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Original Article

ANTI-TYROSINASE AND CYTOTOXICITY ACTIVITIES OF CURCUMIN-METAL COMPLEXES

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ABSTRACT

Objective: The objective of this study is investigating the potential of curcumin metal complexes in pharmaceutical and cosmetic products.

Methods: Curcumin was complexed with five divalent transition metals (Zn(II), Cu(II), Fe(II), Mn(II), and Mg(II)) and then investigated for their anti-tyrosinase activity and their mode of inhibition against mushroom tyrosinase and cytotoxicity against KB and MCF-7 cell lines.

Results: The tyrosinase inhibition of curcumin increased in the presence of Mn (II) and Zn (II); however, the activity was reduced after complexingwith Cu (II), Fe (II), and Mg (II). Curcumin manganese complex (curcumin-Mn) exhibited the highest potent anti-tyrosinase activity with classical noncompetitive inhibitor which showed the inhibition constant (KI)of 3.57 µg/mL(7.58µg/mL for free curcumin). For the cytotoxicity against KB and MCF7 cell lines, free curcumin showed cytotoxicity against both KB (IC_{50} 9.58 µg/mL) and MCF7 (13.86 µg/mL) cancer cell lines; whereas, it was found to be lower in the metal complexes.

Conclusion: This study suggests a potential use of the curcumin-Mn and Zn as a depigmentation agent in cosmetic products.

Keywords: *Anti-tyrosinase, Curcumin* metal complexes, Cytotoxicity.

INTRODUCTION

Curcumin is a polyphenol yellow pigment found in rhizomes of curcuma species. It is widely used as an active substance in food supplements, cosmetics, and pharmaceuticalsdue to its multiple therapeutic activities. These include anti-cancer [1], anti-inflammatory, anti-bacterial, anti-fungal [2], anti-oxidant [3], inhibition of lipid per oxidation and anti-tyrosinase [4]. Curcumin was used in anti-wrinkle, anti-acne, and skin whitening products. However, a major problem in application of this compound in pharmaceutical and cosmetic products is that it rapidly decomposes under high temperature and exposure to light [5-6].

Several studies demonstrated that the stability and biological activities of curcumin can be enhanced by complexing with transition metal ions. Complexation of curcumin with Zn(II) and Mg(II) can enhanced the thermal and UV stability in solution and emulsion systems [7]. Lipid peroxidation and superoxide dismutase (SOD) activity increased six- and ten-fold, respectively, when curcumin was complexed with Cu (II) [8-9]. The antioxidant activities of curcumin complexes with Zn(II), Cu(II), Fe(II), Mg(II), and Mn(II)) have better DPPH radical scavenging and ferrous reducing power activities than free curcumin with the same dosage [10].

Skin whitening products are very popular, especially in Asian countries. These products focus on lightening skin color and treating the production of abnormal pigmentation such as melasma, freckles, agespots, liver spots, and other forms of melanin hyperpigmentation [11]. Depigmentation agents act at various levels of melanin production in the skin. Many of them are known as competitive inhibitors of tyrosinase, which is the key enzyme in melanogenesis. This enzyme is a copper-containing enzyme that catalyzes the production of melanin from tyrosine by oxidation reaction [12]. Hence, the compounds that inhibit tyrosinase activity leading to skin lightening have been the subject of much research [13-15]. In order to investigate the potential of curcumin metal complexes in pharmaceutical and cosmetic products, this study will evaluate anti-tyrosinase and cytotoxicity activities of curcumin metal complexes.

MATERIALS AND METHODS

The chemicals and reagents were analytical grade. Curcumin, kojic acid, L-tyrosine and tyrosinase enzyme, zinc acetate ($Zn(OAc)_2$), copper acetate ($Cu(OAc)_2$), manganese dioxide (MnO_2) and magnesium chloride ($MgCl_2$)were purchased from Sigma. Ethanol, methanol and DMSO were purchased from Merck. Sodium dihydrogenphosphate ($NaH_2PO_4 \bullet 2H_2O$), and disodium hydrogen phosphate ($Na_2HPO_4 \bullet 12H_2O$) were purchased from Prolabo.

