

CHAPTER I

INTRODUCTION

Nowadays, medicinal herbs have been widely used as food supplement, nutraceutical, cosmoceutical and medicine. Herbs and those compounds that are isolated from herb are believed to be safer or less toxic than those synthetic chemicals. However, prove of the beneficial efficacy of those herbs or isolated natural compounds which are considered to be the active principle, are problematic and complicated. In addition, consistency of the quality of natural derived products is also inadequate, which may affect by several factors, such as the variation of the content of biologically active compounds due to different climate, location or optimum time of collecting sample, different extraction process, inadequate procedure in the quality control system. To develop the herbal drug scientifically, active principle(s) contained in the herb should be identified and quantified. The consistency of the presence of the active principle should also be monitored and controlled during the process of manufacturing of such herbal drug.

To identify and quantify the interest active principle, an appropriate analytical method(s) which can simultaneously detect those analytes, must be developed. The chromatographic method is usually applied to separation and quantitation of the analyte in the complex matrix of natural product extract. High performance liquid

chromatographic (HPLC) method is the most effective, rapid and reliable to do the analysis of such a complex mixture extract, in addition, thin layer chromatographic (TLC) method is also popular due to the simplest and rapid analysis.

Centella asiatica Linn. (CA) or Gotu kola plant is a small member of the family Apiaceae (Umbeliferae) and widely distributed in Asia, Africa, North and South America (1-3). It is widely used to treat various illnesses, particularly in traditional eastern medicine. Historically, Gotu kola has been used to treat syphilis, hepatitis, stomach ulcers, mental fatigue, epilepsy, diarrhea, fever and asthma (2-5). Today, American and European herbalists use Gotu kola for disorders that cause connective tissue swelling, such as scleroderma, psoriatic arthritis (arthritis occurring in conjunction with psoriasis) and rheumatoid arthritis. Recent studies confirm some of the traditional uses and also suggest possible new application for Gotu kola, such as lowering high blood pressure, treating venous insufficiency (pooling of blood in the veins, usually in the legs), boosting memory and intelligence, easing anxiety, gastric ulceration (6) and speeding wound healing. Asiaticoside, asiatic acid and madecassic acid injected to wound chambers were characterized by increased dry weight, DNA, total protein, collagen and uronic acid contents (7). An aqueous extract of *C. asiatica* is effective in preventing the cognitive deficits, as well as the oxidative stress, caused by intracerebroventricular (ICV) streptozotocin (STZ) in rats (8-9).

The plant contains several natural compounds including the triterpenoid glycosides, madecassoside (MS), asiaticoside (AS) and their aglycones, madecassic acid (MA), and asiatic acid (AA) (12-13) which are considered to be the active principles of CA.

Various techniques, including titrimetric, colorimetric, Thin-layer chromatography (TLC) and reversed-phase gradient high-performance liquid chromatography (HPLC) methods have been used to determine the triterpenoid glycosides and their aglycones.

The purposes of this research work are to develop the chromatographic method(s) (both HPLC and TLC) for quantitative and qualitative analysis of the main active principles which are MS, AS, MA and AA presence in CA simultaneously. The developed method will also be validated following The International Conference of Harmonization (ICH) guideline. The distribution and variation of those four compounds in the plants, in the CA extract will also be monitored in order to obtain data for setting the specification of plant and its extract.

Gradient elution technique has been reported in several HPLC report, this may due to the extreme different in chemical and physical properties, especially the polarity of the analyte in the sample. MS and AS are very hydrophilic, where as MA and AA are very lipophilic. However the gradient elution technique is usually suffer from drift and unstable baseline during the chromatograph. Ideally, to find the suitable analytical

method, here in this research, an isocratic reversed phase HPLC system which can determine MS, AS, MA and AA in a single run will be developed

Another problem in analysis of natural derived sample is the interference by impurities matrix which may lessen the efficiency and ultimately deteriorate the expensive column. Therefore, in this research, a sample clean up procedure using the solid phase extraction (SPE) was also developed in order to protect and prolong the column life.

For the TLC analysis, due to the lack of chromophore in the bioactive analyte structure, an anthrone spraying reagent was used to develop colorimetric analysis by densitometry. The developed TLC system will also be validated according to the ICH guideline and applied for analysis of interested samples.

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