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Research Article

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Green Synthesis of Copper Oxide Nanoparticles Using Glycyrrhiza glabra Extract: Structural Characterization, Antioxidant Activity, and Catalytic Application in 1,2,3-Triazole Synthesis

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Abstract: In this study, copper oxide nanoparticles (CuO NPs) were synthesized using an aqueous extract of Glycyrrhiza glabra (licorice) as a natural reducing and stabilizing agent. The synthesized CuO/GLY nanoparticles were thoroughly characterized using UV–Vis spectroscopy, FT-IR, XRD, FE-SEM-EDS, BET, and TGA analyses. The CuO/Gly nanoparticles exhibited mesoporous structure, spherical morphology, and good thermal stability. The CuO/Gly nanoparticles were applied as an efficient heterogeneous catalyst for the synthesis of substituted 1,2,3-triazoles under mild and environmentally benign conditions. The catalytic system demonstrated high efficiency, operational simplicity, low cost, and good recyclability. In addition, the antioxidant activity of the CuO/Gly nanoparticles was evaluated using the DPPH radical scavenging assay. The nanoparticles exhibited moderate free radical scavenging activity, indicating potential applications beyond catalysis. Overall, this study presents a sustainable and cost-effective strategy for the synthesis of biologically relevant triazole derivatives using a green nanocatalyst.

Keywords: Green biosynthesis; Plant extract–mediated synthesis; 1,2,3-triazole derivatives; CuO/Gly nanocatalyst; Antioxidant activity.

Introduction

Triazole derivatives are an important class of heterocyclic compounds widely recognized for their diverse biological activities, including antifungal, anticonvulsant, anticancer, and antioxidant properties [1]. Among them, 1,2,3-triazoles have attracted considerable attention due to their structural versatility and extensive applicability in medicinal chemistry [2-5]. The development of efficient and environmentally friendly synthetic strategies for triazole derivatives remains a significant objective in contemporary organic chemistry [6-8]. Click chemistry and 1,3-dipolar cycloaddition reactions have emerged as reliable methodologies for the synthesis of 1,2,3-triazoles [9-13]. These approaches provide high regioselectivity, mild reaction conditions, and excellent functional group compatibility [14]. However, conventional synthetic procedures may involve toxic reagents, harsh conditions, or expensive catalytic systems, emphasizing the need for sustainable alternatives.

Nanocatalysts have demonstrated significant potential in promoting green and efficient chemical transformations [15]. Copper oxide nanoparticles (CuO NPs) are particularly attractive due to their unique physicochemical properties, High catalytic activity, and ease of preparation [16-22].

Plant-mediated nanoparticle synthesis has emerged as an eco-friendly approach that utilizes bioactive phytochemicals as reducing and stabilizing agents. Glycyrrhizin, a major bioactive component of Licorice root extract (from Glycyrrhiza glabra), has been reported as an effective natural stabilizer in nanoparticles synthesis. Moreover, it contains several bioactive compounds, the most important being glycyrrhizin, a triterpenoid saponin responsible for many of its pharmacological effects. It also includes flavonoids such as liquiritin and isoliquiritigenin, as well as chalcones and various phenolic compounds, which contribute to its antioxidant and anti-inflammatory properties. Regarding antimicrobial activity, licorice extract has demonstrated inhibitory effects against a range of bacteria, fungi,

and some viruses. As a result, licorice extract is considered a promising natural agent for supporting the treatment of infections and for use in pharmaceutical and oral-care formulations [23].

In the present study, CuO nanoparticles (CuO/Gly nanocatalyst) were biosynthesized using Glycyrrhiza glabra extract and applied as a heterogeneous nanocatalyst for the synthesis of 1,2,3-triazole derivatives (Scheme 1, 2). The structural characteristics, antioxidant activity, catalytic efficiency, and recyclability of the CuO/Gly nanocatalyst were systematically investigated.

<Scheme 1>

<Scheme 2>

Experimental

In this study, the synthesis of 1,2,3-triazole derivatives was investigated through a one-pot three-component reaction of sodium azide, phenylacetylene or propargyl ethers, and various benzyl or alkyl halides in the presence of a CuO/Gly nanocatalyst. The nanocatalyst was prepared via a green and sustainable approach using Glycyrrhiza glabra extract as a natural reducing and stabilizing agent. The physicochemical properties of the synthesized nanocatalyst were characterized using several analytical techniques. The crystalline structure and phase composition were determined by X-ray diffraction (XRD). The morphology and particle size distribution were examined using field emission scanning electron microscopy (FE-SEM), while the elemental composition was analyzed by energy-dispersive X-ray spectroscopy (EDS). The specific surface area and pore size distribution were evaluated using the Brunauer–Emmett–Teller (BET) method. In addition, thermogravimetric analysis (TGA) was performed to investigate the thermal stability of the nanocatalyst. The melting points of the obtained compounds were determined and compared with reported values. Structural characterization of the synthesized triazole derivatives was performed using Fourier

transform infrared spectroscopy (FT-IR) as well as ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

Preparation of Glycyrrhiza glabra Aqueous Extract

Fresh licorice roots were thoroughly washed with double-distilled water, shade-dried at room temperature (25 °C), and ground into a fine powder. Five grams of the powder were mixed with 100 mL of distilled water and stirred using a magnetic stirrer at 25 °C for 15 min. The mixture was then heated at 70 °C for 10 min to facilitate extraction. After cooling, the solution was filtered through Whatman filter paper and centrifuged at 8000 rpm for 30 min to

Green synthesis of CuO/Gly nanoparticles

10 milliliters of licorice extract were mixed with an aqueous solution of $Cu(NO_3)_2 \cdot 3H_2O$ (0.2 M) under vigorous stirring at 70 °C. Subsequently, 50 mL of Na_2CO_3 solution (0.5 M) was added dropwise over 15 min. The reaction mixture was stirred at 70 °C for 24 h, resulting in the formation of a black precipitate. The precipitate was filtered, washed repeatedly with distilled water, dried at 100 °C for 1 h, and calcined at 300 °C for 2 h to obtain CuO/Gly nanoparticles.

