UNIVERSITI PUTRA MALAYSIA

DESIGN AND OPTIMIZATION OF NANOEMULSION FORMULATION CONTAINING Clinacanthus nutans *LINDAU LEAF EXTRACT FOR COSMECEUTICAL APPLICATION*

INTAN SORAYA BINTI CHE SULAIMAN

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By

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DESIGN AND OPTIMIZATION OF NANOEMULSION FORMULATION CONTAINING *Clinacanthus nutans* LINDAU LEAF EXTRACT FOR COSMECEUTICAL APPLICATION

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Chairman: Professor Mahiran Basri, PhD
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Plant-based cosmetics have gained more attention compared to chemical-based product in the cosmetic industry due to its safety and efficacy. However, the primary challenges are incorporation of plant based extract in the formulation owing to its hydrophobicity, instability against oxidation and also difficulties in formulating a stable carrier system. Palm kernel oil esters (PKOEs) with great skin-penetrating abilities were selected to be blended with non-ionic surfactants in the formulation process. The structure of this nanoemulsion is defined as dispersions of self-assembled PKOEs and surfactants molecules in water resulting from solubilization of surfactant micelles. A newly developed nanoemulsion system was designed to incorporate of *Clinacanthus nutans* (*C. nutans*) leaves extract for transdermal application. *C. nutans* or also known as Belalai Gajah is a well-known medicinal plant in tropical Asian countries that has recently attracted attention for its therapeutic characteristics. Numerous reports have documented the biological activity of *C. nutans*, including its anti-inflammatory, anti-viral, antioxidant and anti-cancer properties. Extract of *C. nutans* have been well explored for their potential as pharmaceutical agents. However, work on its use in cosmeceutical application is unknown.

Formulating nanoemulsion containing *C. nutans* extract has much promise as an effective delivery system and leads to the potential use of this plant extract in cosmeceutical application. Preparation of *C. nutans* leaves extract were obtained via sequential extraction and the optimization of the extraction process were carried out using Response Surface Methodology (RSM). The optimal condition suggested by the RSM model were extraction temperature of 60 °C, at 120 min with solvent ratio (water: ethanol) of 90: 10 v/v% which yielded 23.51% of extract. However, the insolubility of this extract in the methanol testing system has limited their accessibility to antioxidant assay. Thus, in this study extracts from sequential extraction with better solubility in testing system were chosen for further used. The properties of the extract showed their suitability to be used

as source of antioxidants and exhibited non-toxicity against fibroblasts cells (3T3) which established their safe properties.

Design of nanoemulsions system containing *C. nutans* extract involved several steps including screening of the level of variables, determination of the extract solubility and finally preparation of the nanoemulsions system. Under centrifugal force, all mixture of PKOEs: guava seed oil (GSO) in ratios of 9:1, 8:2 and 7:3 containing the extract did not show any precipitation at the bottom of the test tube. The ratio of 9:1 (PKOEs: GSO) was chosen as the suitable amount of oil mixture to be used in the formulation. The effect of composition on nanoemulsions; oil and surfactant on variation of particle size was investigated using Mixture Experimental Design (MED) and Artificial Neural Network (ANN). The nanoemulsion compositions predicted by different optimization methods were different. MED (labelled as CN1) suggested an optimal formulation containing 8.13% surfactant, 5.00% oil, 1.00% xanthan gum, 0.10% bioactive extract, 0.80% preservative, and 84.97% water to produce particles with a size of 97.38 nm. Meanwhile, ANN (labelled as CN2) suggested an optimal formulation containing 10.32% surfactant, 8.00% oil, 1.00% xanthan gum, 0.10% bioactive extract, 0.80% preservative, and 79.78% water to produce particles with a size of 125.40 nm. Although the particle size obtained by MED optimization was smaller than ANN, the residual standard error (RSE) for ANN was lower than MED which were 1.17% and 2.61%, respectively. This suggested that predicted and actual values of ANN had good correlation, implying that in this work ANN approach was an effective quantitative tool to be used in optimizing formulation design.

