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Characterization and in vitro permeation study of microemulsions and liquid crystalline systems containing the anticholinesterase alkaloidal extract from *Tabernaemontana divaricata*

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ABSTRACT

The aims of the present study were to characterize the microstructure and study the skin permeation enhancement of formulations containing the alkaloidal extract from *Tabernaemontana divaricata*. The extract was loaded in the formulations composed of *Zingiber cassumunar* oil, Triton X-114, ethanol and water with the oil:surfactant ratios of 1:5 and 2:5. The formulations were characterized by photon correlation spectroscopy, polarizing light microscopy, differential scanning calorimetry, and viscosity measurement. A reverse micellar phase, w/o microemulsions, liquid crystalline systems, liquid crystal in microemulsion systems and coarse emulsions were formed along the aqueous dilution line of both oil:surfactant ratios. Formulations with the ratio of 1:5 containing 0.1 μg/ml extract showed a significantly higher acetylcholinesterase inhibition than those with the ratio of 2:5. The skin of stillborn piglet was used in the permeation study. The liquid crystalline and microemulsion systems significantly increased the transdermal delivery of the extract within 24 h. It was concluded that the alkaloidal extract from *T. divaricata* stem loaded in liquid crystalline or microemulsion systems comprising *Z. cassumunar* oil/Triton X-114/ethanol/water may act as an alternative percutanous formulations for enhancing the acetylcholine level in Alzheimer's patients.

1. Introduction

Alzheimer's disease (AD) is a progressive degenerative neurologic disorder resulting in impairment of memory and behavior. Approximately 10% of people 65 years old or older suffer from dementia, and about 70% of these individuals have AD, making it the most common form of dementia (Szekely et al., 2007). It has been determined that the global prevalence of AD was approximately 26 million people in 2006 and that it will grow to more than 106 million by 2050. By that time 1 in 85 persons worldwide will be living with the disease (Ghochikyan, 2009; Brookmeyer et al., 2007). Besides increasing demands on both public health systems and medical services due to the growing number of older adults, AD places an additional economic burden on countries in terms of health care resource usage (direct costs) and reduced or lost productivity (indirect costs) (Brasnjevic et al., 2009). About half the patients with AD need high-level care, equivalent to that in a nursing home (Ghochikyan, 2009). Therefore, if even modest advances in preventing AD or delaying its progression can be made, this would have a large impact on global public health.

The actual cause of AD is still unknown. However, there are many hypotheses including the cholinergic hypothesis, glutamergic hypothesis, amyloid cascade hypothesis, etc. (Lane et al., 2005; Greig et al., 2001; Sommer, 2002; Vassar, 2002; Kokjohn and Roher, 2009; Danysz and Parsons, 2003). Most of the drugs currently used for treating the cognitive impairments in AD are based on effects on neurotransmitters and provide symptomatic benefits (Brasnjevic et al., 2009). One of the most promising approaches for treating this disease is to enhance the acetylcholine (ACh) levels in the brain using acetylcholinesterase (AChE) inhibitors (based on the cholinergic hypothesis) (Ingkaninan et al., 2006). The principal role of AChE is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of ACh. There are a few synthetic (tacrine, donepezil) as well as natural product-based drugs, including rivastigmine and galantamine, for treatment of cognitive dysfunction and memory loss associated with AD (Martin and Farlow, 2007; Mukherjee et al., 2007). Nevertheless, none of these drugs can cease the disease and these compounds have been reported to have adverse effects including

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gastrointestinal disturbances, as well as problems associated with bioavailability, which explains the interest in finding better AChE inhibitors (e.g. from natural sources) and better ways to deliver these compounds.

Tabernaemontana divaricata (L.) R. Br. Ex Roem. & Schult has been reported to have an inhibitory effect against the enzymes associated with AD (Ingkaninan et al., 2003, 2006; Chattipakorn et al., 2007; Nakdook et al., 2010). Alkaloids found in *T. divaricata* show higher inhibitory activity on AChE compared to galantamine, a well-known AChE inhibitor (Ingkaninan et al., 2006). Therefore, *T. divaricata* is an attractive source for the development of therapeutic agents for treating AD.

Currently, most approved pharmacological treatments for dementia are delivered orally (Lefevre et al., 2008). Since cholinesterase inhibitors (ChEIs) are associated with gastrointestinal side effects (Johnson et al., 2000), patients encounter nausea, vomiting, diarrhea, weight loss, dizziness, decreased appetite, headache and asthenia (Wentrup et al., 2008; Winblad et al., 2007). The incidence of these adverse effects depends on the degree and duration of enzyme inhibition and on the daily fluctuations in enzyme activity. For these reasons, it is believed that transdermal application may reduce daily fluctuations and improve overall tolerability while maintaining efficacy (Lefevre et al., 2008). Transdermal application offers some advantages over conventional oral administration, including continuous drug delivery, less frequent administration, reduced C_{max} and steadier systemic drug levels. This may improve the tolerability profile of the medication, allowing easier access to optimal therapeutic doses (Lefevre et al., 2008).

