ANTIOXIDANT AND ANTITYROSINASE ACTIVITIES OF METAL COMPLEXES OF *Curcuma petiolata* EXTRACT FOR COSMETIC APPLICATIONS

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Abstract

Wan-Bao-Chan (*Curcuma petiolata*), one of *Curcuma* species, contain high amount of curcumins which is worldwide use for food supplement, cosmetic and pharmaceutical purposes due to multiple therapeutic activities. However, there are restriction on the use of the compound due to its instability to light and heat which affect to its activities and the cosmetic products characteristics. The efficacy and stability of curcumins can be enhanced by coordinated with transition metal ions (Zn^{2+}, Mg^{2+}, and Mn^{2+}) which has been reported enhancing the antioxidant and anti-tyrosinase activities of curcumins. The present study, these divalent transition metals were complexed with curcumin rich extract from *C. petiolata.* Then the complexes were characterized by spectroscopic technique and were evaluated for their antioxidant and anti-tyrosinase activities. The results showed that the DPPH radical scavenging, ferrous reducing power, and anti-tyrosinase activities was enhanced by complexing with Zn^{2+}, Mn^{2+}, and Mg^{2+}. Our findings provide evidence that the metal complexes of curcumin rich extract from *C. petiolata* can be the potential sources of active ingredient for cosmetics application.

Keywords: anti-oxidant, anti-tyrosinase, *Curcuma petiolata*, curcumin, complexes

Introduction

Curcuma species belong to the zingiberaceae family which widely distributed in Asia. About 30 species were found in Thailand but few are of economic importance. The rhizomes of these species are used in traditional medicines (Perry, 1980) which contain curcuminoids, a yellow pigment, as a major constituent. It is a generally regarded as the most active constituent which a wide range of biological and pharmacological activities including; anti-cancer (Hatcher *et al*., 2008; Yang, 2005), anti-inflammatory (Ammon *et al*., 1992), inhibition of bacterial and fungal growth (Srinivasan *et al*., 2001; Roth *et al*., 1998) efficient in anti-oxidant activity (Jayaprakash *et al*., 2006) and inhibition of lipid peroxidation more than ascorbic acid and vitamin E. Moreover, it showed 75 folds more potent in anti-tyrosinase activity than arbutin and showed similar effect as kojic acid (Shirota *et al*., 1986; Khunlad *et al*., 2008). With these properties curcumins was used as active ingredients in many products such as anti-wrinkles, anti-acne and whitening products. However, there are restriction on the use of curcumin due to its instability to light and heat (Tonnesen *et al*., 2002; Sowbhagya *et al*., 2005) which affect to efficacy and appearance of cosmetics.

The efficacy and stability of this compound can be enhanced by coordinated with transition metal ions (Hesham *et al*., 2004). We previously reported the increasing antioxidant and anti-tyrosinase activities of curcumin when coordinated with Zn^{2+}, Mn^{2+}, and Mg^{2+} (Thakam and Saewan, 2011).

Curcuma petiolata is one of *Curcuma* species which widely cultivated as an ornamental plant and has long been used as a folk botanical in Asia (Perry, 1980). The pseudo stem of *C. petiolata* has been used as traditional medicine for anti-inflammatory (Prasad *et al*., 2008). The *C. petiolata* rhizome extract contain high amount of curcumins with potent DPPH radical scavenging, ferrous reducing power and inhibition of lipid peroxidation activities (Thakam and Saewan, 2012). In addition to *Curcuma longa*, this plant has been suggested as an alternative source of curcumin.

The objectives of this study were to evaluate the DPPH radical scavenging, ferrous reducing power, and antityrosinase activities of curcumin rich extract from *C. petiolata* and its metal complexes.

Methodology

Extraction
The air dried rhizomes of *C.petiolata* (100 g) was powdered and twice extracted with ethanol. The solvent were evaporated using rotary evaporator and kept at 4°C until further analysis.

Determination of total curcumins content
The curcuminoid content of extract was determined according to the method from Xu (Xu et al, 1984) with some modifications. Varies concentration of standard curcumin solution was measured at 434 nm and the calibration curve was plotted between absorbance and concentration of the curcumin. The absorbance of the extract was measured.

