VIRGIN COCONUT OIL BASED
NANOSTRUCTURED LIPID CARRIER LOADED WITH
FICUS DELTOIDEA EXTRACT FOR SKIN BARRIER IMPROVEMENT

NOR AZRINI NADIHA BT AZMI

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To the most special ones, mama and ah.
ACKNOWLEDGEMENT

In the name of ALLAH, the Most Gracious and The Most Merciful, who showered me with his blessings and giving me strength to finally complete this study.

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**ABSTRACT**

*Ficus deltoidea* (FD) is a medicinal herb which had been discovered to possess antioxidant and anti-inflammatory activities. Thus, it has the potential to be a good active ingredients for improving skin barrier function. Nanostructured lipid carrier (NLC) is a promising delivery system to transport FD extract through the skin as it is composed of biocompatible lipids, which are similar to the intercellular part of the stratum corneum. The objective of this research is to formulate and characterize physicochemical and biological properties of virgin coconut oil (VCO) based NLC loaded with FD extract to improve skin barrier function. The FD loaded NLC (FDNLC) formulation was prepared using melt emulsification homogenization technique with glycerol monostearate as solid lipid, VCO as liquid lipid and combination of Tween 80 and soy lecithin as surfactant. The delivery system had the particle size of 158.033\(\pm\)10.53 nm, polydispersity index of 0.153\(\pm\)0.06, zeta potential of \(-42.32\pm1.54\) mV, and encapsulation efficiency of 87.401\(\pm\)7.33%. Transmission electron microscopy images indicated that the prepared NLCs were spherical in shape with even size distribution. The formulation was stable for at least 40 days. Diffusion experiments showed that FDNLC diffused through the skin had the steady state flux of 12.727 mg/cm\(^2\)h, lag phase of 0.023 h, and permeability coefficient of 0.098 cm/h. The results showed better penetration in FDNLC compared to non-encapsulated FD. The skin test study in term of transepidermal water loss, hydration, elasticity, melanin and erythema showed significant improvement between week 0 and week 4 with the usage of cream loaded with FDNLC. Taken together, the formulation of FDNLC can be concluded to be efficient in improving skin barrier function.
**ABSTRAK**

*Ficus deltoidea* (FD) adalah sejenis ubatan herba yang telah dikenal pasti untuk mempunyai aktiviti antioksidan dan anti-radang yang tinggi. Ini menjadikan ia sesuai sebagai bahan aktif untuk meningkatkan fungsi rintangan kulit. Pembawa lipid nanostruktur (NLC) adalah pembawa yang sesuai untuk ekstrak FD memandangkan ianya terdiri daripada lipid bioserasi sama sifat dengan bahagian ruang antara sel pada lapisan *stratum corneum*. Objektif penyelidikan ini adalah untuk merumuskan dan mencirikan sifat fizikokimia dan sifat biologi NLC yang dimuatkan dengan ekstrak FD berasaskan minyak kelapa dara (VCO) untuk meningkatkan fungsi rintangan kulit. FD yang dimuatkan ke dalam NLC (FDNLC) telah disediakan menggunakan teknik penghomogenen pengemulsi cecair dengan menggunakan gliserol monostearat sebagai lipid pepejal dan VCO sebagai lipid cecair serta gabungan Tween 80 dan lestitin soya sebagai surfaktan. Sistem penghantaran ubat ini mempunyai ciri-ciri berikut iaitu saiz partikel 158.033 ± 10.53 nm, indeks poliselerakan 0.153 ± 0.06, potensi zeta -42.32 ± 1.54mV dan kecekapan pengkapsulan 87.401 ± 7.33%. Imej NLC daripada mikroskop elektron penghantaran menunjukkan partikel NLC berbentuk sfera dengan agihan saiz yang sekata. Formulasi ini stabil dalam tempoh sekurangnya selama 40 hari. Kajian resapan menunjukkan FDNLC yang diresap ke dalam kulit mempunyai fluks berkeadaan mantap 12.727 mg/cm2/jam, fasa lag 0.023 jam dan pekali kebolehtelan 3.19 ± 0.098 cm/jam. Keputusan kajian menunjukkan FDNLC adalah lebih bagus dari segi penyerapan berbanding FD yang tidak dienkapsulasi. Kajian kulit pula mendapati kehilangan transepidermal air, hidrasi, keanjalan kulit, melanin dan eritema kesemuanya menunjukkan peningkatan yang signifikan antara minggu kosong dan ke empat dengan penggunaan krim FDNLC. Kesimpulannya, formulasi FDNLC adalah efisien untuk meningkatkan fungsi rintangan kulit.
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Percentage change of melanin value after 4 weeks of application

