SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF COPPER(II)-CURCUMIN COMPLEX AGAINST STAPHYLOCOCCUS AUREUS

Le Dang Khoa, Hoang Minh Hao
Ho Chi Minh City University of Technology and Education, Vietnam

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ABSTRACT

Curcumin, a phytochemical from turmeric, and its derivatives have been extensively investigated from both chemical and biological strategies. However, the main problem encountered while using curcumin in clinical trials is its poor solubility and rapid degradation, resulting in its low levels in tissues, thus decreasing the medicinal effect of curcumin. To overcome these problems several synthetic approaches have been carried out to prepare new derivatives possessing better properties. Curcumin as a β-diketone ligand can act as chelating ligands toward a variety of metals to form stable complexes. Some studies showed that a metal-curcumin complex displayed potential medicinal activities. In this work, a copper(II)-curcumin complex was synthesized in a two-step procedure: (i) curcumin was separated from commercial turmeric powder using chromatography techniques and (ii) the copper(II) chloride (1 eq.) and pure curcumin (2 eq.) were mixed together in ethanol. The mixture was stirred at 60 ^oC for 3 hours to afford a stoichiometric copper(II)-curcumin complex. The curcumin ligand and its copper(II) complex were characterized by UV-Vis, IR, NMR spectroscopic methods, from which it was found that copper atoms are coordinated through keto-enol groups of two curcumin molecules. The ground state spectral features of the copper(II)-curcumin complex were consistent with that of the 1:2 copper(II)-curcumin complex. The antibacterial activities of curcumin ligand and its complex were evaluated against Staphylococcus aureus (ATCC 6538) using a well diffusion method on nutrient agar. The results showed that the inhibitory activity was not observed for free curcumin at any concentrations while the copper(II)-curcumin complex exhibited the inhibition zones (mm) of 7.8, 11.6 and 14.9 at various concentrations (mg/mL) of 1.0, 5.0 and 15.0, respectively.

Keywords: *Copper(II)-curcumin; curcumin; metal-curcumin complex; antibacterial activity; Staphylococcus aureus.*

1. INTRODUCTION

Curcumin (Figure 1), a yellow pigment isolated from *Curcuma longa* L., has been shown to be the main active compound responsible for various biological effects of turmeric, including anti-inflammatory, anti-angiogenic, anti-oxidant, wound healing and anti-cancer effects [1]. Having diverse biological activities, curcumin has become one of the most favorite subjects for many branches of chemistry including organic, inorganic, and physical chemists. In organic chemistry field the extract and synthesis of curcumin and its derivatives have been extensively studied while inorganic chemists have used its chelating ability through the β-diketone group to form new structures with promising biochemical activities.

The main problem encountered while using curcumin in clinical trials was its poor bioavailability, resulting in its low levels in tissues, thus decreasing the medicinal effect of curcumin [2]. Hydrophobic nature of this polyphenolic compound along with its rapid metabolism, physicochemical and biological instability, were the main factors limiting its bioavailability. To overcome these problems several approaches have been proposed like

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the use of adjuvant like piperine that interferes with glucuronidation [3], encapsulation of curcumin in liposomes [4] and polymeric micelles [5], inclusion complex formation with cyclodextrin [6], formation of phospholipid–curcumin conjugates [7], and use of nanoparticles [8].

In recent years, curcumin complexation with transition metals has emerged as a highly promising and innovative approach to improve the bioavailability of curcumin and to explore its more potential health benefits. Curcumin as a β-diketone ligand can act as chelating ligands toward a variety of metals to form stable complexes. Some studies showed that a metal-curcumin complex can have higher potential medicinal effect than curcumin itself [9-12] such as selective cytotoxicity [13-14], anticancer activity [15], anti-Alzheimer's disease activities [16], antioxidative effects [17] and antibacterial activities [18] of such complexes. In present work, the copper(II)-curcumin complex was synthesized by mixing curcumin and copper(II) chloride in ethanol at molar ratio of copper(II): curcumin (1:2). The curcumin ligand and its complex were characterized by UV-Vis, IR, NMR techniques. The antibacterial activities of free curcumin and copper(II)-curcumin complex were evaluated against *Staphylococcus aureus* (ATCC 6538).

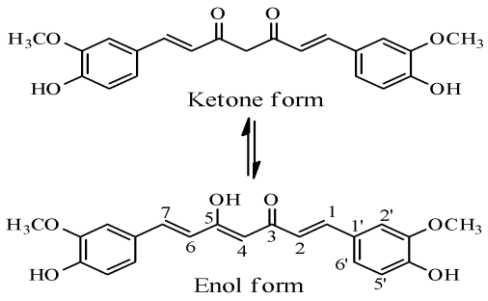

Figure 1. *Keto-enol equilibrium tautomerism of curcumin.*

2. EXPERIMENTAL

2.1. Materials

Copper(II) chloride dihydrate (99%), ethanol (99.5%) and dimethyl sulfoxide (DMSO, 99.7%) were purchased from ACROS Organics. Hexane (97%) and ethyl acetate (97%) were received from local companies. Thin layer chromatography (TLC) was performed on silica gel (Merck, Kialselgel 60 F_{254}, 250 μm). Column chromatography (CC) was carried on silica gel (40–63 μm, Merck). Spots were visualized by spraying 10% H_2SO_4 and UV light (254 and 365 nm). *Staphylococcus aureus* (ATCC 6538) was supplied by Laboratory Applications in Microbiology, Institute of Tropical Biology, Vietnam Academy of Science and Technology, Linh Trung, Thu Duc, Ho Chi Minh City.

Isolation of curcumin from commercial turmeric powder: The turmeric powder was received from Vietnam Academy of Science and Technology. The powder (20 g) was subjected to CC using gradient elution of hexane-ethyl acetate to give pure curcumin (500 mg) for synthetic procedure. *Synthesis of copper(II)-curcumin (Cu(II)-curcumin):* The 1:2 Cu(II)-curcumin complex was synthesized following a published procedure with minor modification [19]. The ethanolic solution of curcumin (2 eq, 50 mg) and $CuCl_2.2H_2O$ (1 eq, 11,6 mg) was stirred under reflux at 60 ^oC for 3 hours. The dark precipitated complex was filtered, washed several times with cold water and ethanol and dried in vacuum to obtain a complex (32.8 mg, 60.5% yield). *Agar well diffusion assay:* Antibacterial activities of curcumin and copper (II)-curcumin complex were evaluated using well diffusion method on nutrient agar (0.5% Peptone, 0.3% yeast extract, 0.5% NaCl, distilled water). The inhibition zones were reported in millimeters (mm). *S. aureus* (ATCC 6538) was used as references for the antibacterial assay of curcumin and Cu(II)-curcumin. The bacterial strain was cultivated in nutrient agar slants and preserved at 4 °C. Eighteen to 24 hours single colonies on agar plates were used to prepare the bacterial suspension with the turbidity of 0.5 McFarland. Turbidity of the bacterial suspension was measured at 600 nm. This suspension was diluted to the working bacterial suspension (equal to

1.0×10^6 colony-forming units (CFU)/ml) by sodium chloride 0.9% solution. Briefly, nutrient agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter = 6 mm) were filled with 60 µl of the test samples and incubated at 37 °C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured. Dimethyl sulfoxide was used as solvent control while tetracycline (50 µg/mL) used as positive control. All tests were performed in triplicate.

2.2. Apparatus

^{1}H (500 MHz, $CDCl_3$, TMS), ^{13}C (125 MHz, $CDCl_3$, TMS) NMR spectra were recorded on a Bruker Avance III spectrometer. FTIR spectrum was recorded by KBr pellet method with JASCO-8000 Fourier Transform Infrared Spectrophotometer in the range 400-4000 cm^{-1}. UV-Vis spectra were measured using HITACHI UH5300 spectrophotometer in DMSO. Melting point was determined on a Kruss melting point meter model: M5000.

3. RESULTS AND DISCUSSION

Curcumin is a yellow crystal with the melting point of 182-183 ^{o}C. When visualized under UV light (365 nm), it gives a yellow spot on TLC with R_f = 0.4 after eluted with hexane:ethyl acetate (1:1). Curcumin is a symmetric molecule, the ^{1}H-NMR spectrum of curcumin in $CDCl_3$ thus displays signals of protons of a half moiety. A singlet at δ 3.94 refers to the methoxy group (-OC**H**$_3$). A broad singlet at δ 3.94 is a signal of a proton of hydroxyl group (-O**H**) attached to the aromatic ring. Two doublets, each one proton, at δ 6.48 and 7.60 with coupling constant *J* = 15.5 Hz revealed the *trans* configuration of the >C=C< group corresponding for H_2 and H_1 of an α, β-unsaturated carbonyl group (Figure 1). The region δ 6.9÷7.3 showed the presence of three aromatic protons and trisubstituted benzene ring. The peaks at δ 6.93 ($H_{5'}$, 1H, d, *J* = 8.0 Hz), 7.12 ($H_{6'}$, 1H, dd, *J* = 8.0; 2.0 Hz) and 7.04 ($H_{2'}$, 1H, d, *J* = 2.0) confirmed that these three protons coupled each other in *ortho* and *meta* positions. A singlet at δ 5.79 denoted one proton adjacent to the carbonyl group of enol form (H_4). The ^{13}C-NMR spectrum showed 14 carbons including 12 unsaturated carbons in the region of δ 100-170, a conjugated carbonyl group at δ 183.31 and a methoxy group (-**C**H_3) at δ 56.00. These evident spectra in comparison with literature confirmed the chemical structure of curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) [18].