General procedure for the synthesis of 1,4-disubstituted-1,2,3-triazoles

For the synthesis of 1,2,3-triazole derivatives, 0.06 g of the catalyst was initially added to a round-bottom flask containing a mixture of phenylacetylene or propargyl ethers (1 mmol), benzyl or alkyl halides (1 mmol), and sodium azide (1.1 mmol) in 5 mL of ethanol. The reaction mixture was thoroughly stirred using a magnetic stirrer and then heated under reflux conditions. The progress of the reaction was monitored by thin-layer chromatography (TLC) using a solvent system of n-hexane and ethyl acetate (6:2). Upon completion of the reaction, the mixture was filtered to remove the catalyst. The crude product was then recrystallized from hot ethanol to afford the pure compound.

Results and Discussion

Catalyst characterization

Copper oxide nanoparticles were synthesized through a green and sustainable approach using glycyrrhizin extracted from Glycyrrhiza glabra as a natural reducing and stabilizing agent. The gradual change in color of the reaction mixture during synthesis indicated the formation of CuO nanoparticles. This was further confirmed by UV–Vis spectroscopy, which showed a characteristic absorption band at 232 nm, consistent with previously reported CuO nanostructures. These results demonstrate the effective role of plant-derived glycyrrhizin in the biosynthesis of CuO nanoparticles [28, 29]. The formation of biosynthesized copper oxide nanoparticles was preliminarily confirmed using UV–visible spectrophotometry. As shown in Figure 1, the UV–Vis absorption spectrum of the CuO/Gly nanoparticles exhibited a distinct absorption band at around 232 nm, which is characteristic of CuO nanostructures. In contrast, the glycyrrhizin extract used as the control showed no comparable absorption peak in this region. The appearance of the absorption band within the range of 200–300 nm is consistent with previous reports and is commonly attributed to the electronic transitions associated with CuO nanoparticles, confirming the successful biosynthesis of CuO/Gly nanocatalyst [30, 31].

<Figure 1>

To investigate the functional groups involved in the formation and stabilization of CuO/GLY nanoparticles, FT-IR spectroscopy was performed. The FT-IR spectra of glycyrrhizin extract and the biosynthesized CuO/Gly nanoparticles are shown in Figure 2. In the spectrum of glycyrrhizin, a broad peak at 3306 cm^{-1} corresponds to O–H stretching vibrations, and a peak at 1635 cm^{-1} is attributed to C=O stretching. In the CuO/Gly nanoparticles, these peaks shifted to 3436 cm^{-1} (O–H) and 1495 cm^{-1} (C=O) with reduced intensity, indicating coordination of copper oxide with glycyrrhizin molecules. Additionally, a peak observed at 855 cm^{-1} is characteristic of Cu–O bond vibrations. These results suggest that the functional groups of glycyrrhizin act synergistically in the reduction and capping of

CuO nanoparticles, confirming the successful formation of stabilized CuO/GLY nanostructures (Figure 2) [27].

<Figure 2>

The crystalline structure of the biosynthesized CuO/Gly nanoparticles was examined by XRD analysis, as shown in Figure 3. The diffraction peaks observed at 36.48°, 38.7°, 48.7°, 53.28°, 62.66°, 70.68°, and 75.17° correspond to the (111), (200), (220), (311), and (222) planes of copper oxide, confirming the formation of crystalline CuO nanoparticles. The average crystallite size calculated using the Debye–Scherrer equation for the most intense peak was approximately 30 nm. These results are consistent with previously reported data and indicate the successful synthesis of well-crystallized CuO nanoparticles [32].

<Figure 3>

The morphology and elemental composition of the synthesized CuO/Gly nanocatalyst were investigated using field emission scanning electron microscopy (FE-SEM) coupled with energy-dispersive X-ray spectroscopy (EDS). As shown in Figure 4 (a–d), the FE-SEM images recorded at different magnifications (200 and 500) reveal the formation of predominantly spherical copper oxide nanoparticles with a noticeable tendency toward agglomeration. The average particle size was estimated to be below 30 nm, confirming the successful formation of nanoscale CuO particles. The elemental composition of the CuO/GLY nanocatalyst was further analyzed by EDS (Figure 5). The EDS spectrum confirms the presence of copper as the main element, along with oxygen and carbon originating from the copper oxide structure and the plant-derived organic moieties, respectively. No additional impurity elements were detected, indicating the high purity of the biosynthesized nanocatalyst. According to the EDS quantitative analysis, the copper content in the CuO/GLY nanocatalyst was estimated to be approximately 15 wt %, which is

consistent with the successful incorporation of copper oxide nanoparticles stabilized by Glycyrrhiza glabra extract [33, 34].

<Figure 4>

<Figure 5>

The specific surface area and porosity of the CuO/Gly nanoparticles were evaluated using the BET method. The N_2 adsorption–desorption isotherm (Figure 6) corresponds to type V according to IUPAC classification, with a H1-type hysteresis loop, indicating mesoporous structure. The BET results showed a specific surface area of 39.44 m^2/g and an average pore diameter of 16.29 nm. The pore size distribution obtained from the BJH method revealed a narrow distribution around 7.99 nm (Table 1). The hysteresis observed in the isotherm can be attributed to interactions between the gas phase and the nanoparticle surfaces, as well as particle aggregation during calcination. These results confirm the mesoporous nature of the CuO/GLY nanocatalyst, which is favorable for catalytic applications due to increased surface area and accessibility of active sites.

<Table 1>

<Figure 6>

The thermal stability and decomposition behavior of the biosynthesized CuO/Gly nanoparticles were investigated using thermogravimetric analysis (TGA). The TGA curve (Figure 7) shows a slight weight loss below 150 °C, which can be attributed to the evaporation of physically adsorbed water molecules. A more pronounced weight loss in the range of 200–400 °C corresponds to the decomposition of organic compounds and secondary metabolites from Glycyrrhiza glabra that act as capping and stabilizing agents on the nanoparticle surface. Beyond 400 °C, the weight remains relatively constant, indicating the formation of stable copper oxide nanoparticles. These results confirm the effective capping of

CuO nanoparticles by plant-derived biomolecules, which enhances their thermal stability [35].