Qualitative analysis of curcumins
Analytical HPLC analysis for determine the curcumins content was carried out using a model 1090 M series II DR5 ternary pumping system with integral variable volume autosampler, column oven and model 1040 series II photodiode array detector with HP workstation (Hewlett–Packard, Stockport, UK). All samples were filtered through a 0.2µm membrane syringe filter prior to analysis. The injection volume was 20 µl. The column used was a 250 × 4.6 mm HRPB 5 µm (octyl-/octadecylsilane, fully end-capped 14% carbon loading; HiChrom, Theale, UK) and the mobile phase consisted of solvent A (0.4% v/v acetic acid in acetonitrile) and solvent B (0.4% v/v aqueous acetic acid) delivered at 1 ml/min. The mobile phase was running by gradient elution. The starting mobile phase ratio was 50%A:50%B which was increased linearly over 30 min to 20%A:80%B and held for 10 min. Photodiode detection was carried out at 425 nm for curcumin.

Synthesis of metal complex
The metal complexes of curcumin rich extract from C. *petiolata* were prepared by refluxing the ethanol solution of the extract and each metal compound (zinc acetate ($Zn(CH_3COO)_2$), manganese dioxide (MnO_2), and magnesium chloride ($MgCl_2$)) in ratio 1:1 mol at 60 °C for 3 hours. The precipitates were washed with cold ethanol-water mixture and the metal complexes of curcumins rich extract from C. *petiolata* powders were obtained.

UV absorption spectrum

The absorption spectra of C. *petiolata* extract and their metal complexes were scanned on the wavenumber range of 350–600 nm.

FT-IR Spectroscopy

FT-IR spectra of C. *petiolata* extract and their metal complexes were performed on FT-IR equipped with resolution are fixed to 4 cm^{-1}. The pellets of sample and potassium bromide were prepared by compressing the powders and the spectra were scanned on the wave number range of 4000–400 cm^{-1}.

Determination of DPPH radical scavenging activity

The analytical procedure was modified from Rangkadilok method (Rangkadilok *et al*., 2005). This assay detects scavenging of free radicals of the sample through the scavenging ability of the stable DPPH free radical. The 5 µl of each sample was reacted with 195 µl of 100 µM DPPH in a 96-well microplate and incubated at 37 ºC for 30 minutes in dark. Then, the absorbance was measured at 515 nm. The percentage of DPPH radical scavenging inhibition was calculated using the formula given below:

$$\%\ \text{DPPH radical scavenging} = \frac{Ac - As}{Ac} \times 100$$

where Ac is the absorbance of the control and As is the absorbance of samples

Determination of ferrous reducing power activity

Reducing power of C. *petiolata* extract and their metal complexes were determined according to the ferrous reducing power method of Takashi (Takashi *et al*., 2009) with some modifications and expressed as ascorbic acid equivalents (AAE). Each 25 µl of sample or ascorbic acid (a positive control), 25 µl of 0.1 M phosphate buffer (pH 7.2) and 50 µl of 1% potassium ferricyanide were added to 96-well microplate. After incubation at 37ºC for 60 minutes, 25 µl of 10% trichloroacetic acid and 100 µl of DI water were added. Then, the absorbance was measured at 700 nm (A1). After 25 µl of 0.1% ferric chloride was added to the mixture and the absorbance was measured again (A2). The reducing power of samples was determined using the standard ascorbic acid calibration curve.

Anti-tyrosinase activity

Anti-tyrosinase activity of C. *petiolata* extract and their complexes was determined according to the method from Rangkadilok with some modifications (Rangkadilok *et al*., 2005). L-tyrosine solution (40 µl) at 1.7 mM was dissolved in 40 µl of 0.1M phosphase buffer (pH 6.8) and then added to 40 µl of samples (dissolved in DMSO). After 10 minute of incubation at room temperature, 40 µl of mushroom tyrosinase (245 U/mL in phosphase buffer (pH 6.5)) was added. The absorbance was recorded after 20 minute of incubation at room temperature at 490 nm using microplate-reader. The percentage inhibition of tyrosinase was calculated by following equation