The main challenge in transdermal drug delivery, however, is the barrier function of the skin which is difficult for most drugs to overcome (El Maghraby, 2008). Therefore, effective formulations that are able to deliver the therapeutic agents through this barrier are required. Many strategies have been employed to enhance transdermal delivery, including encapsulation or solubilization of the drug in colloidal delivery systems, e.g. in microemulsions which provide a promising alternative for transdermal delivery of both hydrophilic and lipophilic drugs. Higher diffusion and skin penetration rates were observed for drugs solubilized in microemulsions, compared to conventional formulations (Schwuger et al., 1995). Microemulsions are thermodynamically stable systems which require no specific or sophisticated technology to be formulated. Therefore, low cost of production is one of the economic benefits of microemulsion development. In addition, large scale production is easily achievable and storage stability is high in microemulsion systems (Schwuger et al., 1995).

Therefore, development of microemulsions (or related colloidal systems) loaded with *T. divaricata* extract, as a transdermal delivery system would be an attractive and worthwhile option. The aims of the present study were to develop and characterize microemulsion formulations loaded with alkaloidal extract from *T. divaricata* stem. In vitro permeation studies of the selected formulations were also investigated, using a piglet skin model.

2. Materials and methods

2.1. Plant material

T. divaricata was collected from the Northern part of Thailand during 2009. The voucher specimen (collection no. 0010115) was deposited at a herbarium, Faculty of Pharmaceutical Sciences, Chiang Mai University. *Zingiber cassumunar* Roxb. and *Cymbopogon citratus* Stapf. were collected from a local farm located in Chiang Mai, Thailand during January 2009.

2.2. Extraction of alkaloidal extract from T. divaricata

The dried powder of *T. divaricata* stems was macerated in 95% ethanol for 3 days. After filtration, the filtrate was collected and the residue was again macerated in 95% ethanol for 3 days. Finally, the total filtrate was evaporated under reduced pressure until dryness to give *T. divaricata* crude extract. The *T. divaricata* crude extract was then dissolved in acetate buffer (pH 3) and washed with ethyl acetate (EtOAc). The aqueous part was basidified with sodium bicarbonate solution (pH 10) and then extracted with EtOAc. The organic layer was dried under reduced pressure to give the alkaloidal extract.

2.3. Extraction of the essential oils from C. citratus and Z. cassumunar

The fresh stem of *C. citratus* and the rhizome of *Z. cassumunar* were cut into small pieces and subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The essential oils obtained were dried over anhydrous sodium sulphate and stored in a refrigerator and protected from light until further use. The density of each essential oil was analyzed by using a pycnometer.

2.4. Selection of components for construction of the phase diagram

Surfactant and co-surfactant were mixed in a 2:1 weight ratio to obtain a surfactant mixture (Smix). Microemulsions were then prepared by adding water to the mixture of oil and Smix when oil:Smix:water was 2:5:3. Two types of essential oils (*C. citratus* stem oil and *Z. cassumunar* rhizome oil), two surfactants (Tween 20 and Triton X-114) and two co-surfactants (ethanol and propylene glycol) were used for microemulsion formation.

2.5. Preparation of alkaloidal extract loaded microemulsions

Alkaloidal extract was firstly dissolved in ethanol. The ethanolic alkaloid solution was then mixed with Triton X-114 and *Z. cassumunar* essential oil, respectively. Finally, water was added and mixed by vortex mixing. The weight ratio of oil:Smix:water was 2:5:3 or 1:5:4. The final concentrations of alkaloidal extract in the formulations were varied from 0.05 to 5 mg/ml to investigate the effect of the extract on the colloidal structure of the formulations.

2.6. Photon correlation spectroscopy

Particle size analysis was carried out using photon correlation spectroscopy (Zetasizer® version 5.00, Malvern Instruments Ltd., Malvern, UK). Measurements were carried out at a fixed angle of 173°. Results are reported as the mean and standard deviation (S.D.) of at least ten measurements on the sample.

2.7. Phase diagram

The phase behavior of *Z. cassumunar* oil/Triton X-114/ethanol/water system was investigated by constructions a pseudoternary phase diagram, in which the oil was used as one component, water as the other, and the third component was Smix, which was a mixture of Triton X-114 and ethanol (2:1). The ternary phase diagram was constructed adopting a simple titration method. Smix was first prepared by combining the required amount of the surfactant (Triton X-114) and co-surfactant (ethanol). Then an appropriate quantity of the oil was added and water was the titration component. The phase boundaries were