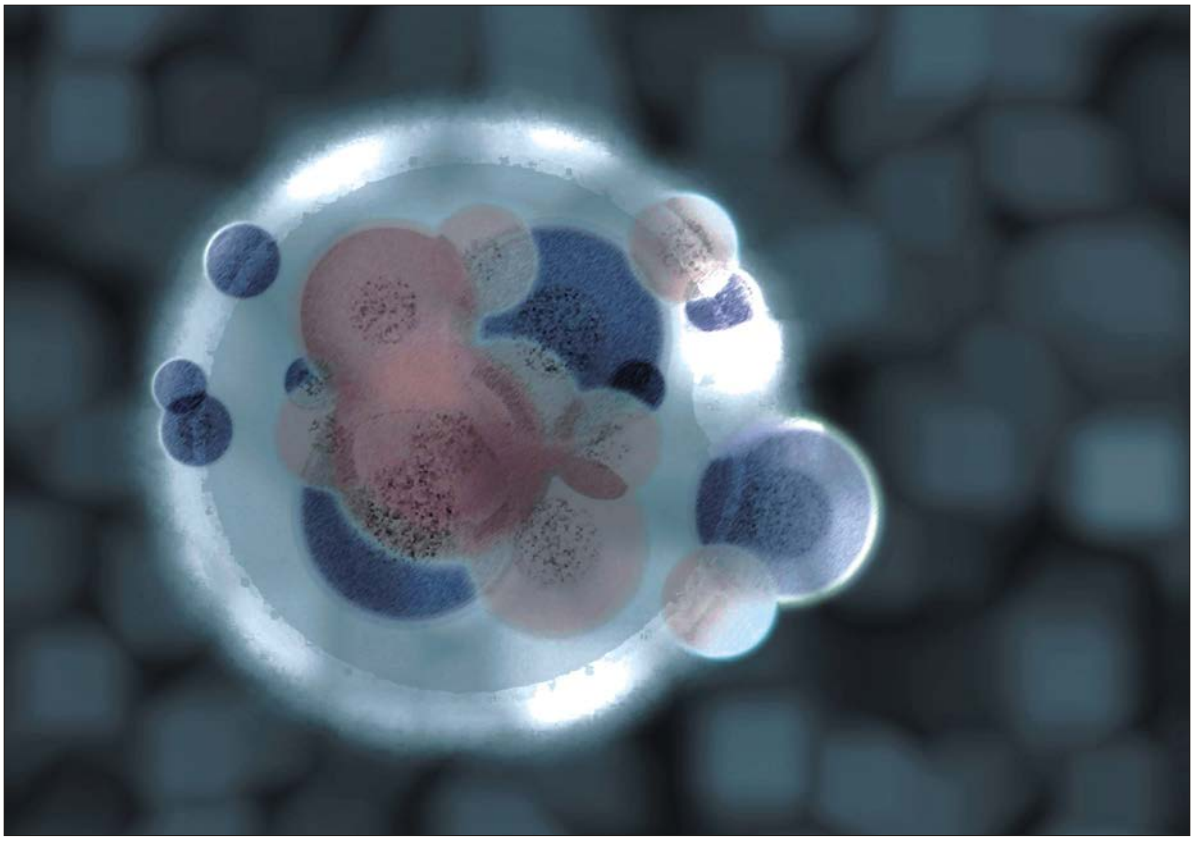

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 12 (4): 239-245, 2012
ISSN 1727-3048 / DOI: 10.3923/jbs.2012.239.245

Tamarind Seed Coat Extract Reduces Melanin Production via Tyrosinase in Melanocyte

[1]K. Phetdee, [2]K. Rattanamanee, [2]T. Teaktong and [1,3]J. Viyoch
[1]Department of Pharmaceutical Technology,
[2]Department of Pharmacy Practice, Naresuan University, Phitsanulok, 65000, Thailand
[3]Cosmetics and Natural Products Research Center (CosNat),
Center of Excellence for Innovation in Chemistry, Faculty of Pharmaceutical Sciences,
Naresuan University, Phitsanulok, 65000, Thailand

