# CHAPTER I

# INTRODUCTION

**Background of the study**

Free radicals are generally very reactive molecules possessing an unpaired electron. During normal cellular activities, various processes produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) which induce free radical formation. The ROS and RNS including free radicals in a high enough concentration can damage cellular proteins, lipids or DNA and are related to many kinds of diseases (Seifried, et al., 2007). Antioxidants are substances that can stop the formation and cascade effect of free radicals, repair damage caused by oxidation and lipid peroxidation. The dietary antioxidants have been mostly used in the prevention and treatment of several diseases (Seifried, et al., 2007). Oxidative stress was defined as a disturbance in the prooxidant-antioxidant balance in favour of the former, leading to potential damage (Seis, 1997).

Reactive oxygen species (ROS) along with reactive nitrogen species (RNS) including free radicals are also well recognized for playing a dual role in biological systems, since they can be either harmful or beneficial to living systems (Valko *et al.*, 2006). On one hand, they prevent disease by assisting the immune system, mediating cell signaling and playing an essential role in apoptosis. On the other hand, they can damage important macromolecules in cells and may have a role in carcinogenesis and other diseases (Seifried, et al., 2007).

The antioxidant phytochemicals such as polyphenols including flavonoids have been commonly used and commercially available. Flavonoids including proanthocyanidins contain multiple hydroxyls which are readily oxidized. The proanthocyanidins, known as condensed tannins, represent a ubiquitous group of plant phenolics and are widely distributed in the plant kingdom and well known as one of the most attractive materials for health care because of their strong antioxidative and free radical scavenging activity (Takahata, et al., 2001).

Grape seed (GS) has been used as one of the most representative natural sources for oligomeric proanthocyanidins (OPC), and the grape seed extract (GSE) has been extensively studied in chemistry (Labarbe, et al., 1999; Kennedy, et al., 2000; Saucier, et al., 2001), biological activities (Kusuda, et al., 2006; Kim, et al., 2005; Bagchi, et al., 2000), and pharmaceutical applications (Saito, et al., 1998; Yamakoshi, et al., 2002). However, the supplement from GSE is very expensive and rarely used in under-developed countries.

Tamarind (*Tamarindus indica* Linn.) belongs to the family of Fabaceae and is widely distributed throughout tropical and sub-tropical regions. The ripened fruit pulp is edible and popularly used. Fresh stem bark and fresh leaves have been used as a traditional herbal medicine. The polysaccharides mainly consisting of xyloglucans prepared from the tamarind seed kernel have been used for cosmetics. Tamarind seed husk (TaSH) is a wasted product from fruit industry. TaSH had high antioxidant activity among the hundreds of seeds and herbs as determined by Soong, et al. (2004). Antioxidative activities of TaSH have been reported by Pumthong (1999), Soong, et al. (2004) and Tsuda, et al. (1994). Komutarin, et al. (2004) reported that polyphenolic flavonoids from TaSH could inhibit nitric oxide production in macrophages. Suksomtip and Pongsamart (2008) reported protective effects of TaSH extract on the oxidation of human low-density lipoprotein and plasmid DNA strand breakage. However, the studies of TaSH to elucidate their antioxidant mechanism and biological property are still limited.

**Statement of problem**

The seeds of tamarind are an abundant source of phytonutrient including flavonoids which have high antioxidant activity (Pumthong, 1999, Soong, et al., 2004 and Tsuda, et al., 1994). The main compounds in TaSH are monomer, oligomer and polymer of proanthocyanidins (Sudjaroen, et al., 2005). However, the profile of polymer proanthocyanidins or condensed tannins in TaSH has not been fully studied. The oligomeric proanthocyanidins (OPC) in grape seed and pine bark are commercially used as powerful antioxidative supplements, but these must be imported, and they are expensive. The tamarind seed husk extract which also contains the OPC may be used as a cheaper powerful antioxidant in place of grape seed extract.

The mechanisms of antioxidative compounds in TaSH are still not clearly understood. The elucidation of the chemical structure and in TaSH is important for at least two different reasons. The first reason is to provide a biochemical and molecular background for their safe and proper use, and the second reason is to find the biological effects of flavonoids of TaSH that will provide a better understanding of the preventive mechanism of the antioxidant supplement. However, the biological properties of the related chemical structure have not been extensively investigated and reported.

In this thesis, the flavonoids from TaSH will be isolated, partially purified and chemically characterized. Their biological effects on antioxidant action, preventive activities on cell viability, inhibition of lipid peroxidation and hemolysis, prevention of reduced glutathione loss in erythrocytes, iron chelation and DNA oxidative damage will be investigated.

**Objectives of the study**

1. To characterize the chemical structures of flavonoid compounds with high antioxidative activities present in the extract from TaSH.
2. To investigate the biological properties related to antioxidative activities of the flavonoids prepared from TaSH.

**Scope of the study**

In the thesis research, seeds from tamarind bought from a local market in Chiang Mai were used in the study. The TaSH will be prepared as powder by drying in an oven, separating and grinding. The TaSH powder will be extracted by acetone/ water (7:3, v/v) or ethanol/ water (3:2, v/v). The active antioxidant compounds in TaSH will be partially purified and chemically identified. The antioxidant activity of the main polyphenolic compounds in TaSH will be determined by various methods. The biological and protective effects of the antioxidative extract isolated from TaSH on iron chelation, hemolysis, DNA damage and cell viability will be investigated.

**Basic agreement**

The solvent extraction and partial purification of flavonoid-rich fractions from TaSH will be performed. Many molecular species of oligomeric and polymeric proanthocyanidin have been reported according to the presence of different monomers, linkages, branching, and number of subunits in proanthocyanidins (Appeldoorn, et al., 2009). The main difficulty in studies on oligomeric and polymeric proanthocyanidins is to obtain them in an individual molecular form. The complete purification of proanthocyanidins with a degree of polymerization (DP) above five is difficult to achieve.

**Hypothesis**

It is hypothesized that the polyphenolic compounds including flavonoids in TaSH, which have high antioxidative activity, would be able to protect oxidant-induced cell damage, prevent normal cell death, and hemolysis, prevent glutathione loss in erythrocytes, chelate iron ions and protect DNA oxidative damage.

**Expected benefits**

1. The chemical structures of antioxidant flavonoids, which are the main proanthocyanidins isolated from tamarind seed husk, will be characterized.
2. The antioxidantive and biological activities of proanthocyanidins isolated from tamarind seed husk will be elucidated.