Percentage change of erythema value after 4 weeks of application
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<tr>
<td>DL</td>
<td>Drug loading</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified Eagle’s medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-diphenyl-2-picrylhydrazyl</td>
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<tr>
<td>EE</td>
<td>Encapsulation efficiency</td>
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<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
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<td>FD</td>
<td><em>Ficus deltoidea</em></td>
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<td>FDNLC</td>
<td><em>Ficus deltoidea</em> loaded Nanostructured Lipid Carrier</td>
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<tr>
<td>GCMS</td>
<td>Gas chromatography mass spectroscopy</td>
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<td>GMS</td>
<td>Glyceryl monostearate</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
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<td>SLN</td>
<td>Solid lipid nanoparticles</td>
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<td>VCO</td>
<td>Virgin Coconut Oil</td>
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<td>ZP</td>
<td>Zeta potential</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>μg/ml</td>
<td>Microgram per millilitre</td>
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<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>°C</td>
<td>Degree celcius</td>
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<tr>
<td>mg/ml</td>
<td>Milligram per millilitre</td>
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<td>μL</td>
<td>Microlitre</td>
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<td>nm</td>
<td>Nanometer</td>
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<tr>
<td>mM</td>
<td>Millimolar</td>
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<td>μM</td>
<td>Micromolar</td>
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<td>mm</td>
<td>Millimetre</td>
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<tr>
<td>ml</td>
<td>Millilitre</td>
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<td>mV</td>
<td>Millivolts</td>
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<td>g</td>
<td>Gram</td>
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<tr>
<td>rpm</td>
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1.1 Background of Study

Skin is the largest organ in human which covers almost the whole body. The skin is a protective barrier against external mechanical, chemical, microbial and physical harms (Desai et al., 2010). It is made up of three layers: epidermis, dermis and hypodermis. Stratum corneum (SC) is the outermost layer of epidermis and it is very important in permeation of drugs into our body. SC acts as formidable barrier to dermal absorption which specifies the rate of dermal penetration (Eman et al., 2016). SC is also the barrier to loss of body water. In addition, SC produces a protective shield against the admission of harmful materials, such as bacteria or allergens, from the surrounding environment. This barrier fulfills an entire set of critical defensive functions including deflecting the ultraviolet light, refining most of it before it penetrates even deeper into the skin.
Luebberding et al. (2012) stated that the skin barrier function is the natural boundary between the inner organism and the surroundings, and this function is mainly formed by the epidermis. However, skin barrier function could be depleting due to many factors such as chronic stress. Animal studies performed by Denda et al. (1998; 2000) proved that stress can weaken the regaining of skin barrier function from barrier disruption by tape stripping. A study by Altemus et al. (2001) proposed the deterioration of skin permeability barrier homeostasis caused aggravation of inflammatory skin diseases.

Skin barrier disruption is related with both an increase in keratinocyte proliferation (Proksch et al., 1991; Denda et al., 1996) and an increase in production of inflammatory cytokine in the local tissue (Nickoloff and Naidu, 1994; Wood et al., 1997). Both of these consequences could probably worsen inflammatory skin diseases such as psoriasis, eczema, and atopic dermatitis, and also aggravate contact dermatitis (Altemus et al., 2001). Besides, the skin barrier function could be depleted as it is exposed to various exogenous sources of oxidative stress, such as ultraviolet radiation and pollutants. Reactive oxygen species (ROS) and other free radicals are generated in the skin as response to these oxidative attacks, (Dreher and Maibach, 2001). To curb the harmful effects of ROS, antioxidants is present in the skin and is responsible for the balance between pro-oxidants and antioxidant (Thiele et al., 2000). An impairment of this balance, due to an increased exposure to exogenous sources of ROS, has been defined as “oxidative stress” and involves oxidative damages of lipids, proteins and DNA (Sies, 1985). On top of that, age is one of the factors which contributes to the declining of skin barrier functions. Although researches by Ghadially et al. (1995); Roskos and Guy (1989), showed that there is no change in water loss with ageing, barrier in aged skin has been claimed to be more vulnerable to penetration. The susceptibility to damage increased and there is setback in barrier recovery (Ghadially et al., 1995).

