Journal of the Iranian Chemical Society
https://doi.org/10.1007/s13738-021-02378-7

ORIGINAL PAPER

Biosynthesis, characterization, and application of Cu_2O nanoparticles originated from Cressa leaf extract as an efficient green catalyst in the synthesis of some chromenes

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Received: 4 June 2021 / Accepted: 11 August 2021

Abstract
Cu_2O as a new heterogeneous green nanocatalyst is biosynthesized from Cressa leaf extract and characterized by some techniques including XRD, FTIR, UV–Vis, SEM, and TEM. Then, the prepared nanocatalyst was applied in the synthesis of some chromeno[4,3-b]chromenes under aerobic conditions. Reusability studies warrant acceptable reproducibility of the nanomaterial. Low reaction time, easy workup, cost-effectiveness, wide substrate scope, good to excellent yield, and complete atom economy are significant features of this new method. At the final part of this study, the in vitro cellular toxicity of Cu_2O nanoparticles is examined on SKOV3 human ovarian carcinoma cell line via an MTT assay. The reduction in cell viability proved the cytotoxicity of the nanoparticles toward the performed ovarian cancer cell line.

Keywords Heterogeneous · Nanocatalysis · Cu_2O · Chromenes · SKOV3

Introduction

During the past few decades, cancer has been known as the main reason for death globally and WHO announced that cancer is the chief cause of ~ 26% death per year in 2030 [1]. Among all cancers, ovarian cancer is an important case with the annual percentage increase in occurrence and remained as the most lethal gynecological malignancy [2, 3]; therefore, new treatments are necessary to overcome this type of cancer. Nanotechnology provides new treatments with better therapeutic outcomes, fewer side effects, and lower systemic toxicity. In this regard, various environmental benign methodologies are introduced to prepare a large number of pharmaceutically important chromene compounds such as preparation of pyrazolopyridines [4], 2-amino-tetrahydro-4H-chromene-3-carbonitriles [5], 2-amino-4H-chromenes [6, 7], and other substituted chromenes [8, 9] that have diverse applications as important drugs.

Although some effective routes are opened to prepare chromeno[4,3-b]chromenes [10–15], some deficiencies are combined with most of them such as using toxic solvents, precious catalysts, long reaction time, corrosive mediators, tedious workup, non-recyclable nature of the catalysts, high temperature, and low yields. Thus, progress of new effective and environmental benign protocols is demanding to synthesize these important compounds. Among all the recommended methods, plant-based routes for the biosynthesis of nanocatalysts are important because of low cost, being easy to handle, and eco-friendly nature of these methods. Therefore, these environmental benign methods are favored over most chemical and physical routes [16].

In the extension of our previous studies on the development of green synthetic protocols to synthesize heterocyclic compounds [17–22], herein, we report a new convenient and simple method to synthesize chromeno[4,3-b]

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Published online: 17 August 2021

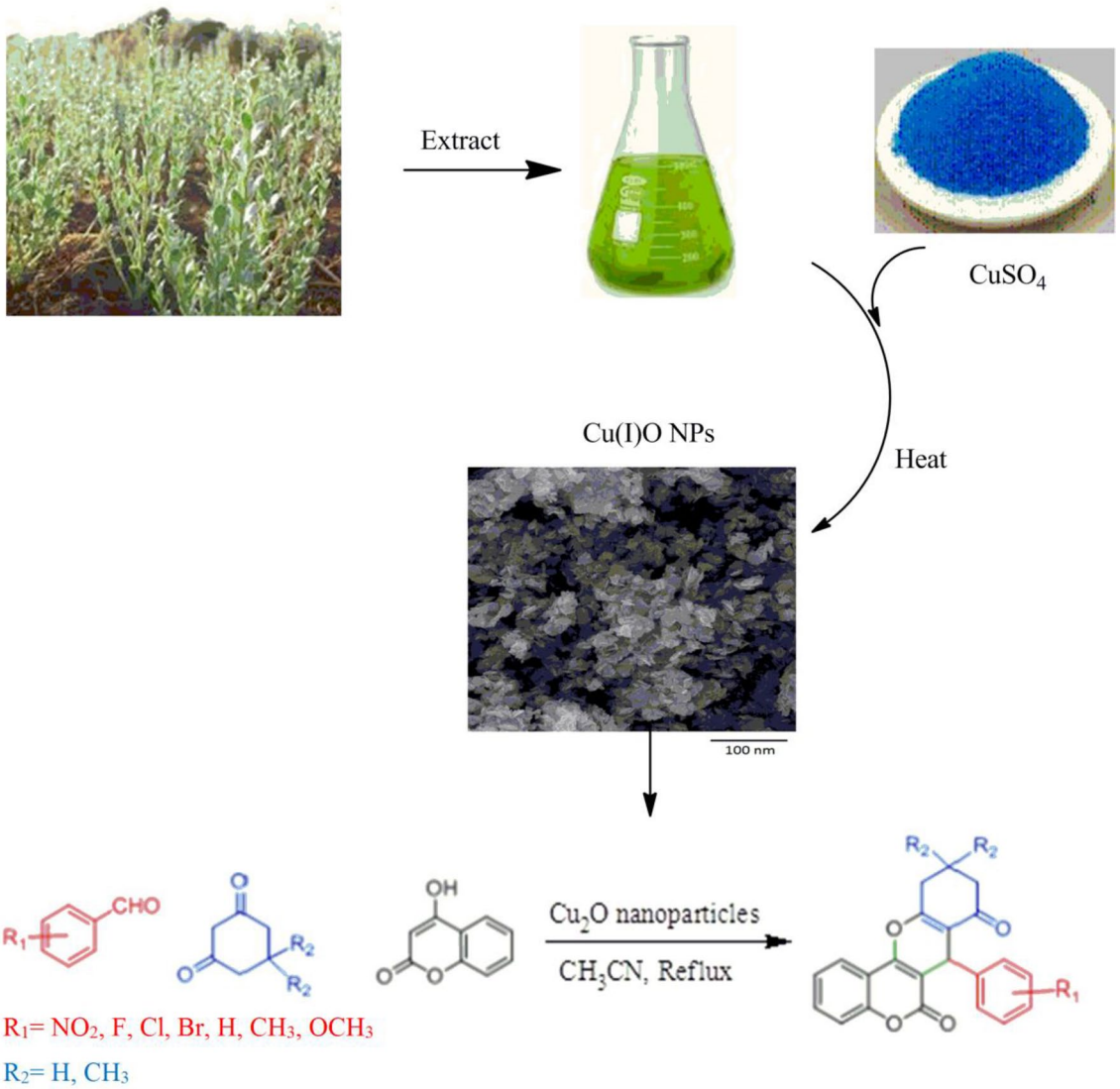
chromenes by means of Cu_2O nanoparticles biosynthesized from Cressa leaf extract as a green heterogeneous nanocatalyst (Scheme 1). To the best of our knowledge, there is no report on the synthesis of Cu_2O nanoparticles using Cressa leaf extract as an effective reducing agent for Cu^{2+} ions.

