RESEARCH ARTICLE

Biosynthesis of copper(II) oxide nanoparticles using *Murayya koeniggi* aqueous leaf extract and its catalytic activity in 4-nitrophenol reduction

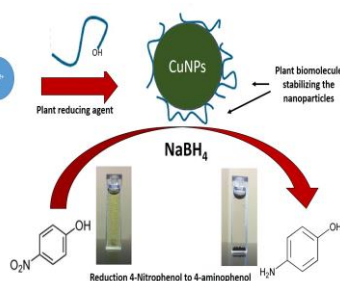Nurulhuda Raja Nordin, Mustaffa Shamsuddin[*]

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Graphical abstract

Abstract

Copper(II) oxide nanoparticles (CuO NPs) have a wide range of applications as catalysts. The natural abundance of copper and its relatively low cost make it a viable alternative to catalysts that made from expensive precious metals such as platinum and palladium. In this study, a rapid, simple and green method was developed for the synthesis of CuO NPs using an aqueous extract of *Murayya koenigii* leaves. Several parameters were optimized, namely, the volume of leaf extract, pH, reaction temperature and reaction time. The optimum condition for the biosynthesis was obtained by using 3 mL of leaf extract; 10 mL of 5 mM $CuSO_4$, at pH 11, at room temperature. The biosynthesis was completed within 50 minutes. The synthesized CuO NPs were characterized using Ultraviolet-visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Transmission Electron Microscope (TEM) analyses. The UV-Vis absorption spectra confirmed the formation of CuO NPs with characteristic peak at 634 nm. The FTIR spectroscopic analysis of the biosynthesized CuO NPs confirmed the surface adsorption of the bioactive components in the leaf extract that acted as the reducing agent and stabilizing agent for the metal nanoparticles. XRD analysis showed a series of diffraction peaks at 2θ of 32.5°, 35.5°, 38.6°, 48.8°, 53.4°, 58.1°, 61.5°, 66.3°, 68.0°, 72.4° and 75.0°, corresponding to (110), (002), (111), (202), (020), (202), (113), (311), (220), (311) and (222), respectively. From TEM images, CuO NPs were of spherical shape with a mean diameter of 8.4 nm. The biosynthesized CuO NPs demonstrated good catalytic activities on the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) in the presence of sodium borohydride, $NaBH_4$ and could be reused three times without significant decreases in the catalytic activities.

Keywords: Copper oxide nanoparticles, biosynthesis, *Murayya koenigii*, catalytic properties

INTRODUCTION

Nanomaterials prepared from earth-abundant and inexpensive metals have attracted considerable attention because of their potential as promising alternatives to expensive noble metals that used as catalysts in many commercials chemical processes [1] . These metal nanoparticles often exhibit activity that different from the corresponding bulk materials due to their different sizes and shapes, resulting in unique catalytic, electronic, optical and other structural properties [2]. In this context, copper nanoparticles (Cu NPs) are attractive because of their natural abundance and low cost. Thus, the applications of Cu NPs that is based on the earth-abundant and inexpensive copper metal, have generated a great deal of interest in recent years, especially in the field of catalysis [3].

Currently, there are well-developed synthesis methods for Cu NPs, including thermal evaporation, sonochemical, sol-gel, hydrothermal, and electrochemical methods [4]. However, these methods are quite expensive and involved toxic reductants, polymers and solvents such as $NaBH_4$, polyvinylpyrrolidone and ethylene gylcol which are potentially hazardous to the environment.

In recent years, the biogenic synthesis for metal nanoparticles synthesis have been gaining attention due to its simplicity and environmental friendly. Biogenic synthesis is also known as green chemical method in metal nanoparticles preparation, which employs non-toxic multi-functional reactants derived from natural, biological resources that ranging from microrganisms to plants [5]. Metal nanoparticles that synthesized from the biological route have good stability due to the presence of capping agents such as alkaloids, flavanoids and polyphenols, which are the major constituents presented in the natural resources.

Table 1 shows various biological methods that have been reported in synthesizing copper oxide nanoparticles (CuO NPs). The *Murraya koenigii* is commonly known as curry leaf or 'daun kari'in Malay. It belongs to the family Rutaceae [14]. Fresh and dried curry leaves are extensively used in Malaysian culinary practices for seasoning and flavouring dishes. Different parts of *Murayya koenigii* have been used in traditional Ayurveda medicine [15]. Previous phytochemical investigations on this plant revealed that the *Murraya koenigii* leaves are rich in phenolic acids (especially gallic acid) and flavonoids (especially myricetin, epicatechin, and quercetin) contents [16]. Rajendran *et al* reported that the essential oil from *Murraya koenigii* leaves showed anti-bacterial effect against bacteria *Corynebacterium tuberculosis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Klebsiella pneumonia* and *Enterobacter aerogenes* [17]. In addition,

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the naturally-occuring polypheols/flavanoids in *Murraya koenigii* have been reported to exhibit anti-tumor [18], anti-oxidant [19], [20], anti-inflammatory [21], anti-hyperglycemic [22], anti-histamnie [23], and hypoglycemic effects [24].

