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### 5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one Functionalized CuO Nanoparticles: Synthesis, Characterization and Antioxidant Activity

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#### Abstract

Naringenin (NaR) is a flavonoid, mostly found in citrus fruit. Its IUPAC name is 5,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one. Naringenin is lipophilic in nature and possessing antioxidant property. In present study,7-Dihydroxy-2-(4-hydroxyphenyl)chroman-4-one was incorporated onto CuO nanoparticles (CuO-NPs) for enhancing its antioxidant activity. The CuO-NPs and its functionalized form (CuO-NaR) were characterized by Fourier-transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), Scanning electron microscope (SEM) and Energy dispersive X-ray (EDX) analysis. The CuO-NPs, CuO-NaR and NaR were examined for their comparative antioxidant activities followed by DPPH (2,2-Diphenyl-1-picrylhydrazyl) method. The results revealed that the antioxidant activity of CuO-NaR was enhanced significantly.

Keywords: CuO nanoparticles, Naringenin, Antioxidant activity, DPPH

#### Introduction

Any material possessing at least one dimension <100 nm is called nanomaterial [1]. The properties (physical and chemical) of nanomaterials are strongly depending on their compositions, size, shape and structure [2]. The shape of nanomaterial depends on the length of three dimensions. Based on the dimensions, the nanomaterials are classified as zero-dimension (0D), one-dimension (1D), two-dimension (2D) and three-dimension (3D) materials. In 0D material, three dimensions are in nano range and materials can be nanoparticles and quantum dots. In 1D material, one dimension is out of the nano range, while two dimensions are in nano range and material can be nanorods, nanotubes and nanowires. In 2D material, two

dimensions are out of the nano range, while one dimension is in nano range and material can be nanofilms, nanolavers and nanocoatings. In 3D material, all the dimensions are out of the nano range and material can be core shells, multi nanolayers, bundles of nanowires, bundles of nanotubes [3,4]. The nanomaterials with unique properties can be synthesized by modifying their size and shape [5-9]. Based on their constituents, morphology size, and properties, the nanomaterials are classified as carbon-based nanomaterials. metal based nanomaterials. semiconductor nanomaterials, lipid-based nanomaterials and polymeric nanomaterials [10]. Different methods such as laser ablation [11],

electron beam irradiation [12], in-situ chemical synthetic route [13] include thermal decomposition [14] and chemical reduction [15] have been employed for the preparation of metal based nanoparticles. Chemical reduction method is the most attractive because of simple and cost effective and size of the material can be vary by optimizing experimental factors such as precursor salt, solvent, capping agent, reducing agent, solvent, reaction time, and temperature [16,17]. The nanomaterial can be tailored by functionalization strategies [18]. This study will focus on the synthesis of CuO NPs and its functionalization with antioxidant moieties for enhancing its activity.

# Materials and Methods *Materials*

Analytical reagent-grade chemicals and double distilled water (18.2 M ohm cm) were used throughout experimental work. CuSO<sub>4</sub>.5H<sub>2</sub>O, NaOH,  $H_2SO_4$ , KH<sub>2</sub>PO<sub>4</sub>,  $K_2HPO_4$ and Dichloroacetic acid (Cl<sub>2</sub>CHCOOH), Sodium dodecyl sulfate (SDS), L- Ascorbic acid and Naringenin (NaR) were purchased from Merck, Germany. FT-IR Spectrometer (Thermo Scientific, Nicolet iS10, UK) was used for recording FT-IR spectra. Bruker X-Flash 4010 133ev (made in Germany) using Cu K $\alpha$  (1 = 1.5406 Å) radiation was used for EDX analysis.

#### Synthesis of CuO NPs

CuO-NPs were synthesized by chemical reduction method [16] using CuSO<sub>4</sub>.5H<sub>2</sub>O as precursor salt and SDS as a capping agent. Experimentally, 1.0 mL of SDS solution (1.0 M) was added to 20 mL of 0.1 M CuSO<sub>4</sub>.5H<sub>2</sub>O solution and stirred vigorously for 30 min and 50 mL of 0.2 M L-Ascorbic acid solution was added with continuous stirring. Furthermore, 30 mL of 1.0 M NaOH solution was slowly added to the mixture with constant stirring and heated at 80 °C for 2 h. For work-up of the reaction, the mixture was settled overnight at room temperature, and the supernatant solution was discarded and filtered. The precipitates were washed subsequently with water and ethanol for three times and dried at room temperature. The precipitates were calcined

by heating in furnace at 300 °C for 3 h for complete conversion of  $Cu(OH)_2$  into CuO NPs.

