Anti-Tyrosinase and DPPH Radical Scavenging Activities of Selected Thai Herbal Extracts Traditionally Used as Skin Toner

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ABSTRACT

Introduction: Skin darkness may be cosmetically undesirable for some people. Plant-derived materials traditionally used for skin lightening may produce satisfactory results. Besides innate tyrosinase activity, oxidative stress also plays an important role in skin darkness by activating tyrosinase. Therefore, herbal extracts with strong anti-tyrosinase and antioxidant activities could be considered as efficacious skin lightening agents. The aims of the present study were to determine the anti-tyrosinase and antioxidant activities of Thai medicinal plant extracts indigenously used as skin toners. **Methods:** The activities of seven Thai medicinal plants; *Zingiber cassumunar* Roxb., *Phyllanthus emblica* Linn., *Tagetes erecta* Linn., *Centella asiatica*, *Raphanus sativus*var. *Longipinnatus* Linn., *Cassia fistula* Linn. and *Butea monosperma* (Lam.) Taub. were investigated using the DOPA-chrome method to determine anti-tyrosinase activity and the DPPH free radical scavenging assay to determine antioxidant activity. **Results:** With respect to tyrosinase inhibitory activity, *P. emblica*, *B. monosperma* and *R. sativus*var. *Longipinnatus* extracts at a concentration of 1.67 mg/mL showed strong activities with the percentage of tyrosinase inhibition at 48.38 ± 4.77, 46.92 ± 3.77 and 42.85 ± 6.54%, respectively. In addition, *P. emblica* and *B. monosperma* extracts also exhibited high antioxidant activities with the IC_{50}values on 33.47 ± 1.24 and 33.57 ± 1.92 µg/mL, respectively. **Conclusion:** Based on tyrosinase inhibition activity and DPPH radical scavenging assays, the herbal extracts of *P. emblica* and *B. monosperma* show promise as potential skin lighteners in cosmetic formulations.

Key words: antioxidant activity, Dopachrome method, skin lightening, traditional skin toners, anti-tyrosinase inhibitory activity, DPPH radical scavenging assay.

INTRODUCTION

Skin darkness is the result of over expression of melanogenesis induced by UV-irradiation[1], hormones[2] or diseases such as melasma.[3] Melanin production in human skin is primarily generated by melanocytes in the basal layer of the epidermis.[4] Many people, especially in tropical countries, suffer from hyper pigmentation or dark skin blemish.[5] Therefore, suppression of melanin production may be a cosmetic or therapeutic goal.

Several substances known to reduce melanin synthesis; for example, hydroquinone[6] or flavonoids (*i.e.* quercetin[7] and arbutin[8]) have been recommended as skin whitening agents. But the uses of chemicals such as hydroquinone have been associated with toxicity mutagencity and carcinogenicity.[9]

There are several mechanisms related to skin lightening: inhibition of tyrosinase activity, suppression of melanogenesis and inhibition of tyrosinase formation.[10,11] As skin whitening agents, tyrosinase inhibitors and antioxidants are recognized as lightening agents.[12] For instance, UV irradiation which produces oxidative stress by increasing superoxide anion (O_2^-) and activating tyrosinase enzyme result inmelanogenesis.[13] Therefore, free radical

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scavenging is a viable option for skin lightening.

The selected plants, *Zingiber cassumunar* Roxb. (rhizome), *Phyllanthus emblica* Linn. (dry fruits) *Tagetes erecta* Linn. (flowers), *Centella asiatica* (leaves) *Raphanus sativus* var. *longipinnatus* L. (tubers), *Cassia fistula* Linn. (flowers), and *Butea monosperma* (Lam.) Taub. (dry fruits) traditionally use as skin care products which may express wide range of mechanisms. However, the skin lightening activities are mainly associated to anti-tyrosinase and antioxidant activities. This study should provide a scientific basis on herbal extracts traditionally used as skin care products which may exhibit anti-tyrosinase as well as antioxidative properties. Several plants such as *Garcenia magostana*[14], *Glycyrrhiza glabra* and *Morus alba*exhibit high anti-tyrosinase activity.[5] Such plants may offer alternatives to avoid potential toxicities of synthetic chemicals. The aim of the present study was to investigate the anti-tyrosinase and antioxidative activities of Thai herbal extracts indigenously used for skin care.