Abstract: The traditional use of tamarind and its health benefits while the high phenolic content of its seed coat suggest it might be explored as a skin lightener. For this, we focused on the possible role of the seed coat extract in inhibiting melanogenesis. The seed coat of tamarind (*Tamarindus indica* L.) was extracted with ethyl acetate, which among other solvents recovered highest phenolic content (85.6±0.9 mg g^{-1} catechin equivalents-Folin-Ciocalteu assay). B16-F1 melanoma cells were stimulated by α-melanocyte stimulating hormone (α-MSH) for 48 h and the extract added after the first 24 h, it dose-dependently inhibited melanin production by 20-32%. When MSH was added 24 h after the extract, the melanin reduction was about 42-59%. Kojic acid (50 μg mL^{-1}), a well-known hypopigmenting agent had similar effects. Cell viability and morphology were unaffected by any of the concentration used. The extract also inhibited tyrosinase activity (IC_{50} = 152.1±10.2 μg mL^{-1}). This is the first report shows that tamarind seed coat can inhibit melanogenesis and suggests that further refinements may show their benefit in hyperpigmentation improvement.

Key words: Tamarind seed coat extract, melanogenesis, tyrosinase

INTRODUCTION

Oxidative stress caused by UV-generated singlet oxygen is the most significant factor influencing human skin pigmentation (Hensley and Floyd, 2002). The proinflammatory effects from oxidative stress in keratinocytes lead to increased secretion of α-melanocyte stimulating hormone (α-MSH) acting on melanocortin 1 receptor (MC1R) in neighboring melanocytes. Such stimulation results upregulation of melanogenic proteins such as tyrosinase, leading to increased melanin production. Moreover, the oxidative stress increases the activity of protease activated receptor-2 (PAR-2) which stimulates distribution of melanosomes and their uptake into keratinocytes (Seiberg *et al*., 2000a). Accordingly, appropriate antioxidant compounds might reduce skin pigmentation.

There is increasing demand for active ingredients derived from natural sources, which are perceived as safe and effectiveness. Plant phenols and polyphenols are promising naturally-occurring compounds which are capable of reducing oxidative stress (Bravo, 1998) and thus prevent pigmentation when applied on skin (Cos *et al*., 1998). Indeed, this has recently been shown that some phenolics can reduce skin pigmentation via tyrosinase inhibition. For examples, catechin and epicatechin gallate can inhibit tyrosinase activity by chelating the cupric ion within its active site (Kim *et al*., 2004).

Tamarind (*Tamarindus indica* L., family Leguminosae) grows wild in Thailand and its fruit pulps have been widely used as cosmetics purpose for many centuries. In addition, various health benefits of the tamarind seed coat have also been reported (Siddhuraju, 2007; Pumthong, 1999) but any effects on skin pigmentation remains unexplored. Extracts of tamarind seed coat contain many polyphenols, including catechin, procyanidin B2, epicatechin, procyanidin trimer, procyanidin tetramer, procyanidin pentamer and procyanidin hexamer (Sudjaroen *et al*., 2005) and thus may influence melanogenic and melanosomogenesis function activity. Therefore, the present study aimed to assess the effect of tamarind seed coat extract on melanin production in α-MSH-stimulated B16-F1 mouse melanoma cells. To gain some insight into its action, tyrosinase and PAR-2 activities in primary human skin cell were also investigated. The inhibitory effects obtained support the idea that extract of tamarind seed coat might be a useful skin-hypopigmenting agent.

Corresponding Author: J. Viyoch, Cosmetics and Natural Products Research Center (CosNat),
Center of Excellence for Innovation in Chemistry, Faculty of Pharmaceutical Sciences,
Naresuan University, Phitsanulok, 65000, Thailand Tel: 66 55 963682 Fax: 66 55 963731

MATERIALS AND METHODS

Materials: Ethyl acetate was purchased from LabScan Asia, Co. Ltd., Bangkok, Thailand. Folin-Ciocalteu reagent, alpha-melanocyte-stimulating hormone (α-MSH), catechin, Triton x-100, L-DOPA, ethylene diamine tetraacetic acid (EDTA), Dulbecco's Modified Eagle's Medium (DMEM, low glucose), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and sulfinpyrazone were purchased from Sigma-Aldrich, Inc., Missouri, USA. Kojic acid was purchased from Sigma-Aldrich, Steinheim, Germany. Fetal Bovine Serum (FBS), serum-free-keratinocyte medium (SFM) with supplements and trypsin (0.25%) were purchased from GIBCO, Paisley, UK. Fluo-3 AM was purchased from Molecular Probes Inc., Oregon, USA.