Table 1 Biological method in synthesis of CuO NPs

Biosynthesis source	Spesies	Morphology and size	References
Fungi	*Stereum hirsutum*	Spherical, 4-5 nm	[6]
Bacteria	*E.coli*	Spherical, 5 nm	[7]
	Salmonella typhimurium	Spherical, 49nm	[8]
Plant/plant parts	*Drypetes sepiaria* (leaves extract)	Spherical, 25 nm	[9]
	Banana (peel extract)	Spherical, 60 nm	[10]
	Abutilon indicum (leaves extract)	Spherical, 16 nm	[11]
	Cassia auriculata (leaves extract)	Spherical, 23 nm	[12]
	Punica granatum (peel extract)	Spherical, 40 nm	[13]

Nitro aromatic compounds such as nitrophenols, are common industrial nitrogen-containing aromatic organic compounds that are commonly exploited in the manufacturing of pharmaceuticals, plastics, dye, agriculture, etc. The direct discharge of these compounds as industrial wastes into the ecosystem is harmful to the biological system as well as human beings because these compounds are oftenly resistant to degradation under normal environmental conditions. Presence of these compounds in the ecosystem, even in small quantities, is highly undesirable and has drastic, adverse effects on the environment and human beings since many of these compounds possess environmental toxic, mutagenic and carcinogenic potentials [25].

The present study would describe an eco-friendly and simple synthesis route of CuO NPs by employing an abundantly available *Murraya koenigii* leaves aqueous extract as the reducing and stabilizing agent. The potential application of the biosynthesized CuO NPs as catalyst in the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) (Scheme 1) in aqueous medium at room temperature in the presence of sodium borohydride, $NaBH_4$ would also be investigated.
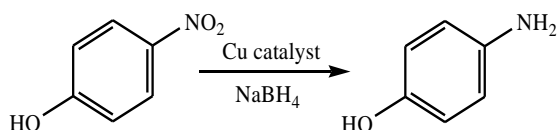
Scheme 1. Reduction of nitrophenol to aminophenol

EXPERIMENTAL

Materials

Copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium hydroxide (NaOH), 4-nitrophenol, 4-aminophenol and sodium borohydride, $NaBH_4$ were purchased from Sigma-Aldrich. All other reagents were of analytical grades. Thin Layer Chromatography (TLC) analyses were performed using Merck pre-coated TLC plates (silica gel 60 GF254, 0.25 mm, Germany). All solutions were prepared in deionized water.

Preparation of *Murayya koenigii* leaves extraction

The collected fresh leaves of *Murayya koenigii* were washed thoroughly with deionized water to remove any dirt particles. The washed leaves were then air-dried. The cleaned and dried leaves were ground into powder using an electrical blender and sieved by using a 20 mesh sieve. In order to prepare the flavonoid rich extract, 2 g of the leaf powder was extracted with 200 mL of deionized water in a Soxhlet apparatus for six hours. After cooling, the leaf aqueous extract was stored at 5ºC for further use. The prepared leaf extract with concentration of 1% w/v was employed throughout the study.

Synthesis of CuO NPs

As for the preparation of CuO NPs, 1 mL of prepared leaf extract was added into 10 mL of 5 mM aqueous copper(II) sulphate, $CuSO_4$ and the pH of the mixture was adjusted to pH 11 using 0.1 M sodium hydroxide, NaOH solution. The mixture was stirred and the colour of the reaction mixture was gradually changed to dark brown, indicating the formation of CuO NPs. The formation of CuO NPs was monitored using Ultraviolet-visible spectroscopy (UV-Vis) for the detection of Surface Plasmon Resonance (SPR) band of CuO NPs after 60 minutes of reaction time. For optimization purposes, the biosynthesis of CuO NPs was carried out using different volumes of leaf extract, pHs and temperatures, while the time taken for the reaction to complete the bioreduction was recorded. The colloidal particles formed were centrifuged at 14800 rpm for 20 minutes and the obtained precipitate was washed with deionized water and then dried in the desiccator for further use.

Characterization of CuO NPs

The biosynthesized CuO NPs were characterized using UV-vis and Fourier transform infrared (FTIR) spectroscopies, X-ray diffraction (XRD) and Transmission Electron Microscope (TEM) analytical methods. UV-vis spectral analysis was recorded on a Shimadzu-1800 double-beam spectrophotometer to ensure the formation of nanoparticles. The shape and size of synthesized CuO NPs were identified by TEM images using JEOL JEM-2000 EX instrument, operated at an accelerating voltage of 100 kV. FTIR analysis was carried out to identify the functional group of the biocapping agents on the CuO NP surfaces. FTIR spectra of the synthesized samples were recorded on a Perkin Elmer 1600 FTIR spectrometer in the spectral range of 4000 cm^{-1} to 400 cm^{-1} using potassium bromide (KBr) pressed disk technique. XRD analysis was done using X-ray diffractometer (D/tex Ultra 250 SmartLab diffractometer, Rigaku, Japan) with Cu Kα radiation (λ = 0.154 nm) in the 2θ range from 10˚ to 90˚. Prior to XRD analysis, the CuO NPs were calcined at 600ºC for 5 hours in order to yield a fine, black CuO NPs which were stored in an airtight container.

