CHAPTER I
INTRODUCTION

1. Background and rationale

Hair is one of a vital part of the body and one of the most important defining characteristic of human. Hair is divided into two parts i.e. shaft and root. Hair shaft is a portion of hair that protrudes from the level of skin epidermis while the hair root portion is deeply buried under skin epidermis level. Hair shaft is elongated and keratinized, it consists of three layers; medulla, cortex and cuticle. Medulla is the innermost layer and present only in large thick hairs. Cortex is the middle layer that provides strength, color and texture of hair. Cuticle, the outermost layer, is thin and colorless and serves as a protector of the cortex (Krause and Foitzik, 2006). Hair root is enclosed within the structure called follicle. Hair follicle is composed of epithelial and mesenchymal compartments and their interactions play important roles in the morphogenesis and growth of the hair follicle (Yang et al., 2010). Hair follicle and hair shaft are complex organs composed of several cells including dermal papilla cells, dermal sheath cells, outer root sheath cells, keratinocyte, melanocyte and sebocyte (Yoo et al., 2009). The cross-talk between dermal papilla cells in dermal papilla portion and keratinocyte in outer root sheath area is thought to be key for successful reconstitution of hair follicles (Fujie et al., 2001). Each hair grows through the cycle that composed of anagen, catagen and telogen, which are the growth phase, regressing phase and resting phase, respectively (Stenn and Paus, 2001). The duration of hair growth cycle in scalp and body hair is different. Moreover, the structure of each hair in the body is practically differing in their physiological responses, especially to hormones. The human scalp, eyebrows and lashes consist of long, thick and pigmented terminal hair shaft, whereas the body is covered with short, thin and unpigmented vellus hairs. Each of people has an estimated total number of 5 million hair follicles; 80,000-150,000 are located on the scalp (Krause and Foitzik, 2006). In general, 50-100 hairs at random are shed everyday and increased of more than 100 hairs per day (Jadhav et al., 2009). Approximately 2-6 years of all scalp hairs are within anagen follicles. Catagen lasts only for a few weeks, followed by 2-4 months

of telogen phase. The usual growth of scalp hair follicles lies between 0.3 and 0.5 mm per day (Jaks et al., 2010). The thickness of the hair shaft is related to the size of the hair follicle (Krause and Foitzik, 2006). During the hair cycle, the follicular pigmentary unit from melanocytes proliferates and migrates within the follicular epithelium towards the hair matrix and dermal papilla and becomes melanogenically active (Botchkareva et al., 2001). Hair follicle melanogenesis directly related to the growth of hair. Cyclic re-construction of hair follicle pigmentary unit occurs in all scalp hair follicle only in the first 10 hair cycles or approximately 40 years of age (Neste and Tobin, 2004). The color of hair relies on the presence or absence of melanin which is the product from melanogenesis. Therefore, lack of the pigmentary potential of each individual hair follicle leads to the formation of true gray and white hair. There are several factors which regulate the pigmentation including melanocortins, α-melanocyte stimulating hormone, stem cell factors, nerve growth factor, and hepatocyte factor (Kauser et al., 2005).

Hair cycles are controlled by changes in the local signaling molecules, based on changes in the expression of a constantly growing number of cytokines, hormones, neurotransmitter, and their cognate receptors (Krause and Foitzik, 2006). These cyclic changes involve rapid remodeling of both the epithelial and the dermal components of the hair follicles (Stenn and Paus, 2001). Various cytokines and growth factors are believed to involve in the regulation of hair growth, including epidermal growth factor (EGF), transforming growth factor-α (TGF-α) and TGF-β, keratinocyte growth factor (KGF), insulin-like growth factor-1 (IGF-1), interleukin-1 (IL-1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (Ozeki and Tabata, 2003). It is of interest that dermal papilla cell-derived factors have chemotactic effects on the surrounding cells, which lead to hair growth promotion (Rho et al., 2005). Moreover, not only growth factors but also androgens are involved in hair cycle. Androgens affect the dermal papilla cells by producing paracrine signals, such as the growth factors that earlier mentioned (Rho et al., 2005). Androgens are known to cause hair regression and balding in genetically individuals. Testosterone and dihydrotestosterone (DHT) are the two major androgens that indirectly control hair growth. DHT binds to androgen receptors of the hair follicle and leads to a shortening of anagen. DHT is derived from