Preparation of the extract from tamarind's seed coat: The seeds of tamarind were purchased from local market in Petchaboon Province, Thailand. The studies were performed between the time periods of June 2009 to October 2010. The seeds were heated in a hot air oven at 140°C for 45 min, cooled and cracked to separate the outside brown layer. Only brown-red seed coats were collected and these were then ground into fine powder (Siddhuraju, 2007). Liquid extraction with ethyl acetate was used for seed coat extraction. After tannin was removed by Sephadex LH20 (Sigma-Aldrich, Inc., Missouri, USA) column, the extract solution was dried under vacuum (Pumthong, 1999). The resultant extract was stored in a tight-amber glass at 4°C for further studies.

Quantification of total phenol in the extract: Phenolic content of the extract was determined using Folin-Ciocalteu assay (Singleton *et al*., 1999). Total phenolic content was expressed as Catechin Equivalents (CE) (mg g^{-1} dry extract).

Effects of extract on melanogenesis in B16-F1 (mouse melanoma cell)

Cells and treatments: This study was designed to primarily screen the extract for inhibitory action on melanin production by using B16-F1 (ATCC No. CRL-6323, American Type Culture Collection, Virginia, USA) mouse melanoma cells, a well-known model for determination of melanogenesis inhibitory activity (Donsing *et al*., 2008; Siegrist and Eberle, 1986). B16-F1 cells were initially cultured in a 25 cm^2 flask with DMEM supplemented with 10% FBS incubated at 37°C with a humid atmosphere containing 5% CO_2. The medium was changed every two days and the number of passages did not exceed 6.

To determine the effect of the extract on melanin production in α-MSH-stimulated melanogenesis, the cell suspension was transferred from a 25 cm^2 flask into a 24-well plate (1×10^5 cells per well). The old medium was then replaced with 1 mL DMEM. Two protocols were tested (i) 1 nM α-MSH (Morandini *et al*., 1998) was present for 48 h and at 24 h, 50-200 μg mL^{-1} catechin equivalents was also added. (ii) 50-200 μg mL^{-1} catechin equivalents was present throughout the 48 h and 1 nM α-MSH added after the first 24 h. In addition, 50 μg mL^{-1} kojic acid, a well-known lightening compound was also tested using the same protocols. Each experiment was performed in triplicate. Control wells contained cells stimulated with 1 nM α-MSH but no extract.

Melanin content assay: The melanin content assay was performed as previously described with slight modifications (Donsing *et al*., 2008; Mun *et al*., 2004). The assay based on destroying the retractile cells leaving behind the melanin granules, which could then be quantified spectrophotometrically. The cells within the wells were trypsinized and washed twice with Phosphate Buffer Saline (PBS), air-dried, dissolved in 200 μL of 1 N NaOH, heated at 80°C for 1 h and then cooled. The melanin content was measured at 490 nm using a microplate reader (Spectra Count®; Perkin Elmer Inc., Boston, Massachusetts, USA). The content of melanin was calculated by comparing the averaged absorbance of the 3 wells with that of the control cells and expressed as percentage.

Melanogenetic activity of the extract in human primary cells

Isolation of cells: Co-culture of isolated keratinocytes and melanocytes were used as an *in vitro* model in this study. Keratinocytes and melanocytes were isolated from foreskin tissues coming from humans aged≤3 years. Tissues were incubated in 10 mL of 5% dispase solution at 4°C for 24 h and the epidermal layer was then separated from the tissue using forceps. This epidermal layer was cut into small pieces (2×2 mm) by using surgical blade and then incubated in 4 mL of 0.25% trypsin solution at 37°C and under 5% CO_2 for 20 min. Four milliliter of DMEM medium with 10% FBS were used to stop trypsin reaction. The cell suspension containing both keratinocytes and melanocytes was centrifuged at 1,500 rpm for 5 min and cells maintained in serum-free medium with supplements under air/5% CO_2 and at 37°C.

Determination of tyrosinase inhibitory activity from keratinocyte/ melanocyte co-cultures: To determine inhibition of tyrosinase, the modified method of

(Huang *et al*., 2005) was used. A suspension of cells from the primary cultures was transferred from a 25 cm^2 flask into a 24-well plate (1×10^4 cells per well) and then incubated with the extract (50-500 μg mL^{-1}) or kojic acid (0.5-500 μg mL^{-1}) at 37°C under 5% CO_2 for 72 h. The cells were washed with ice-cold PBS and then lysed by adding 0.5 mL PBS pH 6.8 containing 1% triton x-100 with sonication. A cell-free supernatant was collected by centrifugation and tested for the tyrosinase activity. Ninety micro litters of each sample solution adjusted to equal protein value were added in a 96-well plate. After incubation at room temperature for 5 min, 10 μL substrate (10 mM L-DOPA) was added to each well. After a further 30 min of incubation, the optical densities of the L-DOPA oxidation product, dopachrome, produced by tyrosinase were measured at 475 nm with the Spectra Count microplate reader. The concentration of the extract giving 50% inhibition (IC_{50}) was determined from plot of percent inhibition against log concentration of the extract or kojic acid using Prism (GraphPad, California, USA). Percent inhibition was calculated by using the following equation:

$$\text{Percent inhibition} = \frac{1\text{-}A_{(\text{treatment})}}{A_{(\text{control})}}\times 100$$

