Copper nanoparticles catalysed an efficient one-pot multicomponents synthesis of chromenes derivatives and its antibacterial activity

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ABSTRACT
In this study, copper nanoparticles (Cu NPs) were synthesised by using diethylenetriamine as a protective agent in chemical reduction method. The obtained nanoparticles were characterised by various spectroscopic techniques like powder X-ray diffraction (PXRD), Fourier transform infrared (FTIR), UV--visible spectroscopy, energy dispersive spectroscopy (EDS), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and thermal analysis (TG/DTA). The structure and composition were estimated by PXRD, FTIR, EDS, UV--visible and TG/DTA techniques, while particles size and morphology behaviours were investigated by SEM and TEM instrumentation. A noteworthy, average particle size of nanoparticles was found around 40 nm with spherical shapes. Furthermore, the applications part of NPs were studied as a catalyst for one-pot solvent-free green synthesis of 3,4-dihydropyrano[c]chromenes from different aromatic aldehydes, malonitrile and 4-hydroxycoumarin by stirring at 80 °C. Moreover, the antibacterial properties of NPs were assessed \textit{in vitro} against human bacterial pathogen such as \textit{Staphylococcus aureus}, \textit{Escherichia coli}, \textit{Klebsiella} sp. and \textit{Pseudomonas aruginosa} using agar well diffusion method. Gram positive bacteria \textit{S. aureus} (18 mm) exhibited a maximum zone of inhibition at 60 µg/ml of Cu NPs. Nonetheless, antibacterial activities of Cu NPs (10–100 µg) were compared with four well-known antibiotics likes amikacin (30 mcg), ciprofloxacin (5 mcg), gentamicin (5 mcg) and norfloxacin (10 mcg). This study indicates that Cu NPs exhibited a strong antibacterial activity against all the test pathogens even at lower concentration.

1. Introduction
Nowadays synthesis of metal nanoparticles is emerging as rapidly growing in the field of nanotechnology due to their tremendous applications. For instance, copper,
zinc, silver, iron, titanium, nickel, tungsten, ruthenium, palladium, bismuth, etc. have immense applications like electronics, photonics, medicines as anticancer, catalysis, and antimicrobial.[1–3] Particularly, copper nanoparticles (Cu NPs) attracted enormous interest in biological as well as pharmacological because of their unique physico-chemical properties. Their properties can be forbidden depending on the synthesis method. One of the main effects in antimicrobial action is enhanced by controlling the particle size.[4,5] An enhancing antimicrobial activity of metal nanoparticles is due to the large surface area of metal nanoparticles is directly contact with microorganisms. For this reason, high surface area of NPs assures a wide range of reactions on the surface of microorganisms inhibiting the normal function of cells or causing cell death.[6] Apart from this, fascinatingly, Cu NPs are used as nanocatalyst for the synthesis of organic compounds through multicomponents reaction. Nowadays, interestingly researchers are trying to develop new synthetic methods by using nanocatalyst to reduce the risks of the environment pollution.[7] On the other hand, organic solvents are high unsafe chemicals since they are used in large amounts, usually volatile liquid, and therefore solvent-free green synthesis of organic compounds have attracted considerable interest [8] as, they have tremendous advantages like high effectiveness, operational cleanness, low costs, mild reaction conditions and pollution-free condition.[9] In this regard, in this paper, we have paid much more attention on the synthesis of various chromenes derivatives in a solvent-free condition by using Cu NPs as a catalyst.

