

CHAPTER I

INTRODUCTION

For the past few years, increasing attention has been paid to herbal plants for developing into modern medicine and cosmetic products. The skin whitening and anti-wrinkle/anti-aging cosmetics from natural products have become very popular. The reason is that the used of herbal plants have their basis on long term application practice. In addition, modern scientific knowledge and technologies have revealed that some new phytochemicals from plants can provide human being with valuable agents having potential use in research, prevention and in treatment of various diseases and health problems.

Various dermatological disorders, such as melasma, age spots and sites of actinic damage, arise from the accumulation of an excessive level of epidermal pigmentation. Tyrosinase inhibitors have been increasingly used in medication and in cosmetics to prevent hyperpigmentation by inhibiting enzymatic oxidation of tyrosine, a precursor of melanin. A number of naturally occurring tyrosinase inhibitors have been described, the majority consisting of a phenol structure or of metal chelating agents. Some of these inhibitors suffer from a number of limitations, such as low activity, high toxicity, and insufficient penetrative ability. Tyrosinase inhibitors also are targeted for development as medicines to treat hyperpigmentation-related problems.

The free radical theory of aging can help us to understand the process of aging and search for effective anti-aging agents. The theory postulates that aging is caused by excessive production of reactive oxygen species (ROS) especially the free radical species. ROS play an important role in the intrinsic and photo-aging of human skin. UV rays from the sun, cigarette smoke, pollutants and the natural process of aging all contribute to the generation of free radicals and ROS that stimulate the inflammatory process in the skin. In addition, the inflammation and ROS can cause oxidative damages to cellular proteins, lipids and carbohydrates, which accumulate in the dermal and epidermal compartments, contributing to the etiology of skin aging. Although the skin possesses certain antioxidant defense mechanisms against oxidative

damages, these mechanisms could be inefficient. Therefore, consumption or using topical preparation of free radical scavengers or antioxidants can support biological resistance against reactive oxygen species and protect the skin from environmental exposure. Although many antioxidants are available in both the synthetic and natural forms, the natural antioxidants are more often used in diets, health food supplements and cosmetics because they originate from natural sources and are expected to have limited toxicity.

Raphanus sativus Linn. (Radish) is an edible plant belonging to the family Cruciferae (Mustard family). It is originally from Europe and Asia. Its roots are thick and of various sizes, forms and colors. Radish roots, which are consumed in several Asian countries. This species is used popularly to treat liver and respiratory illnesses (George and Pandalai, 1949). The antibiotic activity of its extracts and its effectiveness in treating microbial diseases as reported in traditional medicine. The root's juice showed antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella typhosa*. The ethanolic and aqueous extracts of the roots showed activity against *Streptococcus mutans* and *Candida albicans*. In addition, women in Thailand, based on wisdom or traditional beliefs, have long used a fresh white radish root as a treatment of melasma (Chaicharntipyuth, 1980).

Recently, a study has been carried out to determine the antioxidant property of radish roots. The scavenging effect on hydroxyl radical of the methanol extract of radish sprout was compared with that of L-ascorbic acid using the bleomycin-Fe method (Takaya *et al.*, 2003). It was found that the methanol extract exhibited high potency 1.8 times that of L-ascorbic acid.

Although the fresh radish roots contain many compounds such as vitamin C, phenolic compounds, organic acids, enzymes, the various effects of chemical and biological compounds in radish have been reported mostly in terms of nutrient. However, its many other beneficial properties, especially for cosmetic and dermatological applications are not widely known or studied.

This study was focused on the tyrosinase inhibition of fresh radish root extracts (aqueous and methanol extract). Various antioxidant mechanisms of the two extracts were also investigated using different antioxidant evaluation techniques such as DPPH test, NBT test and singlet oxygen inhibition test. The *in vitro* cytotoxicity of both extracts were also determined in human fibroblast cells.

The main objectives of this investigation are as follows:

1. Preparation of the crude extracts (freeze-dried water extract and methanol extract) from fresh roots of *Raphanus sativus* L. locally grown in Thailand and quantitative analysis of the two extracts.
2. Determination of anti-tyrosinase enzyme activity of the two extracts in comparison with other well known anti-tyrosinase agents.
3. Determination of antioxidant and free radical scavenging activities of the two extracts in comparison with other well known antioxidants.
4. Cytotoxicity test of the two extracts on normal human fibroblast cell line by LDH assay.