MATERIALS AND METHODS

Plant materials

The various parts of *Zingiber cassumunar* Roxb. (rhizome), *Phyllanthus emblica* Linn. (dry fruits)*Tagetes erecta* Linn. (flowers), *Centella asiatica* (leaves) *Raphanus sativus* var. *Longipinnatus* L. (tubers), *Cassia fistula* Linn. (flowers), and *Buteamonosperma* (Lam.) Taub. (dry fruits) were collected during March – May 2012 from Mahasarakham province and identified by the author (Dr. M. Phadungkit).The voucher specimens have been deposited in the Herbarium at Faculty of Pharmacy, Mahasarakham University, Thailand. The plant materials were cut into small pieces and dried under the hot air condition at 50∞C to dryness. The dry materials were ground and extracted with 95% ethanol with solid to liquid ratio (1:10) by means of maceration for 7 days. The marc was then filtered and evaporated by rotary evaporator (Heidolph, Schwabach, Germany) to yield the herbal crude extracts. The resultant extracts were kept at 4□C prior to determination of anti-tyrosinase activity and DPPH radical scavenging activities.

Chemicals, reagent and instrumentation

L-DOPA, Phosphoric acid/Sodium dihydrogen phosphate, mushroom tyrosinase enzyme, kojic acid, ascorbic acid and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical were purchased from Sigma Aldrich (St. Louis, MO, USA). Dimethyl Sulfoxide (DMSO) was obtained from Sigma Aldrich Laborchemikalien GmbH (Seelze, Germany). All chemicals and reagents were analytical grade. Absorbance measurements were performed using Jasco V530 UV-spectrophotometer (Tokyo, Japan).

Tyrosinase inhibition assay

Mushroom tyrosinase inhibitory assay was performed using the DOPA-chrome method with some modifications.[15] Briefly, the extracts were dissolved in 10% DMSO in distilled water at a concentration of 5% w/v. Four test tubes (A,B,C,D) were used for each extract. One mL of 2.5 m ML-DOPA and 1.8 mL of 0.1 M phosphate buffer (pH 6.8) were added to each tube and subsequently incubated at room temperature for 10 minutes. After incubation, reagents were added as follows; Tube A (0.1 mL 10% DMSO, 0.1 mL tyrosinase enzyme at a concentration of 605 unit/mL), tube B (0.1 mL water, 0.1 mL of 10% DMSO), tube C (0.1 mL tyrosinase enzyme, 0.1 mL herbal extract), tube D (0.1 mL water, 0.1 mL herbal extract). The final concentration of each extract in reaction tubes was 1.67 mg/mL. After incubation at room temperature for 25 minutes,the absorbance of each tube was measured at 492 nm to monitor the formation of the DOPA-chrome. Each reaction tubes were prepared in 3 replications. Percentage of inhibition of tyrosinase activity was calculated as follows.

$$\% \text{ Tyrosinase inhibition} = 100 \times [(A\text{-}B)\text{-}(C\text{-}D)] / (A\text{-}B)$$

Where; A,B,C,D were the absorbance of mixture of tube A,B,C,D, respectively. Kojic acid at a concentration of 1% was also determined as a positive control. After addition to the reaction tube, final concentration was 0.33 mg/mL.

Antioxidant activity assay: DPPH radical scavenging activity

The radical scavenging activity of extracts and the standard ascorbic acid solutions in absolute ethanol was determined on a basis of their ability to react with the stable DPPH free radical. A 750 □L aliquot of the extracts (50 to 1000 □g/mL, dissolved in absolute ethanol) was added to 750 □L of DPPH in absolute ethanol (152 □M). After incubation at room temperature for 30minutes, bleaching of purple color of DPPH radicals was investigated according to hydrogen atoms or electron donation ability from herbal extracts.[16, 17] The absorbance of each solution was determined at 517 nm with 3 replications using a UV spectrophotometer. The radical scavenging activity was calculated as followed.

$$\% \text{ radical scavenging} = (A_{control} - A_{sample}) \times 100 / A_{control}$$

Where; $A_{control}$ = Absorbance of control which consists of equal volume of 152 □M DPPH and absolute ethanol.

A_{sample} = Absorbance of sample which consists of equal volume of 152 □M DPPH and sample solution.

Percentage inhibitory values of the test solutions were calculated and plotted between concentrations of the extracts or standard against their inhibition percentage to obtain a linear equation.[18,19] The concentration of each sample required for 50% scavenging of the DPPH free radical (IC_{50}) was interpolated from the linear equation.