testosterone by the action of 5α-reductase (Occhiato et al., 2004). There are two 5α-reductase isoenzymes: type I 5α-reductase is widely expressed especially in skin, including scalp, whereas type II 5α-reductase is present in hair follicles and prostate (Eun et al., 2010). Men and women with androgenetic alopecia have a higher activity of 5α-reductase type II and androgen receptors in the frontal scalp area (Price, 2003). Recently, there are highly interested to develop the inhibitors of 5α-reductase for treating hair loss (Faragalla et al., 2003; Cilotti et al., 2001). However, the exact underlying molecular mechanisms in the cycling remain unclear.

Hair loss is not life threatening but often causes severe mental stress and becomes a medical problem when it is excessive, premature and distressing to a patient (McElwee and Sinclair, 2008). It may be caused by genetic factors, follicular microinflammation, precipitating factors, stem cell apoptosis and possibly other factors (Trueb, 2002). Hair loss affects at least 50% of men by the age of 50 years and up to 70% of all males in later life. Its prevalence in women has varied widely, 6% of women aged less than 50 years are affected, increasing to 30-40% of women aged 70 years and over (Norwood et al., 2001). Hair loss often has an underestimated psychosocial impact on an individual's self-esteem, interpersonal relationships and positioning within a society. Telogen effluvium, androgenetic alopecia and alopecia areata, the most frequent type of hair loss are found in clinical practice (Krause and Foitzik, 2006). Statistically, androgenetic alopecia affects approximately 50% of the world's adult population (Whiting, 2001). Currently, there are two available methods commercially employed to treat this problem: drug therapy and human hair transplantation. However, these two methods still have a number of limitations. Drug therapies not only have side effects but after prolong treatment they also accelerate hair loss when stop using. There are 2 commercially available drugs: topical minoxidil and oral finasteride. Minoxidil promotes hair growth through increasing the duration of anagen. It causes hair follicles at rest to grow. Unfortunately, the efficacy of minoxidil is variable and temporary. Serious side effects such as an increase in left ventricular end-diastolic volume, cardiac output and left ventricular mass have been reported (Kumar et al., 2012). Finasteride is a competitive inhibitor of type II 5 α-reductase and inhibits the conversion of testosterone to dihydrotestosterone. Finasteride is contraindicated in women; it may cause malformation of the external

genitalia of male fetuses (Sawaya & Shapiro, 2000; Sinclair, 2004). Recently, dutasteride, a dual inhibitor of both type I and type II 5α-reductase drug is in phase III study. It is approximately 3 times as potent as finasterides at inhibiting type II 5α-reductase and more than 100 times as potent at inhibiting the type I enzyme (Olsen et al., 2006). For hair transplantation, the limitation of this method is the deficiency of transplantable hair, high cost and unpleasing often surgery (McElwee & Sinclair, 2008). The restriction of transplantable hair number in hair transplantation can resolve by hair multiplication technique. Therefore, hair multiplication or hair cloning is a developed technology to harvest donor hair cells, duplicate them and then re-implant into balding areas. The results exhibit the benefits of using this technique. However, it was found that when dermal papilla cells were cultured *in vitro*, they gradually lose their innate hair follicle-inducing ability after about 3-4 passage numbers (Reynolds and Jahoda, 1996). Stem cells therefore is an optional cell source in hair multiplication technique since bone marrow and umbilical cord stem cells can be induced and reconstructed to be dermal papilla-like tissue (Yoo et al., 2010a).

Due to the limitation of hair loss treatment methods, the development of new drugs and therapies to prevent hair loss and to enhance hair growth still is needed. There are many *in vitro* hair growth models such as protein uptake assay, cell proliferation assay, gene or protein determination and enzymatic activity measurement (Yoo et al., 2010b). However, *in vivo* model is still necessary. Various species of animals such as mice, rats, sheeps and monkeys have been used (Datta et al., 2009). Finally, the clinical trial must be performed before launching product to the market.