<Figure 7>

In Vitro Antioxidant Activity

The antioxidant activity of the biosynthesized CuO/Gly nanocatalyst was assessed using the DPPH free radical scavenging assay, with ascorbic acid as a reference antioxidant (Table 2). Various concentrations of CuO/Gly nanoparticles were prepared in methanol, and 1.0 mL of each sample was mixed with 1.0 mL of freshly prepared DPPH solution (0.1 mM in methanol). The mixtures were incubated in the dark for 30 min at room temperature, and the absorbance was measured at 517 nm using a UV–visible spectrophotometer (Figure 1). The radical scavenging activity was calculated using the standard equation, and IC_{50} values were determined from concentration–inhibition plots. The results demonstrated a concentration-dependent antioxidant activity for both CuO/Gly nanoparticles and ascorbic acid. At 200 μg mL^{-1}, the CuO/GLY nanocatalyst exhibited 79.69 ± 5.3% DPPH scavenging activity, whereas ascorbic acid showed a higher inhibition of 93.23 ± 9.2%. The IC_{50} values were calculated to be 97.43 μg mL^{-1} for CuO/Gly nanoparticles and 8.67 μg mL^{-1} for ascorbic acid, indicating a moderate antioxidant capacity for the biosynthesized nanocatalyst [36-39].

<Table 2>

Catalytic activity

The reaction conditions for the synthesis of 1,2,3-triazoles were optimized using a model reaction of phenylacetylene, benzyl chloride, and sodium azide in the presence of CuO/Gly nanocatalyst. The optimization results are summarized in Table 3. The influence of temperature, catalyst loading, and solvent was systematically investigated. At room temperature, the reaction afforded only a trace amount of product, whereas increasing the

temperature significantly improved the yield. The best result was obtained under reflux conditions. Catalyst screening revealed that 0.06 g of CuO/Gly provided the highest yield, and further increase did not enhance the reaction efficiency. Solvent evaluation demonstrated that ethanol was superior compared to other tested solvents. Under the optimized conditions (0.06 g CuO/Gly in ethanol at reflux condition), the desired products were obtained in the highest yield. These conditions were subsequently applied to various alkyl and aryl halides for further studies.

<Table 3>

The generality of the CuO/GLY catalytic system was evaluated using benzyl/alkyl halides and phenylacetylene. Under optimized conditions (0.06 g CuO/Gly in ethanol at reflux), reactions of 1 mmol benzyl/alkyl halides with 1.1 mmol sodium azide and 1.1 mmol phenylacetylene afforded the corresponding 1,4-disubstituted 1,2,3-triazoles in high yields (90–93%). The system showed broad compatibility with both electron-donating (Me, OMe) and electron-withdrawing groups (Cl, Br, NO_2). Results are summarized in Table 4. Further, various propargyl ethers reacted efficiently with benzyl chloride and sodium azide, providing the desired triazole derivatives in yields ranging from 87 to 92%. These results are presented in Table 5. Compared to similar methods reported in the literature, which often show lower yields (80-85%), our method demonstrates superior efficiency and broader substrate compatibility. The structures of all products were confirmed via FT-IR and 1H NMR spectroscopy, and their melting points were compared to reported values.

<Table 4>

<Table 5>

The CuO/Gly nanocatalyst-mediated synthesis of 1,2,3-triazoles is proposed to proceed via the following pathway. Initially, phenylacetylene is deprotonated by the CuO/GLY catalyst, generating a reactive acetylide species. The in situ-formed organic azide, derived

from the reaction of sodium azide with aryl or alkyl halides, subsequently attacks the copper–acetylide complex, forming a six-membered cyclic intermediate. Cyclization then occurs as the lone pair on nitrogen 1 attacks carbon 5, leading to ring contraction and formation of the five-membered 1,2,3-triazole core. Protonation restores neutrality, and the catalyst is released, yielding the desired 1,4-disubstituted 1,2,3-triazole product. The overall process is illustrated in Scheme 3, highlighting the key role of CuO/Gly in both activation and stabilization of the reactive intermediates.

< Scheme 3>

The reusability of the biosynthesized CuO/Gly nanocatalyst was evaluated by performing the model reaction under the optimized conditions. After completion of each reaction cycle, the nanocatalyst was separated by filtration, washed thoroughly with hot ethanol, dried, and reused in subsequent runs. As shown in Table 6, the CuO/Gly nanocatalyst maintained high catalytic activity over five consecutive cycles, with only a slight decrease in product yield, demonstrating its excellent stability and reusability. These results indicate that the biosynthesized CuO/Gly nanocatalyst can be efficiently recovered and reused without significant loss of performance, highlighting its potential for sustainable and cost-effective applications in 1,2,3-triazole synthesis.

<Table 6>

Conclusion

In this study, copper oxide nanoparticles (CuO/Gly nanocatalyst) were successfully synthesized using Glycyrrhiza glabra extract via a green and cost-effective method. The nanoparticles were well-characterized, showing spherical morphology, mesoporous structure, and effective capping by plant bioactive compounds. They exhibited moderate antioxidant activity and high catalytic efficiency in the synthesis of 1,2,3-triazole derivatives. Moreover, these nanoparticles exhibited moderate free radical scavenging activity, indicating potential

applications beyond catalysis. Overall, the biosynthesized CuO/Gly nanoparticles offer a sustainable and promising approach for both biomedical and catalytic applications.

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Declarations

Ethical Approval

We declare that the study was conducted in compliance with international, national and intuitional ethical guidelines governing animal experimentation, clinical research, and biodiversity conservation. No human or animal subjects were involved in this work.

Competing interests

The authors declare that they have no competing interest.

Authors Contributions

We certify that this manuscript has been read and approved by all listed authors. We confirm that no individuals who meet authorship criteria have been omitted from the list. Additionally, the order of authorship presented herein has been mutually agreed upon and approved by all authors.

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Data availability

The data that support the findings of this study are included within the article.