Catalytic activity of CuO NPs

In a typical reaction procedure, freshly prepared $NaBH_4$ (300 µL, 100 mM) was added into 4-NP (3 mL, 0.05 mM) in a quartz cuvette. Both solutions were prepared using distilled water. Then, 1 mg of CuO NPs catalyst was added into the mixture. The colour of the solution would be faded as the reaction was proceeded. The reaction was monitored using UV-vis spectroscopy in which the absorbance of the 4-nitrophenolate ion was recorded every 1.5 minute intervals in the scanning range between 200 nm and 500 nm. After completing the reaction, the mixture was centrifuged at 14800 rpm for 20 minutes to separate the catalyst for recyclability test. The separated catalyst was washed twice with distilled water. The percentage of conversion from 4-NP to 4-AP was determined by calculation from the absorbance data.

Purification and characterization of 4-AP

For the purification of 4-AP, the reaction was scaled up in the ratio of 4-NP (3.75 mM, 50 mL), $NaBH_4$ (750 mM, 50 mL), with 5 mg of CuO NPs catalyst used. The mixture was stirred until the reaction mixture had turned into colourless. After completion of the reaction, the catalyst was separated by centrifugation and hydrochloric acid, HCl solution (2 M, 10 mL) that was added to quench the

reaction. Into this solution, ethyl ether was added and a liquid-liquid extraction was conducted. The extraction step was repeated three times. Ethyl ether fractions containing 4-AP were combined and water was removed using anhydrous sodium sulphate, Na_2SO_4. Then, ethyl ether was evaporated using rotary evaporator. A silica gel column chromatography was performed by eluting with solvent composed of hexane:ethyl acetate in the ratio of 1:2. Each eluent was loaded on a thin layer chromatography, TLC plate together with 4-AP standard. The fractions containing 4-AP were combined and solvents were evaporated. Both standard 4-AP from a commercial source and purified 4-AP from the above procedure were characterized by ^{1}H-NMR.

Recyclability test

Recycle experiments were performed to test the reusability of the CuO NPs catalyst. The recyclability test was performed by carrying out the reaction under the same conditions as the model reaction. After the first run, the catalyst was separated from the reaction mixture by centrifugation. The catalyst was washed twice with deionized water to remove any residue from the catalytic reaction mixture. The separated catalyst was vacuum dried in a desiccator and reused again. In the subsequent run, the same amount of 4-NP and a freshly prepared $NaBH_4$ were used. The percentage of conversion of the 4-nitrophenolate ion was calculated and compared with the first run.

RESULTS AND DISCUSSION

Biosynthesis, optimization and characterization CuO NPs

The formation of CuO NPs was preliminary monitored by UV-Vis spectroscopy, followed by further characterization using FTIR spectroscopy, TEM and XRD analytical data. The formation of CuO NPs was clearly observed by the colour change from pale yellow to dark brown upon the addition of 1 mL of 1% *Murayya koenigii* leaf extract into the 10 mL of 5 mM $CuSO_4$ solution (Fig. 1(a)). The pH of the reaction was adjusted to pH 11 by using NaOH solution because the pH of solution played a vital role in the CuO NPs formation [30]. Fig. 1(b) shows the UV–Vis absorption spectra of the leaf extract and the colloidal solution after 60 minutes of reaction time. The appearance of an absorption maximum at λ_{max} 670 nm could be attributed to the surface plasmon resonance (SPR) band of the CuO NPs, thus indicating the successful formation of CuO NPs. This result was in agreement with a similar study which reported on the biosynthesis of CuO NPs using *aloe vera* leaf extract [31].
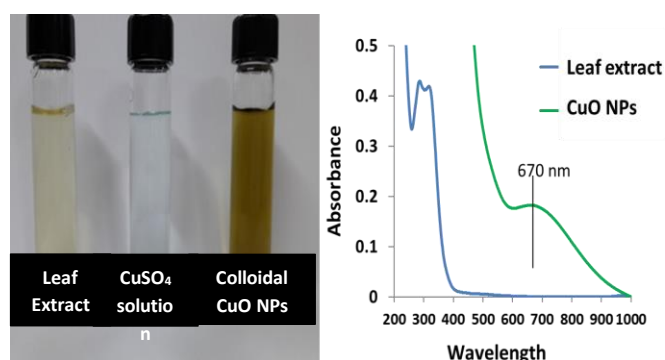

Fig. 1 (a) Photographic image of colour change during the course of the reaction and (b) UV-Vis spectra of leaf extract and colloidal CuO NPs.

