



Antioxidant and Antityrosinase Activities of *Cochlospermum regium* Twig, Petal and Leaf Extracts

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Introduction

Skin pigmentary appearance becomes important focus of cosmetic development. Depigmenting agents are currently used in the treatment of skin pigmentary disorders. Sun and UV radiation exposures can induce the production of melanin from melanocytes and pigment accumulation in keratinocytes to protect the damage from those exposures. Inside the melanosomes, melanin pigments are synthesised by tyrosinase activity. Tyrosinase catalyses two steps of melanin production: 1) the hydroxylation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) and 2) the subsequent oxidation of L-DOPA to L-dopaquinone.¹ Subsequently, L-dopaquinone is precursor in melanin pathway to synthesize black-brownish eumelanin or red-yellow pheomelanin.² In addition, UV radiation also induce the production of reactive oxygen species (ROS) in the skin which contributes to melanogenesis.¹ Antioxidants can interact at the active site of tyrosinase resulting in reduction of that enzyme activity. Moreover, antioxidants can also reduce the direct photooxidation in melanin production.¹ Therefore, inhibitions of tyrosinase activation and oxidation are currently focusing for depigmentation.

Cochlospermum regium (Mart. & Schrank) Pilg. is a plant in family Cochlospermaceae which generally found in Thailand as an ornamental plant. The plant has been used as a Thai traditional medicine for health and skin nourishment. In the southwestern of the Brazil, it is widely used as a folk medicine.³ *C. regium* has been used to treat several illnesses such as internal pain, inflammation, infection disease, rheumatoid arthritis.⁴ The root extract from *C. regium* was reported to contain several phenolic compounds and flavonoids.⁵ In addition, the *C. regium* extract exhibited antibacterial and antifungal activities.⁴⁻⁶ The aims of this study were to characterize the preliminary chemical composition, antioxidant, total phenolic and flavonoid contents and to determine antityrosinase activity of *C. regium* twig, petal and leaf extracts.

Methods

Plant extraction

Twig, petal and leaves of *C. regium* were collected from Burapha University Sakaeo Campus, eastern part of Thailand in March 2016. The dried specimen (C. Rattamanee M274) was deposited in the herbarium at Faculty of Agricultural Technology, Burapha University. The twig was sliced and dried whereas petal and leaves were directly dried by using hot air oven at 50°C. The dried twig, petal and leaves were separately macerated by using 50% ethanol for 7 days at room temperature. The extract was filtrated and concentrated by using rotary evaporator. The extract was dried by using freeze dryer. Yields of twig, petal and leaf extracts were 8.87, 20.54 and 19.00%, respectively.

Thin-layer chromatographic method

One milligram extract was dissolved in 1 ml of 50% ethanol. The extract solution was spotted on TLC Silica gel 60 F254 plates. One dimensional TLC analysis was performed with ethyl acetate: toluene: hexane in volume ratio of 35:30:1, respectively, as a mobile phase. Spots were observed under UV light at 254 nm and 366 nm. The plates were sprayed with 30% sulfuric acid in methanol, anisaldehyde-sulfuric, DPPH solution and observed.

Determination of antioxidant activity

The antioxidant activity assay was modified from Melathi *et al.*⁷ Various concentrations of extracts and gallic acid were diluted with methanol. The extracts or gallic acid solution (0.5 ml) were mixed with 1 ml of 0.3 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) and then kept in the dark for 30 min. The solution absorbance was measured at 516 nm by using UV-Visible spectrophotometer. Radical scavenging activity of gallic acid was calculated and plotted a standard curve. The extract results were compared with the standard curve and then expressed as 50% inhibitory concentration (IC₅₀). The experiments were performed in quadruplicate.