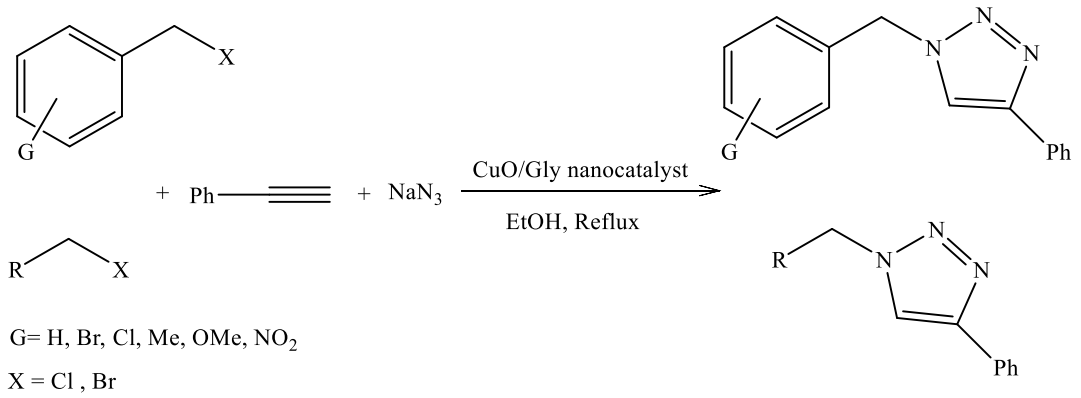
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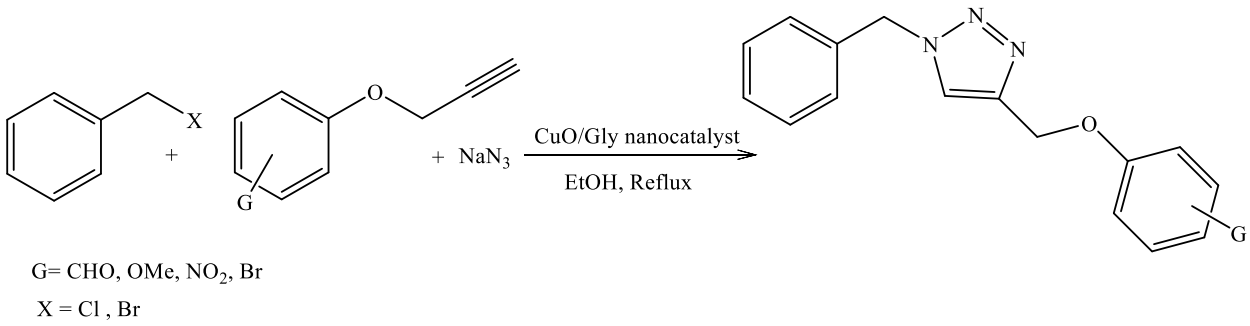
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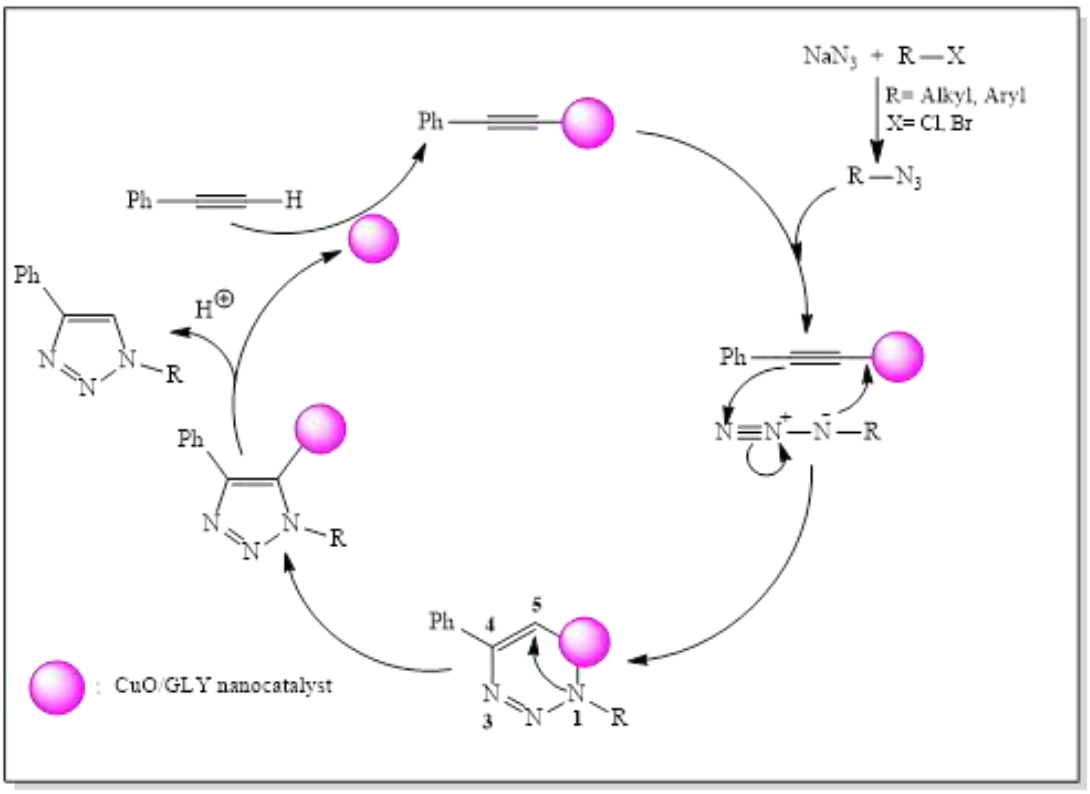
Scheme 1. Synthesis of 1,2,3-triazoles from phenylacetylene, NaN_3 and benzyl or alkyl halides catalyze by CuO/Gly nanocatalyst.



G= CHO, OMe, NO_2, Br

X = Cl , Br

Scheme 2. Synthesis of 1,2,3-triazoles from propargyl ethers, NaN_3 and benzyl halides catalyze by CuO/Gly nanocatalyst.



Scheme 3. The proposed pathway for the synthesis of 1,2,3-triazole derivatives in the presence of CuO/Gly nanocatalyst.

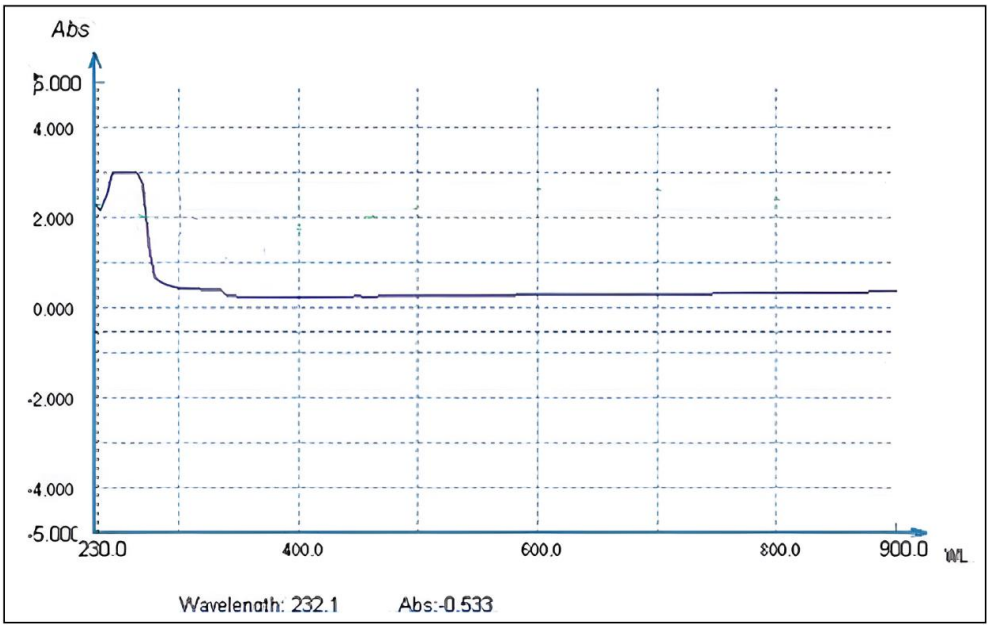


Figure 1. The UV-Vis absorption spectrum of the CuO/Gly nanocatalyst.

