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 herbal plants have their basis for long-term application practice. In addition, modern scientific knowledge and technologies have revealed that some new phytochemicals from natural plants can be a source of valuable agents having strong potential for use in research, prevention and treatment of various health problems including aging.

Although not a disease, aging has increasingly become a serious problem from both the medical and cosmetic points of view. The skin is the most conspicuous tissue where signs of aging develop such as wrinkling and loss of skin elasticity. Other signs include weaker dermal-epidermal junctions. A number of striking changes occur in the dermis where there is a loss of dermal thickness. The concentration of glycosaminoglycans in aged skin becomes progressively lower (Kim et. al., 2003; Scharffetter-Kochanek et al., 2000). There is a marked decrease in the number of elastic fibers resulting in reduction of skin elasticity. These phenomena are accompanied by a decrease in cell activity of the dermis, including a decrease in fibroblast proliferation and a low cellular communication by cytokines. Especially aged skin shows a decrease in collagen production. In the case of photo-aging, the content of collagen is greatly reduced due to concomitant degradation and decreased synthesis of collagen, thereby affecting the dermal architectures as well as changing the stretch and elasticity of the skin, which finally leads to wrinkle formation. More recently, changes in collagen metabolism have been brought into focus as a major factor leading to aging. Specifically, it has been demonstrated that accumulation of elastotic material is accompanied by concomitant degeneration of the surrounding collagenous meshwork, and evidence implicating matrix metalloproteinases (MMPs) as mediators of collagen damage in aging has been presented (Kim et. al., 2003; Jenkins, 2002).

Nowadays, the free radical theory of aging can help us understand the process of aging and search for effective anti-aging agents. The theory postulates that aging is caused by excessive reaction of reactive oxygen species (ROS), especially the free radical species (Wachiranuntasin, 2005). ROS play an important role in both 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, these mechanisms could be inefficient. Therefore, consumption or using topical preparation of free radical scavengers or antioxidants can support biological resistance against ROS and protect the skin from environmental exposure. Although many antioxidants are available in both the synthetic and natural forms, the natural antioxidants are preferred in diets, health food supplements and cosmetics because they originate from natural sources and are expected to have limited toxicity (Wachiranuntasin, 2005).

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 taenicide by Thai traditional doctors for centuries (Farnsworth and Bunyapraphatsara, 1992). 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 an 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 humans have also been investigated (Tanunkat, 1990). Recently, oxyresveratrol from *A. lakoocha* heartwood extract has been reported to have a 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; Tengamnuay et al., 2006). The result of the study clearly demonstrated that the heartwood extract of *A. lakoocha* was able to reduce melanin formation in both guinea pigs and humans. Comparing to other tyrosinase inhibitors commonly used in commercial whitening products like kojic acid and licorice extract, the data were in agreement with the *in vitro* tyrosinase inhibitory effect that 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 activity has recently been reported (Likhitwitayawuid et al., 2003).

Due to its polyphenolic structure, oxyresveratrol may also find applications as an antioxidant. One study was conducted to determine the antioxidant property of oxyresveratrol and its derivatives in *A. lakoocha* extract by evaluating the anti-rancidity in lard using the active oxygen method and Wheerer method (Tiptabiankarn, 1967). Oxyresveratrol could increase the stability of lard by delaying rancidity and was considered to be an effective antioxidant compared to Tenox II©. Recently, the antioxidant and free radical scavenging effects of oxyresveratrol have also 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 suggested that it probably may have important therapeutic applications such as in neuropathologies where oxidative/nitrosative stress is involved. Others have also reported about the inhibitory effect of oxyresveratrol on clyclooxygenase (Shin et al., 1998b) and rat liver mitochondrial ATPase (Nimmanpisut et al., 1976). However, little is still known about the many aspects of the antioxidative/free radical scavenging activities of the extract or oxyresveratrol, especially regarding the cosmetic applications.

Recently, Wachiranuntasin (2005) has evaluated the antioxidant and reactive oxygen species (ROS) scavenging properties of *A. lakoocha* heartwood extract and oxyresveratrol. She found that both the extract and active constituent were able to scavenge ROS like DPPH, superoxide anion, hydroxyl radicals and singlet oxygen, with the scavenging activities comparable, and in some cases, even superior to EGCG and pine bark extract, the antioxidants commonly used in commercial anti-aging

products. Thus, *A. lakoocha* heartwood extract or Puag-Haad may have promising potential for use as an anti-aging agent in cosmetic preparations provided that more evidence is available to support such application.

Therefore, the primary focus of this study was to evaluate the anti-aging activities of *A. lakoocha* heartwood extract (Puag-Haad) and its active constituent oxyresveratrol. Their possible anti-aging mechanisms were also investigated using different *in vitro* techniques such as cell culture and enzymatic assays. The main objectives of this investigation are as follows:

1. To evaluate the proleferative and cytotoxic effects of Puag-Haad and oxyresveratrol on cultured fibroblasts in comparison with other well-known antioxidants.

2. To evaluate the preventive effects of Puag-Haad and oxyresveratrol on cellular and DNA damages induced by oxidative stresses in cultured fibroblasts in comparison with other well-known antioxidants.

3. To evaluate the inhibitory effect of Puag-Haad and oxyresveratrol on collagenase enzyme activity in comparison with other well-known antioxidants.