RESULTS AND DISCUSSION

The tyrosinase inhibitor assay was carried out by the DOPA-chrome method. The enzyme activity was measured on the basis of color formation of DOPA-chrome by UV-Vis spectrophotometer. Tyrosinase inhibition by the herbal extracts and a standard Kojic acid summarized in Figure 1. The three strongest tyrosinase inhibitory activities were the herbal extracts of *Phyllanthus emblica* (48.38 ± 4.77 %), *Butea monosperma* (46.92 ± 3.77 %) and *Raphanus sativus* var. *Longipinnatus* (42.85 ± 6.54 %),respectively. Anti-tyrosinase activity in herbal extracts could be from an amount of flavonoid.[11]The respective flavonoids chelate 2 coppers at the active site of tyrosinase enzyme.[20,21]

Antioxidant activity of herbal extracts was evaluated by DPPH radical scavenging assay. The radical scavenging activity of ascorbic acid was performed as a reference standard. Results are summarized in Figure 2. The three strongest antioxidant activities were the herbal extracts of *P. emblica*, *B.monosperma* and *Z. cassumunar* with the IC_{50} values of 33.47 ± 1.24, 33.57 ± 1.92 and 40.34 ± 0.78 □g/ml, respectively. A strong antioxidant activity of the selected plants could be from phenolic compounds extracted into high polarity solvent.[22]

In case of *P. emblica*, the strongest anti-tyrosinase (48.38 ± 4.77%) and antioxidant (33.47 ± 1.24) activities could be from high level of ascorbic acid[23] and phenolic compounds.[24] Regarding anti-tyrosinase activity, Sripanidkulchai and Junlatat[25] compared the activity of *P. emblica* branches and fruits with ethanol and methanol. The results showed that the activity of the branch extracts expressed much higher activity than fruit extracts. Although, *P. emblica* fruit extract expresses less activity than the branch extracts. A further study with safety considerations of branch and fruit extracts should be evaluated. Comparing with other plants, the activities of *P. emblica* were less active compared

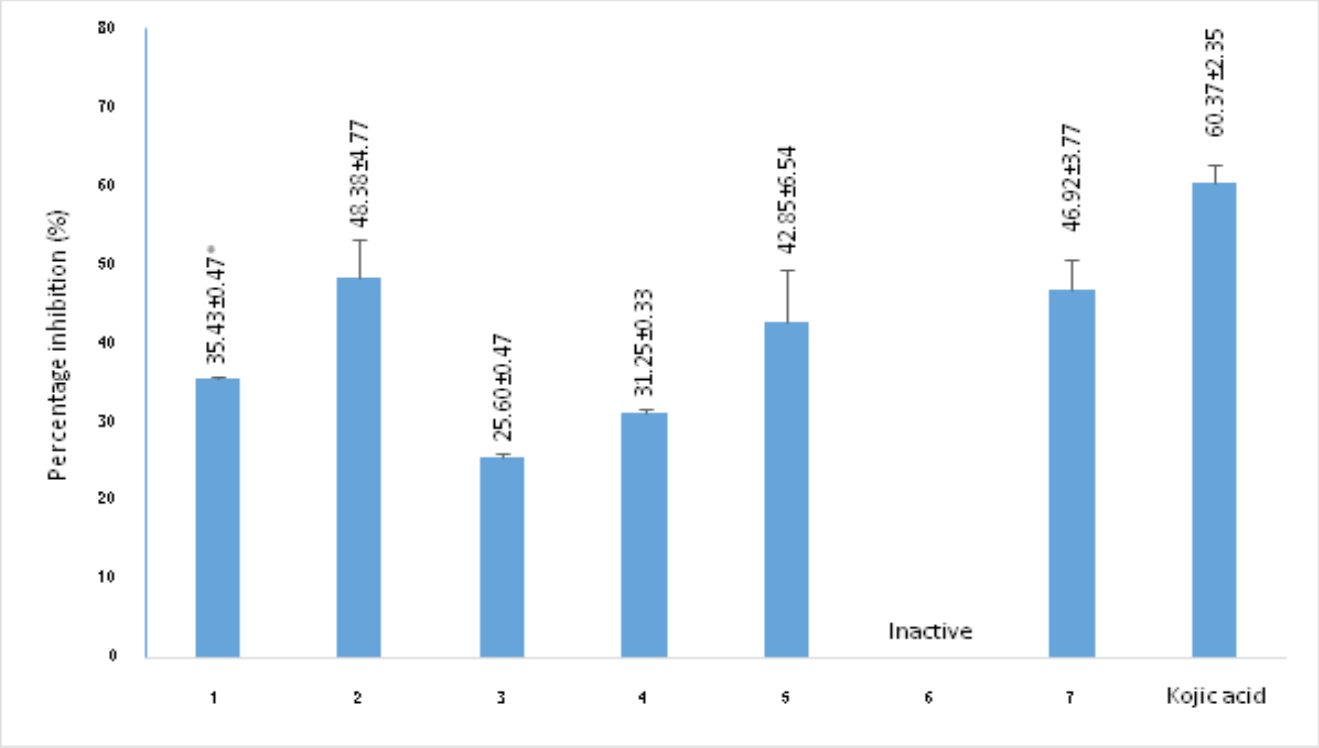

Figure 1: Tyrosinase inhibitory activity of herbal extracts (1.67 mg/mL) and Kojic acid (0.33 mg/mL)