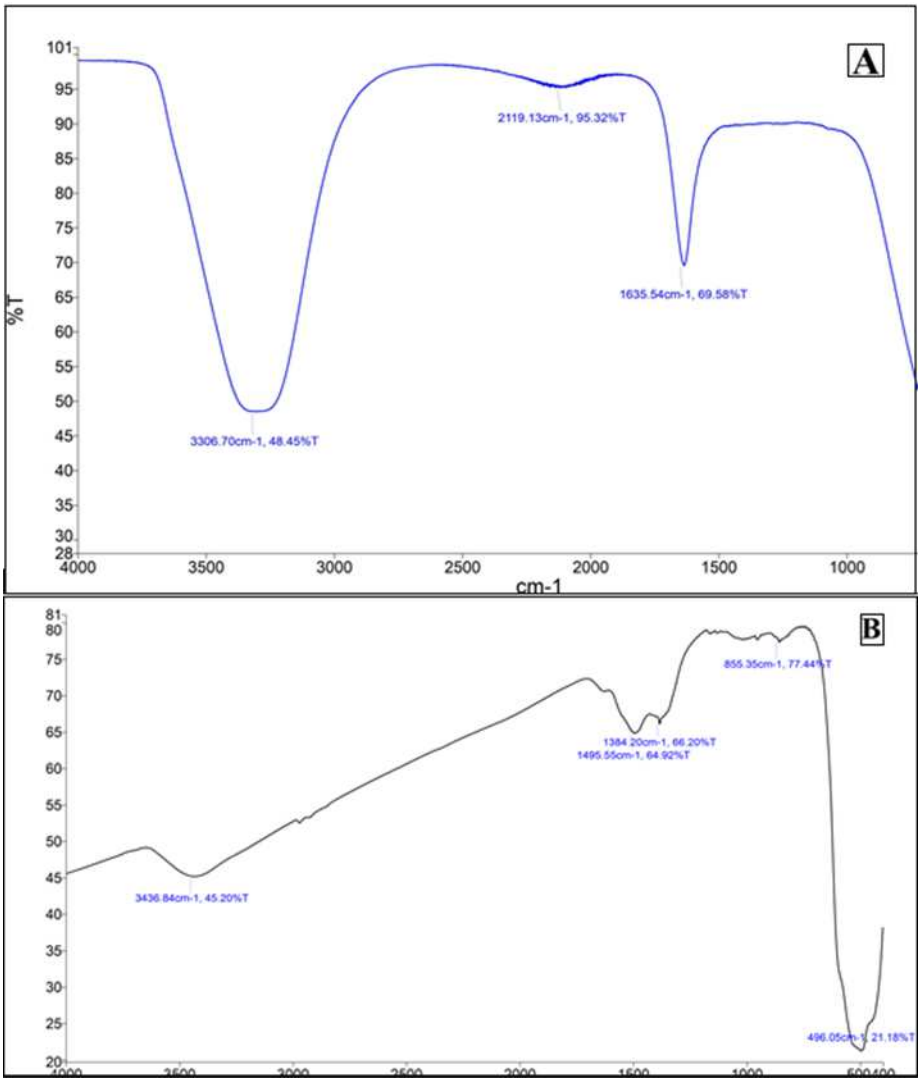


Figure 2. The FT-IR spectra of glycyrrhizin (A) and CuO/Gly nanocatalyst (B).

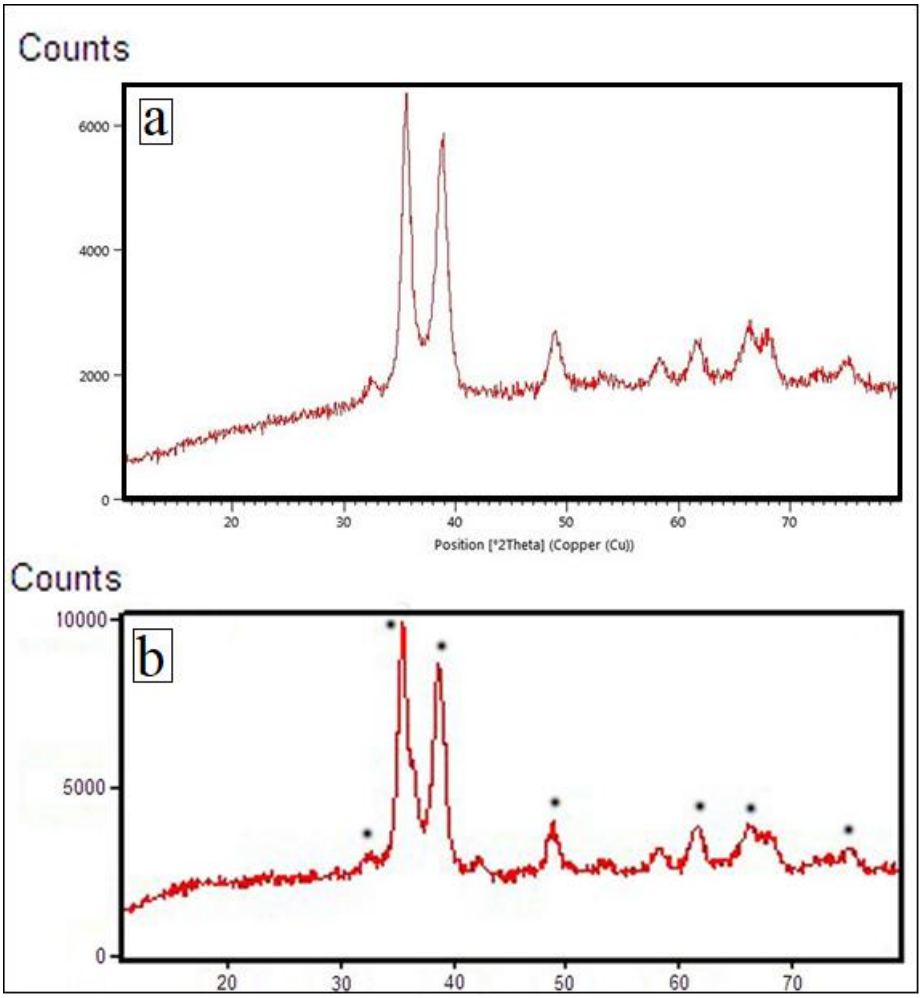


Figure 3. XRD patterns of the nanocatalyst CuO/Gly (a) copper oxide (b).