Also, the literature survey reveals that different chromenes derivatives were synthesised by using various catalysts such as hexamethylenetetramine, sodium bromide, polymer supported sulphanilic acid, sodium dodecylsulphate, piperidine/triethylamine in aqueous media, piperidine-functionalised poly(ethylene glycol) bridged dicationic ionic liquid, ammonium acetate, pyridine, morpholine, thiourea dioxide, etc.[10–19] However, 3,4-dihydropyrano[c] chromenes derivatives were also synthesised by using many metals oxides nanoparticles like ZnO NPs, MgO NPs, α-Fe2O3 NPs, CuO NPs and Al2O3 NPs.[20–24] Nevertheless, there are inadequate works done on the synthesis of chromenes derivatives using Cu NPs. To the best of our knowledge, the synthesis of 3,4-dihydropyrano[c]chromenes through the reaction of different aromatic aldehydes, malonitrile and 4-hydroxycoumarin using Cu NPs as a catalyst is reported for the first time.

In this study, we have reported the synthesis of Cu NPs by using a protective agent and characterised by various spectroscopic and microscopic techniques. Furthermore, we reported Cu NPs catalysed solvent-free green synthesis of 3,4-dihydropyrano[c]chromenes derivative. We have found an efficient and reusability of Cu NPs towards solvent-free green synthesis of 3,4-dihydropyrano[c] chromenes at 80 °C. Interestingly, synthesised nano catalyst exhibited an excellent recyclability and reusability up to 4 times without any additional treatment. Moreover, the antibacterial properties of Cu NPs were evaluated in vitro against strains of human bacterial pathogen and compared with four well-known antibiotics, i.e. amikacin (30 mcg), ciprofloxacin (5 mcg), gentamicin (5 mcg) and norfloxacin (10 mcg). Notwithstanding, in our previous work, we reported synthesis, characterisation and efficient catalytic activity of nickel nanoparticles via Knoevenagel condensation of aromatic aldehydes and malononitrile under solvent-free conditions, while antimicrobial assay of ZnO nanoparticles were successfully investigated using human bacterial pathogens.[25–27]
2. Experimental section

2.1. Materials and microscopic measurements

All the chemicals and solvents were used without further purifications. They include cupric nitrate trihydrate (Sigma Aldrich), ethylene glycol (Merck), diethylenetriamine (DET; Merck), hydrazine hydrate 80% (Sd Fine), aromatic aldehydes (Merck), malononitrile (Merck) and 4-hydroxy coumarins (Merck). The size and morphology of Cu NPs were examined by scanning electron microscope (JEOL model JSM-690LV) whose maximum magnification is 300,000X and the resolution is 3 nm at the Sophisticated Test and Instrumentation Centre, Cochin University Kerala. Transmission electron microscopy (TEM) images were formed using CM200 which can produce magnification details up to 1,000,000X with resolution better than 10 Å at the Indian Institute of Technology, Pawai (Mumbai). The qualitative elemental analysis of the powder sample was studied by energy dispersive spectroscopy (EDS; JEOL Model JED-200) and thermal analyses (TG/DTG/DTA) at a heating rate 10 °C/min under nitrogen atmosphere at the Sophisticated Test and Instrumentation Centre, Cochin University, Kerala. The crystal structure of the sample was characterised by powder X-ray diffraction, Bruker AXS D8 Advance X-ray diffractometer using CuKα radiation. Infra-red spectroscopy was recorded at a 2 cm⁻¹ resolution from 4000 to 400 cm⁻¹ on a Bruker IFS 66v Fourier transform spectrometer using KBr pellets. ¹H-NMR spectroscopy of samples were carried out on NMR Spectrometer model Avance-II (Bruker) at the SAIF Chandigarh, India. The instrument is equipped with a cryomagnet of field strength 9.4 T. Its ¹H frequency is 400 Mhz. Mass analyses organic derivatives were carried out by expression CMS Mass Spectrometer at Synzel laboratory Gandhinagar, Gujarat (India).

2.2. Bacterial pathogens and growth conditions

The pathogens Staphylococcus aureus, Escherichia coli, Klebsiella sp., Enterococcus faecalis and Pseudomonas aruginosa were obtained from the Pathology and Diagnosis Laboratory Department of Microbiology, S.K. Porwal College, Kamptee. The strains were maintained on nutrient agar slants at 4 °C.

