Supplementary Information (SI) for New Journal of Chemistry.

Witch-Hazel-Mediated Hydrothermal Biosynthesis of Copper Nanoparticles: Dye Removal, Antibacterial Potency, and Anticancer Synergy Potential Activities

Supplementary data

1. Instrumentations and characterization

All characterizations and analyses were primarily conducted at the Central Laboratory for Microanalysis and Nanotechnology, Minia University, Egypt, unless otherwise specified. The biosynthesized copper nanoparticles (Cu NPs) were prepared and analyzed using a suite of instrumental techniques to evaluate their physical, chemical, and structural properties. Below, each method is described in detail, including sample preparation, operational parameters, and the purpose of the analysis.

1.1. Mass Measurement (Analytical Balance)

The mass of the Cu NP samples and precursors was measured using an analytical balance (Model ABT 120-5DM, Kern, Germany). This instrument provides high-precision weighing with a readability of 0.01 mg and a capacity up to 120 g. Samples were placed in pre-weighed glass vials or aluminum pans, and measurements were performed at room temperature (25 ± 2°C) under ambient conditions to ensure accurate quantification for subsequent analyses, such as yield calculation and dosage preparation for adsorption studies.

1.2. Fourier-Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy was used to identify functional groups on the Cu NP surface and confirm the involvement of Witch-Hazel bioactive compounds in stabilization. Spectra were recorded on a Thermo Fisher Nicolet iS10 spectrometer in the wavenumber range of 4000–500 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans per sample. Dried Cu NP powder (approximately 1 mg) using ATR module in transmission mode was conducted. This technique helped detect organic capping agents (e.g., O-H, C=O stretches) and verify the absence of Cu-O bonds indicative of oxidation.

1.3. Scanning Electron Microscopy (SEM) with Energy-Dispersive X-ray Spectroscopy (EDX)

SEM was employed to examine the morphology and surface topography of the Cu NPs, while EDX provided elemental composition. Imaging was performed using a JEOL JSM IT 200 microscope equipped with an EDX analyzer. Samples were prepared by dispersing Cu NPs in ethanol (0.1 mg/mL), drop-casting onto a carbon-coated copper grid, and air-drying at room temperature. SEM images were acquired at an accelerating voltage of 15–20 kV and magnifications ranging from 600× to 3300×. EDX spectra were collected over 30–40 seconds at selected regions to quantify elemental ratios (e.g., Cu, O, C), with data processed using built-in software for semi-quantitative analysis (wt% with standard deviations from triplicate scans).

1.4. Transmission Electron Microscopy (TEM)

TEM was used to determine the size, shape, and distribution of individual Cu NPs at high resolution. Analysis was conducted on a JEOL JEM-100 CXII microscope operating at an accelerating voltage of 100 kV. Sample preparation involved ultrasonic dispersion of Cu NPs in distilled water (0.05 mg/mL for 10 min), followed by depositing a drop onto a carbon-coated copper grid and drying under ambient conditions. Images were captured at magnifications up to 40,000×, and particle size distribution was analyzed using ImageJ software.

1.5. pH Measurement

The pH of Cu NP suspensions and reaction mixtures was measured using an AD1030 pH-meter (Adwa, Romania), calibrated with standard buffers (pH 4.0, 7.0, and 10.0) prior to use. Samples (5 mL aliquots) were stirred gently and measured at room temperature, with triplicate readings averaged for accuracy. This was critical for optimizing adsorption conditions and assessing suspension stability.

1.6. Centrifugation

Centrifugation was performed to separate and purify Cu NPs from the reaction mixture using a Sigma 2-16KL benchtop cooling centrifuge (Sigma, Germany). Samples were centrifuged at 10,000–15,000 rpm for 5–30 min at 4°C, depending on the volume (up to 50 mL per tube). The supernatant was discarded, and pellets were resuspended in distilled water for washing (repeated 3 times) to remove unbound biomolecules.