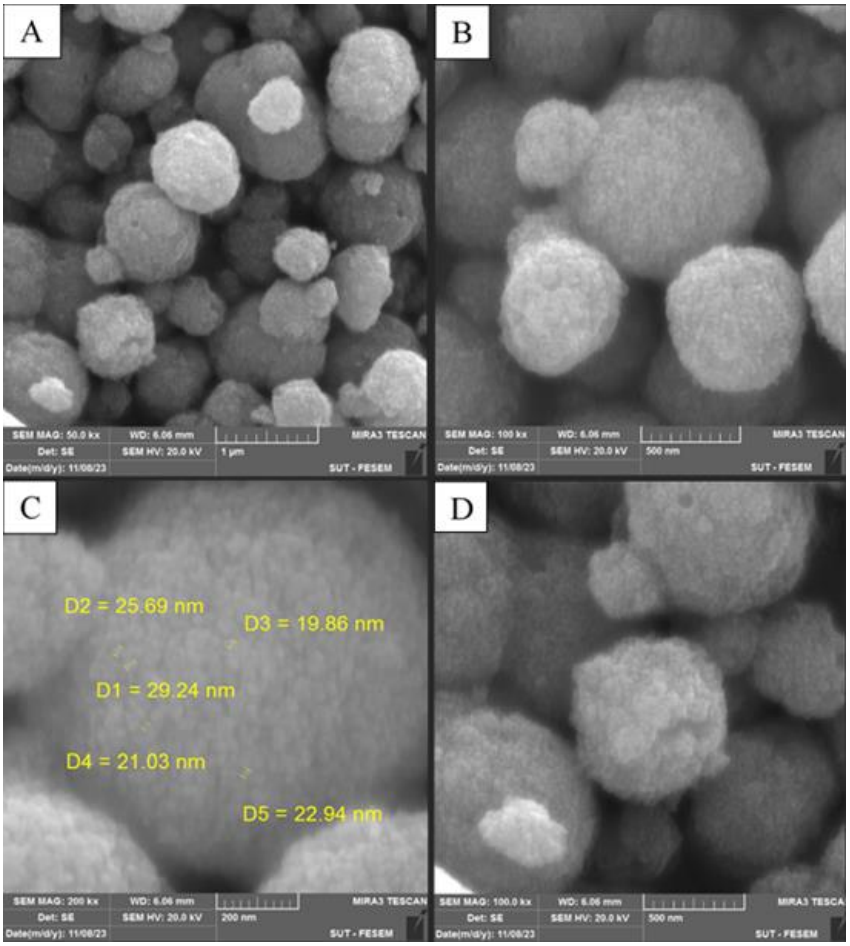


Figure 4. The FESEM images of the CuO/Gly nanoparticles.

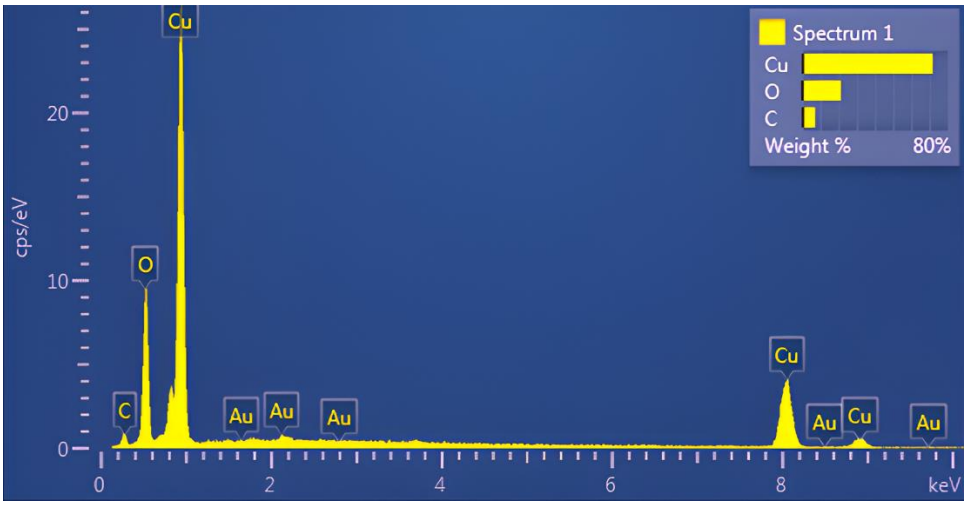


Figure 5. The EDS analysis of the CuO/Gly nanocatalyst.

