



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF NANOEMULSION CONTAINING *Centella asiatica*
(L.) URB CRUDE EXTRACT AS A PROMISING DRUG DELIVERY
SYSTEM FOR EPILEPSY TREATMENT***

SHOBNA A/P L. THURAISSINGAM

FS 2022 5



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By

SHOBNA A/P L. THURAISSINGAM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Master of
Science**

June 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

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Preparations of products containing herbal extracts have grown by leaps and bounds hitting the pharmaceutical industries due to the natural healing approach. *Centella asiatica* (L.) Urb (pegaga) is a famous plant and is commonly served as a salad in Asian diets. It contains various phytoconstituents which plays a vital role in the treatment of various illness. For example, in the treatment of epilepsy, crossing the blood-brain barrier (BBB) has been a challenge (even as in parenteral application) and not all the drugs were able to pass through the membrane and produce a maximum therapeutic effect to the targeted site of action. Thus, nanoemulsion formulation containing *C. asiatica* crude extract needs to be developed in order to penetrate the BBB. *C. asiatica* crude extract was extracted in organic solvent (methanol and acetone) and the highest percentage yield was revealed in methanol extract (33.20 %) followed by acetone (15.90 %). Nanoemulsion was prepared by using the low-energy emulsification method. The particle size, polydispersity index (PDI) and zeta potential of *C. asiatica* crude extract-loaded nanoemulsion were found to be at $57.86 \text{ nm} \pm 0.03$, 0.50 ± 0.03 and $-26.50 \pm 0.03 \text{ mV}$, respectively. The formulation remained physically stable at different storage temperatures (4.0, 25.0 and 45.0 °C) for 90 days. The particle size observed by transmission electron microscopy (TEM) was shown to be at ~50 nm, which correlated well with the Zetasizer analysis. The cytotoxicity study, which was conducted on formulated nanoemulsion towards Vero and 3T3 cell lines showed that the IC₅₀ value indicated that it is nontoxic (>500 µg/ml) when compared to the crude extract alone which was 340 µg/ml against Vero cell line and 365 µg/ml in 3T3 cell line. The formulation was found to be stable based on the good evidence of physicochemical properties and the IC₅₀ value indicate significance for future in vivo and in vitro studies based upon the route of administration.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBANGUNAN NANOEMULSI MENGANDUNGI EKSTRAK *Centella asiatica* (L.) URB SEBAGAI BAHAN YANG BERPOTENSI UNTUK RAWATAN SAWAN