Results and discussion

The morphology and physicochemical properties of the as-synthesized Cu_2O nanoparticles were investigated by means of FTIR, TEM, SEM, UV–Vis, and XRD.

FTIR spectroscopy

FTIR spectroscopy was used to confirm generation of the surface-modified Cu_2O nanoparticles by CLL extract (Fig. 1). The performed FTIR showed the presence of the polyphenols, alkaloids, and carbohydrates in the leaf extract, allowing generation of Cu_2O nanoparticles. Weak absorption bands around 650–750 cm^{-1} may be due to the Cu–O vibrations. The exact position of Cu–O vibration is impossible because of a wide range of hydrogen bondings. Observation of a broad band at ~ 3420 cm^{-1} proved coordination of amine ligands to Cu_2O nanoparticles and confirms the existence of O–H/N–H stretchings due to macromolecular fragments in the extract. In addition, bands at 2920 and 2860 cm^{-1} are attributed to the



Scheme 1. A general scheme for the biosynthesis and application of Cu2O nanoparticles

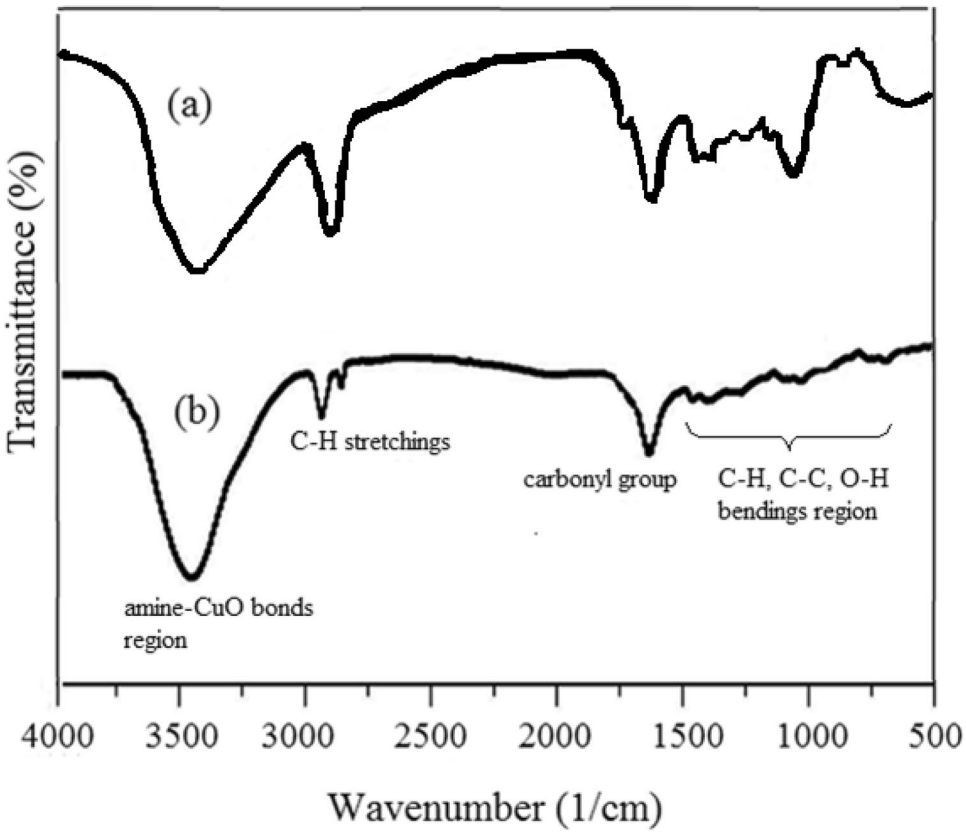


Fig. 1 FTIR spectra of **a** the extract and **b** surface-modified Cu_2O nanoparticles

asymmetric and symmetric C–H stretchings, respectively. The band around 1740 cm^{-1} is due to the carbonyl group in the extract. The weak asymmetric C–H and O–H bendings are also observed at 1450 and 1320 cm^{-1}, respectively. Other bands at 1210–1164, 1073, and 903 cm^{-1} were, respectively, due to C-H bending, skeletal C–C, and bending C–H.