2.3. Preparation of inoculum

Stock cultures were maintained at 4 °C on nutrient agar. Active cultures were prepared by transferring a loopful of culture from the stock to test tubes of Mueller–Hinton broth (MHB) that was incubated for 24 h at 37 °C. The cultures were diluted with fresh MHB to achieve optical densities corresponding to 0.5, i.e. 10⁵ to 10⁶ CFU/ml using MacFarland’s standard.

2.4. Antibacterial assay: agar well diffusion method

The antibacterial activity of the Cu NPs was performed by using well diffusion method. About 20 ml of sterile molten Muller Hinton agar (HiMedia Laboratories Pvt. Limited, Mumbai, India) was poured into sterile petriplates. Triplicates plates were swabbed with
the overnight culture (10^8 cells/mL) of pathogenic bacteria, viz. *Pseudomonas sp.*, *Enterobacter sp.*, *Klebsiella sp.*, *E. coli*, and *S. aureus*. The solid medium was gently punctured with the help of cork borer to make a well. Finally, the nanoparticles samples (20, 40, 60, 80 and 100 μg/ml) were added from the stock into each well and incubated for 24 h at 37±2 °C. After 24 h, the zone of inhibition was measured and expressed as millimetre in diameter.

2.5. Minimum inhibitory concentration (MIC)

About 500 μl of different concentrations (2.5, 5, 10, 15 and 20 μg) of chosen nanoparticles were prepared in dimethylformide and mixed with 450 μl of nutrient broth grown 50 μl of 24 h old bacterial inoculums and allowed to grow overnight at 37 °C for 48 h. Nutrient broth alone served as negative control. The MIC was the lowest concentration of the nanoparticles that did not permit any visible growth of bacteria during 24 h of incubation on the basis of turbidity.[28]

2.6. Preparation of Cu NPs

The Cu NPs were prepared by dissolving 0.1 M cupric nitrate trihydrate in 20.0 mL of ethylene glycol in 250 mL round bottom flask and then 14 mL DET was added to this solution. The system was maintained at room temperature and the mixture was then heated to 80 °C and reduced with 5 mL hydrazine hydrate (80%) followed by 20 ml of 0.1 M solution of sodium hydroxide added into the heated solution to enhance the reducing power. The brownish black colour particles were separated by centrifugation (1500 rpm, 5 min) and then washed several times with methanol, distilled water and acetone to remove the reducing agents. Nanoparticles were obtained after centrifugation kept in vacuum oven at 30 °C for drying. Furthermore, characterisations of Cu NPs were carried out by UV—visible spectra (Figure 3), X-ray diffraction (XRD; Figure 4(a)), EDS (Figure 4(b)), scanning electron microscopy (SEM; Figure 5), TEM (Figure 6), Fourier transform infrared (FTIR; Figure 7) and TG/DTG (Figure 8) in order to determine their formation, size and morphology behaviours. Flow sheet block diagram of Cu NPs synthesis is presented in Scheme 1.

2.7. General procedure for solvent-free green synthesis of chromenes derivative catalysed by Cu NPs

In a typical reaction procedure (Scheme 2), aromatic aldehydes (1 mmol), 4-hydroxy coumarins (1 mmol) and malononitrile (1.2 mmol) were taken in a 25 ml round bottomed flask and 12 mol % Cu NPs was added and stirred on a magnetic stirrer at 70 °C. The reaction progress was followed by thin layer chromatography (TLC) using n-hexane/ethyl acetate (8:2) as an eluent. After the completion of the reaction, 15 ml of ethanol was added to the reaction mixture and heated to remove the catalyst by filtration. The obtained products were characterised by various spectroscopy techniques such as 1H NMR, FTIR and mass analyses and then compared their melting points with authentic samples in the literature.
2.7.1. Spectral data
Selected data for typical compounds are given below.

