CHAPTER I

INTRODUCTION

Pueraria mirifica or "White Kwao-Krua" is a well-known Thai traditional plant which belongs to the Leguminosae family, Papilionoideae subfamily as same as soybean (*Glycine max*) and pea (Chansakaow et al 2000b, Malaivijitnond 2012) in term of woman rejuvenation. Since 1931, Luang Anusan Suntara had published the "Kwao Krua" pamphlet (Suntara 1931). Truly, there are various properties. *P. mirifica* tuberous root was used in the Thai traditional medicines for treatment of menopausal symptoms (Kashemsanta et al 1952). It has been established that the rejuvenating effect of *P. mirifica* was mediated by an estrogenic action of miroestrol (Cain 1960, Pope et al 1958). The key chemicals, as well as crude extract, were tested in, animals and human cells and exhibited estrogenic effects. There are major isoflavonoids such as daidzin, daidzein, genistin, genistein and puerarin which played the estrogenic activity in *P. mirifica* tuber (Cherdshewasart et al 2007b, Cherdshewasart & Sriwatcharakul 2007) and leaves (Jungsukcharoen et al 2014). On the other hand, puerarin and daidzein extracted from the tuber exhibited antioxidant activity as strong as α-tocopherol in DPPH assay (Cherdshewasart et al 2004). One of these major isoflavonoids (puerarin) showed significantly increased activation of casepase-3, a key executioner of apoptosis of cancer cell (Yu & Li 2006). *P. mirifica* extract also showed a biphasic response to MCF-7 cells with a strong binding with estrogen at high dose in term of competition with estrogen receptors (Cherdshewasart et al 2004). Furthermore, *P. mirifica* established strong estrogenic effects *in vitro* test with MCF-7 proliferation/antiproliferation assay (Cherdshewasart et al 2007b), Uterotrophic assay (Cherdshewasart et al 2007a) and YES assay (Boonchird et al 2010). *P. mirifica* extract also induced osteoblast differentiation rather than osteoblast proliferation in an ER-dependent manner (Tiyasatkulkovit et al 2012). In term of *in vivo* assay, *P. mirifica* played preventive effects on bone loss in ovariectomized rats (Urasopon et al 2007, Urasopon et al 2008). Puerariae powder (the root of *P. mirifica* and *P. lobata*) has been reported an additive effect along with microgrooved tonographical stimulation to promote the transformation in the STRO-1+proteome (skeletal stem cell) that effect cell phenotype but did not affect cell growth (or cell survival) and cell viability (Kantawong et al 2010). There have a comparative of chemical constituents of two varieties of *Pueraria* (Yusakul et al 2011) by using developed HPLC method.

Proteins are an important class of biological macromolecules present in all organisms. All proteins are polymers of amino acids classified by their physical size, proteins are nanoparticles (definition: 1-100 nm). The total complement of proteins by a genome, cell, tissue or organism at a certain time is known as "Proteome", and the study of the proteomes called "Proteomics". In plant proteomic studies, a large-scale study on proteomics in soybean (legume family) has been revealed since 2005 (Mooney et al 2004). There are many protein extraction methods have been developed for plant proteome analysis (Sheoran et al 2009, Wang et al 2006, Xu et al 2006) including trichloroacetic acid (TCA)-acetone, phenol, direct iso-electric focusing (IEF) buffer, and Tris-HCl. TCA-acetone and phenol protein extraction methods were found to be superior to other two tested methods because they can remove a large proportion of non-protein materials which can interfere with plant proteomics and separation proteins in 2005, the protein solubilization methods suitable for proteomic analysis in soybean seed proteins were established (Natarajan et al 2005b).The proteomic reference map of soybean leaves was also established in the following year (Natarajan et al 2006) by using 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) as a separation technique and identified by mass spectrometer (MS). The result indicated that this 2D-PAGE, combined with MALDI-TOF-MS and LC-MS/MS, is a sensitive and powerful technique for separation and identification of soybean leaf proteins. The proteomics and functional analysis of soybean was revealed (Komatsu & Ahsan 2009). Many studies of tuber proteomes in plants have been done, for example: Ginger root extract (Fasoli et al 2012), cassava (Owiti et al 2011), curcuma (Boonmee et al 2011, Chokchaichamnankit et al 2009), grape (Niu et al 2013), potato tubers (Yu et al 2012a) and carrot root (Louarn et al 2012). The discoveries of tuberous proteins are including Methionine synthase, cysteine synthase (involved in amino acid biosynthesis) heat shock protein, protein disulfide-isomerase (involved in protein folding), superoxide dismutase (detoxification) are commonly found in carrot root. Specific proteins that involved in potato tuber development included 6-fructokinase, phytoalexin-deficient 4-1, metallothionein II-like protein, and malate dehydrogenase, whereas, Novel stage- specific proteins identified during in vitro tuberization were ferredoxin–NADP reductase, 34 kDa porin, aquaporin, calmodulin, ripening-regulated protein, and starch synthase (Niu et al 2013). Methionine synthase, cysteine synthase (involved in amino acid biosynthesis), heat shock protein, protein disulfide-isomerase (involved in protein folding) and superoxide dismutase (detoxification) are commonly found in carrot root (Louarn et al 2012). Zingipain-2OS, zingipain-1OS and cystein protease (milk coagulating