Fig. 2 shows a schematic diagram of a proposed mechanism for the biosynthesis and stabilizing of the CuO NPs. The initial steps were involved the reduction of Cu^{2+} ions and followed by nucleation of the reduced metal atoms. The growth steps were referred to the spontaneous coalescence of the small adjacent nanoparticles into particles of a larger size, followed by capping with the plant biomolecules (polyphenols).
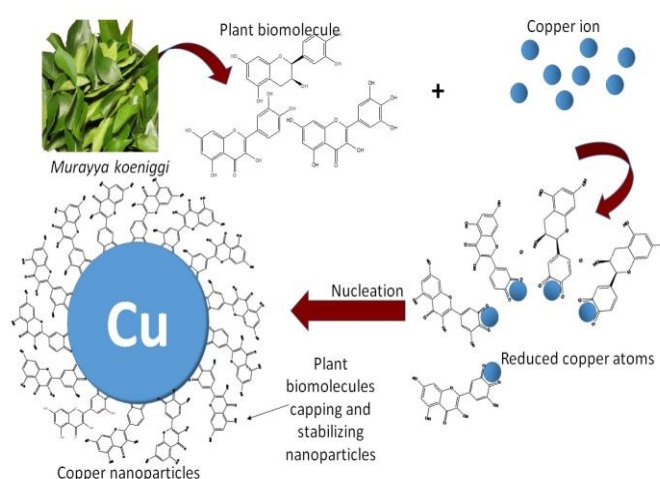

Fig. 2 Proposed mechanism for the formation and stabilization of CuO NPs.

Biosynthesis conditions, such as volume of leaf extract, pH, and temperature are among the significant influencing factors on the size of nanoparticles [26]. In this study, an optimization approach for synthesizing CuO NPs with uniform shape, smaller particle size and narrow size distributions was reported. The intensity and the width of the SPR absorption band are depended on the size of the metal nanoparticles [27]. In order to get the optimum amount of leaf extract for the reduction of Cu^{2+} ion, the volume optimization of *Murayya koenigii* leaf extract was studied. Fig. 3(a) shows the UV-Vis spectra for CuO NPs formation after 60 minutes of reaction time using a fixed $CuSO_4$ concentration (5 mM) with different volumes of *Murayya koenigii* leaf extract added. A blue shift in the wavelength from λ_{max} 670 nm to 658 nm was observed with the increase in amount of leaf extract (3 mL), which could be explained due to the basis of increased nucleation rate in the biosynthesis process. As a conclusion, greater amount of polyphenol would increase the nucleation rate and lead to the generation of smaller CuO NPs in the solution. However, with further increase in the leaf extract volume to 4 mL, the plasmonic band in the visible region became broader. The broadness of the absorption spectra could be attributed to the wide size distribution of CuO NPs. This might be due to the collision between smaller nanoparticles which then led to particle growth during the growth phase.

The work reported by Rajesh *et al* showed that the pH in aqueous media has an influence on the progress of the copper reduction reaction [28]. The biosynthesis of CuO NPs using 3 mL leaf extract and 10 mL of 5 mM $CuSO_4$ was carried out at different pH values, namely pH 8, 9, 10, and 11 (Fig. 3(b). Without any pH modification, after mixing the leaf extract and $CuSO_4$ solution, the pH value of about 5.4 was recorded. At this acidic pH, the particles size was seemed to be larger than at the basic pH, as a red shift (λ_{max} = 770 nm) was clearly observed in the SPR spectra. As the pH was increased from pH 8 to pH 10, it was observed that there was no significant difference on the intensity and the position of the SPR band at λ_{max} 660 nm. Meanwhile, at pH 11, the blue shift in SPR peak position at λ_{max} 634 nm could be attributed to the decrease in the particle size. This result was in good agreement with the previous study on the biosynthesis of CuO NPs at optimum pH of pH 11 [29]. The role of pH on nanoparticles synthesis could be seen in its effect on the capping and stabilizing abilities, and subsequently the growth of the nanoparticles [30]. The presence of OH^- ion in alkaline pH environment might enhance the reducing and stabilizing capabilities of the biomolecules in the leaf extract. The –CHO group on biomolecules was oxidized into $–COO^-$, offering stability and better capping agent for the generated metal particles in the presence of OH^- ion [31].
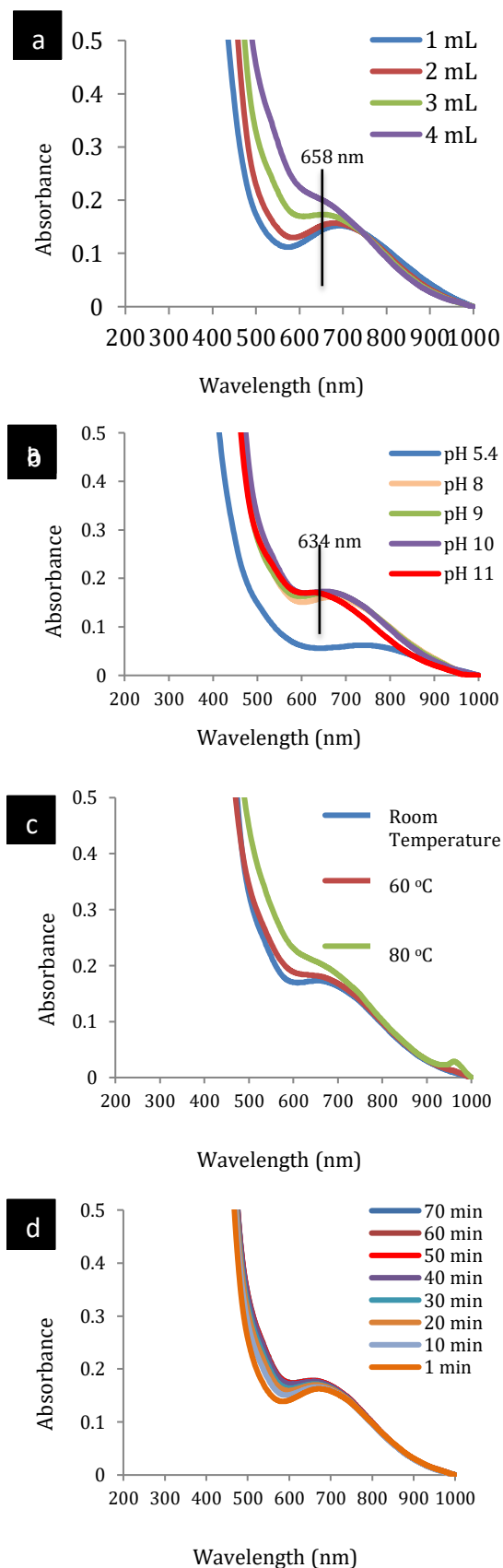
Fig. 3 UV-Vis of bioreduction of CuO NPs at different (a) volume of leaf extract; (b) pH; (c) temperature and (d) reduction time