Determination of total phenolic content

Total phenolic content was analyzed by using Folin-Ciocalteu colorimetric method.⁸ Various concentrations of extracts and gallic acid were diluted with methanol. The extracts or gallic acid solution (10 µl) were mixed with 150 µl of 20% Folin-Ciocalteu reagent in 96-well plate and then kept in the dark for 30 min. The mixtures were added with 50 µl of 75% sodium carbonate and then kept in the dark for 2 hours. The solution absorbance was measured at 765 nm by using UV-Visible spectrophotometer. Phenolic content of extracts was calculated from standard curve of gallic acid and expressed as mg GAE/g extract. The experiments were performed in quadruplicate.

Determination of total flavonoid content

Total flavonoid content was analyzed by using colorimetric aluminum chloride method.⁹ Various concentrations of extracts and quercetin were diluted with methanol. The extracts or quercetin solution (50 µl) were mixed with methanol (150 µl) and 10% aluminium chloride (10 µl) and incubated for 1 min. The mixture was added with 1 M potassium acetate (10 µl) and incubated for 1 min. The distilled water (30 µl) was added and then kept in the dark for 30 min. The solution absorbance was measured at 415 nm by using UV-Visible spectrophotometer. Flavonoid content of extracts was calculated from standard curve of quercetin and expressed as mg QCE/g extract. The experiments were performed in quadruplicate.

Determination of antityrosinase activity

The antityrosinase activity determination was modified from Momtaz, *et al.*¹⁰ The extracts (100 mg/ml) were separately dissolved in dimethyl sulfoxide (DMSO). Standard control, kojic acid, was dissolved in DMSO to 20 mg/ml and then diluted with phosphate buffer (pH 6.8) (PB) to various concentrations. Tyrosinase (167 unit/ml) and L-tyrosine (4 mM) was dissolved in PB. The experiments were divided to 4 groups as described below. All groups were performed in 96-well plate and in triplicate.

Group A: tyrosinase solution (40 µl) was mixed with PB (120 µl) and DMSO in PB (40 µl).

Group B: PB (160 µl) was mixed with DMSO in PB (40 µl).

Group C: tyrosinase solution (40 µl) was mixed with PB (120 µl) and various concentrations of kojic acid or extracts (40 µl).

Group D: PB (160 µl) was mixed with various concentrations of kojic acid or extracts (40 µl).

The mixture plates were incubated at room temperature for 10 min. Four millimolar L-tyrosinase (50 µl) was added to all wells and then incubated at room temperature for 45 min. The optical density was measured at 492 nm. The results were calculated to % inhibition and expressed as IC₅₀.

$$\% \text{ inhibition} = \frac{(A-B) - (C-D)}{(A-B)} \times 100$$

Statistical analysis

All experiments were expressed in mean ± S.D. One-Way ANOVA and multiple comparison (LSD) were analyzed by using SPSS version 16.0.

Results

In Figure 1, the TLC fingerprints revealed that *C. regium* extracts contained several chemicals. Based on the R_f values of standard compounds, the extracts especially leaf extract had the similar R_f values with catechin (R_f of 0.13), gallic acid (R_f of 0.07) and quercetin (R_f of 0.46). From DPPH spray reagent, the extract spots showed antioxidant activity especially leaf extract. And the bleached spots were matched with those standard compounds (Figure 1F).

In Table 1, leaf extracts showed significantly higher in antioxidant activity than twig and petal extracts with IC₅₀ of 11.34±4.52 µg/ml. While the standard compound gallic acid (IC₅₀ of 2.70±0.15 µg/ml) showed approximately 4.2 times higher in antioxidant activity than those of leaf extract. In addition, leaf extract also contained higher total phenolic (253.26±10.68 mg GAE/g extract) and total flavonoid contents (97.37±27.29 mg QCE/g extract) than twig and petal extracts. These results indicated that leaf extract of *C. regium* had high antioxidant activity which may due to the high contents of phenolics and flavonoids in leaf extract. Moreover, leaf extract also showed stronger inhibitory effect (IC₅₀ of 0.42±0.07 mg/ml) on the tyrosinase activity than those of petal and twig extracts, respectively (Table 1 and Figure 2). Kojic acid had approximately 8.4 times higher in antityrosinase activity than those of leaf extract. These results suggested that the high antityrosinase activity of leaf extract may associate with its high antioxidant, phenolic and flavonoid contents.