1. 2-amino-4-(4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile 4(h): whitish yellow powder, mp 242–243 °C, yield 91%; IR (KBr, ν): 3190, 3065, 2199, 1702, 1670, 1604 cm⁻¹; ¹H NMR (400 Hz, DMSO-d₆): δ 3.73 (s, 3H, OCH₃), 4.41 (s, 1H, CH), 6.72–8.33 (m, 10H, Ar, NH₂); MS (ESI): m/z = 346 (M⁺).

2. 2-amino-4-(2,4-dimethoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile 4(g): yellow powder, mp 236–238 °C, yield 93%; IR (KBr, ν): 3406, 3325, 3293, 3258, 3219, 2196, 1709, 1672, 1379, 1237, 1049, 759 cm⁻¹; ¹H NMR (400 Hz, DMSO-d₆) δ: 3.89–3.91 (s, 6H, OCH₃), 4.41 (s, 1H, CH), 6.30–8.28 (m, 9H, Ar, NH₂); MS (ESI): m/z = 376 (M⁺).

3. 2-amino-5-oxo-4-(pyridin-3-yl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile 4(i): brown powder, mp 255–256 °C, yield 90%; IR (KBr, ν): 3371, 3248, 2192, 1613, 1445 cm⁻¹; ¹H NMR (400 Hz, DMSO-d₆) δ: 4.59 (s, 1H, CH), 7.20–8.61 (m, 10H, Ar, NH₂); MS (ESI): m/z = 317 (M⁺).
3. Result and discussion

3.1. Catalytic activity of Cu NPs

The synthesis of 2-amino-4-aryl-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile derivatives was achieved by the one-pot, three-component condensation of aromatic aldehydes, malonitrile and 4-hydroxycoumarin by using Cu NPs as a catalyst under solvent-free condition at 80 °C that give products in excellent yields (Figure 1, Table 2). In our initial study, for optimisation of reaction conditions, the reaction of benzaldehyde, malonitrile and 4-hydroxycoumarin was used as a model reaction to optimise the reaction conditions. First, the reaction was conducted in various solvents using Cu NPs as a catalyst under refluxing conditions and also under solvent-free conditions.

Furthermore, we also investigated the possibility of recycling the nano catalyst. The nano catalyst was recovered by filtration from the model reaction checked in the subsequent runs without further purification (Figure 2). The activity of the catalyst did not get much affected in terms of yields after five successive runs for the model reaction. It revealed that the catalyst displayed very good reusability.
The mechanism of this reaction has not been clearly established. A probable explanation is proposed in Scheme 3. In the first step, aromatic aldehydes are condensed with malononitrile by condensation to afford an α-cyanocinnammonitrile derivative. In the second step, the active methylene group of 4-hydroxycoumarin reacts with electrophilic carbon atom of α-cyanocinnammonitrile giving the Michael adduct which was undergoes cyclised by nucleophilic attack of the carbonyl group on cyano group giving another intermediate. Finally, the expected product 4 is afforded by tautomerisation. Increase in yield of the products in this study may be due to the large surface-to-volume ratio and stability of Cu NPs which may have suppressed the formation of the side products.

The catalytic activity graph with amount of catalyst is shown in Figure 1. The reaction was carried out with various amount of Cu NPs as the catalyst (3 to 18 mol%) for the synthesis of 3,4-dihydropyrano[c] chromenes. The yield of products increases remarkably from 54% to 94% with the increase in the concentration of the catalyst amount from 3 to 12 mol%. When the catalyst amount increased from 12 to 18 mol%, no further increase in the yield of product was observed. Therefore, the amount of 12 mol% of Cu NPs was selected for all the subsequent reaction.