1.7. Muffle Furnace Calcination

Post-synthesis annealing was conducted in a digital muffle furnace (Model FHPX-12, Daihan Scientific Co., Korea) to enhance crystallinity. Dried Cu NP powder (approximately 100 mg) was placed in a porcelain crucible and heated at 500°C for 1 hour under a nitrogen atmosphere (flow rate: 100 mL/min) to prevent oxidation. The furnace was programmed with a ramp rate of 10°C/min and cooled naturally to room temperature.

1.8. Magnetic Stirring with Heating

Mixing and heating during synthesis were achieved using a digital magnetic stirrer with hotplate (Model MSH20D, Daihan Scientific Co., Korea). Reactions were stirred at 250–500 rpm with temperature control up to 60°C, monitored via an integrated thermocouple, ensuring uniform dispersion of precursors and extract.

1.9. Drying Oven

Drying of Cu NP samples was performed in a digital drying oven (Model WON-32, Daihan Scientific Co., Korea) at 60–80°C for 2 hours under ambient air circulation. This step removed residual moisture without altering nanoparticle structure.

1.10. Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS was used to identify bioactive compounds in the Witch-Hazel extract. Analysis was conducted on a Shimadzu LC-MS-8030 system with a BM-20A control bus module, CTO-30A column oven, LC-30AD liquid chromatograph, SIL-30AC autosampler, and ESI interface.

1.11. X-ray Diffraction (XRD)

XRD was employed to determine the crystalline structure and phase purity of Cu NPs. Measurements were performed on a JEOL 60PA-JSDX diffractometer using Cu Kα radiation ($\lambda = 1.5418$ Å) at 42 kV and 36 mA. Powder samples were scanned from $2\theta = 4°$ to 80° with a step size of 0.06° per step.

1.12. Dynamic Light Scattering (DLS) and Zeta Potential

Particle size distribution and zeta potential were measured using a NICOMP Z3000/N3000 ZLS system (Entegris, USA). Cu NPs were dispersed in distilled water (0.1 mg/mL, sonicated for 10 min), and measurements were taken at 25°C with a He-Ne laser ($\lambda = 632.8$ nm) at a scattering angle of 173°. Three replicates were averaged for hydrodynamic diameter, polydispersity index (PDI), and zeta potential.

2. Adsorption equations [1, 2]

2.1. Loading and removal (%) of MB

$$q_e = (C_i - C_e)\, V/M \qquad \textbf{(SEq.1)}$$

where q_e is the equilibrium adsorption capacity, C_i and C_e are the initial and the unbounded concentrations of the MB, V is the volume of the MB solution, and M is the mass of Cu NPs.

The percentage removal of MB has been calculated using the following equation:

$$\text{Adsorption (\%)} = [(C_i - C_e)/C_i] \times 100 \qquad \textbf{(SEq.2)}$$

2.2. Adsorption isothermal models

- *Langmuir isotherm model*

It is a homogeneous monolayer adsorption isotherm. The Langmuir equation can be driven in the linear form as the following:

$$C_e/q_e = (C_u / q_L K_L) + (C_e / q_L) \qquad \textbf{(SEq.3)}$$

where q_e is the equilibrium adsorption capacity of MB on the Cu NPs (mg/g), C_e is the unbounded concentrations (mg/L) of the MB, q_L is the maximum capacity of monolayer onto Cu NPs (mg/g), and K_L is the equilibrium constant (L/mg) relating to the sorption energy.

$$R_L = 1/(1 + K_L C_{max}) \qquad \textbf{(SEq.4)}$$

R_L (separation factor) is a dimensionless parameter that indicates the type of isotherm. Linear adsorption is given if $R_L = 1$. Irreversible adsorption is denoted by $R_L = 0$, while unfavorable adsorption is signified by $R_L > 1$. The reaction is favorable for adsorption if the R_L value is between 0–1.