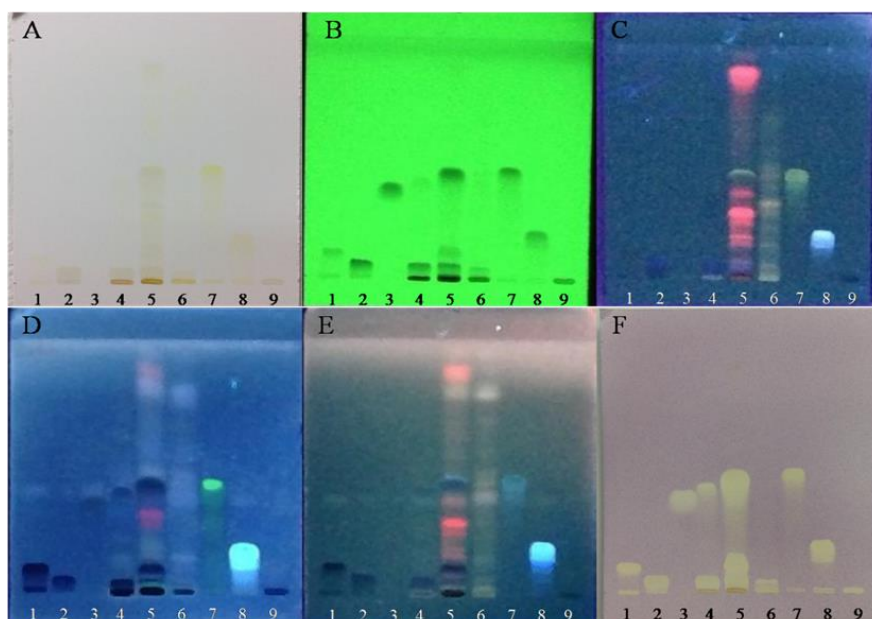


Figure 1 TLC fingerprint of twig, leaf and petal extracts compared with standard compounds. A) Visible light, B) UV254, C) UV366, D) 30% H₂SO₄ in methanol under UV366, E) anisaldehyde under UV366 and F) DPPH under visible light. Lanes: 1) catechin, 2) gallic acid, 3) vanillic acid, 4) twig extract, 5) leaf extract, 6) petal extract, 7) quercetin, 8) caffeic acid and 9) tannic acid

Table 1 Antioxidant activity, total phenolics, total flavonoids and antityrosinase activity of *C. regium* extracts

Extracts or standards	Antioxidant activity (IC ₅₀ ; µg/ml)	Total phenolics (mg GAE/g extract)	Total flavonoids (mg QCE/g extract)	Antityrosinase activity (IC ₅₀ ; mg/ml)
Twig extract	79.73±3.46 ^a	110.48±6.72 ^a	13.88±5.56 ^a	>1.60
Petal extract	114.33±24.00 ^b	148.30±9.31 ^b	62.39±13.10 ^{b,c}	1.28±0.27 ^a
Leaf extract	11.34±4.52 ^c	253.26±10.68 ^c	97.37±27.29 ^c	0.42±0.07 ^b
Gallic acid	2.70±0.15 ^d	ND	ND	ND
Kojic acid	ND	ND	ND	0.05±0.01 ^c