The effect of temperature in solvent-free conditions was studied by carrying out the reaction at 60 °C, 70 °C, 80 °C, 90 °C, 100 °C and 120 °C. The results from Table 1 (entry 6) showed that 80 °C would be the best temperature for all reactions. Under the optimised reaction conditions, a series of 3,4-dihydropyrano[c]chromenes derivatives 4 (a–j) were synthesised. The results are summarised in Table 2. In all cases, aromatic

Scheme 3. Proposed mechanism for the synthesis of 3,4-dihydropyrano [c] chromenes derivative using Cu NPs catalyst.

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Table 1. Synthesis of 3,4 dihydropyrano[c]chromenes derivative in various solvents and solvent-free conditions by using Cu NPs.

| Entry | Solvent      | Time(min) | Yield(%) | Colour  | M.P (°C) | Reference |
|-------|--------------|-----------|----------|---------|----------|-----------|
| 1     | Ethanol      | 80        | 62       |         |          |           |
| 2     | Methanol     | 80        | 58       |         |          |           |
| 3     | CH$_3$CN     | 80        | 57       |         |          |           |
| 4     | CHCl$_3$     | 80        | 53       |         |          |           |
| 5     | Toluene      | 80        | 45       |         |          |           |
| 6$^b$ | Solvent-free condition | 60 | 94,93,91,90 |         |          |           |

$^a$ Isolated yield.  
$^b$ Catalysed recycled four times.

Table 2. Cu NPs catalysed synthesis of 3, 4-dihydropyrano[c] chromenes derivative.

| Entry | Aldehydes | Product | Stirring time (min)$^a$ | Yield (%) | Colour     | M.P (°C)   | Reference |
|-------|-----------|---------|-------------------------|-----------|------------|------------|-----------|
| 4(a)  | CHO       | ![Image](image.png) | 60                      | 94        | Yellow     | 255–256    | 256–257[11] |
| 4(b)  | ![Image](image.png) NO$_2$ | ![Image](image.png) | 80                      | 95        | Yellow     | 256–257    | 257–260[11] |
| 4(c)  | ![Image](image.png) NO$_2$ | ![Image](image.png) | 80                      | 94        | Yellow     | 260–262    | 260–263[11] |
| 4(d)  | ![Image](image.png) Cl | ![Image](image.png) | 70                      | 92        | Whitish yellow | 266–268 | 266–267[11] |
| 4(e)  | ![Image](image.png) OH | ![Image](image.png) | 70                      | 90        | Whitish yellow | 260–262 | 262–266[11] |
| 4(f)  | ![Image](image.png) F | ![Image](image.png) | 80                      | 90        | White      | 278–279    | 278–282[11] |
| 4(g)  | ![Image](image.png) OCH$_3$ | ![Image](image.png) | 95                      | 93        | Whitish yellow | 236–238 | 236–238[11] |

(continued)
aldehydes substituted with either electron-donating or electron-withdrawing groups, which underwent the reaction smoothly and gave the expected products in good to high yields under the same reaction conditions. Moreover, heteroaromatic aldehydes like pyridine also was successfully converted to the corresponding heteroaryl substituted 3, 4-dihydropyra-no[c]chromenes in excellent yields.

3.2. Antibacterial activity

The antibacterial activity of Cu NPs in different concentration (10–100 μg) was quantitatively assessed on the basis of zone of inhibition and compared with standard antibiotics. According to the Clinical and Laboratory Standards Institute,[29] the sample was taken in minimum quantity. There was no increase in the zone of inhibition beyond 60 μg/ml concentration (Table 3). Among the Gram negative bacteria tested E. coli not only

### Table 2. (Continued)

| Entry | Aldehydes | Product | Stirring time (min) | Yield (%) | Colour | M.P (°C) | Reference |
|-------|-----------|---------|---------------------|-----------|--------|----------|-----------|
| 4(h)  | CHO       | ![Product](image) | 80                  | 91        | White  | 242–243  | 242–244[11]|
| 4(i)  | NCHO      | ![Product](image) | 90                  | 90        | Brown  | 255–256  | –         |
| 4(j)  | CHO       | ![Product](image) | 90                  | 89        | Yellow | 265–266  | 265–267[11]|

*a*Used magnetic stirrer for stirring.