Where:
$A_{(treatment)}$: Absorbance intensity of extract-treated group
$A_{(control)}$: Absorbance intensity of untreated group

The study was performed in three batches of keratinocyte-melanocyte co-cultures isolated from one-skin tissues.

Intracellular calcium and PAR-2 activity: PAR-2 activity was assessed by the rise in intracellular calcium (Ca^{2+}) when released from the endoplasmic reticulum by the action of PAR-2 (Bohm *et al*., 1996). Briefly, the co-cultured cells were collected by using EDTA-containing calcium-free isotonic PBS. The cells were suspended in DMEM containing 10%FBS and 200 μL of this was added to black 96 well-plates. 0.25 mM sulfinpyrazone and 1 μM fluo-3 AM were then added. The mixture was incubated at room temperature for 20-25 min and the cells were then washed twice. Two hundred microliters of assay buffer (150 mM NaCl, 3 mM KCl, 1.5 mM $CaCl_2$, 20 mM HEPES, 10 mM glucose and 0.25 mM sulfinpyrazone) (untreated group), assay buffer plus 10 μM trypsin (as a PAR-2 activator) or assay buffer plus 200 μg mL^{-1} extract were added and incubated for a further 60 min.

Fluorescence was excited at 485 nm and emission measured at 530 nm using a multimode detector (Beckman Coulter Inc., California, USA). The emission signal was adjusted to 100% using the untreated cells and intracellular free calcium expressed as a percentage assuming that this is linearly proportional to calcium at low concentrations (<500 nM). The study was performed in three batches of keratinocyte-melanocyte co-culture cells isolated from one-skin tissues.

Cell viability assay: Viability following treatment was determined by counting the number of trypan blue positive cells in each well under x100 microscope. The study was performed in triplicate to obtain average number of viable cells.

Statistic analysis: All quantitative data reported here are expressed as mean±SD of samples for each treatment. Student's unpaired t-test was used for comparison between two groups. The $p<0.05$ was considered significant.

RESULTS

The appearance and phenolic content of the extract: The crude extract from tamarind seed coat after evaporation process was a brownish powder, as shown in Fig. 1. The percent yield of the extract obtained was 25.4±1.2% w/w. As determined by Folin-Ciocalteu assay, the amount of the phenolic compounds contained in the extract was 85.6±0.9 mg CE g^{-1} extract.

Effects of extract on melanogenesis in B16-F1 (mouse melanoma cell): The melanogenesis activity of B16-F1 cells was investigated by determining content of the melanin produced by cells. The extract showed the dose-dependent inhibition and protection of melanin production of B16-F1 cells stimulated by α-MSH, as shown in Fig. 2. Furthermore, the extract did not affect viability of cells within the range of concentrations tested, as shown in Fig. 3. As compared to the α-MSH-stimulated

Fig. 1: The appearance of tamarind seed coat crude extract

cells without extract treatment, the percentage of melanin reduction was about 20-32% in the cells treated with the extract at high concentration (150-200 μg mL^{-1}) after being stimulated with α-MSH (inhibition condition) whereas the melanin reduction was about 42-59% in the cells treated with the extract at the similar concentrations before being stimulated with α-MSH (protection condition). For kojic acid (50 μg mL^{-1}), which was used as a reference inhibitor, the decrease in melanin content was observed at about 50% in both inhibition and protection conditions.

Melanogenetic activity of the extract in human primary cells: In this study, the co-culture of keratinocyte-melanocyte without any melanogenesis stimulator was used in order to mimic the melano-epidermal activity. From the results obtained, IC_{50} value as determined by plotting between the log concentration of the test agent and percent inhibition of dopachrome formation was 152.1±10.2 μdg mL^{-1} for the extract whereas that of the kojic acid was 33.3±2.5 μg mL^{-1} (Fig. 4). Percent inhibition of the extract at the highest concentration used (500 μg mL^{-1}) was 94.7% where as that of kojic acid was 96.6%. Although, the extract showed lower inhibitory activity, it (at high concentrations, 200-500 μg mL^{-1}) did not affect the viability and morphology of human keratinocyte-melanocyte cells, as shown in Fig. 5.