FESEM and TEM of Cu_2O nanoparticles

FE-SEM and TEM imaging are used to justify morphology and average size of the nanoparticles, respectively. The FE-SEM and TEM images of Cu_2O nanoparticles are shown in Fig. 2. Figure 2a shows that Cu_2O nanoparticles are semi-spherical in shape with some agglomeration and irregularities. The exact shape and size of the nanoparticles were investigated by TEM that shows a relatively good dispersion

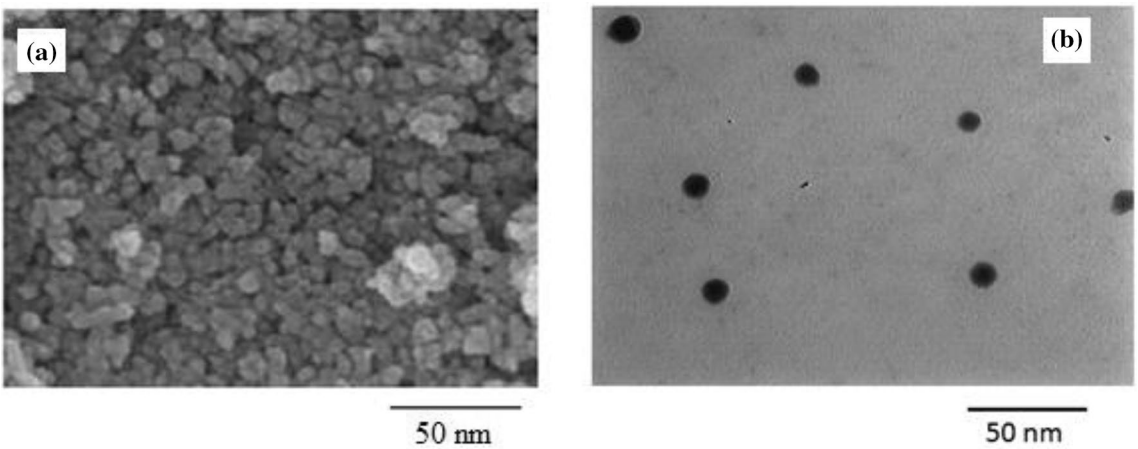


Fig. 2 The FE-SEM (**a**) and TEM (**b**) images of Cu_2O nanoparticles after annealing at 400 °C

of the spherical Cu_2O nanoparticles and the majority of the nanoparticles are small in size (6–15 nm), confirming the high surface catalytic activity of Cu_2O. However, a few nanoparticles have a bigger size (~ 20–50 nm), which may be due to the presence of excessive phytochemicals on the surface of Cu_2O nanoparticles, inducing aggregation and enlargement of the nanoparticles. However, no aggregation in most cases suggested the presence of hydrophobic coatings around the nanoparticles.

XRD

The crystalline nature of annealed Cu_2O nanoparticles was studied by XRD (Fig. 3). The intense reflections around 36.4° and 42.4° are due to the (111) and (200) planes that match well with the ICDD 98-018-0846. The reflections at the 2 theta of 29.8°, 36.7°, 42.3°, 61.4°, 73.3, and 77.4 can be indexed to the (110), (111), (200), (220), (311), and (222) lattice planes of Cu_2O (JCPDS No. 05-0667). In addition, the observed size of the nanoparticles with XRD is bigger than in that of TEM, which may be due to the aggregation of particles during drying before XRD analysis.

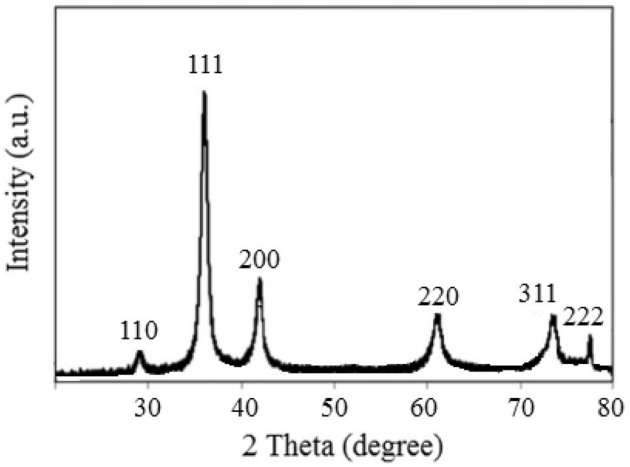


Fig. 3 Wide-angle XRD pattern of (**a**) Cu_2O nanoparticles

UV–Vis and XPS

Figure 4 shows the UV–Vis (Fig. 4a) and high-resolution XPS (Fig. 4b) of Cu_2O nanoparticles. Figure 4a describes that aqueous colloidal solution of Cu_2O nanoparticles confirms a strong fundamental absorption band at 255 nm due to the surface plasmon resonance (SPR) of the nanoparticles. These nanoparticles are quite stable after one month, and no significant change was observed in the UV–Vis spectrum. The high-resolution XPS Cu2p of the as-synthesized Cu_2O nanoparticles showed the photoelectron peaks at 933.2 and 953.4 eV due to Cu2p3/2 and Cu2p1/2, respectively [23]. These signals proved the presence of copper in the nanomaterial that supports the FTIR observations for the Cu–O stretching of Cu_2O.