Physicochemical characterization and stability evaluation were conducted for both formulations. Based on the results, both formulations showed their suitability for transdermal applications. These formulations were found out to be in nano sized with good stability against phase separation. The formulations were also stable under storage temperature of 25 °C and 45 °C for 90 days, freeze thaw cycles and centrifugal force tests. No distinct changes were observed in the particle size of the ANN formulation, indicating that the nanoemulsion prepared was stable at the chosen optimum composition. However, the particle size of the MED formulation was slightly increased in size over the storage period, nevertheless it was still in the nano-sized range (less than 200 nm).

Transmission Electron Microscopy (TEM) images for both formulations showed the spherical shape of the oil droplets in the colloidal system and the encapsulation of the bioactive extract in the oil droplets. The optimal nanoemulsion had a shear-thinning behavior in both rheological experiments (steady state and oscillatory). This direct interaction fitted the Power Law Model indicating the pseudoplastic behavior in the system and gel structure existence which was one of the criteria in the formulation of transdermally applied cosmetics. Both formulations had pH values within the human skin range which was good for transdermal use. Taking into account the physicochemical characterization of both optimal nanoemulsions, ANN formulation labeled as CN2 was chosen to be the optimal composition in designing the *C. nutans* nanoemulsion. CN2 exhibited non-irritant property with a Human Irritancy Equivalent score of 0.13. In vivo ultrasound attributes of the skin study for CN2 showed that the collagen content increased significantly with the application of the *C.nutans* nanoemulsion among all 21 volunteers during the 21 d of the treatment period. The biophysical attributes of skin studies demonstrated that skin hydration increased without any increment in transepidermal water loss. Thus, a stable *C. nutans* nanoemulsion was successfully developed which had the ability to promote collagen production in human skin and improved the skin barrier function and hence could be potentially used as a system for the delivery of natural antioxidant in cosmetic products.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

REKABENTUK DAN PENGOPTIMUMAN FORMULASI NANOEMULSI YANG MENGANDUNGI EKSTRAK DAUN *Clinacanthus nutans* LINDAU BAGI KEGUNAAN KOSMESEUTIKAL

Oleh

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Kosmetik berasaskan tumbuhan mendapat lebih perhatian berbanding produk berasaskan bahan kimia di dalam industri kosmetik. Walaubagaimanapun, cabaran utamanya adalah menggabungan ekstrak tumbuhan ke dalam formulasi kerana kehidrofobikannya, mudah teroksida dan kesukaran untuk memformulasikan sebuah sistem pembawa yang stabil. Ester minyak isirong sawit (PKOEs) yang mempunyai kemampuan hebat menembusi kulit telah dipilih untuk digabungkan bersama surfaktan bukan ion di dalam proses formulasi. Struktur nanoemulsi ini ditakrifkan bilamana penyebaran pengumpulan PKOEs dan molekul surfaktan di dalam air hasil daripada kelarutan misel surfaktan. Sebuah sistem nanoemulsi baru telah dicipta dengan menggabungkan ekstrak daun *Clinacanthus nutans* (*C. nutans*) untuk aplikasi transdermal. *C. nutans* atau lebih dikenali sebagai Belalai Gajah adalah tumbuhan ubatan yang popular di negara Asia tropika yang sudah mula mendapat perhatian ramai kerana sifat-sifat terapeutiknya. Terdapat banyak kajian tentang aktiviti biologikal tumbuhan ini termasuk anti keradangan, antiviral, antioksidan dan anti kanser. Ekstrak *C. nutans* telah dikaji secara mendalam untuk kegunaan sebagai agen farmaseutikal. Walaubagaimanapun, kajian tentang kegunaannya dalam kosmeseutikal masih belum diketahui.

Formulasi ekstrak *C. nutans* dengan menggunakan sistem pembawa nanoemulsi yang efektif akan menjamin potensi aplikasi ekstrak tumbuhan ini untuk kegunaan kosmeseutikal. Penyediaan ekstrak daun *C. nutans* dilakukan melalui pengestrakkan berperingkat dan proses pengoptimuman ekstrak dijalankan menggunakan kaedah gerak balas permukaan (RSM). Kondisi optimum yang dicadangkan oleh model RSM adalah pengekstrakkan pada suhu 60 °C, selama 120 minit dengan nisbah pelarut (air: etanol) 90: 10 v/v% untuk memberikan hasil ekstrak sebanyak 23.51%. Walaubagimanapun, ketidaklarutan ekstrak ini di dalam sistem pelarut methanol telah menghadkan penilaian aktiviti antioksidan ekstrak. Maka, di dalam kajian ini ekstrak

daripada pengestrakkan berperingkat yang mempunyai keterlarutan yang lebih baik di dalam sistem pelarut methanol telah dipilih untuk digunakan dalam formulasi. Sifat-sifat ekstrak menunjukkan kesesuaiannya sebagai bahan antioksidan yang selamat untuk digunakan di mana ia mempamerkan kesan tidak toksik ke atas sel fibroblast (3T3).