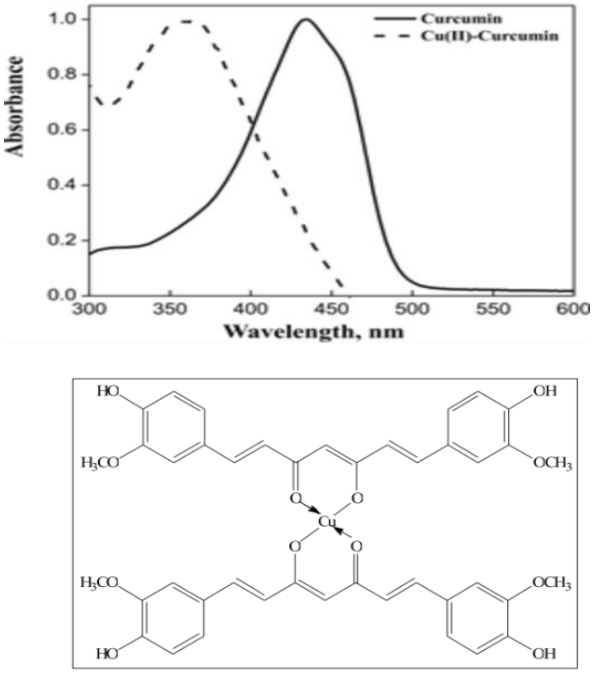

Figure 2. *Top panel refers to the normalized absorption spectra of curcumin ligand and 1:2 Cu(II)-curcumin complex in DMSO. A suggested structure of Cu(II)-curcumin complex is given in the bottom panel.*

Curcumin and Cu(II)-curcumin complexes are soluble in DMSO. The normalized UV-Visible spectra of curcumin and Cu(II)-curcumin complex in DMSO are given in Figure 2. Curcumin exhibits maximum at 435 nm, it is noted that this peak is attributed to the π→π* transition in curcumin in DMSO [18, 20]. At the same time the copper complex shows maximum at 361 nm, and this blue-shift by 74 nm can be attributed to the curcumin-metal charge transfer, indicating the involvement of the carbonyl group of curcumin in complexation with the metal. The ground state spectral

features of the Cu(II)-curcumin complex are consistent with that of the 1:2 Cu(II)-curcumin complex but are distinctly different from that of the 1:1 Cu(II)-curcumin complex reported in the literature [19, 20].