Catalytic tests

To find the best reaction conditions, a model condensation reaction was conducted by using benzaldehyde (1 mmol), dimedone (1 mmol), and 4-hydroxycoumarin (1 mmol) in the presence of Cu_2O nanoparticles (0.005 g) in refluxing acetonitrile, and effects of some important reaction

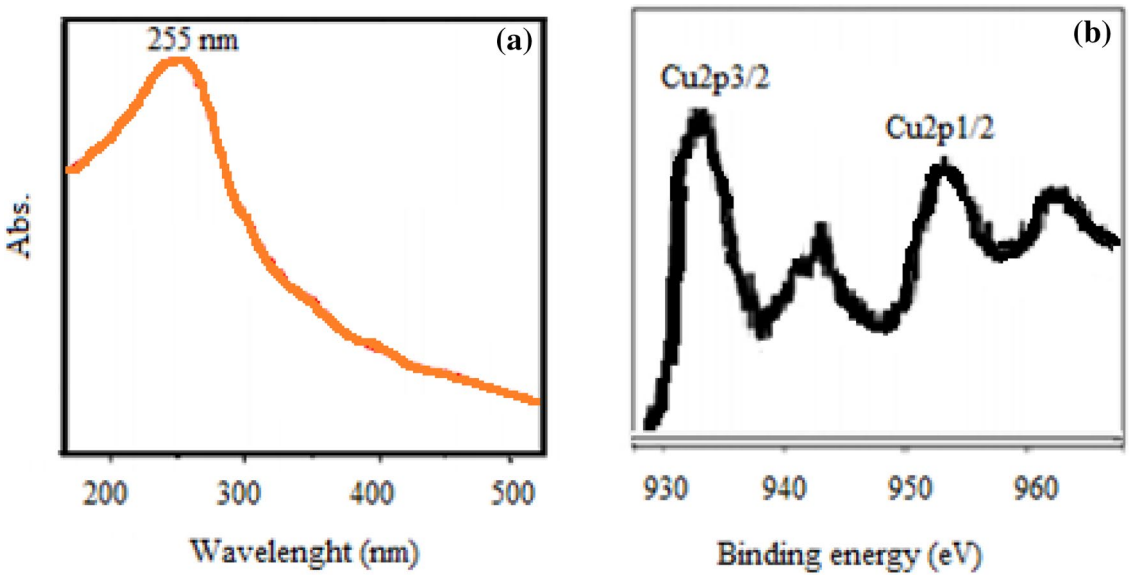


Fig. 4 UV–Vis spectrum (**a**) and high-resolution XPS Cu2p region (**b**) of Cu_2O nanoparticles

parameters were evaluated in the efficacy of condensation reaction.

Catalyst amount

A model reaction was performed with different amounts of catalyst to optimize the optimum quantity of Cu_2O. As seen in Fig. 5, 0.005 g of Cu_2O has been appropriate and gave the yield of 93% under the selected reaction conditions. However, further increase in the catalyst amount had no a significant effect on the yield% and reaction time.

Effect of different solvents

In this experiment, effect of solvent was investigated on the reaction progress (Table 1). Thus, condensation of dimedone, benzaldehyde, and 4-hydroxycoumarin was performed under the optimum conditions in some selected solvents such as CH_3CN, H_2O, toluene, ethanol, and solvent-free condition. According to the obtained data in Table 2, acetonitrile is the best choice between the examined solvents and provided the maximum yield of 93% after 60 min. To compare the catalytic activity of the biosynthesized Cu_2O nanoparticles with pure Cu_2O attained after annealing at 400 °C, entry 6 was run. This finding showed that the pure nanomaterial has a higher catalytic activity, maybe because of the more active surface in the absence of tailed organic functional groups.

Effect of reaction time and temperature

The impact of temperature was also investigated in the condensation of dimedone, benzaldehyde, and 4-hydroxycoumarin by the presence of 0.005 g Cu_2O nanoparticles (Fig. 6). It was found that elevation of temperature significantly affects the reaction progress and the best yield can be achieved under reflux condition (82 °C). Therefore, the temperature 82 °C was chosen as the best for all runs in acetonitrile. Effect of reaction time was also again studied to explore the minimum time needed to acquire the best yield. As Fig. 6 shows, 60 min was adequate to reach the maximum yield of 93%.