Oleh

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Penyediaan produk yang mengandungi ekstrak herba telah berkembang pesat dalam industri farmaseutikal disebabkan pendekatan penyembuhan semula jadi. *Centella asiatica* (L.) Urb (pegaga) ialah tumbuhan terkenal dan lazimnya dihidangkan sebagai 'ulam' dalam diet orang Asia. Ia mengandungi pelbagai fitokonstituen yang memainkan peranan penting dalam rawatan pelbagai jenis penyakit. Sebagai contoh, dalam rawatan sawan, lintasan penghalang darah-otak (BBB) telah menjadi cabaran (walaupun dalam aplikasi parentral) kerana tidak semua ubat mampu melintasi penghalang tersebut dan memberi kesan terapeutik maksimum kepada tapak tindakan yang disasarkan. Oleh itu, formulasi nanoemulsi yang mengandungi ekstrak kasar perlu dihasilkan bagi melintasi BBB. Tujuan kajian ini adalah untuk memformulasi suatu nanoemulsi yang mengandungi ekstrak kasar daun *C. asiatica* untuk rawatan sawan. Nanoemulsi disediakan dengan menggunakan kaedah pengemulsian tenaga rendah. Saiz zarah, indeks poliserakan (PDI) dan nilai keupayaan zeta oleh nanoemulsi yang mengandungi ekstrak kasar *Centella asiatica* (L.) adalah masing-masing $57.86 \text{ nm} \pm 0.03$, 0.50 ± 0.03 and $-26.50 \pm 0.03 \text{ mV}$. Formulasi ini kekal stabil secara fizikal pada suhu simpanan yang berbeza (4.0 , 25.0 and $45.0 \text{ }^\circ\text{C}$) selama 90 hari. Saiz zarah dapat diperhatikan daripada transmisi mikroskopi electron (TEM) adalah $\sim 50 \text{ nm}$, dimana ia berkolerasi positif dengan analisis *Zetasizer*. Kajian kesitotoksikan yang dijalankan ke atas formulasi nanoemulsi terhadap sel Vero dan 3T3 menunjukkan nilai IC_{50} yang tidak toksik ($>500 \text{ } \mu\text{g/ml}$) apabila dibandingkan dengan ekstrak kasar sahaja terhadap sel Vero iaitu $340 \text{ } \mu\text{g/ml}$ dan sel 3T3 iaitu $365 \text{ } \mu\text{g/ml}$. Nanoemulsi didapati stabil dan mempunyai ciri-ciri fizikokimia yang baik dan nilai IC_{50} menunjukkan kepentingan bagi kajian *in vivo* dan *in vitro* pada masa hadapan berdasarkan laluan pentadbirannya.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AED	Anti-Epileptic Drugs
BBB	Blood Brain Barrier
C.asiatica	Centella Asiatica
CA-NE	Centella asiatica loaded nanoemulsion
CNS	Central Nervous System
ED	Effective dose
EEG	Electroencephalographic
EO	Essential oil
i.p	Intraperitoneal
K+	Potassium
Cl-	Chloride ion
Kg	Kilogram
MES	Maximal Electroshock
Mg	Milligram
Na+	Sodium ion
NE	Nanoemulsion
PIC	Picrotoxin
PTZ	Pentylentetrazole
SEM	Standard Error of Mean
TLE	Temporal Lobe Epilepsy
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Epilepsy is a chronic disorder of the brain that affects people worldwide (WHO, 2018). According to Falco-Walter *et al.*, (2018), epilepsy is a transient occurrence of signs or symptoms due to abnormal excessive or synchronous neuronal activity in the brain and this definition was updated by International League Against Epilepsy (ILAE). About 50 million people globally have epilepsy. This number has therefore signified the world's most common neurological diseases.

Adding to the statistics, low- and middle-income countries have the highest percentage of people with epilepsy which is approximately 80 percent. Specifically, it is estimated that epileptic patients make up 1 percent of the overall Malaysian population, with roughly 230,000 diagnosed cases, affecting individuals of all races, ages and gender (WHO, 2018). Anti-epileptic drugs used such as phenytoin and phenobarbital have caused severe side effects such as hepatotoxicity, nephrotoxicity and behavioural changes. Therefore, patient compliance to the drug has reduced as they looked for traditional medicine as an alternative treatment to avoid those side effects. As traditional/ natural product formulation has taken a trend in the pharmaceutical formulation due to the high demands, many researchers are working on formulation containing natural products than synthetic/modern drugs. Interestingly, looking into the alternative treatment for epilepsy using traditional/natural product, *Centella asiatica* was found to be as an Ayurveda remedy used in the treatment of various disorders including epilepsy (Roy *et al.*, 2013).

A study has reported the phytochemical screening of *C. asiatica* aqueous extract contains mostly triterpenes and flavonoids which are responsible for anticonvulsants activity (Manasa and Sachin, 2016). It was concluded that the bioactive compound from those extracts can be used to treat epilepsy and to control seizure generation (Visweswari *et al.*, 2010).

The major obstacle in treating epilepsy is the penetration of the drug across the blood-brain barrier (BBB) (Bennewitz & Saltzman, 2009). Poor penetration to BBB leads to a poor pharmacokinetic profile of the drug (French & Gazzola, 2011). In order for a formulation/drug to pass through BBB, the particle size of the formulation used will be smaller (20-200nm) and therefore, nanoemulsion for parenteral delivery can be developed (Haque *et al.*, 2012).