The effect of temperature on the biosynthesis of CuO NPs was also studied for the 3 mL volume of leaf extract and 10 mL of 5 mM CuSO₄ solution at pH 11 at different temperatures, namely, at room temperature, 60°C and 80°C (Fig. 3(c)). Previous work revealed that temperature of the reaction would affect the size and morphology of

the synthesized nanoparticles due to the fact that the nucleation and growth of nanoparticles were depended on the temperature [32]. The studies on the physico-chemical conditions optimization during biosynthesis by Kumari reported that different temperatures would produce different nanoparticles morphologies such as prism, pentagon and hexagon [33]. In this study, as revealed by the UV-Vis spectra shown in Figure 3(c), both room temperature and 60°C produced similar size of nanoparticles since the plasmonic bands were appeared at the same λ_{max}. However, as the temperature was increased to 80°C, the formation of a new peak near the infra-red region was appeared which indicated to the anisotropic growth of CuO NPs. Thus, the biosynthesis of CuO NPs that carried out at room temperature would be the best condition as it did not show any difference in term of the nanoparticle size obtained as compared to those that performed at higher temperature (60°C).

Furthermore, the time for complete formation of CuO NPs was also investigated. The UV-Vis spectra for the colloidal CuO NPs against time were shown in Fig. 3(d). Meanwhile, the plot of absorbance at λ_{max} versus time for CuO NPs formation was illustrated in Fig. 4, showing that the biosynthesis has reached saturation within 50 minutes. Thus, it was concluded that the formation of CuO NPs was completed within 1 hour.

FTIR spectral analysis was conducted to identify the possible functional groups of the biomolecules presented in the *Muraya koenigii* leaf powder that would be responsible for the reduction of Cu^{2+} ion to CuO NPs. The FTIR spectrum of *Muraya koenigii* leaf powder was shown in Fig. 5(a) and revealed characteristic bands for O−H stretching vibrations at 3396 cm⁻¹ that could originate from alcohol or phenol functionality. The weak absorption bands were observed at 2944 cm⁻¹ that corresponded to C–H stretching in alkanes, while strong stretching vibration of C=O was observed at 1606 cm⁻¹. The medium intesity band observed at 1400 cm⁻¹ was assigned to C–C stretching in aromatic groups. The appearance of these functional groups might be attributed to the presence of biomolecules such as polyphenolic acids and flavonoids in the leaf extract powder. By comparison, the FTIR spectrum of the CuO NPs powder showed almost similar pattern as the FTIR spectrum of the *Murayya koenigii* leaf powder, which probably suggesting the presence of flavonoids and polyphenolic acids on the surface of the CuO NPs (Fig. 5(b)). These phytochemicals were not only acted as reducing agent but also as capping agent which helped to minimize the agglomeration of NPs by controlling the morphology and stabilizing the NPs. The phenolic hydroxyl group of phytochemicals was formed into metal–phenolate complex when hydroxyl group was bound with metal by chelating effect [34]. These complexes would undergo direct decomposition at 600°C and lead to the formation of CuO NPs.
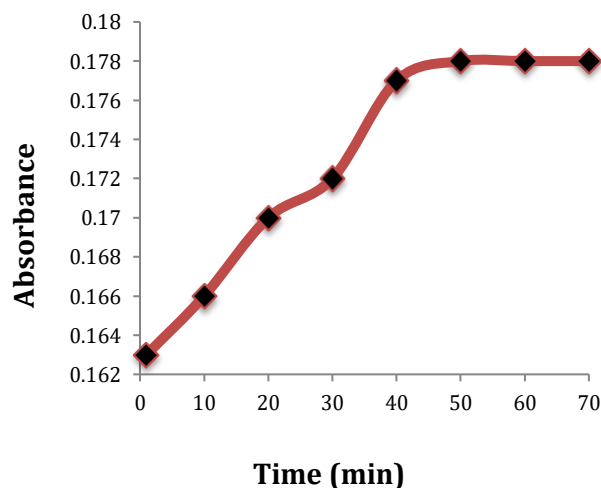

