CHARPTER I

INTRODUCTION

Statement of purpose

Oxidative stress refers to how the process of elements combines with oxygen in our skin and body. This process releases substances called free radicals, which have been shown to cause damage on the cellular level. The major creators of free radicals in the skin are 1) daily chemical processes that give off free radicals as a natural byproduct 2) products applied to the skin that produce free radicals 3) pollution and 4) UV exposure, the most effective creator.

The free radical theory of aging, proposes that the molecular basis of aging derives from accumulation, over a lifetime, of oxidative damage to cells resulting from excess reactive oxygen species (ROS). Although skin possesses extremely efficient antioxidant activities such as the antioxidative enzymes (superoxide dismutases, glutathione peroxidases, glutathione reductase and catalase), it has been demonstrated that during aging, ROS levels rise and anti-oxidant defenses decline. The unifying pathogenic agents for these changes are UV-generated free radicals. In fact, increases in ROS-induced DNA damage are correlated with cell cycle arrest. As well as causing permanent gene mutations, free radicals activate signal transduction pathways that are related to growth, differentiation, senescence, and connective tissue degradation result in upregulates expression of matrix metalloproteinase (MMPs). This scenario provides a mechanism for the observed increased collagen degradation in aged human skin. In addition, free radicals also cause damage to connective tissue components of the dermis, which again is likely to influence cell behavior via cell–matrix interactions.

UV-generated free radicals are the most significant factor influencing human skin pigmentation. The proinflammatory mediators released from keratinocytes oxidative stress lead to increase the secretion of alpha-melanocyte stimulating hormone (α-MSH) and stimulation of melanocortin 1 receptor (MC1R) function in neighboring melanocytes. These results in the expression of melanogenic proteins such as tyrosinase enzyme, lead to increase melanin production. Moreover, this effect also increase the activity of protease activate receptor-2 (PAR-2) in keratinocytes,

which increases uptake and distribution of melanosomes by keratinocytes in the epidermis. Therefore, the hyperpigmentation after photodamage occur.

These skin disorders both aging and hyperpigmentation are not desirable. According to the above-mentioned, to slow the process of skin aging and also decrease the skin pigmentation is reducing the volume of non-essential free radical activity in the skin by antioxidant compounds.

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. It is considered that phenolic compound, which have five basic mechanisms of antioxidant activity: (1) free radical scavenging activity, (2) chelation of transition metals, (3) inhibition of enzymes, (4) enzyme-mimetic activity, and (5) quenching of singlet oxygen, posiibly show the anti-aging and also in skin lightening activities. It has been reported that, they can protect the viscoelasticity of the skin via the mechanism of metallothionein that is the natural antioxidant of skin and also promote the hyarulonic acid production and inhibit proteinases causing proteolytic degradation of extracellular matrix. The anti-inflammatory effect of some polyphenol has been studied. Moreover, the inhibition of enzyme involving in skin pigmentation such as tyrosinase has been reported. They posulated that, tyrosinase which is a copper-containing enzyme that catalyzes the hydroxylation of a monophenol (monophenolase activity) and the oxidation of an o-diphenol (diphenolase activity), the polycondensates of catechin inhibit the tyrosine hydroxylation and L-DOPA oxidation by chelation to the active site of tyrosinase.

Since phenolic compounds are widely distributed in higher plants. Among the interesting phenolic plants, Tamarind (*Tamarindus Indica* L) meets the requirements of being grows naturally in Thailand and its fruit pulps are widely use as cosmetic purpose 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 cosmetic purpose remain unexplored. The major antioxidant components in the seed coat of tamarind are polyphenolic compounds: catechin, procyanidinB2, epicatechin, procyanidin trimer, procyanidin tetramer, procyanidin pentamer and procyanidin hexamer. Therefore, the phenolic constituents,

antioxidant potential and free radical scavenging capacity of tamarind seed coat extract in term of anti-aging and lightening activities should be considered.

Regarding this reason, we hypothesize that tamarind seed coat extract possibly show the multifunctional activities in the treatment of skin aging and hyperpigmentation. Taken into consideration, the extract will be determined the anti-aging and lightening effect by using skin cell cultures. Firstly, cytotoxicity of the extract on skin cell will be investigated. Then, the effect of the extract on the reduction of melanin content from α-MSH stimulation will be studied to assure that the extract has the lightening effect. Furthermore, to clarify the mechanism underlying melanogenesis inhibitory of the extract, tyrosinase in melanocytes and PAR-2 activity in keratinocytes are investigated. For anti-aging evaluation, we will stimulated the oxidative stress in the fibroblast cells by H_2O_2 and study the cell damage prevention capacity of the extract. Moreover, the effect of the extract on skin cell after stimulated with UV irradiation, fibroblast cell cycle, total glutathione that indicate the activities of free radical scavenging enzyme, collagen type-I and MMP-1 content are investigated. In addition, the effect of the extract on the ability of "wrinkled-skin fibroblasts" to reorganize collagen fibers in a collagen lattice will be studied. All obtained results will suggest whether tamarind seed coat extract has potential as a multifunctional cosmetic ingredient.

Objectives of the study

1. To investigate the biological effect of tamarind seed coat extract on melanogenesis.

2. To investigate the biological effect of tamarind seed coat extract on skin aging.

Expected outputs of the study

1. Natural extract for active ingredient from tamarind seed coat, which have multifunctional activities.

2. The developed method for skin evaluation in biomolecular model.