# Functionalization of NaR onto the surface of CuO NPs

For functionalization of CuO NPs, 0.01 mg of CuO NP was dispersed in 20 mL of methanol and 100 mg of NaR was added with stirring at room temperature for 1 h. The reaction mixture was settled for overnight and the supernatant solution was discarded and filtered. The product was washed with methanol for several times to remove unreacted NaR. The final product (CuO-NaR) was dried by evaporating the methanol at room temperature.

#### Antioxidant activity

The free radical scavenging activities (FRSA) of CuO NPs, CuO-NaR and NaR were tested followed by DPPH method [19, 20]. In a typical procedure, 0.2 mg of CuO-NPs was dispersed in 3.20 mL of 100  $\mu$ M DPPH solution (prepared in methanol). The concentration of free radical was monitored after each of 0, 2, 4, 6, 8, 10, 12, 14 and 16 min followed by absorbance recording at  $\lambda_{max}$  of 517 nm. The free radical scavenging activities of NaR and CuO-NaR were also tested followed by the same procedure. FRSA (decolourization) of CuO NPs, CuO-NaR, NaR were calculated by equation 1.

Decolourization (%) =  $[1 - (Absorbance_{(sample)} / Absorbance_{(control)})] \times 100$  (1)

#### Ferric reducing antioxidant potential (FRAP)

FRAP study of CuO NPs, CuO-NaR and NaR was carried out by reported method [21]. Experimentally, the series of test solutions 0.5-3.0 mg/mL of CuO NPs were prepared. For testing FRAP, 0.5 mL of test solution was taken into small glass tube and 2.5 mL of KH<sub>2</sub>PO<sub>4</sub>/K<sub>2</sub>HPO<sub>4</sub> buffer solutions was mixed to the solution for maintaining pH 7.5. Therefater, 2.5 mL of 1.0% (w/v) K<sub>3</sub>[Fe(CN)<sub>6</sub>] (potassium ferricyanide) was mixed to the solution and incubated at 25 ° C for 20 min. Then 2.5 mL of 10% (w/v) dichloroacetic acid was mixed in it and centrifuged at 3000 rpm for 10 min. Then 2.5 mL from upper layer (supernatant) of solution was transferred to another small glass tube containing 2.5 mL of water and 0.5 mL of 0.1% (w/v) ferric chloride solution and placed in dark for 15 min. Then solution was subjected to spectrophotometer at  $\lambda_{max}$  of 700 nm for absorbance recording against blank. Same procedure was applied for testing FRAP of NaR and CuO-NaR.

#### Total antioxidant capacity (TAC)

TAC of CuO NPs, CuO-NaR and NaR was evaluated separately by reported procedure [22]. Experimentally, 1.0 mL of alcohol containing 0.5 mg of CuO-NPs was taken in a glass tube containg 0.5 mL reagent solution (0.6 M H<sub>2</sub>SO<sub>4</sub>, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95 °C for 150 min. The content was cooled at room temperature and subjected to spectrophotometer at  $\lambda_{max}$  of 595 nm for absorbance recording against blank at different intervals of times i.e. 20, 40, 60, 80, 100 and 120 min. Same procedure was applied for testing TAC of NaR and CuO-NaR.

#### **Results and Discussion** *FT-IR spectroscopy*

The Fig. 1 shows FT-IR spectra of CuO-NaR. The characteristic peaks at 3269.32, 1614.01, 15.60.22 and 1049.68 cm<sup>-1</sup> correspond to the stretching vibrations of O-H, C=O, C=C and C-O bonds, respectively of NaR. The peaks at 795.54 and 650.91 cm<sup>-1</sup> correspond to stretching

vibrations of Cu-O bond [23]. This spectral information revealed the successful incorporation of NaR onto the surface of CuO NPs.

#### X-ray diffraction (XRD)

The Fig. 2 depicts the XRD pattern of CuO-NPs. The diffraction peaks at  $2\theta = 31.058^{\circ}$ ,  $35.703^{\circ}$ ,  $38.932^{\circ}$ ,  $48.877^{\circ}$ ,  $53.831^{\circ}$  and  $56.352^{\circ}$  correspond tp (110), (002), (111), (-202), (020) and (202) planes of monoclinic phase of CuO-NPs respectively, which confirmed the formation of CuO-NPs phase [24]. According to Bragg's law (d =  $n\lambda/2Sin\theta$ , where  $\lambda$  is wave length of X-rays (for Cu K $\alpha$ ,  $\lambda = 1.54056$  Å) and n = 1 (order of diffraction) and  $\theta$  is position of peaks in radian) [24]. The Basal spacing of (002) and (111) planes was calculated and found to be 0.25 and 0.23 nm, respectively. According to Debye Scherrer equation [25], the average crystallite size of CuO-NPs was calculated and found to be 16.40 nm.