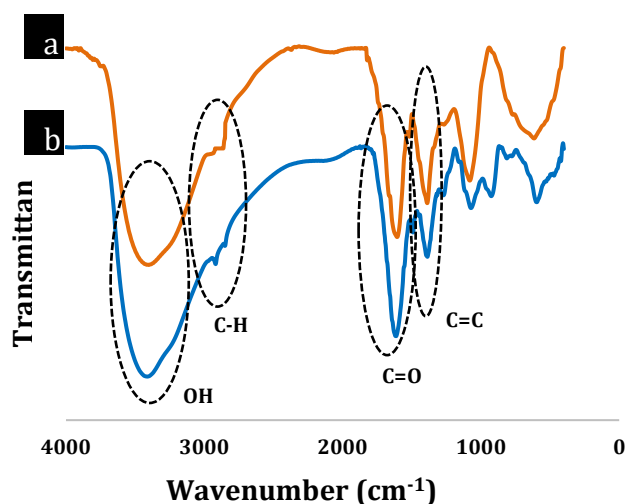**Fig. 4** Absorption at λ_{max} versus time for CuO NPs formation

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Fig. 5 FTIR spectra of (a) *Murayya koenigii* Leaf Extract Powder and; (b) CuO NPs Powder

The CuO NPs biosynthesized from *Murayya koenigii* leaf extract were confirmed by the characteristic peaks observed in the XRD pattern, as shown in Fig. 6. XRD analysis showed intense peaks at 32.5°, 35.5°, 38.6°, 48.8°, 53.4°, 58.1°, 61.5°, 66.3°, 68.0°, 72.4° and 75.0°, which corresponding to (110), (002), (111), (202), (020), (202), (113), (311), (220), (311) and (222), respectively. The observed diffraction reflections were comparable with JCPDS No. 45-0937 and attributed to bulk CuO materials. All diffraction peaks could be indexed as the typical monoclinic structure and no extra diffraction peaks of other phases were observed. Moreover, the well-defined and sharp CuO reflections in the observed XRD patterns were confirmed the well-crystalline nature of CuO NPs.

Fig. 7 demonstrates the TEM images of the CuO NPs at different magnifications which clearly showed spherical morphology of the CuO NPs biosynthesized from *Murayya koenigii* plant. The mean particle diameter, measured by image J software from 50 particles counts for CuO NPs was estimated to be 8.4 nm.
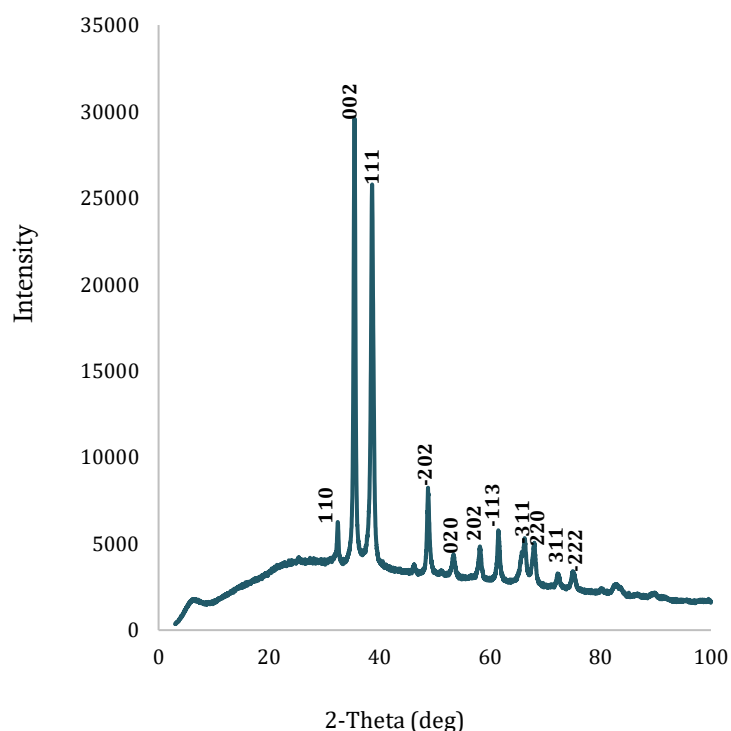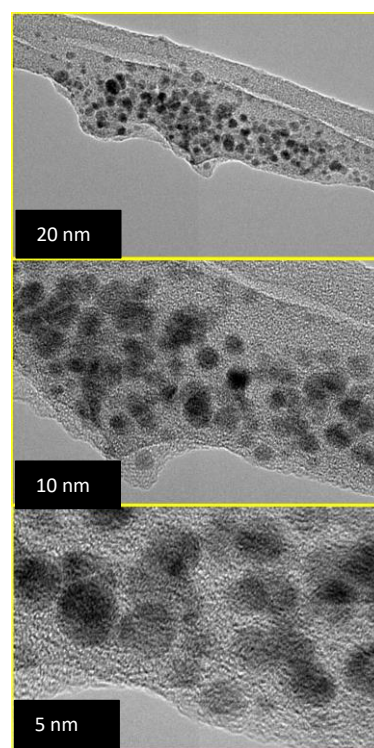

Fig. 7 TEM images of CuO NPs at different magnifications (20, 10 and 5 nm).