Preparation ofcurcumin-metal complexes

Ethanolic solution of 0.1 M metal salt (5 mL) was added dropwise to 5 mL of 0.1 M curcumin solution and reflux at 60 °C for 3 hours. After the reaction was complete (as assessed by the precipitate occurred), the solution was then cooled to room temperature and washed with cold ethanol-water. The precipitates were then dried, weighted and calculated for their yield percent.

Determination of anti-tyrosinase activity

Anti-tyrosinase activity of curcumin and their metal complexes was determined according to the method of Rangkadilok [16]. Briefly, L-tyrosine solution (40 µl; 1.7 mM) was dissolved in phosphase buffer (pH 6.8; 40 µl; 0.1M) and then added to 40 µl of samples in DMSO. After incubation for 10 minute at room temperature, 40 µl of mushroom tyrosinase (245 U/mL in pH 6.5 phosphase buffer) was added and the mixture was then incubated for 20 minute at room temperature. The absorbance of the reaction was recorded at 490 nm using microplate-reader(UVM 340, Biochrom). The percentage inhibition of tyrosinase was calculated by following equation

$$\text{Anti} - \text{tyrosinase activity}\,(\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where, $Abs_{control}$ is absorbance of control at 490 nm, Abs_{sample} is absorbance of sample at 490 nm.

The IC_{50} values were determined from plots of percent inhibition 50 versus inhibitor concentration and were calculated by linear regression analysis from the mean inhibitory values. Kojic acid was used as the reference tyrosinase inhibitor. All tests were performed in triplicate.

The metal curcumin complex with the highest anti-tyrosinase activitywas determined for its inhibition mechanism by using a Lineweaver–Burk plot compared with standard curcumin. The inhibition constant of complex (KI) was determined by plotting the intercept values versus the concentration of the corresponding compound.

Determination of cytotoxicity

The cytotoxicity experiments were based on resazurinmicroplate assay (REMA) as described by Brien [17]. Two cancer cell line including KB and MFC7 cancer cell lines were used. In brief, cell line at logarithmic growth phase were harvested and diluted to $7x10^4$cells/ml fresh medium. The 5µl of test sample diluted in 5% DMSO and 45 µl of cell suspension were successively added to a 96-well plate and incubated at 37 °C in a 5% CO_2 incubator. After 3 days of incubation, 12.5 µl of 62.5 g/ml resazurin solution was added to each well and the plate was then incubated at 37°C for 4 hr. Fluorescence signals were measured using a microplate reader at excitation and emission wavelengths of 530 nm and 590 nm, respectively. Ellipticine and doxorubicin were used as positive controls and 0.5% DMSO as negative control. Percent inhibition of cell growth was calculated with the following equation.

$$\% \text{ Inhibition } = 1 - \frac{\text{FUT}}{\text{FUC}} \times 100$$

Where, FUT and FUC are the mean fluorescent intensity from treated and untreated conditions respectively.

Statistical analysis

Data were expressed as means standard deviations (SD) of three replicate determinations. One way analysis of variance (ANOVA) was used to determine the differences among the means. P values < 0.05 were regarded as significant.

RESULTS AND DISCUSSIONS

In this study, five metals including Zn, Cu, Fe, Mg, and Mn were chosen to complex with curcumin based on their color, toxicity, economic and cosmetic regulation. The complexes were successfully prepared by refluxing the mixture of curcumin and metal salts in ethanol for three hours, resulting in different colored powder (Fig. 1). The color of all complexes was darker than that of free curcuminresulting from the reaction between metals and curcumin ligand. Zn, Mn, and Mg complexes of curcumin were light red-brown colorpowder, while those of Cu and Fe were a solid dark brown color. The yield percent was calculated based on the weight of complexes and decreased in order; curcumin-Fe (87.3%) >curcumin-Cu (79.2%) >curcumin-Mn (75.7%) >curcumin-Zn (68.4%) >curcumin-Mg (67.4%).

a) b) c) d) e) f)

Fig. 1: The physical appearance of curcumin complexes a) curcumin, b) Cur-Zn, c) Cur-Cu, d) Cur-Fe, e) Cur-Mn, f) Cur-Mg

The interaction between the metal and curcumin at β-diketone group of the prepared complexes was confirmed by UV-vis and IR spectroscopic techniques[9]. In our previous study, the stability and antioxidant activities of curcumin-metal complexes were investigated and the results indicated that curcumin-Zn and curcumin-Mg are more stable and have a stronger antioxidant activity than free curcuminat the same dosage. The most powerful metal enhancing antioxidant ability of curcumin is Zn [10].