Rekabentuk nanoemulsi yang mengandungi ekstrak *C. nutans* melibatkan beberapa langkah termasuk saringan julat pembolehubah-pembolehubah, mengenalpasti kelarutan ekstrak dan akhir sekali penyediaan sistem nanoemulsi. Di bawah daya empar, kesemua campuran PKOEs: minyak biji jambu batu (GSO) di dalam nisbah 9:1, 8:2 dan 7:3 yang mengandungi ekstrak tidak menunjukkan sebarang mendakan di dasar tabung uji. Nisbah 9:1 (PKOEs:GSO) telah dipilih sebagai amaun sesuai campuran minyak yang akan digunakan dalam formulasi. Kesan komposisi nanoemulsi; minyak dan surfaktan ke atas perubahan saiz zarah di kaji menggunakan rekabentuk campuran eksperimen (MED) dan rangkaian neural tiruan (ANN). Komposisi nanoemulsi yang diramal menggunakan kaedah pengoptimuman berbeza memberikan ramalan yang berbeza. Kaedah MED (dilabelkan sebagai CN1) meramalkan formulasi optimum mengandungi 8.13% surfaktan, 5.00% minyak, 1.00% gam xantan, 0.10% ekstrak bioaktif, 0.80% pengawet, dan 84.97% air untuk menghasilkan zarah bersaiz 97.38 nm. Manakala, ANN (dilabelkan sebagai CN2) meramalkan formulasi optimum mengandungi 10.32% surfaktan, 8.00% minyak, 1.00% gam xantan, 0.10% ekstrak bioaktif, 0.80% pengawet, dan 79.78% air untuk menghasilkan zarah bersaiz 125.40 nm. Walaupun saiz zarah yang dihasilkan dengan kaedah pengoptimuman MED lebih kecil daripada ANN, ralat piawai residu (RSE) menggunakan ANN lebih rendah berbanding MED dengan nilai 1.17% dan 2.61% masing-masing. Ini menunjukkan anggaran nilai eksperimen dan nilai sebenar menggunakan ANN mempunyai hubungkait yang tepat, membuktikan dalam kajian ini kaedah ANN adalah kaedah kuantitatif yang efektif untuk pengoptimuman rekabentuk formulasi.

Pencirian fizikokimia dan penilaian kestabilan telah dijalankan untuk kedua-dua formulasi. Berdasarkan hasil keputusan, kedua-dua formulasi menunjukkan kesesuaiannya untuk kegunaan transdermal. Kedua-dua formulasi ini didapati bersaiz nano dan mempunyai kestabilan yang baik mencegah pemisahan fasa. Kedua-dua formulasi ini juga stabil di bawah penyimpanan pada suhu 25 ° C dan 45 ° C selama 90 hari, ujian beku-cair dan ujian daya empar. Tiada perubahan ketara diperhatikan ke atas saiz zarah formulasi ANN, menunjukkan formulasi yang disediakan adalah stabil pada komposisi optimum yang telah dipilih. Walaubagaimanapun, saiz zarah formulasi MED sedikit bertambah semasa tempoh penyimpanan, namun masih di dalam julat saiz nano (kurang daripada 200 nm).

