CHAPTER I

INTRODUCTION

Plant cytochrome P450 (CYP or P450) is a heme containing enzyme which is closely associated with the cytochrome P450 reaductase (CPR). Both are membrane bound proteins located on the endoplasmic recticulum (ER) as a type of microsome (Bak et al., 2011; Durst et al., 1992; Nelson and Werck-Reichhart, 2011; Schuler and Werck-Reichhart, 2003; Werck-Reichhart et al., 2002; Werck-Reichhart and Feyereisen, 2000; Werck-Reichhart et al., 1988). Plant P450s are involved in the catalysis of a vast variety of metabolic reactions (Durst et al., 1992; Mizutani, 2012; Mizutani and Sato, 2011). The mechanism of this P450 catalysis has a characteristic of regiospecific and stereospecific oxidative of non-activated hydrocarbons (Bolwell et al., 1994; Durst et al., 1992; Mizutani, 2012; Schuler and Werck-Reichhart, 2003). The catalytic reaction can be generalized by the transfer of two electrons from NADPH related to the cytochrome P450 reductase part to the cytochrome P450 part to form one molecule of water and one molecule of product (Jensen et al., 2011; Jensen and Moller, 2010).

Plant cytochrome P450s are divided into two groups: A-type, which is presumably involved in the secondary metabolic pathways, and Non-A type, which is presumably involved in the house-hold metabolic pathways according to the phylogenetic analysis (Werck-Reichhart et al., 2002). The CPRs are also divided into two groups: CPR1 and CPR2, according to the information obtained from both the dicotyledon and monocotyledon plants (Paquette et al., 2009). The nomenclature of the P450s has been assigned based on the identity of their amino acid sequence (Nelson, 2006; Werck-Reichhart et al., 2002; Werck-Reichhart and Feyereisen, 2000). The family name of the P450s is not only refers to the enzyme identity used for the classification but also to the catalytic reaction type. The characterization of the P450s is normally based on the amino acid conserved motif sequences represented for PxPPx, (A/G)Gx(D/E)T(T/S), and FxxGxRxCxG correspond for hinge, oxygen binding site and the heme binding site, respectively while the CPR characterization is based on the motifs of two flavin adenine (FAD), flavin mononucleotide (FMN) sites, and NADPH binding and one P450 binding site (Nelson and Werck-Reichhart, 2011; Nelson, 2006; Schuler and Werck-Reichhart, 2003; Werck-Reichhart et al., 2002; Werck-Reichhart and Feyereisen, 2000; Werck-Reichhart et al., 1988; Yamazaki et al., 2002).

Functionally, the P450s are biocatalyst enzymes involved in a vast variety of natural metabolic reactions in plants (Poulos, 2005). Among various secondary products, the terpenoids, which represent the largest class of characterized natural plant compounds, are often substrates for P450. Their biosynthesis are required the oxygenation reaction typically catalyzed by P450 enzymes for modulated chemical properties e.g. increase polarity and decrease volatility for further step in pathway (Hamberger and Bak, 2013). The terpenoids are classified into monoterpenes, sesquiterpenes, diterpenes, and tetradepenes, sesquiterpenes depending on the number of isoprene units present in the backbond $(C_5H_8)n$ of the molecule (Bohlmann and Keeling, 2008). The metabolic pathways of these terpenoid subgroups commonly involve the steps of P450-dependent hydroxylation (Bohlmann and Keeling, 2008; Hamberger and Bak, 2013). For example, Taxol®, the triterpenoid used for anti-cancer, uses P450s (CYP725As) as biocatalysts in many steps in its biosynthetic pathway (Croteau et al., 2006; Kaspera and Croteau, 2006).

Among various Thai medicinal plants, *Croton stellotopilosus* Ohba is the only species that produces plaunotol. Plaunotol is an acyclic diterpenoid consisting of 20 carbon backbond and had a hydroxyl group at C_{18} (Tansakul and De-Eknamkul, 1998). Plaunotal biosynthetic pathway has purpose that cytochrome P450 involving in last step (Tansakul and De-Eknamkul, 1998). Gene understanding using cloning and functional analysis of plant P450 and associated CPR enzyme, its might be better to understanding the metabolic pathway of the plaunotol.