Table 1 Optimization of solvent on the condensation of dimedone, benzaldehyde, and 4-hydroxycoumarin

Entry	Cu_2O nanoparticles (g)	Solvent	Time (min)	Temp. (°C)	Yield (%)[b]
1	0.005	–	120	82	80
2	0.005	H_2O	120	Reflux	35
3	0.005	Toluene	120	Reflux	62
4	0.005	Ethanol	120	Reflux	75
5	0.005	CH_3CN	60	Reflux	93
6	0.005	CH_3CN	45	Reflux	97

Reaction conditions: dimedone (1.0 mmol), benzaldehyde (1.0 mmol), and 4-hydroxycoumarin (1.0 mmol) were mixed in acetonitrile (3 mL) under reflux. The prepared surface-modified Cu_2O nanoparticles were used in all experiments, except for entry 6 with pure nanomaterial

[b]Yield refers to the isolated product

Synthesis of different chromeno[4, 3-b]chromenes

The generality of the present methodology was explored in the synthesis of some chromeno[4,3-b]chromene derivatives using a variety of aromatic aldehydes. Aldehydes with different electron withdrawing-releasing substituents provided good to excellent yields according to the optimum reaction conditions (Table 2). Although most aromatic aldehydes

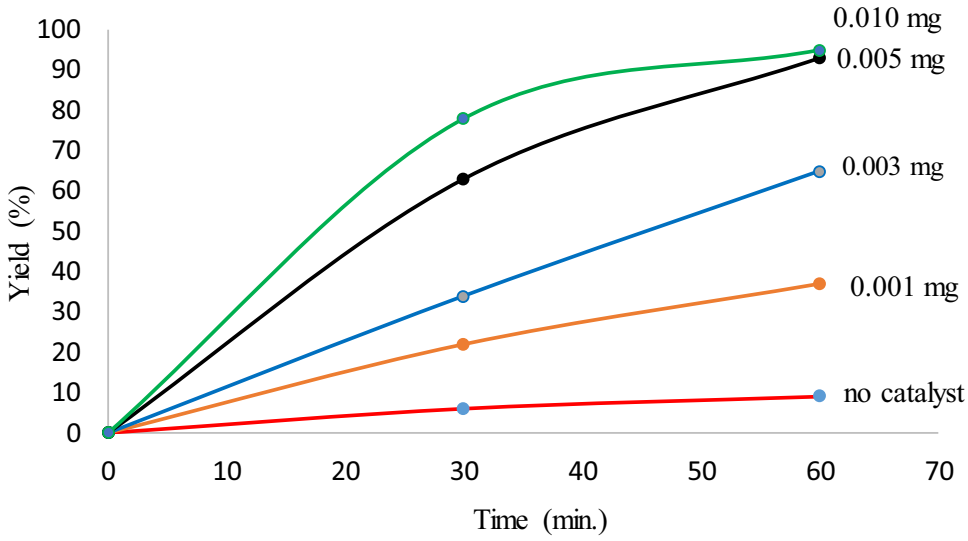
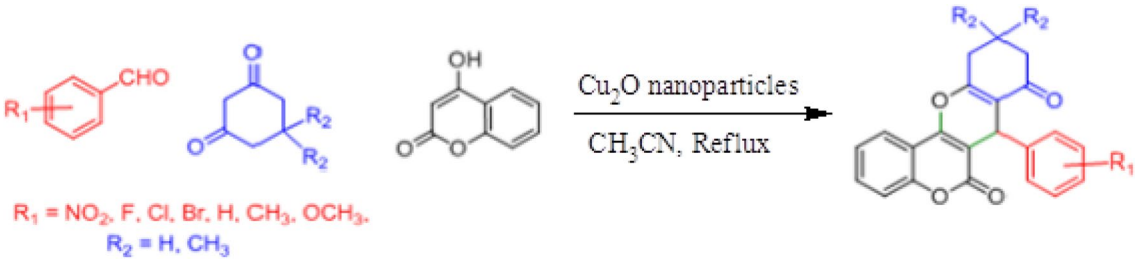


Fig. 5 Effect of catalyst amount on the condensation of dimedone (1.0 mmol), benzaldehyde (1.0 mmol), and 4-hydroxycoumarin (1.0 mmol) in acetonitrile under reflux

Table 2 Substrate scope and generality of the condensation reaction



Aldehyde	Dimedone	Yield (%)	TOF[a]	M.P. (°C) Found/reported
R_1 = H	R_2 = CH_3	95	186	220–222/221–223 [24]
R_1 = 4-NO_2	R_2 = CH_3	100	200	208–210/209–212 [24]
R_1 = 4-Br	R_2 = CH_3	89	178	228–230/229–231 [25]
R_1 = 4-OCH_3	R_2 = CH_3	78	156	187–189/187–188 [25]
R_1 = 3-NO_2	R_2 = CH_3	97	194	240–245/243–244 [24]
R_1 = 3-NO_2	R_2 = H	94	188	218–220/220–221 [24]
R_1 = 4-OCH_3	R_2 = H	83	166	196–197/195–198 [25]

Reaction conditions: dimedone (1.0 mmol), benzaldehyde (1.0 mmol), and 4-hydroxycoumarin (1.0 mmol) were mixed in acetonitrile (3 mL) under reflux. Reaction time was 60 min, and 0.005 g of Cu_2O was used in all cases

[a]TOF = (yield/time (h))/catalyst amount (g)