Nanoemulsion is an emulsion having a droplet size below 200 nm. Nanoemulsion consists of oil, water and surfactants (Jabir *et al.*, 2014). A large

interfacial area in nanoemulsion promotes the transport of drug to the targeted site. The incorporation of drug/crude in oil will increase the permeability of the drug/crude through passive diffusion. It increases drug delivery to the brain by improving lipid solubility (Kulkarni, 2011). The properties of nanoemulsion which have the ability to protect the drug/crude from hydrolysis and enzymatic has make it an ideal vehicle for drug delivery across BBB (Prakash & Thiagarajan, 2011). Therefore, a novel nanoemulsion-based delivery system containing *C.asiatica* leaves (crude extract), will be formulated as an alternative treatment for epilepsy in medicinal plants and the determination of physicochemical characterization will be carried out for use in clinical practice.

1.2 Problem Statement

Anti-epileptic drugs (AEDs) such as phenytoin and phenobarbital are used to treat epilepsy to reduce the frequency and severity of the seizure without causing an adverse effect (Lee, 2014). Unfortunately, those drugs used for the treatment of epilepsy have poor bioavailability as the percentage of drug to reach the targeted site was found to be lesser and requires high dosage and eventually turns ineffective due to drug resistance (Jabir *et al.*, 2014). Some newer AEDs such as levetiracetam are less prescribed due to their serious side effects (Kaur *et al.*, 2016). In present-day AEDs are prescribed for safer and better patient tolerability only.

For older drugs as mentioned (such as phenytoin and phenobarbital) which is the first-generation of AEDs, cause serious side effects that affects the central nervous system (CNS) and other side effects (such as hepatotoxicity, nephrotoxicity, encephalopathy and gingival hyperplasia) which has made it less prescribed by a physician compared to newer AEDs (French & Gazzola, 2011). Besides that, phenytoin also has enzyme inducing effect which contributes to the negative impact on bone metabolism. Poor bone metabolism will result in reduced bone density and increase risk of fracture resulting in poor quality of life (Lee, 2014).

Generally, the disadvantage of modern drugs used for the treatment of epilepsy (both older and newer AEDs) is known and therefore other alternatives such as traditional medicine is hitting a trend in the pharmaceutical industry due to the high demand of natural products then synthetic/modern drugs. According to Liu *et al.*, (2017), traditional herbal medicine does play a vital role in the treatment of epilepsy. *Centella asiatica* is used in Ayurvedic remedy in the treatment of epilepsy (Nagoor *et al.*, 2018). An *in vivo* study conducted by Deka *et al.*, 2017, has proven that the crude extract of *Centella asiatica* possess anti-epileptic activity.

Secondary metabolite compounds which are widely present in the *C.asiatica* leaves such as terpenoids have demonstrated poor absorption and inability to cross the lipid membranes due to large molecular sizes. This has also resulted

in the loss of efficacy in most clinical trials (Gunasekaran *et al.*, 2014). Therefore, a nanoemulsion formulation containing *C.asiatica* crude extract can be developed to formulate a non-toxic formulation, as a parenteral delivery in the treatment of epilepsy which enables the formulation to pass through the blood brain barrier

1.3 Scope of Study

In this research, the formulation of nanoemulsion containing crude extract of *Centella asiatica* was developed as a promising drug delivery system for epilepsy treatment. Phase behaviour study was conducted to obtain the composition of formulation which was found in the isotropic region. The physicochemical properties of nanoemulsions such as particle size, polydispersity index, zeta potential, morphology and stability were evaluated. The greater the physicochemical properties in the desired range, the greater the potential will be in the treatment of epilepsy. The cytotoxicity of the nanoemulsion formulation was studied towards the Vero cell line and 3T3 cell lines as a preliminary study to determine nephrotoxicity. The *in-vitro* drug released from the nanoemulsion formulation was determined by using the dialysis bag method.

1.4 Objectives

This study aims to develop a nanoemulsion containing crude extract of *Centella asiatica* leaves as promising drug delivery system for epilepsy treatment. The specific objectives were:

1. To extract and determine the phytoconstituents present in the crude extract of *C.asiatica* leaves.
2. To formulate and determine the physicochemical characterization of nanoemulsion containing *C. asiatica* crude extract.
3. To evaluate the stability of nanoemulsion containing *C. asiatica* crude extract.
4. To investigate the *in-vitro* cytotoxicity and release studies of the nanoemulsion formulation.

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