Imej Mikroskop Elektron Transmisi (TEM) untuk kedua-dua formulasi menunjukkan titisan minyak yang berbentuk sfera di dalam sistem koloid dan pengkapsulan ekstrak bioaktif di dalam titisan minyak. Nanoemulsi optimal mempunyai sifat ricih penipisan di dalam kedua-dua eksperimen reologi (keadaan stabil dan ayunan). Perkadaran terus ini mematuhi model Hukum Power menandakan sistem bersifat pseudoplastik dengan pembentukan struktur gel yang merupakan kriteria untuk kegunaan transdermal. Kedua-dua formulasi juga mempunyai nilai pH di dalam julat kulit manusia yang sesuai untuk kegunaan transdermal. Dengan mengambil kira pencirian fizikokimia ke

atas kedua-dua formulasi optimal, formulasi ANN yang dilabelkan sebagai CN2 telah dipilih sebagai komposisi optimal di dalam rekabentuk nanoemulsi *C. nutans*. CN2 mempamerkan sifat tidak merengsa dengan skor 'Human Irritancy Equivalent' pada 0.13. Berdasarkan penggunaan CN2, kajian in vivo ultrabunyi kulit menunjukkan kandungan kolagen bertambah secara signifikan ke atas 21 orang sukarelawan sepanjang 21 hari tempoh rawatan. Kajian biofizikal kulit menunjukkan penghidratan kulit meningkat tanpa sebarang kenaikan kehilangan transepidermal air. Maka, nanoemulsi *C. nutans* yang stabil telah berjaya di bangunkan yang berkemampuan untuk meransang penghasilan kolagen kulit manusia dan memperbaiki fungsi penghalang kulit serta berpotensi sebagai sistem pembawa antioksidan semulajadi di dalam produk kosmetik.

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I certify that a Thesis Examination Committee has met on 15 June 2017 to conduct the final examination of Intan Soraya binti Che Sulaiman on her thesis entitled "Design and Optimization of Nanoemulsion Formulation Containing *Clinacanthus nutans* Lindau Leaf Extract for Cosmeceutical Application" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAD	Minimum average absolute deviation
AAPH	2,2'-Azobis (2-methyl propionamidine) dihydrochloride
ANN	Artificial neural network
ANOVA	Analysis of variance
AUC	Area under the curve
BBP	Batch back propagation
BCB	ß-Carotene bleaching assay
C. nutans	*Clinacanthus nutans*
DLS	Dynamic light scattering
DPPH	1,1-Diphenyl-2-picrylhydrazyl
ECM	Extracellular matrix
EFA	Essential fatty acids
EPP	Entry point project
ETP	Economic transformation programme
FCS	Fetus calf serum
FDA	Food and drug administration
GA	Genetic algorithm
GAE	Gallic acid equivalent
GSO	Guava seed oil
HIE	Human irritancy equivalent
HLB	Hydrophilic lipophilic balance
HPLC	High performance liquid chromatography
IBP	Incremental back propagation
IC_{50}	Concentration of sample that caused inhibition of 50% cell growth
IPL	Intense pulsed light
IUPAC	International Union of Pure and Applied Chemistry
LC-MS	Liquid chromatography-mass spectrometry
LM	Levenberg- marquardt
MD	Molecular dynamics
MED	Mixture experimental design
MLP	Multilayer perceptron
MMPs	Matrix metalloproteinases
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
NKEAs	National key economic areas
O/W	Oil-in-water
OD	Optical density
ORAC	Oxygen radical absorbance capacity
OTC	On-the-counter
PKOEs	Palm kernel oil esters
PVPP	Polyvinylpolypyrrolidone
QP	Quick propagation
R&D	Research and development
RE	Rutin equivalent
RF	Radiofrequency
RMSE	Root mean squared error
ROS	Reactive oxygen species
RSE	Residual standard error
S80	Sorbitan monooleate

SB	Stratum basal
SC	Stratum corneum
SG	Stratum granulosum
SS	Stratum spinosum
T20	Polyoxyethylene (20) sorbitan monolaurate
T80	Polyoxyethylene (20) sorbitan monooleate
TE	Trolox equivalent
Teq	Alpha tocopherol equivalent
TEWL	Transepidermal water loss
TFC	Total flavonoid content
TMR	Transparency market research
TPC	Total phenolic content
Trolox	6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid
TTC	Total tannin content
U.S.	United States
UV	Ultraviolet
UVB	Ultraviolet B
W/O	Water-in-oil