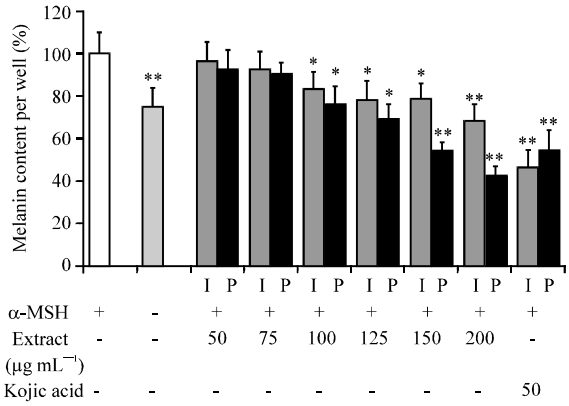

Fig. 2: Tamarind seed coat extract reduced melanin production. B16-F1 melanoma cells were treated with the extract at concentrations in range of 50-200 μg mL^{-1} after (inhibition, I) or before (protection, P) being stimulated with α-MSH. Each bar represents Mean±SD of triplicate study. *p<0.05 and **p<0.01 denote significant differences when compared to control (Student's t-test)

Intracellular calcium and PAR-2 activity: The present study was designed to examine the possibility that tamarind's seed coat extract could also affect pigmentation by inhibiting the PAR-2 activity. The results from fluorescence values of staining calcium expressed as

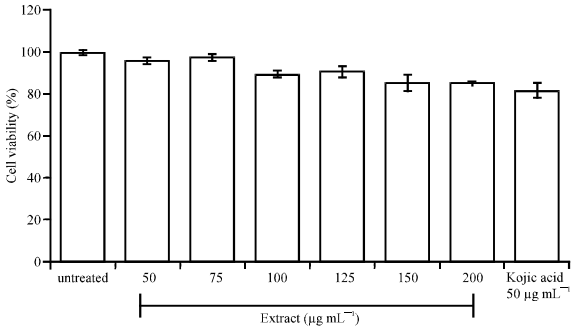

Fig. 3: The viability of B16-F1 mouse melanoma cells treated with tamarind seed coat extract at concentrations in range of 50-200 μg mL^{-1} for 24 h. Each bar represents Mean±SD of triplicate study

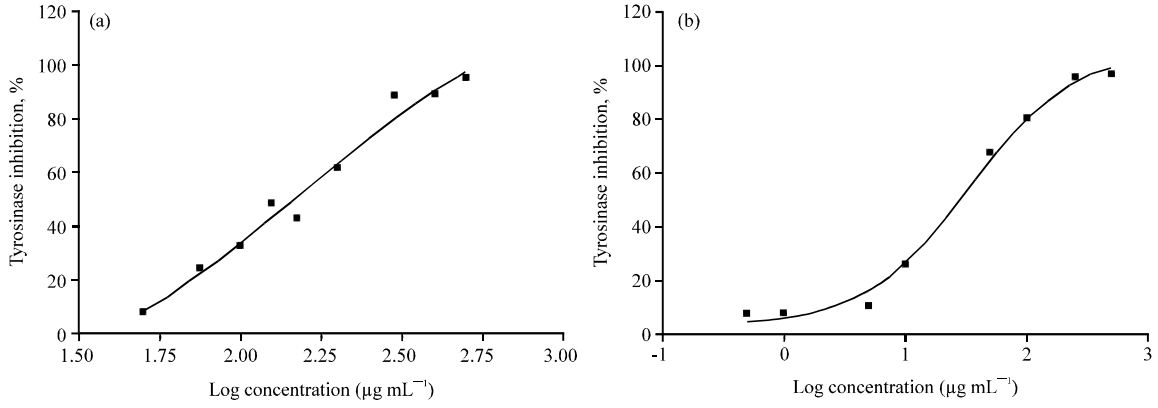

Fig. 4(a-b): Percent inhibition of tyrosinase activity by, (a) Tamarind seed coat extract and (b) Kojic acid. The study was performed in three batches of keratinocyte-melanocyte co-culture cells isolated from one-skin tissues