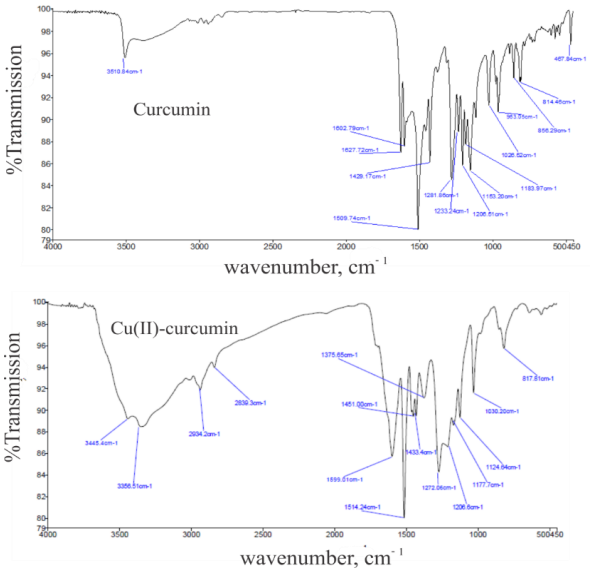

Figure 3. *FTIR spectra of curcumin and Cu(II)-curcumin complex.*

Figure 3 gives the FTIR spectra of free curcumin ligand and Cu(II)-curcumin complex. The infrared (IR) data of curcumin ligand shows O-H stretching vibration at 3510 cm^{-1} (3200–3500 cm^{-1}), >C=C< aromatic stretching vibration at 1429 cm^{-1} and >C=C< aliphatic chain stretching vibration at 1509 cm^{-1}. The band at 814 cm^{-1} referred to the >C=CH- aromatic stretching vibration. An intense band at 1281 cm^{-1} attributed to the bending vibration of the C-O phenolic band. The FTIR spectrum of the Cu(II)-curcumin complex is different from that of the curcumin ligand. The peak at 1599 cm^{-1} of the complex, assigned to the >C=O stretching vibration, is shifted to lower energy in comparison with that of curcumin at 1627 cm^{-1}. This result indicates that the carbonyl groups of the ligand in enol form coordinate to copper ion (right panel of Figure 1) [21]. However, the position of the vibrational band around 3445 cm^{-1} of curcumin remains unchanged on complexation with metal ions. This showed that the phenolic -OH group of curcumin is not involved in complexation with copper ion [21, 22]. In addition, the bands in 3200-3500 cm^{-1} attributed to the strong broadening bands which were caused by four -OH phenolic groups [22].

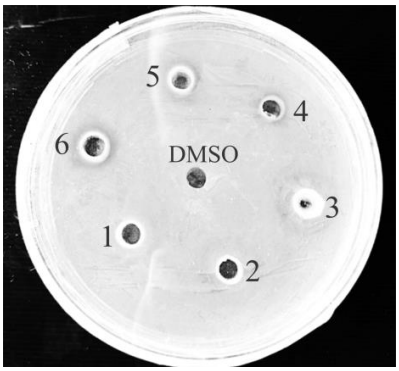

Figure 4. *The antibacterial activities of curcumin ligand and Cu(II)-curcumin complex at various concentrations against S. aureus after 24 hours of incubation. The numbers of 1, 2, and 3 referred to the free curcumin at 1, 5, and 15 mg/mL. The ones of 4, 5, and 6 denoted the Cu(II)-curcumin at the same concentrations with curcumin ligand. DMSO was a negative control.*

The antimicrobial test results of curcumin ligand and its complex by using the well diffusion method were shown and summarized in Figure 4 and Table 1. Curcumin did not exhibit an inhibition zone whereas Cu(II)-curcumin complex clearly showed antibacterial activity against S. *aureus*.

Table 1. *The antibacterial activity against S. aureus of curcumin and Cu(II)-curcumin complex.*

	Curcumin (mg/mL)			Cu(II)-curcumin (mg/mL)			DMSO	Tetracycline (50 µg/mL)
	1	5	15	1	5	15		
Inhibition zone (mm)	0	0	0	7.8±0.3	11.6±0.5	14.9±0.2	0	30.2±0.2

Curcumin was inactive (zone of inhibition = 0 mm) for all treatments in different concentrations. The diameter of the inhibition zone increased with increasing the concentration of Cu(II)-curcumin complex. The zones of inhibition were observed under three concentrations of 1 mg/mL, 5 mg/mL, and 15 mg/mL of Cu(II)-curcumin were 7.8 mm, 11.6 mm, and 14.9 mm, respectively. There being no antibacterial activity was observed under treatment with DMSO.

This result demonstrated that Cu(II)-curcumin complex had antibacterial activity against S. *aureus*. The complexation of curcumin with copper metal plays a significant role in inhibiting bacteria [18].

4. CONCLUSION

In present work, the 1:2 copper(II)-curcumin complex was successfully synthesized in a moderate yield (60.6 % yield) and characterized by spectroscopic techniques. In UV-Vis spectra, the maximum peak of the complex is blue-shifted by 74 nm in comparison with that of curcumin ligand. This shift was attributed to the curcumin-metal charge transfer between carbonyl group of curcumin and metal ion in the complex. The FTIR also confirmed that the carbonyl groups of the ligand in enol form coordinates to copper and the phenolic groups are totally free from complexation with copper ions. The antibacterial activities of curcumin ligand and its copper(II) complex against *S. aureus* demonstrated that the free curcumin was inactive at any concentrations whereas Cu(II)-curcumin complex showed the inhibition zones at various concentrations due to the presence of copper metal.

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Corresponding author:
Hoang Minh Hao
Ho Chi Minh City University of Technology and Education
Email: haohm@hcmute.edu.vn