CHAPTER 1

INTRODUCTION

1.1 Background

By definition, a cosmeceutical is a hybrid category of products lying on the spectrum between drugs and cosmetics (Kligman, 2005). A cosmeceutical product does not merely satisfy the need for beauty but is also enhanced with medicinal or druglike benefits through its biologically active ingredients (Gao *et al*., 2008). The unique ability of cosmeceutical products is their ability to enhance and beautify the skin appearance from inside out. These functioning ingredients able to penetrate and alter the skin structure by improving skin functionality or texture, protect the skin from harmful effect, maintain the skin hydration and remove minor scar and pigmentation (Bissett, 2009). Cosmeceutical actives are potent substances that may provide a biologic effect to skin. These substances may source from synthetic or plant-based elements. Cosmeceutical products also have been reported to have side issues such as skin irritation and toxicity (Draelos, 2009; Gao *et al*., 2008). Efforts have been focused on preparing green formulation which is safer than artificial sources where the source of the active ingredients is mainly from the plant kingdom. The advantage of using plant extract instead of pure compounds is one single plants extract may have a diversity of phytochemical properties such as anti-inflammatory, antioxidant, anti-bacterial, and whitening agent (Bissett, 2009). However, the uses of many active plant-based ingredients have been restricted to poor water solubility, low bioavailability and instability against oxidation and degradation by light.

Consumers are now well educated and demand sophistication in the products that they purchase. This requirement has led to the design of multifunctional cosmetic products which could provide a visible improvement in skin appearances instead of just scent and color to the skin (Draelos, 2009, 2011). In order to formulate a functioning cosmeceutical product mainly for transdermal use, the focus has to be on the ability of the active ingredients to deliver their biologically active forms adequately to the skin. Therefore, an effective carrier vehicle should be addressed to assume the applied product to reach the target site and works efficiently (Draelos, 2011).

One of the most popular use for cosmeceutical products is in improving the appearance of aged skin. Hectic lifestyles and environmental pollution are some of the reasons for cutaneous aging and this is mainly due to the oxidation of skin structure from the generated highly reactive oxygen molecules (Brandt *et al*., 2011). Cosmeceutical antioxidants of plant origin having the advantage of possessing non-irritating ingredients are indeed the best alternative to protect the skin from radiation and oxidation damage and at the same time, are able to improve the appearance of the skin. Malaysia's rainforests are well known for their wealth of plant biodiversity. About 12,000 species of flowering plants have been reported in Malaysian forests, and up to 1,300 species have been identified as having therapeutic potential (Jamal *et al*., 2010). Owing to their beneficial attributes, wide acceptance, and growing awareness of healthcare, green formulations have been of increasing interest among stakeholders, including wide-spread interest in developing cosmeceutical products containing plant-derived sources. Moreover, there has been rising concern on aging and photo damage leading to skin diseases in the population worldwide. The combination of these factors has caused one of the fastest growth of cosmeceutical products in the personal care industry, which mainly involved products designed to diminish visible signs of aging (Brandt *et al*., 2011).

Clinacanthus nutans (*C. nutans*) belonging to the Acanthaceae family, is an herbal plant widely used in folk medicine. This plant is commonly known in Malaysia as Sabah Snake Grass or Belalai Gajah and is widely distributed in tropical Asian countries such as Malaysia, Indonesia and Thailand. Various reports have documented the biological activity of *C. nutans*, including its antioxidant (Yuann *et al*., 2012), anti-inflammatory (Wanikiat *et al*., 2008), anti-viral (Direkbusarakom *et al*., 1998; Kunsorn *et al*., 2013; Yoosook *et al*., 1999) and anti-cancer properties (Yong *et al*., 2013).

Previous investigation has established the presence of numerous polyphenols such as isomollupentin-7-*O*-beta-glucopyranoside, vitexin, isovitexin, shaftoside, orientin, isoorientin, 4-vinylphenol, 7-hydroxyflavone and 2,6-dimethoxyphenol from the leaves extract of *C. nutans* (Mustapa *et al*., 2015; Teshima *et al*., 1997). Furthermore, many plants are rich in endogenous antioxidants as they must survive in an environment that is constantly exposed to ultraviolet radiation (Draelos, 2009) and thus have potential to be used as an alternative to synthetic antioxidants.