This study aimed to investigate the anti-tyrosinase activity of curcumin metal complexes for using as depigmentationagents in cosmetic products,which is of much interest to the pharmaceutical and cosmetic industry. The effect of curcumin metal complexes on tyrosinase activity was determined using the L-tyrosine oxidation assay. The absorbance at 490 nm decreases as a result of the reaction of melanin synthesis was interrupted. The activity expressed as IC_{50} values; the concentration of sample required for 50% inhibition of tyrosinase enzyme. Enzyme activity was directly related to curcumin concentration. Free curcumin showed inhibition of tyrosinase enzyme with IC_{50} 31.52 µg/mL. The anti-tyrosinase activity of metal derivatives was in order of curcumin-Mn>curcumin-Zn >curcumin≅kojic acid ≅curcumin-Mg >curcumin-Cu ≅curcumin-Fe. The inhibition of tyrosinase activity of curcumin-Mn (IC_{50} 10.82 µg/mL) and curcumin-Zn (IC_{50} 25.22 µg/mL) showed significantly higher than those of free curcumin and kojic acid, a commercial whitening agent. Curcumin-Mg showed the anti-tyrosinase activity to be equally strong to free curcumin and standard kojic acid. In contrast, curcumin-Cu and curcumin-Fe showed three-fold lower tyrosinase activity than standard compounds. The tyrosinase inhibition activity of curcumin was enhanced by complexing with Mn(II) and Zn(II), but was decreased in the presence of Mg(II), Cu(II), and Fe(II). This may be due to the effects from various metals give differentplanar structureof the complexesleading to their different chelation patternwith the active site of tyrosinase enzyme [18].

In order to investigate the inhibition mechanism of the complexes, curcumin-Mnwhich showed the highest inhibition value was determined by using a Lineweaver–Burk plot compared with standard curcumin. The inhibition mechanisms can provide a basis for the development of new effective tyrosinase inhibitors. True inhibitors are classified into four types: competitive inhibitors, uncompetitive inhibitors, mixed type inhibitors, and non-competitive inhibitors. The inhibitors that combine with free enzyme and then prevent substrate binding with it are known as competitive inhibitors. The uncompetitive inhibitors can bind to the enzyme-substrate complex. The mixed type inhibitors and non-competitive inhibitors bind with both free enzyme and enzyme-substrate complex with different equilibrium binding constants and with the same equilibrium binding constants, respectively (Fig. 2) [19].

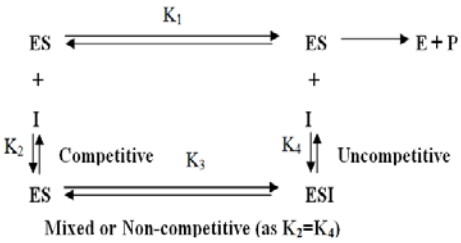

Fig. 2: Action mechanism of reversible inhibition. Complexes are: E = enzyme, S = substrate, I = inhibitor, P = product, ES = enzyme-substrate, EI = enzyme-inhibitor, and ESI = enzyme-substrate-inhibitor

Fig. 3 displayed as a plot of1/V versus 1/[S] gave three straight lines with different slopesand a horizontal line that intersected at the same point. With anincrease in compound concentration, the Vmax value decreased, whereas the Km value remained the same, suggesting that curcumin-Mn complex is a non-competitive inhibitor of tyrosinase as is free curcumin (Fig. 4). The results indicated that curcumin-Mn bind with tyrosinase enzyme (E) and tyrosinae-tyrosine complex (ES) with the same equilibrium binding constants. The inhibition constant of curcumin-Mn was determined by plotting the intercept values versus the concentration of the corresponding