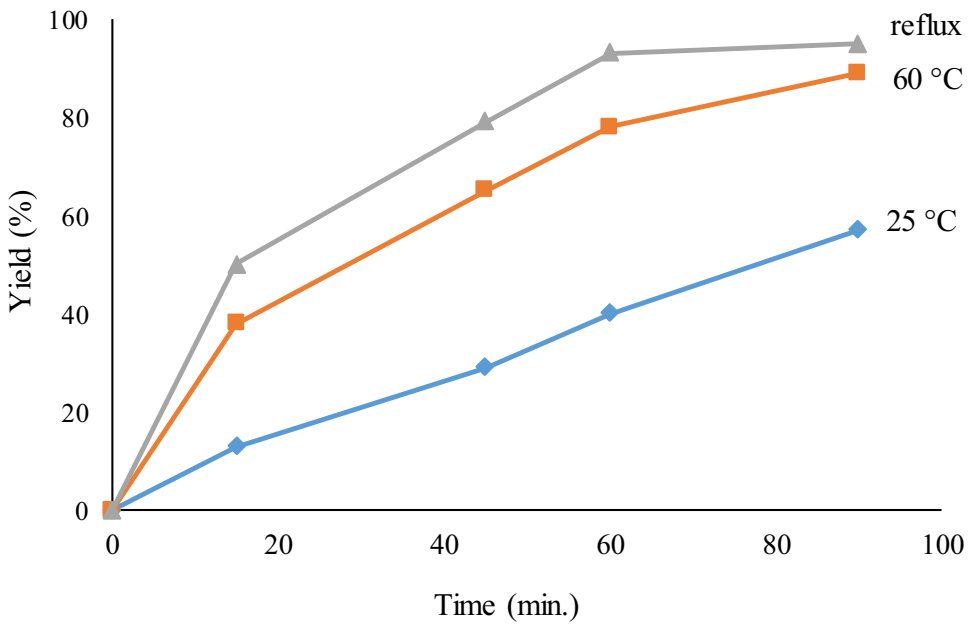


Fig. 6 Studying effect of reaction temperature and time

with electron-poor and electron-rich substituents afforded the desired products in high yields, aldehydes involving electron-withdrawing groups reacted better than with electron-donating groups.

Superiority of the present method

Up to now, a number of catalysts have been documented for the preparation of chromeno[4, 3-b]chromenes under different reaction conditions. Herein, the present protocol is compared to some published methods, as shown in Table 3. In this experiment, the comparison was made in terms of reaction time, catalyst amount, and yield%. Interestingly, the present methodology offers a few advantages including a very low amount of an environmental benign catalyst under mild reaction conditions as well as some reported methodologies. The distinct feature of the introduced Cu_2O catalyst is its simplicity, easy preparation, and cheap nature of the material. Thus, this protocol is a special case, providing a new approach in the single-step, one-pot preparation of the desired chromenes under milder conditions.

Stability and reusability of Cu_2O nanoparticles

To monitor stability and reusability of Cu_2O nanoparticles, Cu_2O nanoparticles were removed after completion of the first run by centrifuge and simple filtration. Then, the recycled catalyst was washed, dried at room temperature, and prepared for the next run. Therefore, the nanocatalyst was reused for the subsequent five runs to prove its good stability and reusability (Fig. 7). This study showed that the activity of Cu_2O has been alleviated after five runs. The FTIR of the nanocatalyst was checked after fifth run. This study showed no significant changes have occurred after five reusing (Fig. 8). However, the decrease in the activity of catalyst after stepwise reusing would be due to the inactivity of the catalyst surface. It seems that these structural changes are not so obvious that can be detected by FTIR.

An elementary MTT assay and in vitro cellular cytotoxicity of Cu_2O nanoparticles

One of the most challenges in achieving a standard cancer therapy is the preparation of biocompatible and effective carriers to prepare drugs with more influencing therapeutic applications to fight cancer cells. The recent cancer statistics analysis by WHO (International Agency for research on cancer) shows that ovarian cancer has been increased in the recent decade. Since anticancer activity of various chromenes is studied extensively, herein, we performed a preliminary cellular cytotoxicity test on the bare Cu_2O nanoparticles against SKOV3 ovarian cancer cell line. This study showed that the metal oxide nanoparticles affect the mean growth of cancer cells in a dose-dependent manner and a significant decrease in the MTT signal was observed in comparison with the untreated cells. Thus, the SKOV3 cell line was incubated with 0–50 µg/mL Cu_2O nanoparticles for 48 h. As seen in Fig. 9, cell survival was decreased with enhancing Cu_2O concentration and the results for amounts greater than 25 µg/mL are comparable. As a result, Cu_2O

Table 3 The comparison of the catalytic activity of Cu_2O nanoparticles with some reported catalysts in the synthesis of chromeno[4, 3-b] chromenes

Catalyst	Catalyst amount	Time (h)	Temp. (°C)	Solvent	Yield (%)
Fe_3O_4@SiO_2@ propyltriethoxysilane@o-phenylenediamine[a]	5 mg	0.16	r.t	EtOH	90 [6]
ZnS/$CuFe_2O_4$/agar[b]	30 mg	0.5	r.t	EtOH	97 [5]
Fe_3O_4@SiO_2–NH_2[c]	20 mg	1.3	60	EtOH	93 [8]
pumice microparticles[d]	30 mg	0.1	ultrasonic	EtOH	97 [9]
Nano-$CuFe_2O_4$@SO_3H	50 mg	2.5	70	EtOH	90 [26]
WO_3/ZnO@NH_2-EY	0.03 g	0.75	25	–	97 [27]
ZrO_2 nanoparticles	10 mol%	0.5	80	H_2O	91 [28]
Eosin Y	2 mol %	3.5	r.t green LED	CH_3CN	77 [29]
Cu_2O nanoparticles	5 mg	1	Reflux	CH_3CN	97 (This work)