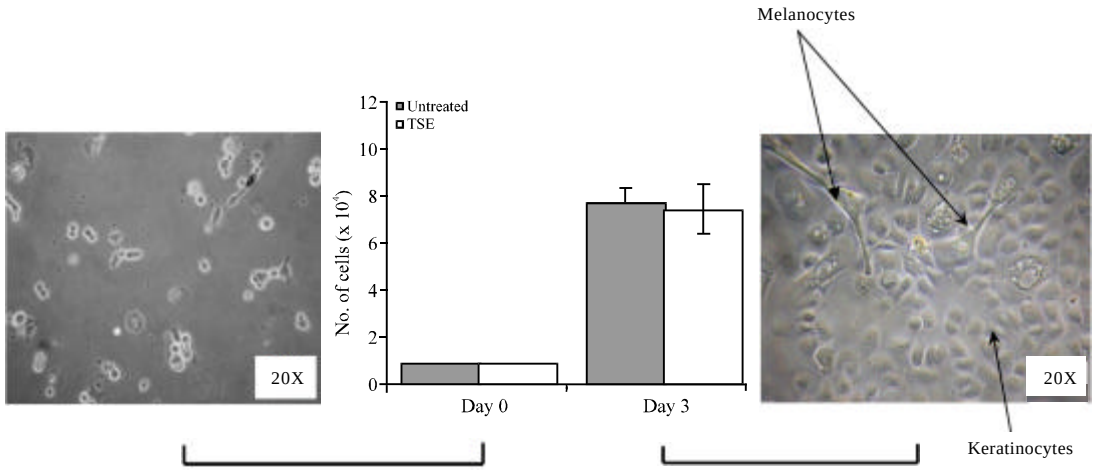

Fig. 5: The viability and morphology of human keratinocyte-melanocyte co-culture treated with 200 μg mL^{-1} (open bar) of tamarind seed coat extract and untreated (control) (filled bar) at day 0 and day 3. Each bar represents Mean±SD of study from three batches of keratinocyte-melanocyte co-culture cells

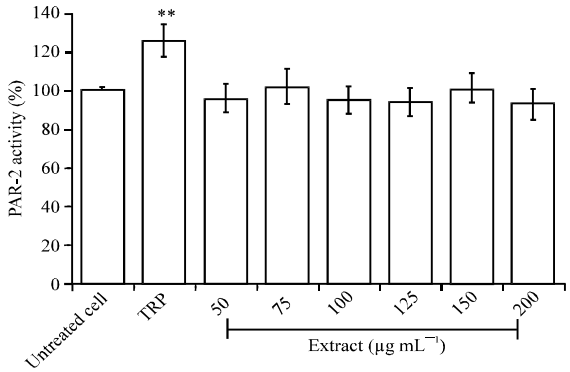

Fig. 6: Tamarind seed coat extract and intracellular calcium related to PAR-2 activity. Trypsin (TRP, 10 μM) was used as a PAR-2 activator. Each bar represents Mean±SD of three batches of keratinocyte-melanocyte co-culture cells. **$p<0.01$ denotes significant differences when compared to untreated cells (Student's t-test)

a percentage are shown in Fig. 6. The activation by TRP significantly promoted PAR-2 activity ($p<0.01$) as compared to the untreated group. Tamarind seed coat extract at any concentration used did not alter PAR-2 activity.

DISCUSSION

Plant phenols and polyphenols constitute an important group of naturally-occurring antioxidants by virtue of the fact that the phenolic group can stabilize free radicals which there is much supporting evidence for an antioxidative benefit to skin (Cherniack, 2010). The fruit pulps of tamarind are used as medicinal plant for centuries. Even though the seed coat possesses antioxidant activity and other health beneficial effects, the activities of bioactive compounds extracted from dry heated tamarind seed coat in hypopigmentation purpose remain unexplored. In this study, the crude extract from tamarind seed coat contained 85.6±0.9 mg phenolics expressed as catechin equivalents per gram extract which accords with a previous study (Soong and Barlow, 2004). The previous study showed that the extract showed antioxidant scavenging activity on a variety of oxidants in a dose dependent manner (50-200 μg mL^{-1}) (Siddhuraju, 2007). Therefore, this concentration range was used in the present study.