compound, as shown in Fig. 4. The inhibition constant (KI) values for curcumin-Mn complex and free curcumin are 3.57 and 7.58 µg/mL, respectively. Curcumin-Mn showed more potent in tyrosinase inhibition than free curcumin. The cytotoxicity of all complexes on two human cancer cell lines, oral cavity cancer (KB) and breast cancer (MCF7) was evaluated. Free curcumin had moderate cytotoxicity against both KB and MCF7 cancer cell lines with IC_{50} 9.58 and 13.86 µg/mL, respectively. On KB cancer cell line, curcumin-Mn and Mg showed moderated cytotoxicity with IC_{50} 11.58 and 10.01 µg/mL. Curcumin-Zn showed weak activity, beingthree-fold lower than free curcumin, with IC_{50} 33.51 µg/mL. On MFC7 cancer cell line, curcumin-Zn, Mn, and Mg showed lower cytotoxicity than free curcumin with IC_{50} 16.73, 30.07 and 26.98 µg/mL, respectively. All curcumin-metal complexes had lower cytotoxicity than free curcumin in both KB and MCF7 cancer cell lines. The lower cytoxicity of the metal complexes might be due to the lower solubility of complexes when compared with free curcumin. These results are in related with the report of Mutasim [20], where curcumin-Fe complex showed lower cytotoxic than free curcumin 80 fold. This result may be caused by the central carbon atomwith the labile hydrogen is locked and unable to produce oxyradicals which result in the cytotoxicity behavior.

Table 1: Bioactivity of curcumin-metal complexes

Sample	Anti-tyrosinase activity[1] (IC_{50} ug/mL)	Anti-cancer activities	
		KB-Oral cavity cancer (IC_{50} ug/mL)	MCF7-breast cancer (IC_{50} ug/mL)
Kojic acid	35.15 ± 0.462[c]	-	-
Curcumin	31.52 ± 0.128[c]	9.58	13.86
Curcumin-Zn	25.22 ± 0.047[b]	33.51	16.73
Curcumin-Cu	99.29 ± 0.018[d]	Inactive	Inactive
Curcumin-Fe	116.51 ± 0.771[d]	Inactive	Inactive
Curcumin-Mn	10.82 ± 0.256[a]	11.58	30.07
Curcumin-Mg	33.77 ± 0.196[c]	10.01	26.98
Ellipticine	-	1.08	-
Tamoxifen	-	-	9.52

[1]Different letters in column indicate significant differences among means of treatments ($P < 0.01$).

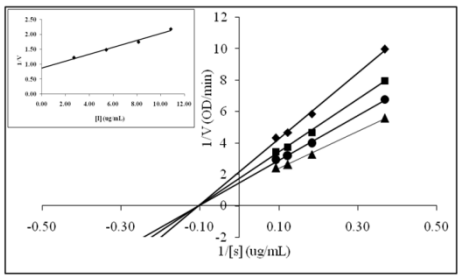

Fig. 3: Lineweaver–Burk plots for inhibition of curcumin on mushroom tyrosinase for catalysis of L-Tyrosine. Concentrations were 2.7, 5.4, 8.1 and 10.9 µg/mL, respectively. The inset represents the secondary plot of 1/Vmax versus concentration of curcumin to determine the inhibition constant (KI).

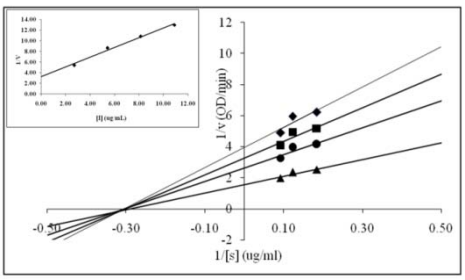

Fig. 4: Lineweaver–Burk plots for inhibition of Curcumin-Mn complex on mushroom tyrosinase for catalysis of L-Tyrosine. Concentrations were 2.7, 5.4, 8.1 and 10.9µg/mL, respectively. The insets represent the secondary plot of 1/Vmax versus concentration of curcumin, to determine the inhibition constant (KI).

CONCLUSION

The study demonstrates that anti-tyrosinase activity of curcumin can be enhanced by complexing with Mn(II) and Zn(II), whereas the activity was reduced when curcumin was complexed with Cu(II), Fe(II), and Mg(II). The highest potential on tyrosinase inhibition was found in curcumin-Mn, which binds toboth free tyrosinaseenzyme and the tyrosinase substrate complex. However, the cytotoxicity against both KB and MCF7 cancer cell lines of metal curcumin complexes are less than free curcumin. In conclusion, this study demonstrates the potential anti-tyrosinase capacity of curcumin-Mn and Zn to be processed as a depigmentation agent for esthetics andtreatment of hyperpigmentation disorders.

CONFLICT OF INTERESTS

Declared None

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