparticles, which affected the antimicrobial properties.

CONCLUSION

In this paper, the synthesis of copper oxide nanoparticles was performed using the aqueous extract of the Hypericum perforatum plant and the aqueous extract of the Marrubium Vulgare plant with the help of copper sulfate salt solution. However, the method used for Marrubium Vulgare plant extract did not lead to the synthesis of oxidant nanoparticles. The reason may be due to the low antioxidant properties of this plant and thus reduced its regenerative power. Therefore, we succeeded in synthesizing copper oxide nanoparticles only from Hypericum perforatum plant extract. UV-Visible spectral studies of copper oxide nanoparticles showed that the color of the samples changed from light blue to brown due to the reduction of copper ions in copper cellulose phosphate salt solution by reducing agents in Hypericum perforatum plant extract and production of nanoparticles. The maximum adsorption of copper oxide nanoparticles was observed between 200-400 nm. The XRD spectrum of the synthesized nanoparticles demonstrated the presence of copper oxide nanocrystals in the Hypericum perforatum plant extract. Also, the study of SEM images of synthesized nanoparticles showed that the synthesized copper oxide nanoparticles were mainly spherical with a thickness of approximately 33 nm. Also, the images showed a uniform size distribution of synthetic particles, which is one of the advantages of using this method. FT-IR spectroscopy was performed to identify the Cu-O bond. The triple peak in the range of 700-500 cm-1 and the strong peak in the range of 1400-2800 cm-1 indicated the presence of copper oxide nanoparticles. The EDS spectrum also showed the purity of the synthesized copper oxide nanoparticles. The results of antibacterial tests revealed the effect of synthesized copper oxide (CuO) nanoparticles against two target bacteria. The mean drop of growth inhibition for the two tested bacteria compared to the two types of antibiotics indicated that the extract containing the synthesized nanoparticles of the Hypericum perforatum had very little antibacterial effect, which could result from changes in the concentration of the extract or salt solution used for the synthesis of nanoparticles and consequently the size of nanoparticles was reduced.

Consent to Publish

The article is approved by all authors for publication.

Authors Contributions

Ashkan Farazin performed the experimental calculations. Ms. Kavehzadeh proof the language of the manuscript. Authors contributing to the final version of the manuscript.

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No conflict of interest exists in the submission of this article.

Availability of data and materials

Data required to reproduce these findings have been given in the text.

Ethical Approval

Not applicable

Consent to Participate

Not applicable

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