CHAPTER 1

GENERAL INTRODUCTION

Alzheimer's disease (AD) is a progressive degenerative neurologic disorder resulting in impairment of memory and behavior. It is the most common cause of dementia since approximately 10% of people 65 years old or older have dementia, and about 70% of these individuals have AD (1). The researchers have determined that the global prevalence of AD was approximately 26 million people in 2006 while it will grow to more than 106 million by 2050 and by that time 1 in 85 persons worldwide will be living with the disease (2-3). The age distribution of the population rapidly shifts upward (1). Beside increased demands on both public health systems and medical services due to the growing number of older adults, AD place additional economic burden on countries in terms of health care resource usage (direct costs) and reduced or lost productivity (indirect costs) (4). About half of patients with AD need high-level care, equivalent to that of a nursing home (2). Therefore, if we can make even modest advances in preventing AD or delay its progression, we could have a huge global public health impact.

The actual cause of AD is still unknown. However, there are many hypothesis of AD pathogenesis included cholinergic hypothesis, glutamate hypothesis, amyloid cascade hypothesis, etc. Nevertheless, most of current drugs for treating the cognitive impairments in AD are based on neurotransmitter which provides symptomatic benefits (4). One of the most promising approaches for treating this disease is to enhance the acetylcholine (ACh) levels in the brain using acetylcholinesterase inhibitors (AChEIs) which is based on the cholinergic hypothesis (5). Principal role of

acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of ACh, inhibition of AChE therefore serves as a strategy for the treatment of AD. There are a few synthetic medicines, e.g. tacrine, donepezil, and the natural product-based rivastigmine and galantamine for treatment of cognitive dysfunction and memory loss associated with AD (6-7). Nevertheless, none of them can cease the disease and these compounds have been reported to have their adverse effects including gastrointestinal disturbances and problems associated with bioavailability, which necessitates the interest in finding better AChEIs from natural resources and better way to deliver these compounds.

Tabernaemontana divaricata (L.) R. Br. Ex Roem. & Schult (TD) which is widely distributed in Thailand, has been reported for its inhibitory effect against the enzyme associated with AD (5, 8-10). Two alkaloids found in TD show higher inhibitory activity on AChE in comparison with galantamine, a well-known AChEIs (5). Therefore, TD would be an attractive source of therapeutic agents for treating AD. In addition, this would be a good chance to support our local plant.

Previously, most approved pharmacological treatments for dementia were delivered orally (11). Since cholinesterase inhibitors are associated with gastrointestinal side effects (12), the patients encounter with nausea, vomiting, diarrhea, weight decreased, dizziness, decreased appetite, headache and asthenia (13-14). The incidence of these adverse effects depends on the degree and duration of enzyme inhibition and on the daily fluctuations in enzyme activity, therefore, it is believed that transdermal application which reduce daily fluctuations will improve overall tolerability while maintain the efficacy (11). Transdermal application offer many advantages over conventional oral medications, including smooth and

continuous drug delivery, reduced C_{max} and steadier systemic drug levels. This may improve the tolerability profile, allowing easier access to optimal therapeutic doses (11).

Transdermal drug delivery offers many advantages over other traditional routes of drug delivery, however, the barrier nature of the skin made it difficult for most drugs to be delivered through it (15). Therefore, there should be the effective formulation that is able to deliver the therapeutic agents through this barrier. Many strategies have been employed to enhance transdermal delivery including encapsulating the drug in vesicular delivery systems especially microemulsion which provides another promising alternative for transdermal delivery of both hydrophilic and lipophilic drugs. Higher diffusion and skin penetration rates were observed for microemulsions in pharmaceuticals, compared to conventional formulations (16). Microemulsion is thermodynamic system which requires no high technology to formulate. Therefore, decrease in cost of production is one of the economic benefits of microemulsion development. In addition, large scale production is very simple and needs no further modifications. Moreover, the storage stability is also improved in the microemulsion system (16).

Therefore, development of microemulsion loaded with TD extract which is able to inhibit the enzyme associated with AD as a transdermal delivery system would be an attractive and worthwhile study. The purposes of thesis study were as followed:

1. To acquire bioactive compounds from TD plant.
2. To determine anticholinesterase activity of TD alkaloidal extract.
3. To do the preformulation study of TD alkaloidal extract.
4. To develop microemulsions containing TD alkaloidal extract.

According to the purposes, the thesis study detail was divided mainly into 4 parts as followings.

Part I describes the acquisition of bioactive compounds from TD including TD identification, extraction and isolation of TD alkaloidal extract. The quantitative and fingerprint analyses of TD alkaloidal extract were also investigated by the mean of thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The quantitative determination method was validated in the term of limit of detection (LOD), limit of quantification (LOQ), intermediate precision, intraday precision and accuracy. The calibration curve for the quantitative determination was constructed.

Part II describes the anticholinesterase activity (anti-ChE) of the TD alkaloidal extract. Ellman's method was used to determine the *in vitro* anti-ChE activity in the term of % inhibition and IC_{50} value was also revealed. Moreover, Morris water maze test (MWM) was also employed to define the *in vivo* anti-ChE in mammals.

Part III describes the preformulation study of the TD alkaloidal extract. The preformulation study was done by the study of physicochemical properties of TD alkaloidal extract such as solubility, partition coefficient and thermal analysis. The stability and cytotoxicity of the TD alkaloidal extract were also investigated in this part.

Part IV describes the development of microemulsions containing TD alkaloidal extract. Pseudoternary phase diagrams were constructed to give the primary information about the proper amount of each component in the microemulsion formulations. Effect of various factors including surfactant types, co-surfactant types,

surfactant to co-surfactant ratio, pH and ionic strength of aqueous phase on the microemulsion region in the phase diagram were also investigated. The microemulsions containing TD alkaloidal extract were characterized by various techniques including photon correlation spectroscopy (PCS), polarizing light microscopy (PLM), freeze fracture transmission electron microscopy (FF-TEM) and differential scanning calorimetry (DSC). The electrical conductivity and rheology properties of the formulations were also investigated. The anti-ChE of the formulations was revealed by Ellman's assay in the term of IC_{50}. The microemulsions containing TD alkaloidal extract were also determined for their stability. Moreover, skin permeation study was also investigated.

The results and discussion are reported in **Chapter 4**; in addition the conclusion is given in **Chapter 5.**