- *Freundlich isotherm model*

It is supposed that multilayer adsorption may have happened, and its adsorption isotherm is expressed by the following equation:

$$\log q_e = \log K_F + (1/n) \log C_e \qquad \textbf{(SEq.5)}$$

where K_f represents the Freundlich constant related to adsorption capacity (mg/g), and n is the adsorption intensity. If an adsorption condition is favorable, the n will be greater than the unit, where the value of (1/n) is used to quantify the adsorption intensity; a value near 0 suggests a more heterogeneous surface of Cu NPs.

- *Temkin*

$$q_e = B_T \ln A_T + B_T \ln C_e \qquad \textbf{(SEq.6)}$$

$$\mathbf{b_T = RT/\ B_T} \qquad \text{(SEq.7)}$$

where $\mathbf{B_T}$: Temkin adsorption constant (KJ/mol) that is related to the sorption heat. **R** is the gas constant (0.008314 kJ/mol K). **T** is the absolute temperature at 298 K, and $\mathbf{b_T}$: Adsorption process constant.

- *Dubinin–Radushkevich (D-R)*

$$\mathbf{\ln q_e = \ln q_m - \beta\ \varepsilon^2} \qquad \text{(SEq.8)}$$

$$\mathbf{\varepsilon = RT\ \ln(1+ 1/C_e)} \qquad \text{(SEq.9)}$$

$$\mathbf{E_D = (-2\ \beta)^{-1/2}} \qquad \text{(SEq.10)}$$

Where $\mathbf{q_m}$ is D-R adsorption capacity of Cu NPs (mg/g), $\boldsymbol{\beta}$ is the coefficient related to the mean free energy (mol^2KJ^{-2}), and $\boldsymbol{\varepsilon}$ is Polanyi potential. $\mathbf{E_D}$ is the adsorption energy per molecule of the MB adsorbate when it is transferred to the surface of the solid Cu NPs from infinity in the solution (kJ/mol).

- *Halsey model:*

$$\mathbf{\ln q_e = (1/n_H)\ \ln (K_H) - (1/n_H)\ \ln (C_e)} \qquad \text{(SEq.11)}$$

2.3. Adsorption kinetics models [3-5]

- *Pseudo-first-order model is by the Lagergren model:*

$$\mathbf{\log (q_e - q_t) = \log q_e - (K_1/2.303)\ t} \qquad \text{(SEq.12)}$$

- *Pseudo-second-order by McKay and Ho model:*

$$\mathbf{(t/q_t) = 1/\ (K_2\ q_e^2) + (1/q_e)\ t} \qquad \text{(SEq.13)}$$

- *Elovich model:*

$$\mathbf{q_t = (1/\beta)\ (\ln \alpha\beta) + (1/\beta)\ \ln (t)} \qquad \text{(SEq.14)}$$

- *Weber's and Moris's / intra-particle diffusion model:*

$$\mathbf{q_t = C + K_{int}\ (t)^{1/2}} \qquad \text{(SEq.15)}$$

C_e is the equilibrium concentration (mg/L) of the unbounded MB in solution after the adsorption process; q_e is the adsorbed amount of MB onto the Cu NPs (mg/g) at equilibrium; q_t is the adsorbed amount of MB onto the Cu NPs (mg/g) at predetermined time interval t; K_1 is the pseudo-first-order rate constant (min^{-1}); t is the time interval (min); K_2 is the pseudo-second-order rate constant (g/mg min); α acts as the initial sorption rate constant (mg/g min); β is the constant activation energy for chemisorption (g/mg); K_{int} is the intra-particle rate constant (mg/g $min^{1/2}$); C is the boundary thickness. The constant n_H is related to the heterogeneity of the adsorbent surface, with values ranging from 2 to infinity. A higher value of n indicates a more homogeneous surface, while a lower value suggests a more heterogeneous surface. The constant k_H is related to the adsorption potential and is dependent on the adsorbent-adsorbate interaction.