ND: no determination

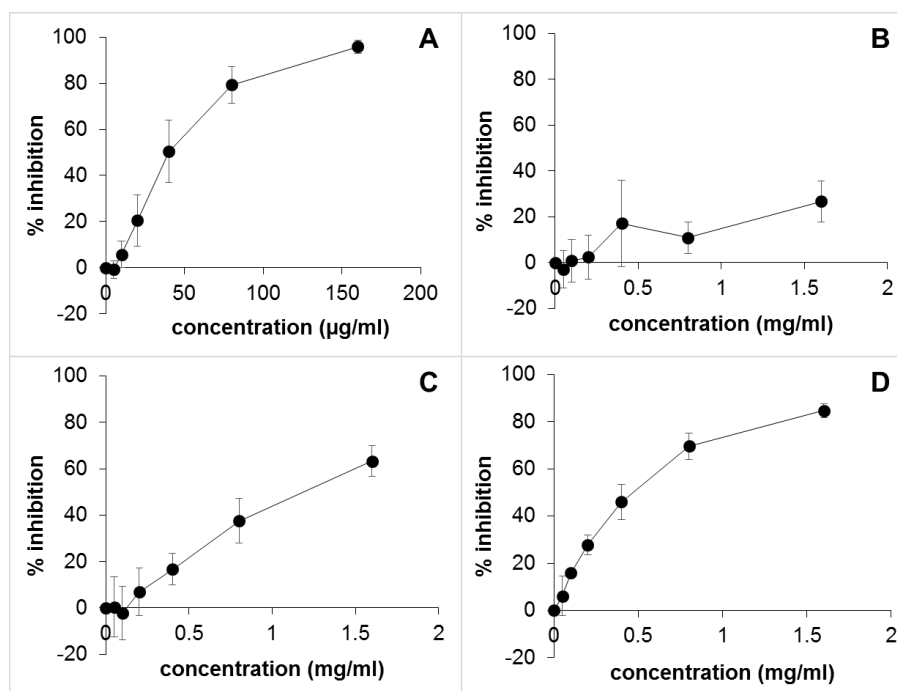


Figure 2 Antityrosinase activity of kojic acid (A), twig (B), petal (C) and leaf (D) extracts

Discussion

The results of TLC fingerprint, total phenolic and flavonoid contents of the *C. regium* leaf and twig extracts in this study correlated with the previous studies. Seven phenolic compounds including ellagic acid, gallic acid, dihydrokaempferol, dihydrokaempferol-3-O- β -glucopyranoside, dihydrokaempferol-3-O-b-(6'-galloyl)-glucopyranoside, pinosresinol and excelsin from *C. regium* root extract was reported.⁵ In addition, *C. religiosum* leaf extract was reported to contain 87 phenolic compounds such as gallic acid, ascorbic acid, quercetin, cynarine, syringaldehyde, thymol, ellagic acid, isorhamnetin and benzoic acid.¹¹ These results indicated that *C. regium* extracts contained high contents of phenolic and flavonoids which correlated with its antioxidant activity. In addition, TLC fingerprint and mobile phase system of the extracts in this study can be used for quality control of *C. regium* extracts. Free radicals and ROS from UV radiation exposure can induce the melanin production in the skin. In addition, oxidation and tyrosinase enzyme involve with the melanogenesis. Therefore, antioxidant and antityrosinase agents are targeted in whitening product development.^{1,12} Kojic acid is common used as skin whitening agent. The strong inhibitory effect on tyrosinase activity of kojic acid causes of melanin content reduction.¹³ *C. regium* leaf extract in this study showed antityrosinase activity nearly the efficacy of kojic acid. Moreover, antioxidants and phenolics showed the inhibitory effect of on melanogenesis.^{14,15} *C. regium* leaf extract also had high antioxidant, phenolics and flavonoids. Therefore, leaf extract of *C. regium* can be a good candidate of an active ingredient in skin whitening product.

Conclusion

This study revealed the chemical composition, antioxidant and antityrosinase activities of *C. regium* twig, petal and leaf extracts. These results indicated that *C. regium* extracts, especially leaf extract, can be developed to further natural skin whitening agent. However, the mode of action in melanogenesis of the extracts and bioactive compound isolation are needed for further studies.

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