CHAPTER I

INTRODUCTION

This chapter contains three parts including the rational for the study, the objectives and the expected outputs of the study. The details of each part are the following:

The Rational for the Study

In cosmetic industry, the demand for multi-functional products and their efficiency are the keys of trend on technology, innovations and cosmetic market.

Skin whitening products are widely used as the cosmetic and therapeutic purposes. When the products containing a chemo-therapeutic agent are applied to the skin and then result in light color of skin. Thus this chemo-therapeutic agent is also called whitening agent (Chang & Chang, 2003. p. 617). The ideal of whitening agents should have a potent rapid and selective bleaching effect on hyperactivated melanocyte cells and carry no short- or long-term side effects.

The interference with melanin synthesis of the whitening agents can be achieved by regulating the activity of melanogenic enzymes, distribution of melanosomes and turnover of pigmented keratinocytes. Most whitening agents act specially to reduce the function of tyrosinase which is the key enzyme in melanin biosynthesis (Briganti, Camera & Picardo, 2003. p. 102).

Nowadays, many research have been reported that the whitening effects relate to the antioxidant properties. Compound with redox properties can have depigmenting effects by interacting with *o*-quinones and avoiding the oxidative polymerization of melanin intermediates. Additionally, the phenolic derivatives of flavanoids are the most important antioxidant that can chelate the copper ions in the tyrosinase. These indicate that the antioxidants can inhibit the activity of tyrosinase enzyme and could be effective as whitening agents (Briganti et al., 2003. p. 104).

Several traditional skin whitening agents such as hydroquinone and mercury compounds are still used in many countries. These compounds result in a serious health affection including irreversible cutaneous damage, accumulation of mercury in the blood and poisoning. These adverse effects have lead to the search for safer whitening agents (Tiedtke, Morel & Marks, 2004. p. 12).

Recently, safe and effective tyrosinase inhibitors extracted from the natural sources have been reported for their potential applications in improving hyperpigmented disorders. For examples, the extracts from plants such as *Glycyrrihiza glabra* (licorice), *Morus alba* L. (white mulberry), *Carthanus tinctorius* L. (safflower), *Arctostaphylos Uva-Ursi* (bearberry) and *Oryza Sativa* (rice bran) have been used as skin whitening agents. These materials are mostly free from harmful side-effects. For this reason, there is an increasing interest in finding natural tyrosinase inhibitors from natural sources.

Artocarpus incisus (breadfruit) belongs to the Moraceae family. This evergreen tree called "Sa-ke" in Thai is found throughout the tropical. Its pulp contains high content of carbohydrate at the amount of 76.7%. In this reason, it has been used as important source of energy over the years (Adebowale et al., 2004. p. 343). There has been reported that the components of the heartwood of *A.incisus* grown in Okinawa, Japan can strongly inhibit tyrosinase activity. The methanol extract of *A. incisus*'s heartwood shows potent inhibitory activity of tyrosinase enzyme. Additionally, the mother liquor by crystallization of *A. incisus* ether extract also shows melanin biosynthesis inhibitory effect on brown guinea pig. The heartwood extract of *A. incisus* consists of several flavonoids including artocarpin, (+)-norartocarpin, artocarpesin, (+)-dihydromorin and cycloartocarpin. Among these compounds, chlorophorin, (+) – norartocapanone, artocarbene and 4-prenyloxyresveratrol show much higher tyrosinase inhibitory activity than kojic acid whereas artocarpin does not shows tyrosinase inhibitory activity (Shimizu et al., 1998. pp. 410-412). However, this compound shows skin lightening effect on the UVB-induced hyperpigmented dorsal skin of brownish guinea pigs (Shimizu et al., 2002. p. 80). These findings lead to the question if the crude extract containing several kinds of flavonoids, will exhibit higher melanogenesis inhibitory activity comparing to the purified

artocarpin, according to the combination of the effects including tyrosinase inhibitory, antioxidant and other possible activity.

Therefore, the purposes of this research are to study the potential of *A. incisus* crude extract for application in skin whitening agent. The heartwood of *A. incisus* will be extracted by using various organic solvents and the obtained extracts is then determined their chemical components by using high performance liquid chromatography (HPLC). The inhibitory effect of the extracts on melanin biosynthesis will be investigated by using *in vitro* DOPAchrome assay and cell culture model. Additionally, *in vitro* DPPH assay will also be performed to determine the antioxidant activity of the extracts.

The Objectives of the Study

The objectives of this thesis are to *in vitro* study the whitening effects of *A. incisus* crude extract. Three objectives are as follows:

1. To determine *in vitro* tyrosinase inhibitory activity of *A. incisus* extract.
2. To evaluate the efficacy of the extracts on melanogenesis inhibitory using cell culture model.
3. To determine *in vitro* antioxidant activity of the extracts.

The Expected Output of the Study

The expected output is to obtain the potent and safe depigmenting agent for the application in skin whitening products. The components of *A. incisus* extract have been found the effects of melanogenesis inhibitory *in vivo* and *in vitro* without any cell toxicity. Therefore, *A. incisus* crude extract is chosen as the skin whitening agent for clarifying the melanogenesis inhibitory and antioxidant activity by using *in vitro* model.