$$\%\text{Inhibition of tyrosinase} = \frac{Ac - As}{Ac} \times 100$$

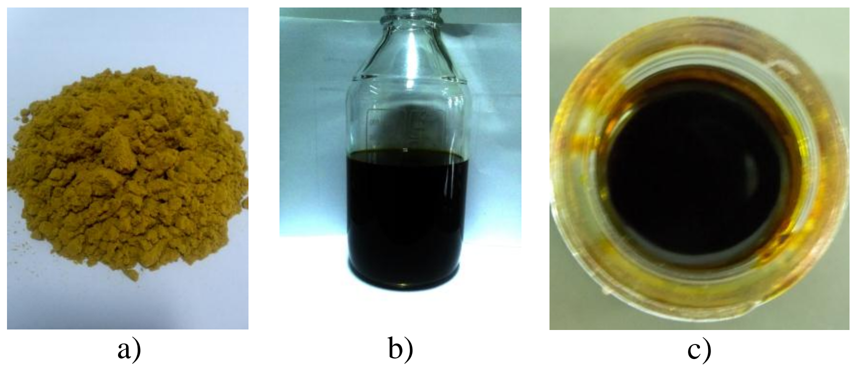
where Ac is the absorbance of the control and As is the absorbance of samples

Statistical analysis

Data were expressed as means ± standard deviations (SD) of three replicate determinations. One way analysis of variance (ANOVA) and Duncan's New Multiple-range test were used to determine the differences among the means. P values < 0.05 were regarded as significant.

Results

The *C. petiolata* rhizome was powdered and extracted with ethanol. The physical appearance of the extract was dark brownish as show in Figure 1.



a) b) c)

Figure 1 The physical appearance of a) *C. petiolata* rhizome powder, b) *C. petiolata* extract c) *C. petiolata* crude extract

The curcumins content of *C. petiolata* extract was determined by UV-Vis spectrophotometer and high liquid performance chromatography. The maximum absorption of the extract was 426 nm. The total curcuminoid content of the extract showed high amount of total curcumins with 133.36 mg/g plant. The yield percentage of total curcumins content per weight of plant was 13.34 %, which was equivalent to that of turmeric (Table 1) (Chavalittumrong & Dechatiwongse, 1988). From qualitative analysis of curcumins using HPLC, the extract contain of bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC), and curcumin (Cur), in order Cur> BDMC > DMC with 90.48, 30.67, and 16.66 mg/g plant, respectively (Figure 2). The extract contain high amount of total curcumins with 137.81 mg/g plant.

Table 1 The curcuminoid content of *C. petiolata* extract

	Content (mg/g plant)
Curcumin content	90.48
Bisdemethoxycurcumin content	30.67
Demethoxycurcumin content	16.66
Total	137.81