*Ficus deltoidea* (FD) or widely known as Mas Cotek in Malaysia is a plant originated from Moraceae family. It had been traditionally used as medicine for many diseases including diabetes and relieving pain. Ramamurthy et al., (2014) stated that
FD is also used for migraines, skin diseases, and diarrhea, pneumonia and heart problems. The active compounds in the extract of the plant are proven to possess anti-inflammatory, antinociceptive, anti-cancer, anti-oxidant and antiphotoaging effects (Bunawan et al., 2014). Haron (2014) reported that among the chemicals found in FD are naringin and polyphenols which are useful for anti-inflammatory effect. There are many mechanisms that are responsible for the anti-inflammatory activity based on the phytochemical compound found in FD. Meotti et al. (2005) found out flavonoids could inhibit protein kinase C and L-arginine/NO pathways. FD also possessed high antioxidant activity. Tannins present in FD might be responsible in preventing lipid oxidization as a reducing agent, free radical scavengers and chelators of pro-oxidant catalytic metals. Tannins are tightened over relaxed tissue, to dry excessive watery secretion and protects damage tissues like eczema or burnt (Ramamurthy et al., 2014).

Many studies were done on the application of FD, for instance, a study by Abdula et al., (2010) showed enhancement in wound healing in rats treated with FD. The results had proved the effectiveness of FD extract to speed up the rate of wound healing enclosure in the experimentally-induced wounds in rats. Along with that, a study by Hasham et al., (2013) proposed that FD extract prevents the inflammatory cytokines production and might be an efficient protective agent to cure skin diseases. The research concluded that FD extract may be beneficial against UVB induced skin damage and is functional for anti-photoaging cosmetic products.

However, using plant extracted with water or ethanol had some limitations. In which the delivery of the extracts is slow and poor dosing of the extracts in human body (Caldorera-Moore and Peppas, 2009). It is also difficult for biologically active water-soluble extracts to permeate through human skin which naturally has hydrophobic characteristics. Another setback of using plant extract is the bioactive substances are unstable when exposed to light and could lose activity during storage (Vinardell and Mitjans, 2015).
Virgin coconut oil (VCO) is defined as the oil resulting from the fresh and mature kernel of the coconut (*Cocos nucifera L.*) through mechanical and natural means, whether with the use of heat or not, as long as it does not lead to alteration of the oil (Mansor *et al.*, 2012). Recently, VCO is recognized for its ample advantages. Marina *et al.* (2009a) confirmed VCO has higher antioxidant activity than refined coconut oil. A research by Nevin and Rajamohan (2006) had shown that VCO is capable in enhancing anti-oxidant enzymes activity and inhibition of lipid peroxidation in mice. In cosmetics industry, VCO is an excellent ingredient which functions as a skin moisturizer and softener (Rohman and Man, 2009). Kim *et al.* (2017) demonstrated that the oil increased expression of cornified envelopes (CE) components, thereby contributing to protective barrier functions of the SC. In addition, VCO had been used as co-carrier for nanostructured lipid carrier (NLC) system in several studies (Pamudji *et al.*, 2016; Rosli *et al.*, 2014). This is because with the listed quality it has, VCO does not only act as liquid lipid in NLC but also complements the system excellently.

