CHAPTER I

INTRODUCTION

The rationale of the study

Androgens are known to involve with some symptoms and diseases such as androgenic alopecia and benign prostatic hyperplasia (BPH), prostate cancer, acne, and hirsutism. Testosterone is the major circulating androgen acting on many androgen-responsive tissues. It is metabolized to a more potent androgen, dihydrotestosterone (DHT) by 5α-reductase. These androgens can bind to androgen nuclear receptors and cause various hormonal actions [1-3]. Thus, the anti-androgens which exhibit inhibitory activity on 5α-reductase and/or androgen receptor may be useful for treatment of these androgen-dependent disorders. Finasteride has been approved by the U.S. Food and Drug Administration since 1992 for BPH treatment [4]. In addition, this group of drugs is used to treat male pattern baldness. However, finasteride causes various adverse effects such as gynecomastia, muscle growth impairment and severe myopathy [5]. Therefore, new selective anti-androgenic compounds are needed and natural products may fulfill this requirement.

Our group recently identified high anti-androgenic activity in a hexane extract of *Curcuma aeruginosa* Roxb. (Zingiberaceae) rhizomes both *in vitro* and *in vivo*. It was proved to be an effective ingredient in hair tonic for androgenic alopecia [6,7]. *C. aeruginosa* is a native plant of the tropical areas. It is commonly known as "Wan mahamek" in Thai, or "pink and blue ginger" in English. It is perennial with oblong tuber roots and the fresh rhizome emits the ginger-like aroma [8,9]. These rhizomes are used in traditional medicine for gastrointestinal remedies such as the treatment of diarrhea and fungal infections. In Thai traditional medicine, maceration of the rhizomes with alcohol is used to treat uterine pain, uterine inflammation, postpartum uterine and perimenopausal bleeding. Reported effects include postcoital contraception, anti-HIV actions, hepatoprotection, antimicrobial effects, antioxidation, reduced platelet-activation and antinociceptive effects [8,10-13]. *C. aeruginosa*

contains essential oils and useful sesquiterpenes including germacrone, dehydrocurdione, curcumenol, isocurcumenol and zedoarondiol [14-16].

Although the anti-androgenic effects from *C. aeruginosa* have been described, the active constituents have not been determined yet. Therefore, we aimed to isolate and identify the anti-androgenic compounds using the *in vitro* enzymatic assay to direct initial fractionation. The most active compound was then characterized by: (i) the anti-proliferation assay on androgen-sensitive human prostate cancer cells (LNCaP), (ii) the *in vivo* hamster flank gland model and (iii) the androgen receptor binding assay. Moreover, the methods for analysis the chemical constituents in *C. aeruginosa* extract i.e. thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC) were developed. Chemical components of *C. aeruginosa* extracts from different sources were also analysed. Finally, the stability profiles of the crude extract and its chemical constituents were studied using the HPLC and GC method developed. The obtained information would be useful for the development of products containing *C. aeruginosa* extract for medical use.

The objectives of this study

1. To isolate and identify the anti-androgenic compounds from the rhizome extract of *C. aeruginosa*

2. To investigate anti-androgenic activity of the isolated compounds using

2.1 an *in vitro* enzymatic assay for screening the inhibitory activity against the conversion of testosterone

2.2 anti-proliferation assay in testosterone-induced cell proliferation of LNCaP cells

2.3 an *in vivo* assay to determine growth suppression of hamster flank gland by topically application of the test compounds

3. To study the potential mechanism of the potent anti-androgenic compound from *C. aeruginosa* by determining the binding capacity on androgen receptor

4. To test the cytotoxic effect of the isolated compounds on the cell viability of human fibroblast cells and LNCaP cells

5. To develop methods for analysis of chemical constituents in *C. aeruginosa* extract

6. To study the stability of *C. aeruginosa* extract and its chemical constituents

The expected outputs

1. The anti-androgenic compounds from *C. aeruginosa* were successfully isolated and identified.

2. The anti-androgenic effect of the isolated compounds from *C. aeruginosa* was investigated on *in vitro* enzymatic assay, testosterone-induced cell proliferation of LNCaP cell and *in vivo* assay.

3. The binding capacity of the potent anti-androgenic compound on androgen receptor was investigated.

4. The cytotoxic effects of the isolated compounds on cell viability of human fibroblast cells and LNCaP cells were studied.

5. The HPLC and GC methods for quantitative analysis of chemical constituents in *C. aeruginosa* extract were developed.

6. The stability profiles of *C. aeruginosa* extract and its chemical constituents were demonstrated.