CHAPTER 1

INTRODUCTION

1.1 General introduction

In recent years, there has been increasing evidence that free radicals are associated with pathological conditions such as atherosclerosis, carcinogenesis and aging. Basically, free radicals and other reactive oxygen species including hydroxyl radical, super oxide radical and other singlet oxygen are continually formed in the human body since they are by products of a variety of pathways in aerobic metabolism. In addition, free radicals derived from the environment, especially ultraviolet radiation are important extrinsic factors accelerating aging (Ames *et al*., 1993; Rieger, 1993; Halliwell and Gutteridge, 1989; Wickens, 2001 and Bokov *et al*., 2004). They also cause damage to connective tissue components of the dermis, particularly collagen, leading to premature skin aging and deepening wrinkles (Jenkins, 2002). Generally, the defense mechanisms of the skin against oxidative damage need antioxidant compounds such as ascorbic acid, tocopherols, selenium and antioxidant enzymes such as glutathione peroxidase and glutathione reductase. Such compounds can, therefore, diminish with aging. Currently, various antioxidants such as ascorbic acid, tocopherols and coenzyme Q_{10} have been added to products of body and skin care cosmetics touted as anti-aging to provide the skin directly with antioxidant nourishment.

Today, medicinal plants are gaining more popularity as ingredients in cosmetic formulation because of their antioxidant properties. Several medicinal plants have been reported for their antioxidant activities including *Curcuma longa* (Motterlini *et al*., 2000), *Aloe barbadensis* (Lee *et al*., 2000), *Hibiscus sabdariffa* Linn. (Wang *et al*., 2000) and *Punica granatum* Linn. (Ricci *et al*., 2006).

Pomegranate (*Punica granatum* L.) fruits are widely consumed fresh and in commercially products as juice, jam and wine. Pomegranate fruit husk/peel is a rich source of hydrolysable tannin called ellagitannins. In the commercial pomegranate juice industry, these ellagitannins are extracted from the husk in significant quantities into the juice due to their

hydrophilic properties. Pomegranate fruit peel, a by-product of the pomegranate juice industry, is therefore an inexpensive and abundant source of ellagitannins. Although the fruit peel has also been used in folklore medicine as astringent for treatment of diarrhea and dysentery (Alanis *et al*., 2005), it has been consumed in very small amount. Pomegranate extracts are also being investigated for their potential use as the food biopreservatives and nutraceuticals (Negi *et al.*, 2003).

It has been reported that the methanol extract of the fruit peel that contained total phenolic content of 19.22 %w/w exhibited antioxidant activity with Trolox equivalent antioxidant capacity (TEAC) values of 394.66 and 316.29 mmol/100 g, evaluated by DPPH and ABTS assay, respectively. Based on HPLC-DAD identification, the major types of phenolic compounds were reported as hydrolysable tannin (punicalin, punicalagin), gallagic acid, ellagic acid and gallic acid. Bioassay-guided isolation of the antioxidant active constituent from pomegranate fruit peels found that ellagic acid is the major active compound (Panichayupakaranant *et al*., 2005). On the basis of DPPH radical scavenging assay, ellagic acid exhibited antioxidant activity with ED_{50} value of 2.1 μM. The aqueous and ethyl acetate extracts of the fruit peels also showed antioxidant activity when evaluated by DPPH radical scavenging assay, 5-lipoxygenase assay and chemiluminescence assay (Ricci *et al.,* 2006). Since pomegranate fruit peel extracts are reported to show antioxidant activity, we are interested in preparation of a potent antioxidant active extract from pomegranate fruit peels using DPPH radical scavenging assay-guided fractionation, and use the obtained extract as the active ingredient for preliminary study on formulation of an anti-aging cream.

1.2 Objectives

The objectives of this study were as follows:

1.2.1 To establish the procedure for preparation of the potent antioxidant extract of pomegranate fruit peels using DPPH radical scavenging assay-guided optimization

1.2.2 To set up the standardization method for the potent antioxidant extract of pomegranate fruit peels by consideration of the ellagic acid content and its antioxidant activity

1.2.3 To study on solubility and stability of the potent antioxidant extract of pomegranate fruit peels

1.2.4 To study on preliminary formulation of anti-aging cream using the potent antioxidant extract of pomegranate fruit peels as an active ingredient