

## **CHAPTER I**

### **INTRODUCTION**

In biological systems, oxidation reaction is an important metabolic part for producing ATP and oxygen is the electron acceptor in the electron flow system [1]. Problems may occur when electron flow and energy production become uncoupled and produce reactive oxygen species (ROS)[2]. ROS is continuously produced within cells as a result of mitochondrial electron transfer processes and bioproducts from xantine oxidase, lipoxygenases and cyclooxygenases enzymes [3]. Moreover, ROS can be generated from xenobiotics metabolism including, toxins, drugs by cytochrome P450, monooxygenases and exposure to environmental factors such as excessive iron salts and UV irradiation [4]. Other ROS sources including macrophages and neutrophils containing NADPH oxidase complex enzyme, can generate superoxide radicals and hydrogen peroxide [5]. When the ROS level exceeds and the antioxidant capacity of the cells is decreased resulting in oxidative stress proceeds. ROS can damage biological macromolecules such as lipids, proteins and DNA [6].

Oxidative stress plays a pivotal role in the pathogenesis of aging and several degenerative diseases, such as atherosclerosis, cardiovascular disease, diabetes and cancer [7-9]. Human have developed sophisticated mechanisms in order to maintain redox homeostasis against an excess of free radicals produced upon oxidative stress.

Protective mechanisms including both scavenge and detoxify ROS, block ROS production, sequester transition metals, induce enzymatic and nonenzymatic antioxidant defenses in the body[10,11], and others supplied with the diet [12].

Polyphenol compounds have been largely studied for their strong antioxidant capacities by many research groups. New antioxidants with better antioxidant capacity and less toxicity, which are beneficial for prevention of diseases and food preservation, are very desirable.

Caffeic acid (3, 4-dihydrocinnamic acid, CAF) is one of the most common phenolic acid which is found in fruits, grains, dietary supplements and Chinese medicinal herbs [13]. CAF derivatives including amides, esters, sugar esters and glycosides are also widely distributed in coffee beans, olives, propolis, fruits and vegetables. The physiological function of CAF and its derivatives have been studied by many researchers. They possess many properties including antibacteria, antiviral, antiinflammatory, antiatherosclerotic, antioxidant, antiproliferative, immunostimulant and neuroprotective properties. CAF and its derivatives are potential natural antioxidants with multi mechanism involving free radical scavenging, metal chelating and inhibition of lipid hydroperoxide formation [14].

The structural feature responsible for the antioxidative and free radical scavenging activity of CAF is the *ortho*- dihydroxyl functionality in the catechol ring. The antioxidative activity of CAF derivatives depends on the electron-donating and withdrawing substituent on catechol ring, the number of hydroxyl groups or catechol moieties, the involvement of other H-donating groups, the chemical stability and the hydrophobicity of the compounds [14]. However, there are only few studies regarding modification of CAF derivatives by changing the functional group and side

chain groups to relate the structure with their properties including the antiradical and antioxidant, inhibition of free radical production via cytochrome P450, induction of microsomal enzymes and inhibition of cell growth [15].

Therefore, this study was carried out to investigate CAF and its derivatives in term of antioxidant, effect on drug-metabolizing enzymes and cytotoxicity. The specific aims of this study were as followed:

1. To synthesize caffeic acid ester and amide derivatives.
2. To study antioxidant properties of caffeic acid derivatives
3. To investigate the effects of caffeic acid and its derivatives on microsomal enzymes using *in vitro* models and Hep G2 cell line.
4. To investigate cytotoxicity of caffeic acid and its derivatives on Hep G2 cell line.