3. Scherrer equation [6-8]

$D_{scher} = 0.9\lambda / \beta_{hkl} \cos(\theta_{hkl})$ (SEq.16)

Where 0.9 is a Scherrer constant;

λ is the radiation X-ray wavelength (CuKα= 1.541838 Å);

β_{hkl} is the full width at half maxima "FWHM" of the measured peaks, which were estimated and corrected according to the pseudo-Voigt function [9, 10] of the line profile relation.

θ_{hkl} is the degree of observed distinct diffraction peak in the XRD diffraction pattern.

4. Various statistical parameters [6, 11-14]

$$\chi^2 = \sum \frac{(q_{\mathrm{exp}} - q_{\mathrm{cal}})^2}{{q_{\mathrm{cal}}}^2} \qquad \textbf{(SEq.17)}$$

$$SSE = \sum (q_{\mathrm{exp}} - q_{\mathrm{cal}})^2 \qquad \textbf{(SEq.18)}$$

$$MSE = \frac{1}{N_{exp}} \sum (q_{\mathrm{exp}} - q_{\mathrm{cal}})^2 \qquad \textbf{(SEq.19)}$$

$$HYBRID = \frac{100}{N_{exp} - N_{para}} \sum \frac{(q_{exp} - q_{\mathrm{cal}})^2}{q_{exp}} \qquad \textbf{(SEq.20)}$$

Where q_{cal} is the calculated adsorption capacity in mg/L, q_{exp} is the experimental adsorption capacity in mg/L, N_{exp} is the number of the data points, N_{para} is the number of the model parameters, x^2 is the Chi-square, SSE is the residual sum of squares error , MSE is the mean sum of squares error , and HYBRID is the hybrid fractional error function.

References

[1] M.F. Al-Hakkani, G.A. Gouda, S.H.A. Hassan, M.M.A. Mohamed, A.M. Nagiub, Surfaces and Interfaces, 2022, **30**, 101877.

[2] M.F. Al-Hakkani, G.A. Gouda, S.H.A. Hassan, M.M.A. Mohamed, A.M. Nagiub, Scientific Reports, 2022, **12**, 10970

[3] M. Temkin, Acta physiochim, 1940, **12**, 327-356.

[4] S.K. Lagergren, Sven. Vetenskapsakad. Handingarl, 1898, **24**, 1-39.

[5] Y.-S. Ho, G. McKay, Process Biochem., 1999, **34**, 451-465.

[6] B.R. Alsehli, M.H.A. Hassan, D.S. Mohamed, M.S. Saddik, M.F. Al-Hakkani, J. Mol. Struct., 2024, **1318**, 139261.

[7] M.S. Saddik, M.F. Al-Hakkani, A.M. Abu-Dief, M.S. Mohamed, I.A. Al-Fattah, M. Makki, M.A. El-Mokhtar, M.A. Sabet, M.S. Amin, H.A. Ahmed, K. Al-Ghamdi, M.K. Mohammad, M.H.A. Hassan, International Journal of Pharmaceutics: X, 2024, **7**, 100245.

[8] M.H.H. Omar, A.E.M. Elfarash, H.M. El-Aref, M.F. Al-Hakkani, Process Biochem., 2024, **142**, 162-174.

[9] G. Madhu, V.C. Bose, K. Maniammal, A.A. Raj, V. Biju, Phys. B (Amsterdam, Neth.), 2013, **421**, 87-91.

[10] M.F. Al-Hakkani, G.A. Gouda, S.H.A. Hassan, A.M. Nagiub, Surfaces and Interfaces, 2021, **24**, 101113.

[11] J. Wang, X. Guo, J. Hazard. Mater., 2020, **390**, 122156.

[12] A.A. Oladipo, E.O. Ahaka, M. Gazi, Environmental Science and Pollution Research, 2019, **26**, 31887-31899.

[13] H. Ansari, A.A. Oladipo, M. Gazi, Int. J. Biol. Macromol., 2023, **246**, 125736.

[14] N. Ayawei, A.N. Ebelegi, D. Wankasi, Journal of Chemistry, 2017, **2017**, 3039817.