Catalytic activity test of 4-NP reduction reaction

The 4-NP reduction to 4-AP was generally selected as a model reaction for evaluating the catalytic activity of CuO NPs. This reaction could be easily monitored by UV-vis spectroscopy. In this study, the catalytic activity of the biosynthesized CuO NPs in the reduction of 4-NP at room temperature in aqueous medium was evaluated. When 4-NP was mixed with NaBH₄, the solution became yellowish and has a maximum absorbance at 400 nm due to the formation of 4-nitrophenolate anion. Without the addition of CuO NPs as a catalyst, the peak of 4-nitrophenolate anion at 400 nm did not change even after 1 hour of reaction time (Fig. 8(a)). Upon the addition of CuO NPs, the peak at 400 nm was started to decrease while a new peak at 300 nm was simultaneously appeared (Fig. 8(b)). The new peak at 300 nm was exhibited to an absorbance corresponding to the reaction product of 4-AP. The reaction was completed within 9 minutes, in which there were no more changes in 4-nitrophenolate absorbance observed.
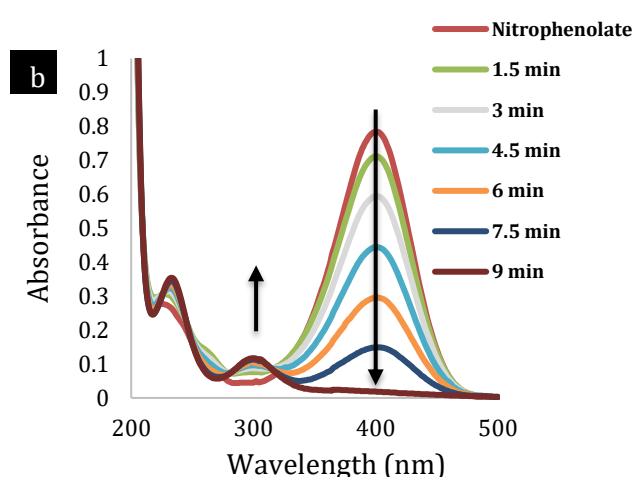

Fig. 6 XRD pattern of biosynthesized CuO NPs.
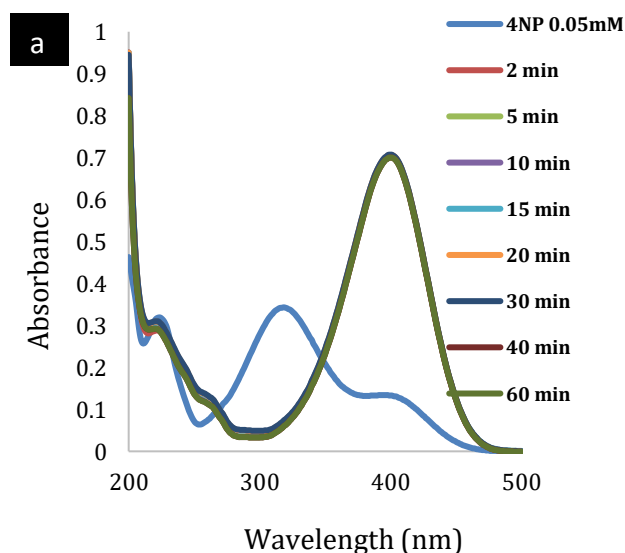
Fig. 8 UV-Vis spectra for the reduction of 4-NP (a) without Catalyst; (b) with CuO NPs Catalyst.

For quantitative analysis, a calibration curve was constructed with 4-NP solutions and a linear regression equation of y=12.968x + 0.0087 with correlation coefficient of 0.9995 in the concentration range of 0.01–0.09 mM was obtained (Fig. 9(a)). Based on this equation, the conversion yield of 4-NP to 4-AP was measured to be 95.8%. A reaction rate was measured from the plot between $\ln(A_t/A_0)$ versus time (sec). Herein, the 4-NP concentrations at time t=0 and time, t were expressed as A_0 and A_t, respectively. It was shown that there was a linear relationship between $\ln(A_t/A_0)$ and time (sec), suggesting that the reaction was followed a pseudo-first-order kinetics with a rate constant value of 9.2×10^{-3} s^{-1} (Fig. 9(b)).
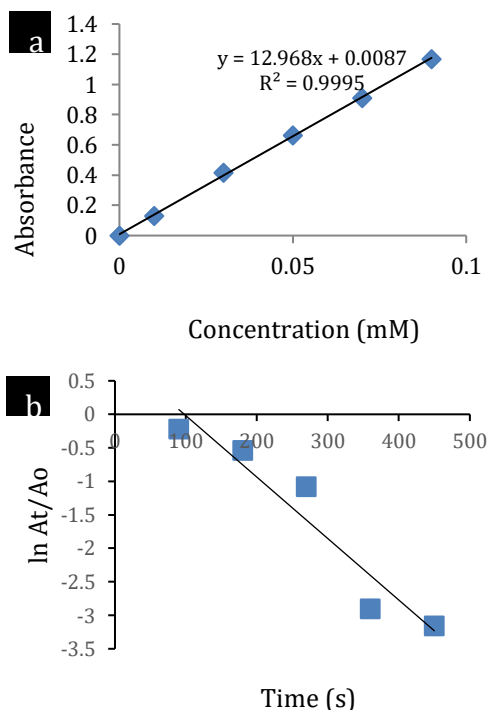

Fig. 9 (a) Calibration curve of 4-NP at different concentrations (0.01 mM – 0.09mM) (b) ln A_t/A_0 vs time for 4-NP reduction reaction.