Reaction conditions are described in Table 1

[a]Due to preparation of 2-amino-4-phenyl-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile

[b]Due to preparation of 2-amino-tetrahydro-4H-chromene-3-carbonitrile

[c]Due to preparation of methyl 2-amino-3-cyano-4-(2-methoxy-2-oxoethyl)-7,7-dimethyl-5-oxo-5,6,7,8-tetrahydro-4H-chromene-4-carboxylate

[d]Due to preparation of 7-amino-2,4-dioxo-5-phenyl-1,3,4,5-tetrahydro-2H-pyrano[2,3-d]pyrimidine-6-carbonitrile

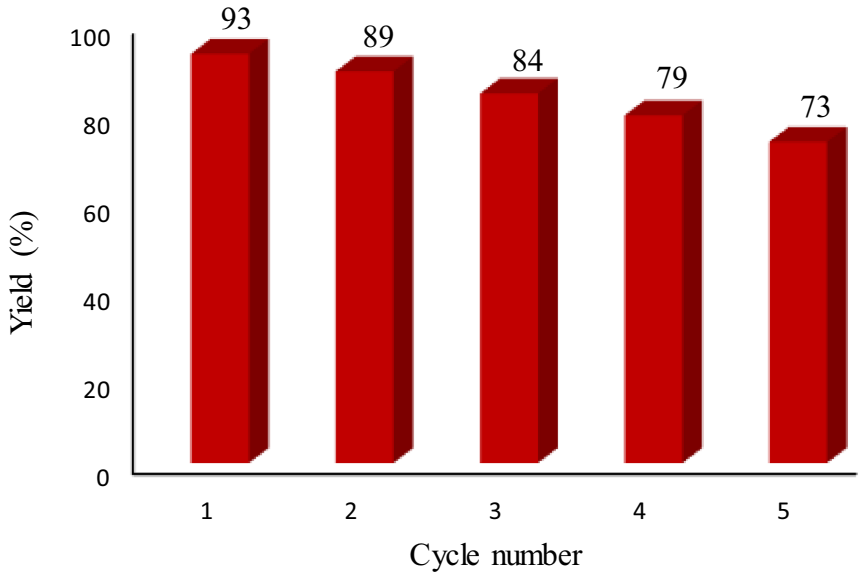


Fig. 7 Yield% *vs* cycle number to study reusability of Cu_2O nanoparticles

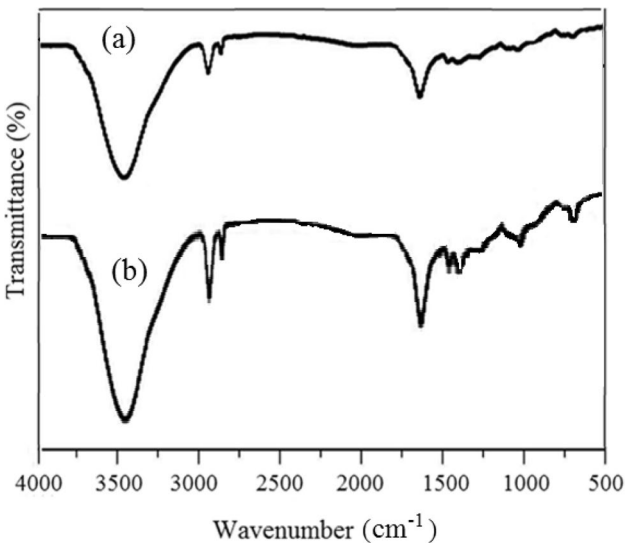


Fig. 8 FTIR spectra of fresh (**a**) and reused (**b**) Cu_2O nanoparticles after the fifth run

nanoparticles can be regarded as effective toxic agents against SKOV3 cell line and the IC50 of ~ 20 µg/mL was achieved in this experiment.

Conclusion

Herein, we opened a new green method to prepare Cu_2O nanoparticles from the leaf extract of Cressa and progressed an ecofriendly and efficient protocol to achieve a range of chromeno[4,3-b]chromenes under mild conditions. Thus, the nanocatalyst Cu_2O is prepared and characterized by means of XRD, FTIR, UV–Vis, XPS, SEM, and TEM. Then, the nanomaterial is used as a superior green nanocatalyst toward the desired heterocyclic condensation reaction. The mild reaction conditions, wide substrate tolerance, easy workup, high atom economy, and good to excellent yields are the major important features of this new catalytic system. The distinct feature of the introduced Cu_2O catalyst is its simplicity, simple preparation, and cheap nature of the material. The Cu_2O nanoparticles could be frequently recovered up over four runs with a little loss of the catalytic activity. Eventually, the in vitro cellular cytotoxicity of Cu_2O nanoparticles by MTT assay showed good inhibitory effect of the performed nanoparticles against SKOV3 human ovarian carcinoma cell line.