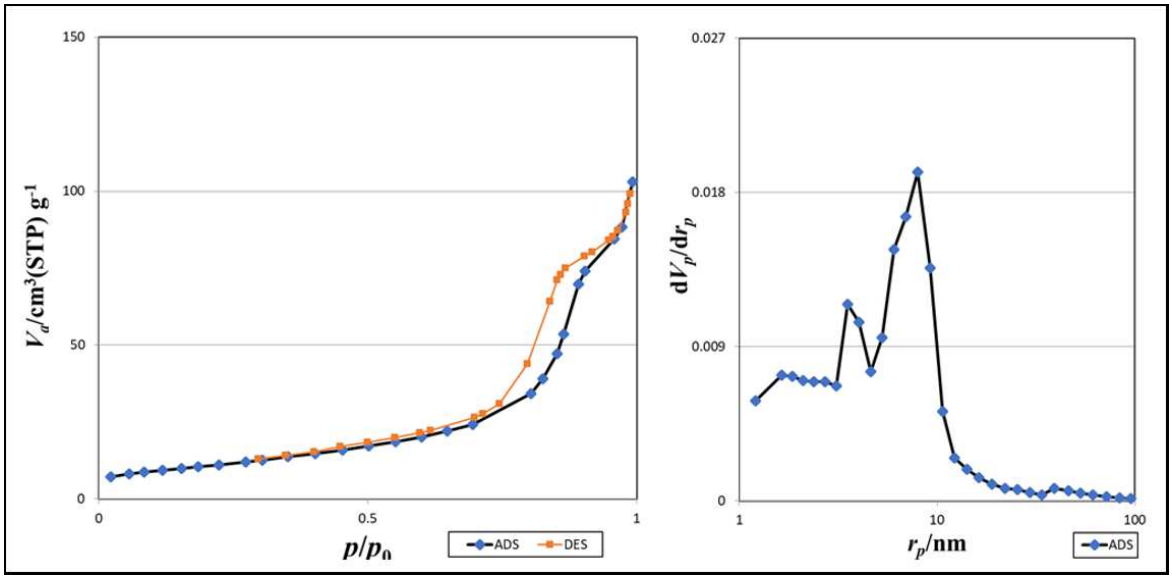


Figure 6. The BET analysis of the CuO/Gly nanocatalyst.

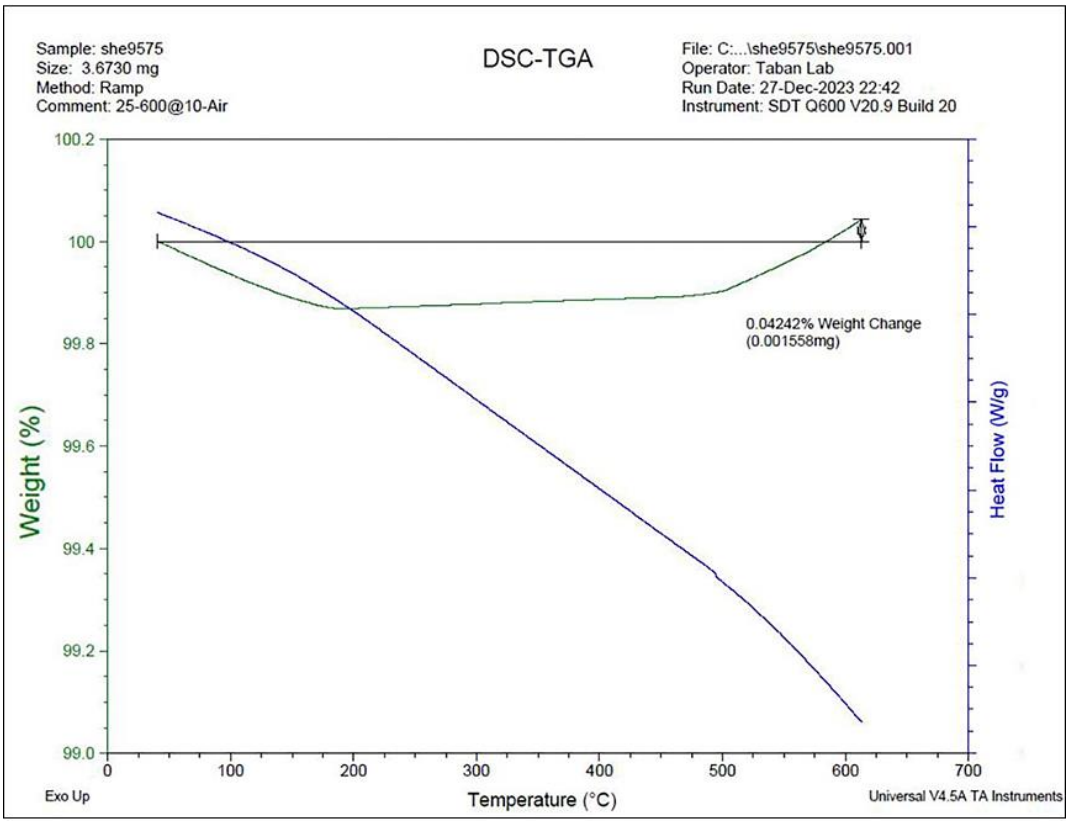


Figure 7. The TGA-DSC analysis of the (CuO/Gly) nanocatalyst.

Table 1: BET and BJH structural parameters of CuO/Gly nanocatalyst.

Sample	**Specific surface area** (m^2g^{-1})	**Mean pore diameter** (nm)	**Pore volume** (cm^3g^{-1})
CuO/GLY nanocatalyst	39.044	16.029	0.1565

Table 2: DPPH radical inhibition percentage of Ascorbic acid, biosynthesized CuO/Gly.

Concentration (µg/mL)	**Ascorbic acid**	**CuO/Gly**
25	56.39±2.7	31.57894737
50	58.64±1.5	37.59398496
100	68.42±2.3	48.12030075
125	71.42±1.8	57.14285714
150	81.20±5.6	63.15789474
175	89.47±5.6	69.92481203
200	93.23±9.2	79.69924812
IC_{50} value	8.67	97.43

Data are presented as mean ± SD (n = 3). ($P < 0.05$). Antioxidant activity of the biosynthesized CuO/GLY nanocatalyst and ascorbic acid at different concentrations (50–200 µg/mL).

Table 3: Optimization of reaction conditions for the model reaction catalyzed by CuO/Glynanocatalyst.

Entry	Condition	Catalyst amounts (g)	Time (min)	Yield (%) [a]
1	Solvent-free/80 °C	0.06	60	Trace
2	H_2O/Reflux	0.06	60	65
3	DMF/80 °C	0.06	60	Trace
4	MeCN/Reflux	0.06	60	Trace
5	EtOH /60 °C	0.06	60	75
6	EtOH/Reflux	0.06	30	94

[a] Isolated yield.

