Title	ANTI-AGING AND LIGHTENING EFFICACIES OF TAMARIND SEED COAT EXTRACT
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Academic Paper	Thesis Ph.D. in Pharmaceutical Sciences (International Program) Naresuan University, 2012
Keywords	tamarind seed coat, phenolic compound, melanogenesis, anti-aging

ABSTRACT

The traditional use of tamarind (*Tamarindus indica* L.) and its health benefits notwithstanding the high phenolic content in its seed coat suggest it might be explored as a skin refinement. For this, we focused on the possible role of the seed coat extract in inhibiting melanogenesis and slowing the effects of skin cell aging. The seed coat of tamarind was extracted with ethyl acetate, which among other solvents recovered highest phenolic content (85.6 ± 0.9 mg/g catechin equivalents - Folin-Ciocalteu assay). The strong antioxidant activity of the extract (DPPH inhibition, EC_{50}= 12.92 µg/ml catechin equivalents) was found higher than L-ascobic acid and tocopherol. The cytotoxicity of HaCaT haman keratinocyte cells was not occurred when treated with effective dose at 50-200 µg/ml of the extract. Nevertheless, our study indicated the melanogenesis inhibitory of the extract via melanin synthesis pathway. 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%. Cell viability and morphology were unaffected by any of the used concentrations. The extract also inhibited tyrosinase activity (IC_{50} = 152.1 ± 10.2 µg/ml). Furthermore, the improvements of skin aging by the extract were studied. For this, we focused on the evidence of cellular oxidative stress stimulation. The obtained result showed the prevention of the extract on H_2O_2 –induced fibroblast

damage. Although 100-1000 μM/ml H_2O_2 induced rapid cell death (62-92%), the extract treated cell remained viable 34-45%, as determined by XTT assay. Coincidence to this, the extract shows the improvement of photoaging results from the study of UVA-induced fibroblast aging. The extract inhibited the cell death that occurred in the fibroblast after acute UVA exposure (5-40 J/cm^2), 85% and 84% of cells were viable upon pre-treated and post-treated UVA irradiation, respectively. Moreover, the results from flow cytometry showed that the reduction of cell cycle arrest in S and G2/M phase and progression of G0/G1 phase also exhibited with tamarind seed coat extract treatment. We also found that fibroblasts treated with tamarind seed coat extract resulted in prevention of UVA-induced depletion of antioxidant defense molecules such as glutathiones. Intracellular total glutathione of UVA-induced cells treated with the extract increased to 10-25% compared to the UVA untreated group at 24-72 h cultivation. Also, the treatment of tamarind seed coat extract prevented UVA-induced MMP-1 secretion and UVA-modulated collagen synthesis by fibroblasts. MMP-1 secretion decreased up to 91% at 48 h of cultivation, 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 untreated group as determined by enzyme-immunoassay and immunofluorescence assay. Finally, the contractile of fibroblasts embedded in collagen lattice was studied. Our findings indicated that tamarind seed coat extract reversed the fibroblast deficiencies in reorganization of collagen and may underlie a wrinkle treatment. This is the first report shows that tamarind seed coat can inhibit melanogenesis and improves skin cell aging suggests that further developments may show their benefits as active ingredient with multifunctional activities.