Product purification and characterization

For the purification of the 4-NP reduction product, the reaction was scaled up with 4-NP (3.75 mM, 50 mL), NaBH$_4$ (750 mM, 50 mL), and CuO NPs catalyst (5 mg). After the work-up procedures, the isolated product was purified by silica gel column chromatography and its structure was determined by ^{1}H-NMR spectroscopy. In comparison with the standard 4-AP (Fig. 10(a)), the purified product was confirmed to be 4-AP (Fig. 10(b)).

4-Aminophenol (standard):
^{1}H NMR (400 MHz, DMSO): δH = 8.37 (s, 1H), 6.50 (d, J = 8.4 Hz, 2H), 6.44 (d, J = 8.4 Hz, 2H), 4.39 (s, 2H).
4-Aminophenol (purified):
^{1}H NMR (400 MHz, DMSO): δH = 8.36 (s, 1H), 6.49 (d, J = 8.4 Hz, 2H), 6.42 (d, J = 8.4 Hz, 2H), 4.39 (s, 2H).
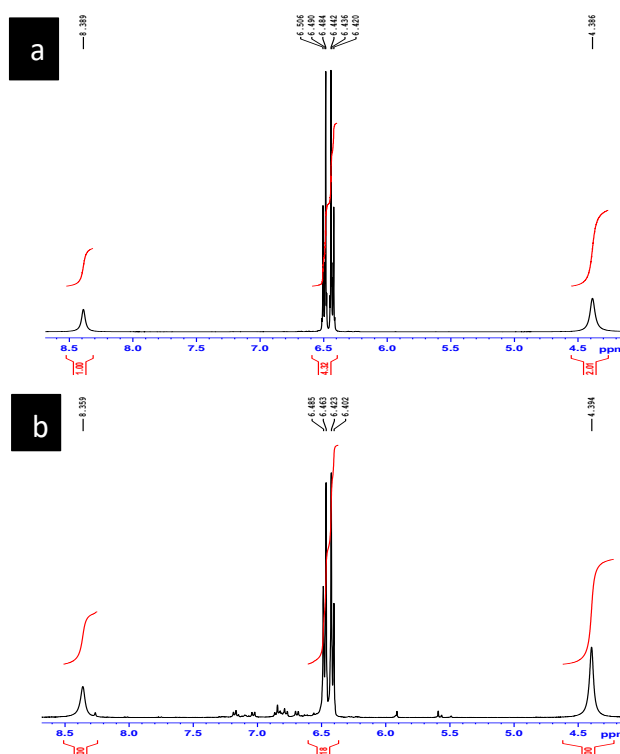

Fig. 10 ^{1}H-NMR spectra of (a) standard 4-AP (b) purified product (4-AP)
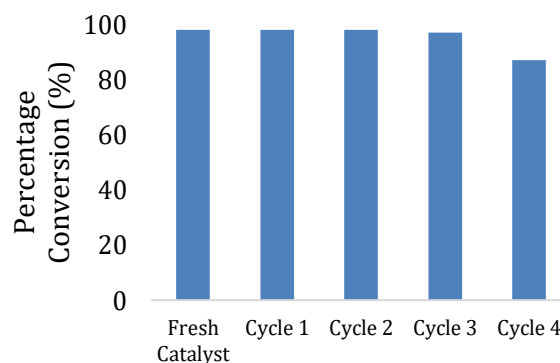
Recyclability test

Fig. 11 Recycle test of CuNPs catalyst (Reaction conditions: 1 mg of 3 mL 4-NP (0.05 mM), 300 µL NaBH4 (100 mM), room temperature, 15 minute).

The recyclability of the CuO NPs catalyst for reduction of 4-NP organic molecule was also investigated by applying the used CuO NPs catalyst for successive cycles of reaction. Used catalyst was collected by centrifugation and washed thoroughly with deionized water to remove residues of catalytic reaction for every subsequent run. The recyclability test results for the CuO NPs catalyst were illustarted in Fig. 11. The catalytic experiments were carried out under similar conditions, indicating that CuNPs have a relatively good activity even in the 3rd cycle. It was shown that the CuNPs could maintain their catalytic activity within 98% conversion after three cycles, indicating that there was no significant loss of catalytic activity. In cycle 4, a slight decrease in conversion of about 10% was observed. This could be probably due to the loss of catalyst mass during the catalyst recovery process.

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CONCLUSION

The green synthesis was successfully employed to synthesize CuO NPs from the leaves of *Murraya koenigii* plant in aqueous medium. This method was simple, low cost and eco-friendly without using any toxic chemicals. The synthesized CuO NPs were mostly spherical in shape with mean particle size of about 8 nm and possessed well crystalline nature which perfectly matched to monoclinic structure of bulk CuO. The biosynthesized CuO NPs were utilized as catalyst for the reduction of 4-NP to 4-AP. The product was obtained in excellent yield and the catalyst could be recycled up to four cycles with almost consistent activity.

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