Table 4: Synthesis of 1,2,3-triazole derivatives catalyzed by CuO/Gly nanocatalyst.[a]

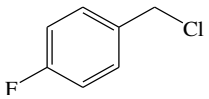
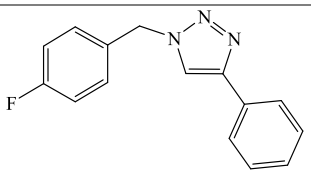
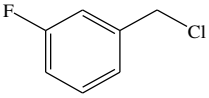
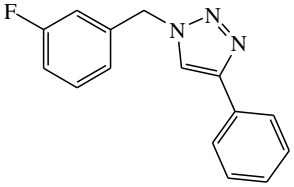
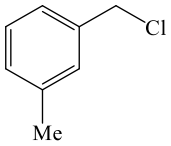
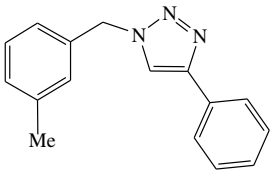
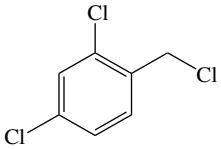
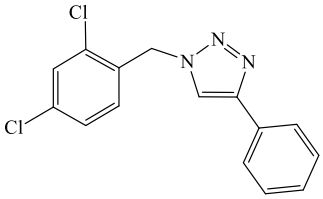
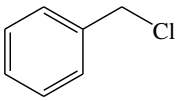
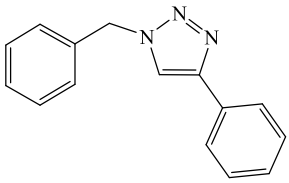
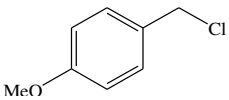
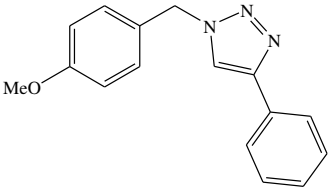
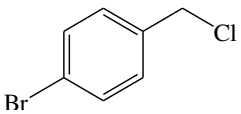
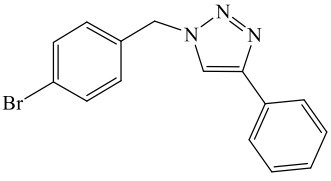
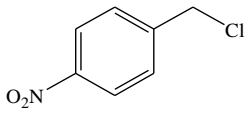
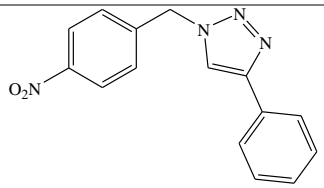
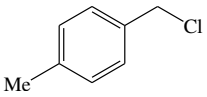
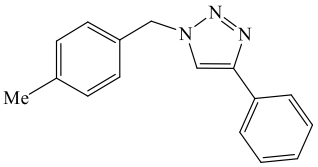
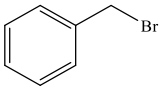
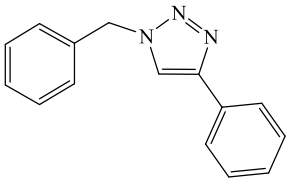
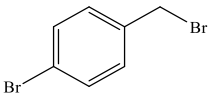
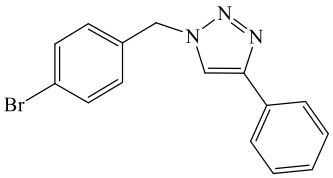
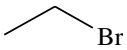
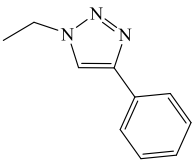
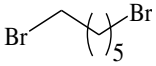
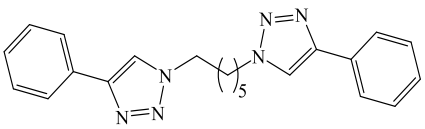
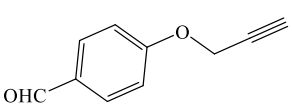
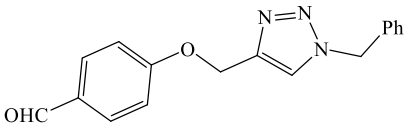
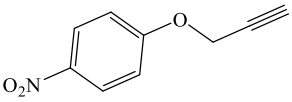
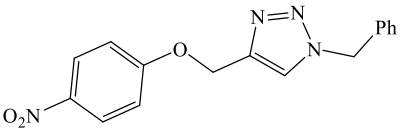
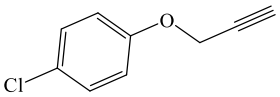
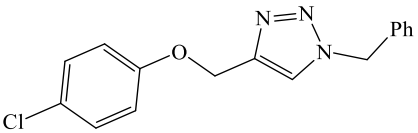
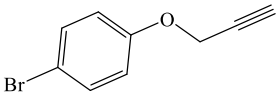
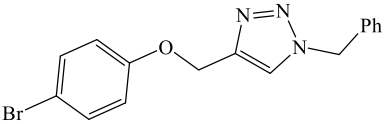
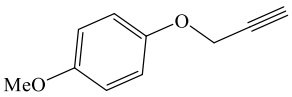
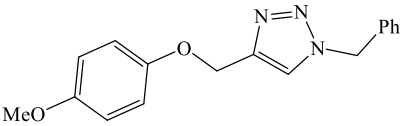
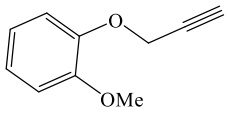
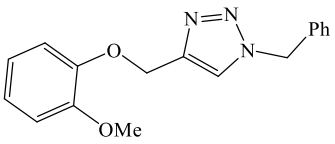
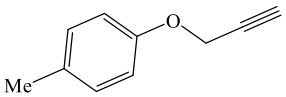
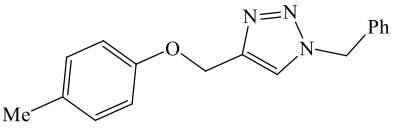
Entry	Aryl/Alkyl halides	Product	Time (min)	Yield (%) [b]
1			20	93
2			30	92
3			25	91
4			20	92
5			30	94
6			19	93
7			20	93

Table 4. *Continued.*

Entry	Aryl/Alkyl halides	Product	Time (min)	Yield (%) [b]
8			20	92
9			20	93
10			30	92
11			25	91
12			20	92
13			30	94

[a] The reaction was performed in the presence of CuO/Gly nanocatalyst in ethanol under reflux conditions. [b] Isolated yields.

Table 5. Synthesis of 1,2,3-triazole derivatives from various propargyl ethers catalyzed by CuO/Gly nanocatalyst.[a]

Entry	Propargyl ethers	Product	Time (min)	Yield (%) [b]
1			30	87
2			25	90
3			45	91
4			30	92
5			20	91
6			45	88
7			30	90

[a] The reaction was performed in the presence of CuO/Gly nanocatalyst in ethanol under reflux conditions. [b] Isolated yields.

Table 6. Recyclability of the CuO/Gly nanocatalyst.

Run	1	2	3	4	5
Time (min)	30	30	30	30	30
Yield (%) [a]	93	92	91	91	89

[a] Isolated yield.

Supplementary Files

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- Supplementaryfile.docx