Experimental section

Materials and methods

All materials and solvents used in the present report including copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$), aromatic aldehydes, dimedone, and 4-hydroxycoumarin were purchased from commercial resources and utilized as received without extra purification (analytical grade). Morphology studies were performed on a Mira 3-XMU FE-SEM instrument. Fourier transform FTIR spectra were drawn on a Shimadzu 8700 Fourier transform spectrophotometer with KBr pellets. UV–visible spectroscopy was run on a Photonix UV–visible array spectrophotometer. X-ray diffractions (XRDs) were studied on an Xpert diffractometer with Cu K_α radiation at 40 keV, 30 mA, and a scanning rate of 2° min^{-1} in the 2θ domain 5°–80.

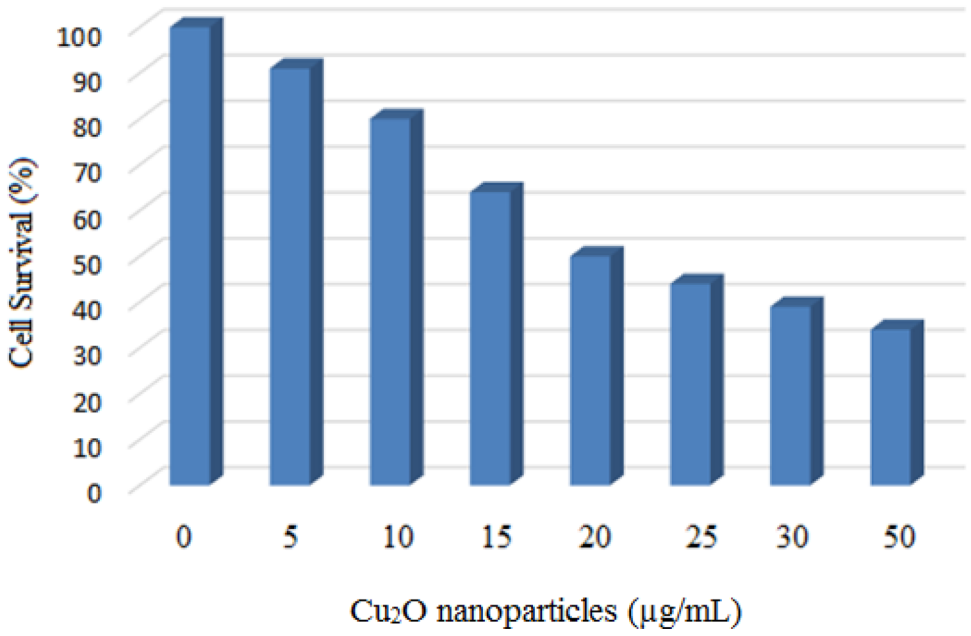


Fig. 9 The cytotoxicity assay of Cu_2O nanoparticles by the MTT assay

Biosynthesis of copper oxide nanoparticles Cressa leaf extract

The extraction of phytochemicals was performed by a standard method commonly used for extraction [30]. Thoroughly washed and air-dried leaves of Cressa (900 g) were crushed into small pieces and transferred to a 150-mL flask containing deionized water (80 mL) in a Clevenger apparatus and heated to 65–70 °C for 45 min. Then, the obtained extract was filtered and stored at 4°°C for further use. For the synthesis of Cu_2O nanoparticles, 5 mL of the leaf extract was added slowly to 25 mL of $CuSO_4$ solution, 10 mM, followed by heating at 90 °C 5 h for 5 h with continuous stirring. The formation of Cu_2O nanoparticles was indicated by a change of the color to green.

A general instruction for the synthesis of chromenes

Benzaldehyde (100 mg, 1 mmol), dimedone (140 mg, 1 mmol), 4-hydroxycoumarin (162 mg, 1 mmol), and the desired amount of Cu_2O (5 mg) were successively added to a round-bottomed flask equipped with a magnet containing acetonitrile (3 mL). Then, the reaction mixture was heated up to reflux in an open-air atmosphere, and completion of the reaction was monitored by TLC. After completion, the mixture was quenched with H_2O (10 mL) and the desired chromene product was dissolved in EtOAc. Finally, the attained organic layer was dried over sodium sulfate and the crude accomplished product was purified by column chromatography to afford the pure chromene.

Cancer cell culture

The cell line SKOV3 (human ovarian cancer) was purchased from NCCS (National Centre for Cell Sciences), India. This cell line was grown and maintained in a suitable Minimum Essential Medium (MEM) and sub-cultured in the medium involving 1% glutamine, 10% fetal bovine, and 1% penicillin solution. Then, the cells were trypsinated by trypsin EDTA solution and seeded at the density of 1×10^5 cells/ well in 96-well plates and cultured at 37 °C for 24 h (incubation time 48 h). Finally, the newly prepared Cu_2O nanoparticles were monitored for the in vitro cytotoxicity effects on SKOV3 by the standard MTT assay. The plates were incubated for a further 5 h at 37 °C in a humid chamber including 5% CO_2. The formazan crystals generated via reduction of the dye by viable cells were dissolved in 200 µl dimethyl sulfoxide (DMSO), and the absorbance was seen at 490 nm.

Acknowledgements The authors would like to acknowledge the financial support of Hakim Sabzevari University.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Data availability statement The original contributions presented in the study are included in the article/supplementary material, and further inquiries can be directed to the corresponding author.

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