The potential of any substance that can improve hyperpigmentation is reducing total melanin content in the skin. To prove the potential of tamarind seed coat's extract in reducing melanin content, mouse melanoma cell line (B16-F1) is a proper model because its released melanin can be clearly observed spectrophotometrically. In the presence of extract (50-200 μg mL^{-1}), there was a concentration dependent reduction in melanin production, which depended on the protocol used. The results show that extract dose-dependently caused some reduction in melanin content of B16-F1 melanoma cells pre-stimulated by α-MSH. Furthermore, the extract had no clear effect on cell viability within the range of concentrations tested. This indicated that the reduction of melanin production was not due to cell death. Interestingly, as compared to the α-MSH-stimulated cells without extract treatment, the

percentage of melamin reduction was about 20-32% in the cells treated with the extract at high concentration (150-200 μg mL^{-1}) after being stimulated with α-MSH (inhibition condition) whereas the melanin reduction was about 42-59% in the cells treated with the extract at the similar concentrations before being stimulated with α-MSH (prevention condition). For kojic acid (50 μg mL^{-1}), which was used as a reference inhibitor, the decrease in melanin content was observed at about 50% in both inhibition and protection conditions. Our findings indicate that the protection effect of the extract was greater than the inhibition effect. This implies that the extract competes with both the downstream activation of α-MSH and the upstream control of melanogenesis perhaps including α-MSH-MC1R binding.

Other plant extracts and their bioactive constituents have been explored previously for tyrosinase inhibitory activity (Yoon *et al*., 2011; Momtaz *et al*., 2008). Polyphenols, such as epicatechin, epigallocatechin and epicatechin-3-gallate, isolated from plants proved to be effective inhibitors of tyrosinase. Thus polyphenols in our extract may also inhibit tyrosinase and/or inhibit L-DOPA auto-oxidation. Here, keratinocyte and melanocyte were co-cultured without any melanogenesis stimulator thus mimicking *in vivo* melano-epidermal activity using dopachrome formation from L-DOPA. The extract was clearly less potent (IC_{50} 152.1±10.2 μg mL^{-1} compared to kojic acid (33.3±2.5 μg mL^{-1}) although the maximum effects were similar (94.7% for extract and 96.6% kojic acid). The morphology of both the keratinocytes and melanocytes appeared to be unaffected by the extract (200 μg mL^{-1}). Furthermore, the cell counts were unchanged in the culture conditions suggesting that the reduced tyrosinase activity did not result from cell death or reduced cell replication. This might include chelation of copper ion within the tyrosinase or the phenolic hydroxyls binding to the enzyme causing stearic hindrance (Yoon *et al*., 2011). For kojic acid treated group, we found that kojic acid at high concentrations (≥100 μg mL^{-1}) caused changes in cell morphology and such changes might influence the tyrosinase amount and activity.

The melanogenesis steps in skin include melanin synthesis and transportation of melamin from melanocyte to keratinocyte. For this reason, the present study was designed to examine the possibility that tamarind's seed coat extract could also affect pigmentation by inhibiting the PAR-2 activity. PAR-2 is expressed mainly in keratinocyte and increased activity of PAR-2 in keratinocytes causes increasing uptake and distribution of melanosomes by keratinocytes in the epidermis. Keratinocyte-melanocyte co-culture was therefore used to mimic the physiological situation happening when melanocytes and keratinocytes co-operate for the transfer of melanins. The natural PAR-2 activator (trypsin, TRP) was used to determine the receptor activity and also consistency of keratinocyte-melanocyte distribution of each batch of isolated co-culture. The results from fluorescence values of staining calcium expressed as a percentage are shown in Fig. 6. As similar to another study (Paine *et al*., 2001), the activation by TRP significantly promoted PAR-2 activity ($p<0.01$) as compared to the untreated group. Tamarind's seed coat extract at any concentration used did not alter PAR-2 activity. Although the direct inhibitory effect of the extract on PAR-2 was not observed, it cannot not be concluded that the extract does not affect melanosome transfer. Recent studies indicate that the skin depigmentation of soymilk and the soybean-derived serine protease inhibitors is not directly involved with receptor inhibition but correlates with blocking action of trypsin, resulting in a decreased transfer of melanosome (Seiberg *et al*., 2000b; Paine *et al*., 2001). Therefore, further study should be performed to determine depigmentation by extract via inhibition of the PAR-2 pathway.

In conclusion, this study clearly showed that melanogenesis could be inhibited by an extract of the seed coat of tamarind in both a melanoma cell line (B16-F1) and in primary cells from human melanocytes co-cultured with physiological partners, keratinocytes. The extract was also able to completely inhibit tyrosinase prepared from the human keratinocyte/melanocyte cultures over the same concentration range. This extract can form the basis of further purification and refinement with the ultimate goal of creating skin depigmentating products.

