CHAPTER I

INTRODUCTION

Centella asiatica (L.) Urb. (CA) or Gotu Kola, a well known medicinal plant has been in use since prehistoric time. The therapeutic value of this herbal remedy with its wide range of applications has been well known in Asia such as China, India, Indonesia and Thailand for centuries. This plant continues to be used as crude medicinal herb within the framework of folk medicine as an effective remedy. Different uses were claimed for this plant, the more common being its use as a wound healing agent **(1-2)**, the treatment of leprosy **(3)** and the constituent of brain tonics for the mentally retarded **(3)**. The active substances of CA were proposed to be the triterpenoids the constituents of which include: asiaticoside (AS), madecassoside (MS), asiatic acid (AA) and madecassic acid (MA) **(4-6)**.

The crude medicinal herb has long been cultivated and traded in many countries around the world. The crude drugs are either supplied in dried or powdered form. The crude medicinal herb is a biosynthetic laboratory as it contains number of chemical compounds like glycosides and their aglycones, pectin and resins; thus can be complicated to quality control.

Nowadays, medicinal herb such as CA has been popularly used as food supplement, nutraceutical, cosmetic and medicine in extracted form. Herbal extracts are believed to be safer or less toxic than those synthetic chemicals. However, prove

of the beneficial efficacy of those extracts or isolated natural compounds which are considered to be the active substances, 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 plant selection, different climate, location or optimum time of collecting sample, different extraction process, inadequate procedure in the quality control system. To develop and make the more value added herbal drug scientifically, active substances contained in the herb should be isolated, identified, quantified and standardized. The consistency of the presence of the active substances should also be monitored and controlled during the process of manufacturing of such herbal drug.

The absence of standards of active ingredient in herbal drug such as asiaticoside and madecassoside (the active compounds of CA) is an important problem for the quality control system. The preparation of extracts from plant is the starting point for the isolation and purification of chemical constituents present in plant tissues as standards for the quality control. Various techniques have been used to extract and isolate and prepared the pure triterpenes of CA. The classical chromatographic method such as column chromatography has been common used to separate the interested active compounds from the extracts. In addition, asiaticoside was separated first from CA extracts by eluted the appropriate mobile phase through the column chromatography, which packed with silica gel as the stationary phase, finally madecassoside was eluted last. The separation of these active compounds in CA extracts was depended on their chemical structures. However, this separation method was inadequate consistence, lack of reproducibility because it was affected by

various factors such as the size of column, the small loading capacity, time-consuming, and the need of personal skill and finally the method is not suitable for scaled up in the manufacture.

The extraction procedure is other important step in studies involving the discovery and isolation of active compounds of plant materials. For the sample preparation, the extraction process prior to the quantitative determination of active compounds presented in plant that revealed not only to remove and separate compounds of interest from the insoluble high molecular weight parts of the plant but also from other extractives, which could interfere with later steps.

Since CA extracts are widely used in the pharmaceutical and cosmetics industries. Extraction techniques have been widely investigated to obtain such valuable active compounds from CA for commercialization. The traditional extraction methods, such as reflux, which have been used for many decades, are very time-consuming and require relatively large quantities of solvents. There is an increasing demand for new extraction techniques with shortened extraction time, reduced the degradation of active compounds due to a long time of extraction.

The novel extraction techniques have become relatively mature and some potential applications for the extraction of active compounds from other various solid plant matrices have been reported, and found that ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) were a fast and efficient method for extracting chemicals from solid plant matrices. These techniques have the possibility of working at elevated temperatures and pressures with greatly decreasing of extraction time **(7-8)**.

The sample, prepared by the procedures described above, was simultaneously removed interferences by the technique such as solid-phase extraction (SPE). The cleaned up sample was subjected to separation and final determination by Thin-layer chromatography (TLC) and High performance liquid chromatography (HPLC). These chromatographic methods were performed as qualitative and quantitative analysis for the quality control system; thus can be increased the valuable and acceptance of medicinal herbs among the physicians and patients.

The purposes of this research work are to develop the extraction of the main active principles, which are MS, AS, MA and AA presence in CA simultaneously by the novel extraction method described as above and isolation of these compounds will also be developed by fractional crystallization method. The developed method will be a much simpler and more effective than a conventional method. Furthermore, the distribution and variation of the active compounds in many CA accessions, collected from various locations will be monitored and a comparative study of triterpenes content profile in CA, collected monthly during the year from a commercial crop will also be evaluated. Information obtained may be utilized as criteria for plant variety selection, appropriate sampling time and commercial cultivars of high triterpenes content for further isolation of pure triterpenes.