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### Table 3. Antibacterial activity of Cu NPs against pathogenic bacteria.

| Cu NPs | Staphylococcus aureus | Pseudomonas aeruginosa | Escherichia coli | Klebsiella sp. |
|--------|-----------------------|------------------------|-----------------|---------------|
|        | Zone of inhibition μg/ml (mm) against pathogenic bacteria (n = 3) |                        |                  |               |
|        | 20 μg | 40 μg | 60 μg | 20 μg | 40 μg | 60 μg | 20 μg | 40 μg | 60 μg | 20 μg | 40 μg | 60 μg |
| Amikacin (AK) 30 mcg | 14±0.57 | 17±0.40 | 18±0.34 | 14±0.58 | 16±0.46 | 19±0.23 | 15±0.33 | 17±0.57 | 18±0.33 | – | 13±0.33 | 16±0.57 |
| Ciprofloxacin (CF) 5 mcg | 29±0.16 | 26±0.66 | 33±0.16 | 29±0.22 | 29±0.22 | 29±0.22 | 29±0.22 | 29±0.22 | 29±0.22 | – | 29±0.22 | 29±0.22 |
| Gentamicin (G) 30 mcg | 21±0.18 | 23±0.22 | 17±0.57 | 18±0.33 | 18±0.33 | 18±0.33 | 18±0.33 | 18±0.33 | 18±0.33 | – | 18±0.33 | 18±0.33 |
| Norfloxacin (Nx) 10 mcg | 24±0.458 | 23±0.34 | 30±0.529 | 27±0.503 | 27±0.503 | 27±0.503 | 27±0.503 | 27±0.503 | 27±0.503 | – | 27±0.503 | 27±0.503 |

Note: ±Standard deviation, (n = 3) three replicates.
show strong inhibition by CU nanoparticles at higher concentration (18 mm) but also shows strong activity at low concentration (15 mm). Similarly, Klebsiella sp. (16 mm) and P. aruginosa (19 mm) were moderately inhibited by Cu NPs. Besides, among the Gram positive bacteria tested, S. aureus (18 mm) exhibited a maximum zone of inhibition at 60 µg/ml of Cu nanoparticles. This study indicates that Cu nanoparticles exhibited a strong antibacterial activity against all the tested pathogens even at lower concentration.

On comparing the effect of standard antibiotics among the tested pathogens, it was found that P. aruginosa exhibits resistance to amikacin and gentamicin, but inhibited by norfloxacin (24 mm) (Table 3). Similarly, S. aureus exhibited high sensitivity against norfloxacin (30 mm), but minimum zone of inhibition was obtained against amikacin and gentamicin. This shows that even the amikacin- and gentamicin-resistant P. aruginosa was strongly inhibited by Cu NPs at concentration 40 µg. The MIC revealed that the Cu NPs showed sensitivity at the conc. of 20 µg/ml, against E. coli and Klebsiella sp. and P. aruginosa at the conc. of 15 and 20 µg/ml against S. aureus.

The observed antimicrobial effect of Cu NPs is not only due to their release of metal ions but can also be attributed to their morphology, mainly their small size and high surface area-to-volume ratio, which allows them to interact strongly with microbial membranes of each bacterium.[30] An instance, in a similar way, the antibacterial activity of silver NPs has been reported on the same bacterial strains.[31] Also, the effect of copper oxide nanoparticles against S. aureus and E. coli has been confirmed by Ren et al. [5] with concentrations ranging from 100 to 5000 µg/ml . According to the literature, the better inhibitory effect observed in P. aruginosa than in S. aureus is related to the difference in the outer covering of these bacteria.[32,30] A Gram positive bacterium S. aureus has a thick layer of peptidoglycan (a sugar-protein shell) wherein the Cu ions can penetrate. A Gram negative bacterium P. aruginosa has an outer membrane covering the thin layer of peptidoglycan on the outside. This outer membrane prevents the Cu ions from penetrating. The released Cu NPs ions may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death.[6]