ACKNOWLEDGMENTS

Financial and facility supports from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education are gratefully acknowledged. In addition, we also thank Dr. C. Norman Scholfield for useful discussions.

REFERENCES

Bohm, S.K., W. Kong, D. Bromme, S.P. Smeekens and D.C. Anderson *et al*., 1996. Molecular cloning, expression and potential function of the human protease-activated receptor-2. Biochem. J., 314: 1009-1016.

Bravo, L., 1998. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutr. Rev., 56: 317-333.

Cherniack, E.P., 2010. The potential influence of plant polyphenols on the aging process. Forsch Komplementmed., 17: 181-187.

Cos, P., L. Ying, M. Calomme, J.P. Hu and K. Cimanga *et al*., 1998. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J. Natural Prod., 61: 71-76.

Donsing, P., N. Limpeanchob and J. Viyoch, 2008. Evaluation of the effect of Thai breadfruit's heartwood extract on melanogenesis-inhibitory and antioxidation activities. J. Cosmet. Sci., 59: 41-58.

Hensley, K. and R.A. Floyd, 2002. Reactive oxygen species and protein oxidation in aging: A look back, a look ahead. Arch. Biochem. Biophys., 397: 377-383.

Huang, K.F., Y.W. Chen, C.T. Chang and S.T. Chou, 2005. Studies on the inhibitory effect of *Graptopetalum paraguayense* E. Walther extracts on mushroom tyrosinase. Food Chem., 89: 583-587.

Kim, Y.J, H. Uyama and S. Kobayashi, 2004. Inhibition effect of (+) catechin-aldehyde polycondensates on proteinases causing proteolytic degradation of extracellular matrix. Biochem. Biophys. Res. Commun., 320: 256-261.

Momtaz, S., B.M. Mapunya, P.J. Houghton, C. Edgerly, A. Hussein, S. Naidoo and N. Lall, 2008. Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. J. Ethnopharmacol., 119: 507-512.

Morandini, R., J.M. Boeynaems, S.J. Hedley, S. MacNeil and G. Ghanem, 1998. Modulation of ICAM-1 expression by alpha-MSH in human melanoma cells and melanocytes. J. Cell. Physiol., 175: 276-282.

Mun, Y.J., S.W. Lee, H.W. Jeong, K.G. Lee, J.H. Kim and W.H. Woo, 2004. Inhibitory effect of miconazole on melanogenesis. Biol. Pharm. Bull., 27: 806-809.

Paine, C., E. Sharlow, F. Liebel, M. Eisinger, S. Shapiro and M. Seiberg, 2001. An alternative approach to depigmentation by soybean extracts via inhibition of the PAR-2 pathway. J. Invest. Dermatol., 116: 587-595.

Pumthong, G., 1999. Antioxidative activity of polyphenolic compounds extracted from seed coat of *Tamarindus indica* Linn. Ph.D. Thesis, Chiang Mai University, Thailand.

Seiberg, M., C. Paine, E. Sharlow, P. Andrade-Gordon, M. Costanzo, M. Eisinger and S.S. Shapiro, 2000a. Inhibition of melanosome transfer results in skin lightening. J. Invest. Dermatol., 115: 162-167.

Seiberg, M., C. Paine, E. Sharlow, P. Andrade-Gordon, M. Costanzo, M. Eisinger and S.S. Shapiro, 2000b. The protease-activated receptor 2 regulates pigmentation via keratinocyte-melanocyte interactions. Exp. Cell. Res., 254: 25-32.

Siddhuraju, P., 2007. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT: Food Sci. Technol., 40: 982-990.

Siegrist, W. and A.N. Eberle, 1986. *In situ* melanin assay for MSH using mouse B16 melanoma cells in culture. Anal. Biochem., 159: 191-197.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178.

Soong, Y.Y. and P.J. Barlow, 2004. Antioxidant activity and phenolic content of selected fruit seeds. Food Chem., 88: 411-417.

Sudjaroen, Y., R. Haubner, G. Wurtele, W.E. Hull and G. Erben *et al*., 2005. Isolation and structure elucidation of phenolic antioxidants from tamarind (*Tamaridus indica* L.) seed and pericarp. Food Chem. Toxicol., 43: 1673-1682.

Yoon, K.N., N. Alam, K.R. Lee, P.G. Shin, J.C. Cheong, Y.B. Yoo and T.S. Lee, 2011. Antioxidant and antityrosinase activities of various extracts from the fruiting bodies of *Lentinus lepideus*. Molecules, 16: 2334-2347.