#### Scanning electron microscope (SEM)

CuO-NPs CuO-NaR and were characterized by SEM at different resolutions as shown in Fig.3a-f. The SEM images a-c evidently discovered the morphology of the CuO-NP as spherical in shape, clustered and porous. After the functionalization of CuO-NPs, the morphology of nanoparticles was changed to crystalline and smooth as shown in images d-f, which indicated that NaR smoothen the surface of nanoparticles and acted as capping agent to prevent agglomeration and functionalized the CuO-NPs.



Figure 1. FT-IR Spectrum of CuO-NaR



Figure 2. EDX pattern of CuO-NPs



Figure 3. SEM Images of CuO NPs (a-c) and CuO-NaR-NPs (d-f) at different resolutions

#### Energy dispersive X-ray (EDX)

CuO-NPs and CuO-NaR were characterized by EDX analysis for elemental quantification. For analysis, the electron-beam was bombarded onto the surface of sample, which caused the generation of K $\alpha$  x-rays. The energy of x-rays is the characteristic of elements present in the sample. EDX (Fig. 4a) of CuO NPs depicts that the contents of Cu: 75.06%, C: 4.72% and O:

18.6%, in CuO-NPs are closely related to the theoretical contents of Cu: 79.89% and O: 20.11% in CuO, confirmed the formation of CuO phase. While EDX (Fig. 4b) of CuO-NaR shows the contents of Cu: 28.83%, C: 28.83% and O: 46.18% in CuO-NaR. The increased in contents of C and O elements in CuO-NaR, indicated the successful incorporation of NaR onto the CuO-NPs.



### DPPH radical scavenging

DPPH was used as source of free radical while CuO NPs and CuO-NaR were acted as free radical scavenger. Fig. 5 shows comparative FRSA of CuO-NPs, NaR and CuO-NaR. The results revealed that CuO-NaR possessed greater FRSA than CuO-NP and NaR

#### Ferric reducing antioxidant potential (FRAP)

FRAP study of CuO, NaR and CuO-NaR-NP was carried out in the concentration range of 0.5-3.0 mg/mL. In this method, iron in ferricyanide complex is  $Fe^{3+}$ , which was reduced to  $Fe^{2+}$  by gaining electron from antioxidants [24].

Fig. 6 depicted that with FRAP was increased with increasing of antioxidants. FRAP of CuO-NaR was greater that CuO-NPs and NaR at all the levels of concentration.

#### Total antioxidant capacity (TAC)

TAC of CuO-NPs, CuO-NaR and NaR were evaluated. Fig. 7 depicts that after the 20 min, absorbance of content was not enhanced significantly. The CuO-NaR based content has greater absorbance value tham CuO-NPs and NaR. Therefore, CuO-NaR possessed comparatively greater TAC.

![](_page_5_Figure_1.jpeg)

Figure 5. FRSA of CuO NPs, CuO-NaR and NaR

![](_page_5_Figure_3.jpeg)

Figure 6. FRAP of CuO NPs, CuO-NaR and NaR

![](_page_5_Figure_5.jpeg)

Figure 7. TAC of CuO NPs, CuO-NaR and NaR

#### Conclusion

CuO nanoparticles (CuO-NPs) were synthesized by chemical reduction method using CuSO<sub>4</sub>.5H<sub>2</sub>O as precursor salt and sodium SDS as a capping agent. The formation of monoclinic phase of CuO-NPs was confirmed by X-ray powder diffraction and the average crystallite size was found to be 16.40 nm. The morphology of the CuO-NPs was spherical in shape, clustered and porous. CuO-NPs were functionalized with NaR. The characteristic peaks in FT-IR spectrum at 3269.32, 1614.01, 15.60.22 and 1049.68 cm<sup>-1</sup> corresponded to the stretching vibrations of O-H, C=O, C=C and C-O bonds, respectively of NaR. The peaks 795.54 and 650.91  $\text{cm}^{-1}$  are associated with vibration of to the C-O bond. This spectral information revealed the successful incorporation of NaR onto the surface of CuO-NPs. Antioxidant activity of NaR was enhanced significantly by functionalization with CuO-NPs. The mechanism of antioxidant activity may be in dose-dependent manner. It can also be fulfilled by free radical reduction, depending on the structure of metal oxide as well as structure of adduct of functionalized CuO-NaR.

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