CHAPTER I
INTRODUCTION

In Thailand, skin whitening products are very popular. They are used to lighten the skin to treat freckles and skin hyperpigmentation. The development of successful whitening skin care products depends on the use of effective whitening or depigmenting ingredients that inhibit melanin formation in melanocytes (Majudar et al., 1998).

Skin color variation depends on racial background, environment, genetics and hormonal influence. Even a single individual does not exhibit the same color on all parts of the body. While skin thickness, hemoglobin and minor pigments like carotenoids affect perceived color, the amount of melanin produced by the melanocytes primarily determines skin color (Lee and Kim, 1995). For this reason, research for the development of whitening products has focused on reducing melanin production in the melanocytes.

Melanin is the main factor determining skin color. Its major function is providing protection against UV irradiation. Melanogenesis occurs in melanocytes, which are found in the epidermal basal layer. Tyrosinase, the enzyme controlling melanogenesis, is initially synthesized on the surface of the rough endoplasmic reticulum. Tyrosinase is then transferred to the Golgi complex incorporated with the lysosome. There, it is activated by the addition of a sugar chain before being secreted into a coated vesicle. A premelanosome released from the Golgi complex fuses with the coated vesicle to form the melanosome. In the melanosomes, tyrosinase converts tyrosine to eumelanin (black) or pheomelanin (yellowish of reddish). Melanocytes eventually transfer the melanosomes to keratinocytes, where they are metabolized during the keratinization process. Finally, they completely disappear with desquamation (Musuda, Tejima and Zuzuki, 1996).

Because tyrosinase plays a key role in melanin biosynthesis, many people have tried to seek substances that can either block the synthesis and/ or processing of tyrosinase or inhibit its activity so that melanogenesis is prevented (Prota, 1996).

Therefore, many tyrosinase inhibitors have been reported and tested as cosmetic and pharmaceutical ingredients to prevent overproduction of melanin in epidermal layer.

Hydroquinone is one of the most widely prescribed skin whitening agents in the world (Dooley, 1997; Clays and Barel, 1998). However, with reports of potential mutagenicity and epidermics of ochronosis, there has been increasing impetus in finding alternative herbal and pharmaceutical depigmenting agents which are non-toxic (Hemsworth, 1973; Tabibian, 2000).

Current trend is toward herbal usage. Natural sources have been evaluated for the development of new melanogenesis inhibitors for skin whitening. We expect that they have relatively lower side effects and a good choice as a cosmetic ingredient for prolonged use (Lee, Kim and Kim, 1997; Kim and Lee, 1998). Some of these, such as kojic acid and azelaic acid are well known to most dermatologists (Farmeco, 1998; Tabibian, 2000). Several plant extracts have showed mushroom tyrosinase inhibition activity (Matsuda, Nakamura and Kubo, 1994; Iida et al., 1995; Lee and Kim, 1995; Masuda et al., 1996; Lee et al., 1997; Jang et al., 1997; Lee and Choi. 1999; Likhitwitayawuid, Sritularak and De-Eknamkul, 2000). Examples of these extracts include *Chaenomeles speciosa*, *Dryopteris crassirhizoma*, *Gastrodia ellata*, *Glycyrrhiza glabra* (licorice extract), *Morus alba*, *Myristica fragrans*, *Rheum palmatum*, *Sophora japonica*, *Areca catechu*, *Broussonetia kazinoki* (paper mulberry), *Rheum officinale*, *Artocarpus incisus* and *A. gomezianus* (Haadnun). Recently, several natural products have already been developed as skin whitening agents in cosmetic preparations such as kojic acid, azelaic acid, licorice extract and *Morus alba* extract (Kim and Lee, 1998).

Artocarpus lakoocha Roxb. is the big tree growing in Thailand and known locally as Mahaad (Tanunkat, 1990). This plant belongs to family Moraceae. According to Thai medicinal plant descriptions (Farnsworth and Bunyapraphatsara, 1992), its morphology is as follows: a large size tree, 15-20 m tall. The crown is dense, rounded. The bark is brownish grey or dark brown and scaly. Leaves are simple, alternate, 10-30 cm long and 5-20 cm wide. Flowers are monoecious, the males and females crowded on

separate receptacles. The male inflorescences are irregularly oblong, yellow, solitary in the axils of the leaves, consisting of short peduncles. The female inflorescences are usually irregularly globular; consisting of peduncle slightly longer than the male one. Fruit is a compound fleshy syncarp, irregularly rounded, about 5-8 cm in diameter, velvety puberulous, when fully ripe yellow, edible. Seeds are oblong, lodged within the fleshy enlarged perianth-parts.

Mongkolsuk, Robertson and Towers (1957) reported that the main component of the dried aqueous extract of the heartwood of *Artocarpus lakoocha*, known as Puag-Haad was 2,4,3′,5′-tetrahydroxystilbene (oxyresveratrol). Yodhabandu (1960) extracted Puag-Haad powder with ether, treated the crude extract with charcoal and isolated 2,4,3′,5′-tetrahydroxystilbene in 51 percent yield. Poopyruchpong et al. (1978), on the other hand, found 2,4,3′,5′-tetrahydroxystilbene in 70 percent yield of Puag-Haad.

Puag-Haad is commonly used in traditional Thai medicine as an anthelmintic and antipruritic agent. Tiptabiankarn (1967) found that the main constituent of Puag-Haad (phenolic stilbebe) possessed strong reducing properties. Recently, Sritularak et al., (1998), reported a potent inhibitory effect of the methanolic extract of *A. lakoocha* on enzyme mushroom tyrosinase *in vitro* using L-DOPA as a substrate. Further comparison of its active constituent, 2,4,3′,5′-tetrahydroxystilbene (oxyresveratrol), showed that the compound had a concentration exhibited of 50% inhibition (IC_{50}) about 1.5 μM, which was 17.9 times higher than kojic acid and 12.9 times higher than norartocarpetin, the active component of *A. gomezianus* (Haadnun) root extract (Sritularak, 1998; Likhitwitayawuid et al., 2000). The IC_{50} value for 2,4,3′,5′-tetrahydroxystilbene was in agreement with Shin et al. (1998) and Kim et al. (2002), who reported the value of 1.0 and 1.2 μM, respectively.

Recently, พรทิพย์ นิมมานนิตย์, ภาคภูมิ เต็งอำนวย และ กิตติศักดิ์ ลิขิตวิทยาวุฒิ (2543) have tested the whitening effect of *Artocapus lakoocha* in limited female volunteers. The preliminary results, although obtained from a short-term study period of 4 weeks, indicated that *A. lakoocha* had a potential to produce significant *in vivo*

whitening activity in a larger and more prolonged study. Thus, the extract of *A. lakoocha* (Puag-Haad), were investigated in this work with the following objectives:

1. To evaluate the *in vivo* skin whitening efficacy of the extracts of *Artocapus lakoocha* heartwood (Puag-Haad) and *A. gomezianus* root (Haadnun) in guinea pigs and compare the results with kojic acid

2. To evaluate the *in vivo* skin whitening efficacy of Puag-Haad after 12-week application in female volunteers and compare the results with kojic acid and licorice extract

3. To assess the physical and biochemical stability of Puag-Haad solutions in 20% propylene glycol, with and without antioxidants, upon storage at room temperature and 45 ^{o}C.