protease) were found in ginger root extract (Hashim et al 2011). Sporamin (storage protein) was mainly found in sweet potato *(Ipomoea batatas)* (Maeshima & Asahi 1978) and was highly expressed in the dormant period in curcuma rhizome (Boonmee et al 2011, Chokchaichamnankit et al 2009) . UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UGT), which is a key step for anthocyanin biosynthesis, involved protein found in grapevine (*Vitis vinifera*) (Niu et al 2013).

The climate changes have been one of the most important in growing plants and plant production. The climatic factors include photosynthetic stress, air pollutants, thermal stress (heat and cold), osmotic stress (drought, salt, flooding stress) and metal stress (Hashiguchi et al 2010). Some evidence shows high temperature and humidity (HTH) stress play important role in differentially expressed proteins in soybean (Wang et al 2012). Protein alteration in different season in Legume plant has never been reported. Low temperature stress (less than 20°C) cause considerable agricultural yields loss in crops (maize, rice and chickpea) (Thakur et al 2010). Low temperature exposure coordinately induces the accumulation of PAL (Phenylalanine Ammonia Lyase) and CHS (Chalcone Synthase) mRNAs only in the light (Leyva et al 1995, Timperio et al 2008), beside; high temperature stress (heat stress) causes oxidative damage that manifests in lipid peroxidation. Plant response to heat stress by produce detoxification enzymes such as glutathione S-transferase, catalases, superoxide dismutase(SOD) and ascorbate peroxidases (APX) for protection (Timperio et al 2008). The optimal temperature is 22°C and the maximum is about 40°C (Nieuwelink 2005). In this study, *P. mirifica* proteomics pattern will be revealed. In addition, the climatic change will be investigated.

The end products of plant cellular functions are "plant metabolites" and their levels can be viewed as the response of biological systems to environmental or genetic manipulation (Fiehn 2002). The study of metabolomics is aimed at linking these differences to that caused them, however indirectly. Collaboration the genome and the proteome to the metabolome is one of the major interests of modem plant science. The challenges are how to measure the chemicals simultaneously and how to make sense of the vast amount of measurements (Jenkins et al 2004). Due to *P. mirifica* is important in agricultural and commercial harvest, the metobolomics especially isoflavonoids revealed in roots (Cherdshewasart & Sriwatcharakul 2007), leaves (Jungsukcharoen et al 2011) and cell suspension cultures (Udomsuk et al 2009). These objectives are to compare the concentration of major isoflavonoids in plant *P. mirifica* that involved in isoflavonoid biosynthetic pathway in annual and climatic change such as rain fall amount and seasonal temperature.

Aims of the study are as followed:

To determine the major isoflavonoid contents in *P. mirifica* leaves and tubers.

To determine the proteomic patterns in *P. mirifica* leaves and tubers.

To correlate the major isoflavonoid contents with some specific proteins involved in isoflavonoid biosynthetic pathway.