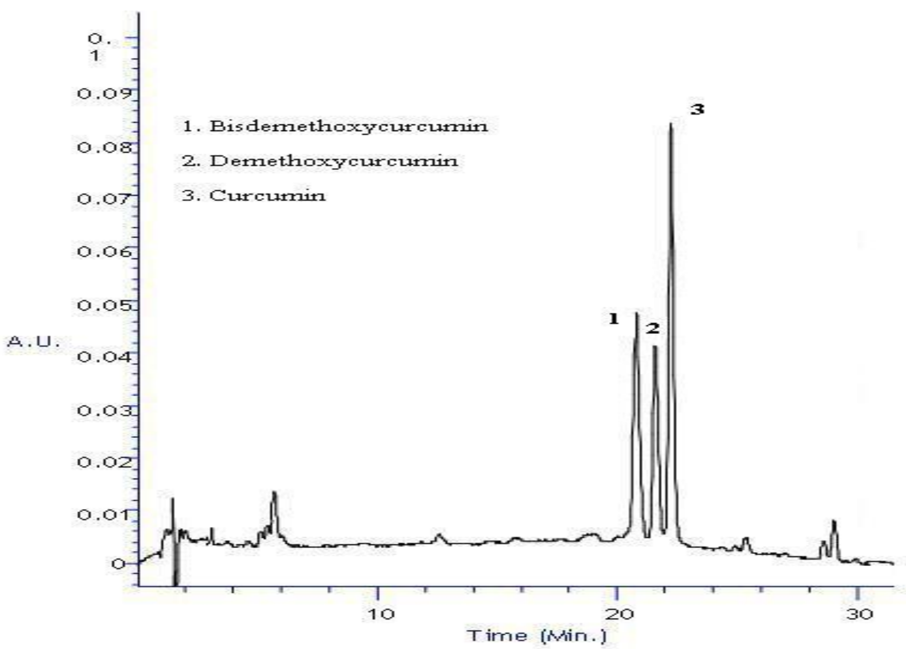


Figure 2 HPLC chromatogram of curcumins rich extract from *C.petiolata* rhizome

The metal complexes of curcumins rich extract from *C. petiolata* were successfully prepared by refluxing the ethanol solution of the extract and each metal compound (zinc acetate ($Zn(CH_3COO)_2$), manganese dioxide (MnO_2), and magnesium chloride ($MgCl_2$)) for 3 hours. The complexes were obtained as difference color powder resulting from the reaction between metals and curcumins. The yield percent was calculated base on the weight of the substrate and curcumin-Mn showed highest yield percent as showed in **Table 2**

Table 2 The percentage yield of curcumins from rich *C. petiolata* and its complexes

Compound	% yield	Physical appearance
Curcumins rich extract from *C. petiolata*	-	
Curcumin-Zn complexe	72.3	
Curcumin-Mn complexe	77.6	
Curcumin-Mg complexe	68.9	

The UV absorption spectra of *C. petiolata* extract and its metal complexes were recorded between 200-600 nm. Curcumin showed two absorption bands at ~360-430 nm ($n \rightarrow \pi^*$ transition) and at ~240-290 nm ($\pi \rightarrow \pi^*$ transition) (Barik et al., 2007). The UV-Vis spectrum

of the extract exhibits a maximum absorption band at 426 nm. The complexes showed a shifting of maximum absorption by 3-18 nm compare with free extract due to the reaction between *β*–diketone with metal ions (Zhao *et al*., 2005) (Figure 3). The maximum absorption of Zn, Mn, and Mg complexes were 422, 442.5, and 421.5 nm, respectively (Figure 4).

Figure 3 The theoretical structure of metal complexes of curcumins from rich *C. petiolata*

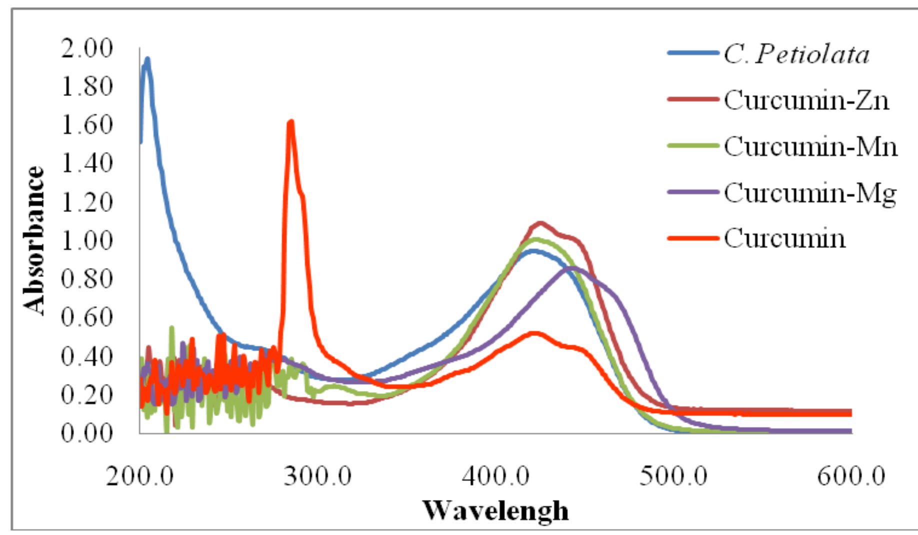


Figure 4 Spectrum of curcumin and its complexes

The FT-IR spectrum of the extract at 3,432 cm-1 is attributed to vibrations of free hydroxyl-group of phenol (Ar-OH). At 1,709, 1667, and 1,645 cm^{-1} are attributed to vibrations of the carbonyl bond (C=O). Compared with the spectrum of the extract, all complexes showed a decrease in the intensity of (C=O) carbonyl band, by more shift wave values (Δv =40-55 cm^{-1}) (Figure 5 and Table 3). These results indicating the some interaction has occurred at these sites between metal and curcumin.