Interestingly, people around the world pay attention in the health of hair. As shown that the world hair care products industry witnessed 3% expansion in 2010, generating revenue of almost $49 billion and volume sales of more than 15 billion units. The market is expected to reach almost $58 billion in 2015 (ReportLinker, 2012). Recently, the hair care products which are available on the market mostly contained herbal substances. Many Asian herbal products have been acclaimed with hair growth-promoting activity (Adhirajan et al., 2003; Jadhav et al., 2009) therefore in this study 15 herbal plants were chosen based on their traditional uses for treating hair loss and promoting hair growth. They were purchased from the local market in

Amphoe Meuang, Khon Kaen Province. Then, these plants were extracted with 50% ethanol and screened for hair growth promoting activity on dermal papilla cells (DPCs) by using MTT assay. Among 15 herbal plants, *Carthamus tinctorius* extract (CTE) exhibited highest proliferative effect on DPCs and also high yield of the extraction therefore it was selected for further study.

C. tinctorius L. (Kham foi or Safflower or Flores Carthami or Saffron) is a member of the family Asteraceae. The florets of Safflower have been used as a remedy for stroke, gynecological disease, coronary heart disease, angina pectoris, anti-inflammation, anti-oxidation, cardio-protective activity and hypertension (Fu et al., 2005; Jun et al., 2011; Kanehira et al., 2003; Tien et al., 2010). In China, it has been used as a food additive, a natural pigment and a famous traditional Chinese medicine to promote blood circulation by removing blood stasis (Fan et al., 2009). In Korea, the Safflower seed extract has been used for blood stasis treatment and supported the bone formation and prevented osteoporosis (Huh et al., 2001; Kim et al., 2002). In Thailand, Safflower has traditionally been used for the hair growth promoting activity. It is contained in the herbal formulations that effectively promote hair growth and has been reported to exhibit anti-hair loss by inhibiting 5α-reductase function (Kumar et al., 2012).

CTE was selected to study for its potential on hair growth both *in vitro* and *in vo* model. *In vitro* study included the study of proliferative effect on hair cells and the effect on the length of cultured hair follicles. After that, the effect of CTE on hair growth factors at mRNA level was examined in hair cells. Besides, the hair growth promoting effect of CTE also was investigated in mice and rats. Moreover, the chemical constituents and stability of CTE also were measured. Finally, the skin permeability of CTE was studied. This study aimed to investigate the prototype natural substance for treating hair loss. The obtained results may provide the valuable information of CTE for product development in the future. In addition, the pattern of the study might be a guideline or informative suggestion for applying to other herbal substances that are needed to be evaluated before further development of hair care products.

2. Objectives of the study

To achieve the goal of searching a potential herbal plant for relieving of hair loss and promoting hair growth, the study is designed with four specific objectives:

2.1 Screen the high potential herbal extracts on hair growth

2.2 Study on the chemical constituents and stability of CTE

2.3 Investigate the safety and skin permeability of CTE

2.4 Study on the effect of CTE on hair growth both in *in vitro* and *in vivo*

3. The scope of the study

This study is composed of five experimental sections:

3.1 Screening of 15 traditional hair growth promoting plants

- 3.1.1 Preparation of ethanolic extract of the plants
- 3.1.2 Proliferative effect of the extracts on dermal papilla cells and keratinocytes

3.2 Study of chemical constituents and stability of CTE

- 3.2.1 Investigation of chemical constituents of CTE
- 3.2.2 Study on the stability of CTE

3.3 Study of the safety of CTE

- 3.3.1 Evaluation of the safety of CTE on white blood cell and bacterial pre-incubation mutation assay

3.4 Study of skin permeation of CTE

3.5 Study of hair growth promoting effect of CTE

- 3.5.1 The effect on hair growth-related gene expression
- 3.5.2 The effect on hair melanogenesis
- 3.5.3 The effect on the development of cultured hair follicle
- 3.5.4 The hair growth promoting effect in animal models

4. Anticipated outcomes

4.1 To establish the potential of herbal plant on hair growth

4.2 To obtain the chemical constituents and appropriate chemical marker for quality control of CTE

4.3 To obtain the safety and skin permeability of CTE

4.4 To understand the effect of CTE on hair growth-related factors

4.5 To confirm the potential of CTE on hair growth in animal models

5. Location of research conducting

The research was conducted at the Center for Research and Development of Herbal Health Products, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand.