

CHAPTER I

INTRODUCTION

For the past few years, public attention has been increasing in the area of development of modern medicine and cosmetic products than herbal plants. Skin whitening and anti-wrinkle/anti-aging cosmetics from natural products have become very popular. The reason is that herbal plants have their basis of long term application practice. In addition, modern scientific knowledge and technologies have revealed that some new phytochemicals can provide valuable agents with potential use in research as well as for prevention and treatment of various diseases and health problems.

At present, the free radical theory of aging can help us to understand the process of aging and search for effective anti-aging agents. It has been postulated that aging is caused by reaction of excessive 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 aetiology of skin aging. Although, the skin possesses certain antioxidant defense mechanisms against oxidative damages. However, these antioxidant 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.

Artocarpus lakoocha Roxb. (Ma-Haad) is a tropical tree belonging to the family Moraceae. It is widely distributed in the northern, northeastern and central part of

Thailand. The dried aqueous extract of *A. lakoocha* heartwood is called Puag-Haad, which is commonly used as an anthelmintic by Thai traditional medicinal practitioner for centuries. The main constituent of purified Puag-Haad is 2, 4, 3', 5'-tetrahydroxystilbene or oxyresveratrol (Mongkolsuk, Robertson and Towers, 1957). Oxyresveratrol has been reported to exert anthelmintic activity (Charoenlarp, Radomyos and Harinasuta, 1981; Preuksaraj et al., 1983) and exhibit good safety profile in cytotoxicity test (Nilvises, Permpipat and Sithisomwong, 1985; Ngamwat et al., 1987). Moreover, the pharmacokinetic properties in human studies have been investigated (Tanunkat, 1990). Recently, oxyresveratrol from *A. lakoocha* heartwood extract has been shown to possess potent inhibitory effect on tyrosinase enzyme, which catalyzes rate-limiting steps of melanin biosynthesis (Sritularak, 1998; Sritularak, De-Eknamkul and Likhitwitayawuid, 1998). Following, the in vitro study, the in vivo skin whitening efficacy of the extract was evaluated in guinea pigs and human volunteers (Tengamnuay, Pengrungruangwong and Likhitwitayawuid, 2003). The result of the study clearly demonstrated that the heartwood extract of *A. lakoocha* could reduce melanin formation in both guinea pigs and humans. When compared with other tyrosinase inhibitors commonly used in commercial whitening products such as kojic acid and licorice extract, oxyresveratrol demonstrated the highest anti-tyrosinase activity (Sritularak, 1998; Sritularak et al., 1998; Shin et al., 1998a; Kim et al., 2002). Also, the anti-HIV and Herpes simplex virus activities have recently been reported (Likhitwitayawuid et al., 2005). Despite the above findings about *A. lakoocha* heartwood extract, its many other medicinal properties especially in cosmetics and dermatological applications are not widely known or studied.

Due to its polyphenolic structure, one study has been carried out to determine the antioxidant property of oxyresveratrol and its derivatives from *A. lakoocha* (Tiptabiankarn, 1967). The extract was evaluated in terms of its anti-rancidity in lard using the active oxygen method and Wheeler method. It was found that oxyresveratrol can increase the stability of lard by delaying rancidity and is considered to be an effective antioxidant compared to Tenox II. Recently, the antioxidant and free radical scavenging effects of oxyresveratrol have been reported (Lorenz et al., 2003). They found that oxyresveratrol was a more potent scavenger of DPPH (2, 2-diphenyl-1-picryl-hydrazyl) and nitric oxide radicals than resveratrol, a related substance well known for its strong

antioxidant activity. They thus suggested that it may have important therapeutic applications such as in neuropathologies where oxidative/nitrosative stress is involved. Others have reported about the inhibitory effect of oxyresveratrol on cyclooxygenase (Shin et al., 1998b) and rat liver mitochondrial ATPase (Nimmanpisut et al., 1976). However, little is known about the many aspects of the antioxidative/free radical scavenging activities of the extract or oxyresveratrol, especially regarding the cosmetic applications. Since there are many possible antioxidant mechanisms, particularly those involving free radical scavenging pathways, the anti-oxidative capacity of the *A. lakoocha* extract should be investigated in more detail and compared with the commercial antioxidants used for cosmetic purposes.

Considering the high content of active constituent ($\approx 50\text{-}70\%$ oxyresveratrol) in the extract (Sritularak, 1998; Sritularak et al., 1998) and its many useful properties, the extract appears to have a very promising potential for further development into a safe, effective and economical ingredient for the cosmetic industry.

This study was focused on the stability of Puag-Haad (the dried aqueous extract) dissolved in aqueous solution in the presence of various antioxidants. The various antioxidant mechanisms of Puag-Haad were also investigated by different antioxidant evaluation techniques such as the DPPH test, NBT test, Fenton reaction and hemolysis test.

The main objectives of this investigation are as follows:

1. Extraction and isolation of active constituents from the aqueous extract of *Artocarpus lakoocha* heartwood (Puag-Haad).
2. Stability evaluation of aqueous solution of *Artocarpus lakoocha* heartwood extract (Puag-Haad) with respect to its antityrosinase activity.
3. Determination of antioxidant and free radical scavenging activities of *Artocarpus lakoocha* heartwood extract (Puag-Haad) in comparison with other well known antioxidants.