Note : 1 = *Z. cassumunar*, 2 = *P. emblica*, 3 = *T. erecta*, 4 = *C. asiatica*, 5 = *R. sativus* var. *longipinnatus*, 6 = *C. fistula*, 7 = *B. monosperma* [*mean ± sd (n = 3)]

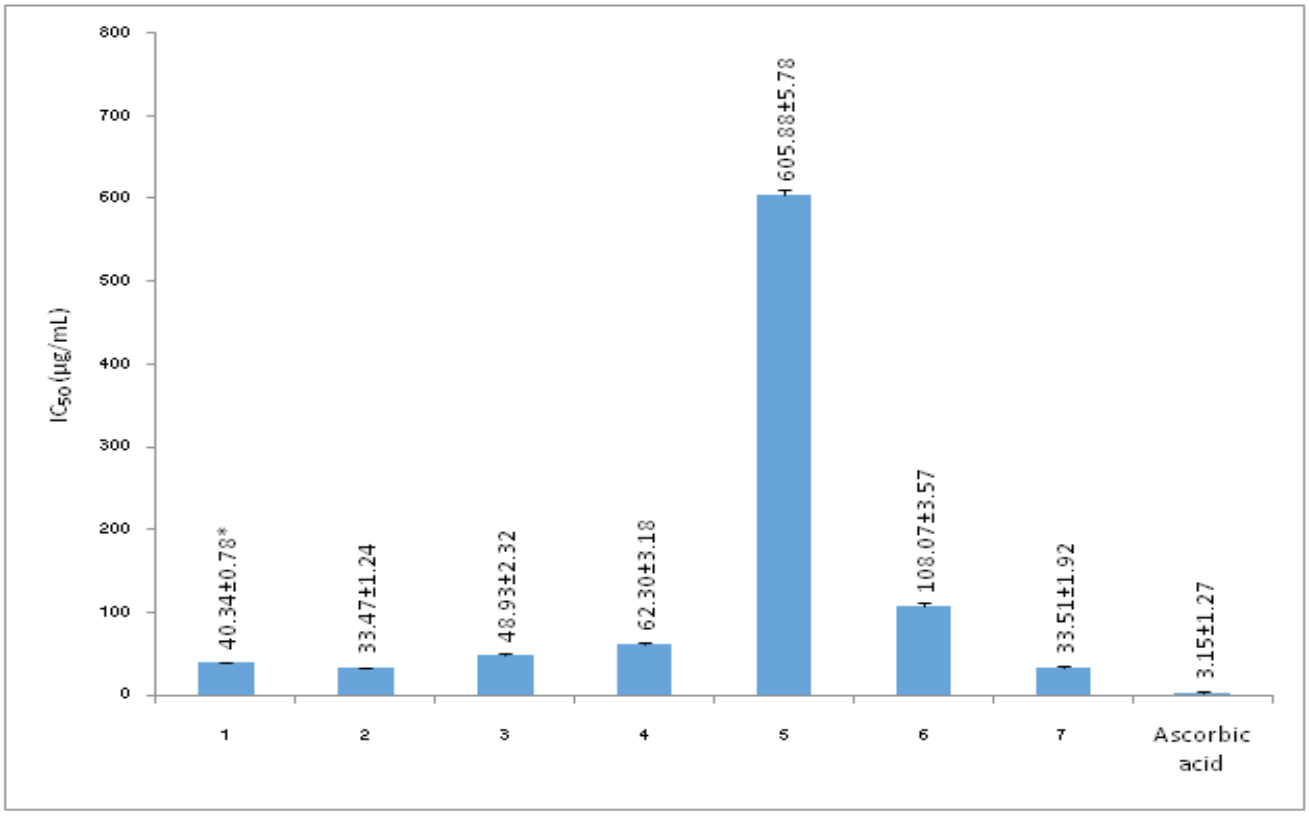

Figure 2: IC_{50} DPPH radical scavenging assay of herbal extracts and ascorbic acid

Note : 1 = *Z. cassumunar*, 2 = *P. emblica*, 3 = *T. erecta*, 4 = *C. asiatica*, 5 = *R. sativus* var. *longipinnatus*, 6 = *C. fistula*, 7 = *B. monosperma* [*mean ± sd (n = 3)]

with the anti-tyrosinase (74.55 ± 7.31%) and antioxidant (4.03 ± 1.51 µg/mL) activities from *G. mangostana* Linn[14]. Therefore, anti-tyrosinase and antioxidant activities of *P. emblica* constituents should be further investigated on skin whitening process as described previously.

CONCLUSION

P. emblica and *B. monosperma* extracts exhibited strong anti-tyrosinase and antioxidant activities. These extracts or active constituents could be further studied and developed as skin whiteners in cosmetic formulations.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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