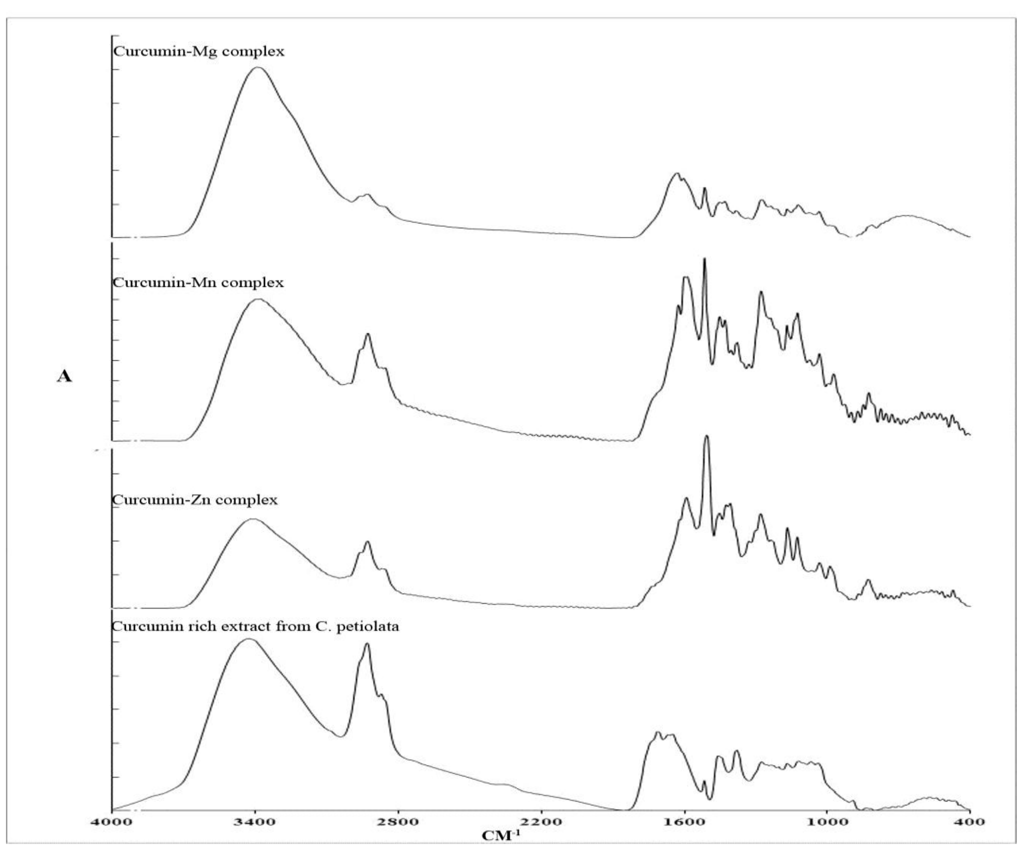


Figure 5 FT-IR spectra of curcumin rich extract from *C. petiolata* and its complexes

Table 3 Wave length changes from infrared (KBr pellets) spectral data of curcumin rich extract from *C. petiolata* and its metal complexes.

Compounds	IR (cm^{-1}) Ketone $_{(C=O)}$		
Curcumin rich extract from *C. petiolata*	1709	1667	1645
Curcumin-Zn	-	1619(Δv = 48)	1590(Δv = 55)
Curcumin-Mn	-	1623(Δv = 44)	1599(Δv = 46)
Curcumin-Mg	-	1627(Δv = 40)	1602(Δv = 43)

The antioxidant activities of complexes were tested by DPPH radical scavenging assay and reducing power assay.

For DPPH radical scavenging assay, the absorbance at 515 nm decreases as a result of DPPH color changes from purple to yellow due to the radical is scavenged by antioxidants was donated the hydrogen atom to form the stable DPPH-H. The lowest absorbance of reaction mixture indicates highest anti-oxidant activity. Curcumin-Zn showed the highest inhibition of DPPH radical scavenging (IC_{50} 9.33 μg/mL) which was significantly higher than standard curcumin and ascorbic acid (IC_{50} 12.36 and 12.46 μg/mL, respectively ($P<0.05$)). The DPPH radical scavenging activity was followed by curcumin-Mn and curcumin-Mg with IC_{50} 11.82 and 15.52 μg/mL, respectively. All of metal complexes showed higher DPPH radical

scavenging activity than that of *C. petiolata* extract due to the metal ions change the chemical properties of the curcumin ligand which more effective to donate hydrogen atom to DPPH radical than free curcumin.