### 3.3. Synthesis and microscopic characterisation of Cu NPs

The preparations of Cu NPs in nanometre range can be effectively carried out through the chemical reduction of Cu salts by using hydrazine hydrates under the basic condition at 80 °C in the presence of DET as a capping agent. The basic condition enhanced the reducing character of hydrazine hydrate. The DET acts as a very good capping agent and surface directing agent. The formation of metallic Cu NPs in this work was based on the following redox reaction under the basic condition (Reaction (1)):

\[
Cu^{2+} + N_2H_4 + 4OH^- \rightarrow Cu + N_2 + 4H_2O. \tag{1}
\]

The reduction is thermodynamically possible because redox potential of the hydrazine hydrate reducer was more negative than that of the cupric nitrate oxidiser (metal precursor). Ethylene glycol used in the reaction could help to avoid coagulation because it can be adsorbed and produce steric stabilisation which in turn inhibits agglomeration of the particles during growth.
A major problem in utilising these Cu NPs is their inherent tendency to oxidise in ambient conditions. Recently, there have been several reports presenting various approaches which demonstrated that Cu NPs can resist oxidation under ambient condition, if they are coated by a proper protective layer. The addition of DET to the reaction solution helps to avoid oxidation and promote the growth of Cu NPs. Temperature was kept constant (80 °C) throughout the reaction for control over particle size. The freshly prepared nanoparticles were found in reddish brown in colour. The UV–visible absorption spectrum of the Cu NPs is recorded in the wavelength region of 200–700 nm (Figure 3). It exhibits a strong intense peak around 275 nm and another low intense peak around 610 nm. The peak at ~275 nm is due to interband transition of copper electron from deep level of valence band while peak at ~ 610 nm is due to interband transition of copper electron from the upper level of valence band, which is also known as surface plasmon resonance peak.[33.

The XRD patterns of Cu NPs (Figure 4(a) display three peaks at 2θ values of 43.274°, 50.427° and 74.123° corresponding to (111), (200) and (220) planes and which were compared with the standard powder diffraction card of JCPDS, Copper file no. 04-0836. The XRD study indicates that the resultant particles of copper nanopowder are face cubic crystals. Furthermore, an average crystalline size of Cu NPs was found out around 40 nm by

![Figure 3. UV–visible spectra of Cu NPs.](image)

![Figure 4. Spectra of Cu NPs sample (a) XRD pattern and (b) EDS analysis.](image)
Figure 5. SEM images of Cu NPs.

Figure 6. TEM image of Cu NPs.
the Debye–Scherer method. Moreover, the present nanocatalyst was characterised by the EDS technique to find out chemical composition (Figure 4(b)). Fascinatingly, total composition of Cu NPs was found in the present sample.

Nonetheless, nanocatalyst was further characterised by microscopic techniques including SEM (Figure 5) and TEM (Figure 6). Images 5 and 6 reveal that the product consists of spherical shape and all nanoparticles dispersed very well. The average diameter was estimated from the TEM image to be around 40 nm (Figure 6). TEM result shows the influence of protecting the agent on the shape and size of Cu NPs. When analysing a TEM image, both light and dark areas were visible. The lighter areas were due to the less dense and more electrons that passed through it, while the darker areas in the material have greater electron density and favour an electron transmitted through it. Actually, in this work, the material electrons passing through were via both organic coating (DET) as well as Cu NPs. The light areas were showing the coating of DET as less dense areas of the materials which will allow more electrons to penetrate. Cu NPs tend to be dense agglomerations of atoms with relatively higher atomic weights than organic materials, they will impede the flow of electrons through the sample. According to above results, it is clear that Cu NPs with a uniform spherical shape and 40 nm size are fruitfully synthesised.