In line with the Economic Transformation Programme (ETP) as announced in the 10th Malaysia Plan, towards transforming Malaysia into a high income country by 2020; ETP was embodied by the 12 National Key Economic Areas (NKEAs). Agriculture was one of the selected industries chosen due to its potential economic growth and development. Their entry point project (EPP) consisted of capitalizing on the potential of Malaysia to become a supplier of world-class, high quality nutraceutical and cosmeceutical products by leveraging the country's natural biodiversity. Efforts have therefore been focused on improving product quality including research and development (R&D) and clinical trials using Malaysia's native plants. *C. nutans* or also known as Belalai Gajah which was included in the ten leading herbs identified as potential plants for R&D (PEMANDU, 2013).

1.2 Problem Statement

Formulating a product with the beneficial attributes of the bioactive ingredients has been challenging due to various reasons including poor solubility and low bioavailability which may hinder effective transdermal delivery. Moreover, antioxidant bioactive substances are naturally unstable, and easy to oxidise and degrade when exposed to the light (Khatri *et al*., 2010). An effective transdermal application requires the product to pass through the skin's inner layers successfully. However, as the biggest organ of the body, the skin's barrier presents a great challenge for the efficient delivery of therapeutics to the skin dermis. The stratum corneum located on the outer layer of the skin, prevents the loss of internal body components, particularly water to the external environment (Escobar-chávez *et al*., 2012). Thus, an effective delivery system was crucial in order to increase the therapeutic effects in transporting agents to target locations by considering the side effects.

Emulsion stability is a primary concern for formulation design. In nanoemulsion development systems, the utmost challenge has been to maintain the particle size in the nanometer range while remaining physically stable for a period of time. Therefore, the composition of formulations developed is very important in order to obtain stable nanoemulsions with characteristics suitable for transdermal use. This process has been time consuming as the factors affecting interactions between each component had to be individually determined. Thus, well-designed data collection processes through optimization using multivariate statistical technique would allow the experiments to achieve high success rates.

1.3 Scope of Study

This study focused on the design and optimization of nanoemulsion systems containing extract of *C. nutans* leaves for cosmeceutical application. The aim was to capitalize on the antioxidant biological activity of *C. nutans* leaves extract, in order to improve human skin texture. The work involved two stages of optimization processes. The earlier stage was optimization of the extraction process using Response Surface Methodology, to obtain optimal extract with highest of antioxidant activity. The work was then further carried out to find the optimal composition for formulation by using two optimization methods (Mixture Experimental Design and Artificial Neural Network). The developed formulation was evaluated for its physicochemical properties and efficacy on skin.

This study was limited to the amount of *C. nutans* extract of 1g/kg of formulation (0.1% w/w). The acute toxicity study of ethanol extract of *C. nutans* leaves at concentrations as high as 1.3 g/kg of body weight did not exhibit toxicity in the mice model (Chavalittumron *et al*., 1995). The use of 1.3 g/kg of *C. nutans* ethanol extract for mice was reported to be equivalent to 5.44 g/kg of body weight for humans (Kamarudin *et al*., 2017). Moreover, sub-chronic oral administration (90 days) of *C. nutans* demonstrated that 1 g/kg body weight in rats showed no any abnormalities of their internal organs (equivalent to 4.18 g/kg of body weight in humans) (Chavalittumrong *et al*., 1995). In sub-acute toxic effect, after 14 days of oral administration with methanol extract of *C. nutans* leaves (0.3 g/kg, 0.6 g/kg and 0.9 g/kg) did not cause any adverse effects and organ damages in Sprague Dawley rats (P'ng *et al*., 2013). Furthermore, the acute oral toxicity study of *C. nutans* extract demonstrated that acute exposure of 1.8 g/kg of *C. nutans* was safe in mice without causing any adverse effects or mortality. The oral LD_{50} of methanol leaves extract of *C. nutans* was suggested to be greater than 1.8 g/kg body weight in male mice (P'ng *et al*., 2012).

1.4 Objectives

The specific objectives of this work are:

1) To optimize the extraction process of *C. nutans* leaves.
2) To determine the antioxidant activity of the extract.
3) To conduct optimization studies of the formulation compositions of nanoemulsion systems containing extract of *C. nutans* leaves.
4) To characterize the physicochemical properties of the newly developed nanoemulsions.
5) To evaluate the safety of the nanoemulsions and their biophysical attributes to human skin for transdermal use.

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