The ferrous reducing power activity was measured the reduction of $[Fe(CN)_6]^{3-}$ to $[Fe(CN_6)]^{4-}$ by antioxidants, which is formed in blue complex from excess Fe^{3+} ions and then expressed as ascorbic acid equivalents (AAE). The more value of ascorbic acid equivalents indicated higher reducing power activity. All of complexes showed 2 to 4 folds higher reducing power activity that that of free *C. petiolata* extract. Curcumin-Mg showed the highest of reducing power with 0.21 mg AAE/mg extract. The reducing power was followed by curcumin-Mg and curcumin-Zn with 0.14 and 0.12 mg AAE/mg extract, respectively. All of complexes showed 6 folds lower reducing power than standard curcumin (1.25 mg AAE/mg). The correlation coefficient between DPPH radical scavenging activity and reducing power is rather small, indicating some compounds which showed high DPPH scavenging activity may not show reducing power activity due to the ferrous reducing power is a method for measuring total reducing power of electron donating substances, while DPPH assays are methods for measuring the ability of antioxidant molecules to quench DPPH free radicals (Wang *et al*., 1998 and Wangcharoen *et al*., 2007).

The tyrosinase activity of curcumin rich extract from *C. petiolata* and their complexes 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 result was expressed in IC_{50} value. Lower IC_{50} value indicated higher antityrosinase activity. All of complexes showed the strong inhibited of monophenolase activity than standard curcumin and kojic acid ((IC_{50} 31.32 and 35.15) µg/mL, respectively ($P<0.05$)). The enhancement of monophenolase inhibition of complexes might be due melain structure was modified by metal ion in melanogenesis pathway (Jara *et al*., 1990)

Table 4 Show the bioactivity of metal complexes of curcumin from *C. petiolata*

Sample	Antioxidant activity		Antityrosinase activity (IC50 ug/mL)
	DPPH radical scavenging (IC50 ug/mL)	Reducing power (mg AAE/mg extract)	
Ascorbic acid	12.36 ± 0.141^{b}	1.00 ± 0.013^{b}	-
Kojic acid	-	-	35.15 ± 0.462^{b}
Curcumin	12.46 ± 0.320^{b}	1.25 ± 0.013^{a}	31.52 ± 0.128^{b}
Extract	23.24 ± 0.128^{c}	0.06 ± 0.001^{e}	56.47 ± 0.012^{c}
Curcumin-Zn	$9.33.16 \pm 0.108^{a}$	0.12 ± 0.005^{d}	11.22 ± 0.044^{a}
Curcumin-Mn	11.82 ± 0.048^{b}	0.21 ± 0.006^{c}	10.82 ± 0.048^{a}
Curcumin-Mg	15.52 ± 0.104^{b}	0.14 ± 0.001^{d}	13.77 ± 0.112^{a}

Different letters in the same column indicate significant differences among means of treatments ($P < 0.05$).

Discussion and Conclusion

The metal complexes of curcumin rich extract from *C. petiolata* were obtained by reflux method. The reaction between curcumin and transition metals was established by comparing the color, UV-Vis and FTIR spectra of complexes spectrums to that of the extract. The antioxidant and anti-tyrosinase activities of complexes were investigated. All of metal complexes showed higher DPPH radical scavenging, ferrous reducing power, and anti-tyrosinase activities than that of free *C. petiolata* extract due to the metal ions change the chemical properties of the curcumin ligand which more effective to donate hydrogen atom than free curcumin and to modify melain structure in melanogenesis pathway. The results suggested that the metal complexes of curcumin rich extract from *C. petiolata* can be active ingredient for cosmetics application and the cosmetic effect of products could be further study.

Acknowledgements

Authors are grateful to Mae Fah Luang University for providing the space and facilities to carry out this research project.

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