Also, the interaction of DET and Cu NPs were investigated by FTIR spectrometry. The FTIR spectra of DET capped Cu NPs is represented in Figure 7. The spectrum shows transmissions at 3445 and 3387 cm$^{-1}$ assigned to the overlap of N–H stretching vibrations, 2863 cm$^{-1}$ due to C–H stretching, 1598 cm$^{-1}$ and 1524 cm$^{-1}$ due to $\text{-NH}_2$ bending, 1413, 1349 and 1344 cm$^{-1}$ due to C–H bending. However, an absorption peak was observed at 580 cm$^{-1}$ corresponding to the interaction between Cu NPs with DET which indicates DET capped on the Cu NP surface.

![Figure 7. FTIR spectra of Cu NPs.](image-url)
From FTIR, EDS and UV-visible spectroscopy was obtained a noteworthy evidence for the presence of organic moiety attached with Cu NPs, however it could be assumed due to the presence of a carbonaceous organic residue which resulted from presence of protective or capping agents. From pertaining this view to identify the carbonaceous organic moiety, the sample was further investigated by thermogravimetric analysis (TG/DTG/DTA). The thermal degradation curves (TG/DTG) of Cu NPs is presented in Figure 8 and interpreted concisely.

The initial and final decomposition temperature and total mass losses for each step in the thermal decomposition curves of Cu NPs were investigated at a heating rate of 10 °C/min under nitrogen atmosphere over the temperature range 39 °C–730 °C. The peak temperatures, initial and final decomposition temperatures were identified at distinct steps by DTG analysis, whereas the DTA techniques supported for the evaluated endothermic or exothermic weight loss. From Figure 8, it is clearly indicated that the thermal decomposition behaviour of Cu NPs occurred via a two-step decomposition process. The first step of decomposition is at the range 39 °C–180 °C associated with $T_{\text{DTG}}$ peak at 65 °C and $T_{\text{DTA}}$ peak at 100 °C corresponding to mass loss due to lattice water molecule, significantly these values were supported by EDS and FTIR spectroscopy. In general, water of hydration may be considered either as lattice water or coordinated water.[34–37] The low-temperature range corresponding to this transformation indicates the presence of crystallisation of water. However, the second step decomposition displayed at a range 180 °C–730 °C which may be associated with $T_{\text{DTG}}$ peaks at 249 °C and 341 °C and strong broad endothermic $T_{\text{DTA}}$ peak at 441 °C due to the mass loss of protective agents. This temperature range noteworthy demonstrated a thermal stability of nanoparticles. Hence, significantly, the surface bound carbonaceous moiety attached to Cu NPs from the surface oxidation is revealed. Eventually, after the loss of organic moiety there was no loss of weight further which might be due to the formation of metallic copper.
4. Conclusion

In conclusion, we have successfully synthesised Cu NPs with average crystalline size 40 nm through chemical reduction method by using hydrazine hydrate as a reducing agent and DET as a capping agent. Furthermore, Cu NP was used as a catalyst for an efficient and very simple solvent-free green synthesis of 3,4-dihydropyrano[c]chromenes derivatives by using the stirring method with maintaining the temperature at 80 °C. Significantly, this catalyst is expected to contribute for the development of more environment-benign methods and forms part of nano-metal chemistry. The mildness of conversion, experimental simplicity, compatibility with various functional groups, excellent yields, shorter reaction time and the easy work-up procedure makes more attractive in the synthesis of chromenes derivatives. Furthermore, the antimicrobial activity of nanoparticles showed better bacteriostatic activity against all the tested pathogens. The zone of inhibition observed against gram-positive and gram-negative bacteria suggested that nanoparticles may be the promising antibacterial agents. The study also reveals that the antibacterial activity increases with the decreasing particle size and the increasing concentration of nanoparticles.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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