CHAPTER V

CONCLUSION

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 and thus prevent skin aging and pigmentation when applied on skin. Our study reported the effects of the crude extract of *Tamarindus indica* seed coat on oxidation activities, melanogenesis and skin cell aging.

A dominant phenolic compound in tamarind seed coat extract was catechin as investigated by thin layer chromatography. However, there are many benefit phenolic compounds in the extract such as epicatechin and procyanidin. The crude extract from tamarind seed coat (without tannins) contained high phenolic content, which was 85.6 ± 0.9 mg catechin equivalents per gram extract as determined by Folin-Ciocalteu assay. The antioxidant potential of tamarind seed coat extract with 50% DPPH inhibition (EC_{50}) was 12.5 μg/ml catechin equivalents therefore higher than L-ascobic acid and tocopherol (25.3 and 30.4 μg/ml, respectively).

Preliminary screening of skin cytotoxicity of the extract was studied. The 97-91% viability of HaCaT (normal human keratinocyte) after treated for 24 h with 50–200 μg/ml extract was not significantly different from control (untreated cell) and the morphology did not changed. This indicates that the efficacious concentrations of tamarind seed coat extract are non-toxic to skin cell *in vitro*.

Steps into the skin refining properties of tamarind seed coat extract, the lightening property of the extract was studied. The melanogenesis activity of B16-F1 mouse melanoma cell line was investigated to prove the potential of tamarind seed coat extract in reducing melanin content. The extract showed the dose-dependent inhibition and protection of melanin production of B16-F1 cells. As compared to the α-MSH-stimulated 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) 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). Our findings indicate that the protection effect of the extract was greater than the inhibition effect. Although, the positive control which was 50 µg/ml kojic acid also showedstrong melanogenesis activities, it seemed to be more toxic than tamarind seed coat extract due to the changed in morphology after treated.

Taken into consideration, the inside mechanisms underlying the melanogenesis inhibition of tamarind seed coat extract was study. Keratinocyte-melanocyte co-culture isolated from human skin tissue was developed without any melanogenesis stimulator thus mimicing in vivo melano-epidermal activity. The effect of tamarind seed coat extract on tyrosinase inhibition was study using dopachrome formation from L-DOPA. The extract shows tyrosinase inhibition (IC_{50} = 152.1 ± 10.2 µg/ml). The extract was clearly less potent compared to kojic acid (IC_{50} = 33.3 ± 2.5 µg/ml) although the maximum effects were similar (94.7% for extract and 96.6% kojic acid). However, the tyrosinase inhibition activity of tamarind seed coat extract did not result from cell death or reduced cell replication whereas kojic acid 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 melanin from melanocyte to keratinocyte. In this reason, PAR-2 activity that causes increasing uptake and distribution of melanosomes by keratinocytes was study whether the possibility that tamarind seed coat extract could also affect pigmentation by inhibiting the. PAR-2. However, tamarind seed coat extract at any concentration used did not alter PAR-2 activity. Although the direct inhibitory effect of the extract on PAR-2 did not exhibit, it cannot not be concluded that the extract does not affect melanosome transfer. Further study should be performed to determine depigmentation by extract via inhibition of the PAR-2 pathway.

Furthermore, the antioxidant properties and others benefit effects of tamarind seed coat extract might useful in the improvement of skin aging. The anti-aging property of the extract was evaluated using fibroblastsisolated from human skin tissue.

Firstly, the prevention of hydrogen peroxide induced cellular damage to oxidative stress of tamarind seed coat extract in primary human skin fibroblasts was

studied. Doses of H_2O_2 (100-1000 μM/ml) induced rapid cell death (62-92%) whereas the protective effect of tamarind seed coat extract expressed as a percentage of the cell viability from 34-45%. Our results suggest that the cellular protective properties of tamarind seed coat extract against oxidative stress might improve the skin cell aging.

Secondly, an understanding of the acute UVA-induced cellular responses to oxidative stress will provide useful insights into the effects of tamarind seed coat extract on photoaging.

The decrease of cell viability was UVA-dose-dependent, resulting in 96 - 71% of remaining survivals at UVA exposure 5 - 40 J/cm^2. However, the decrease was reversed by the treatment of tamarind seed coat (pre and post treated with 200 μg/ml). Approximately 85% and 84% of cells were viable upon pre-treated and post-treated UVA irradiation, respectively.

Cell cycle regulation plays an important role in maintaining the genetic integrity of the cell. In this study, UVA irradiated skin fibroblast cells resulted in cell cycle alterations by increasing G0/G1 phase to 78% from normal condition (59%), and decreased G2/M phase (9%) from 16%. Interestingly, treatment of UV-irradiated cells with tamarind seed coat extract significantly restored the suppressed G0/G1 progression (64%), an also increased G2/M phase. This could indicate the sign of cell proliferation.

The study of intracellular glutathiones deficiency demonstrated the antioxidant effects of tamarind seed coat extract by improving the activity of glutathiones thus the balance of intracellular GSH/GSSG after acute UVA-irradiation. Intracellular total glutathiones of UVA-induced cells treated with tamarind seed coat extract increased to 10, 20 and 25% compared to the UVA untreated group at 24, 48, 72 h cultivation, respectively.

The biosynthesis and secretion of type-1 collagen and MMP-1, could evaluated the skin aging byproducts. In the skin simulation after UVA-irradiation, MMP-1 was found higher than control cells (non-irradiated) ongoing with the decreasing in type-1 procollagen synthesis. The anti- oxidative effect of the extract against ROS damage reverse this effect by decreasing MMP-1 secretion to 89%, 91%, 56% at 18, 48 and 72 h of cultivation, respectively compared to the UVA-irradiated untreated group. While the type-I procollagen synthesis increased up to 25-63% at 18-

72 h of cultivation following treatment of extract after irradiation compared to UVA-irradiated untreated group..

Finally, the skin aging improvement of tamarind seed coat extract could be clearly represented in the study of contraction of fibroblast embedded collagen lattice. The effects of tamarind seed coat extract on promoting the reorganization of fibroblasts could be clearly visualized in model mimicking the skin-aging pattern. The diameter of collagen fibroblast decreased up to 70% of initial diameter in young (non-wrinkle) fibroblast and 53% of initial diameter in old (wrinkled) fibroblasts at day 7 with the treatment of tamarind seed coat extract which was about 2-fold higher than control (untreated cells).

In conclusion, this all finding indicated the strong antioxidant activity, melanogenesis inhibitory and improvements of skin aging of tamarind seed coat extract. This extract can form the basis of further development with the ultimate goal of creating active ingredient with skin multifunctional activities.