Some procedures have been examined to increase the penetration of therapeutic molecules through the skin (Desai *et al.*, 2010). Solid lipid nanoparticles (SLN) were developed at early 1990s as a possible substitute carrier system to emulsions, liposomes and polymeric nanoparticles (Muller *et al.*, 2002). However, some problems were identified in SLN which are drugs load are too low and drug expulsion could happen during storage (Pardeshi *et al.*, 2012). Nanostructured lipid carrier system (NLCs) is composed of liquid lipid blended with a solid lipid to form a nanostructured solid particle matrix. Liquid lipid in NLC is used to alter the formation of SLN. This structure enhances loading of the drug and incorporates the drug securely during storage. However, there is not much study on association of lipid nanocarriers with plant medicine in the bio-nanotechnology field (Lacatusu *et al.*, 2014). With the health advantages and improved delivery features it brings, usage of suitable renewable vegetable sources is highly possible to comply with both industrial and customer demands. Final solid lipid core should contain structural imperfections to increase the incorporation of the drugs into the system. The system has been portrayed as an effective system to enhance skin hydration, due to their physiological lipid composition and occlusive effect properties (Tichota *et al.*, 2014). The small size of
NLCs ensures a close contact to the stratum corneum and can increase the amount of the active compound penetrating the skin (Li and Ge, 2012). NLCs are novel drug delivery systems for the delivery of actives with high solubility, stability, powerful skin penetration, and low skin discomfort (Muller et al., 2007).

NLC incorporated with FD could be a promising treatment for improving skin barrier function. The incorporation of VCO as liquid lipid further added the value of the system. Therefore, the combination of all the powerful ingredients supposed to give good effect to skin in term of barrier function

1.2 Problem Statement

The function of skin barrier is to act as natural boundary between the inner organism and the environment. Nevertheless, the skin barrier function could decline due to several factors such as aging and stress. When the skin barrier function is damaged or depleted by application of harsh products, handling, or environmental conditions, the skin loses water and gets dried out faster, thus becomes more permeable to irritants and allergens. Once irritants or allergens penetrate the epidermis, they may trigger inflammation and this would lead to many skin problems such as dry skin, atopic dermatitis and psoriasis.

There are currently a lot of treatments and ingredients available in the market which could improve skin barrier function. However, the ingredients such as corticosteroids sometimes might also give off side effects to the body such as inflammation, wrinkling and thinning of skin to the site of application (Abraham and Roga, 2014). As these side effects are of synthetic drugs, there is a need for safer and efficient treatment of these conditions. So, natural actives from herbs and plants could be an alternative for the treatment. This is because they produce no or fewer side
effects compared to synthetic drugs. In addition, natural drugs from the plants are gaining popularity because of several advantages such as better patient tolerance, being relatively less expensive and acceptable due to a long history of use (Tabassum and Hamdani, 2014). Also, an increased reliance on the use of medicinal plants in industrialized societies can be attributed to the extraction and development of a number of drugs and chemotherapeutics from plants, as well as herbal remedies. In industrial societies, herbal remedies have become more common in the treatment of minor ailments, due to increasing of cost of maintaining personal health (Hoareau and Dasilva, 1999). Other than that, Malaysia had an extensive variety of herbs and plants which possessed a lot of phytochemical constituents that has not been fully explored. It would be a waste if local researchers did not utilize the existing treasure in the country.

In this research, FD, a Malaysian herbs had been chosen to be encapsulated into NLCs as it had shown no toxicity and it is rich in anti-inflammatory and antioxidant compounds. It had been proved that FD can induce wound healing and act as protective agent in healing skin diseases. However, FD extract exhibits hydrophilic behavior which will make it difficult to penetrate human skin which are lipophilic in nature. To overcome this problem, NLC which are lipophilic will be used to encapsulate FD and it is expected to penetrate well into the human skin. NLCs had been chosen because of its efficacy to deliver the active ingredients with high solubility, stability, powerful skin penetration, and low skin discomfort. Furthermore, the small size of NLCs will establish a close contact to the stratum corneum as well as improve the quantity of the active compound penetrated into the skin. To improve the system, virgin coconut oil (VCO) is used as the liquid lipid in NLC system. VCO is highly known for its antioxidant effects and as moisturizer. The unique properties of VCO as co-carrier, excellently complement NLC system. In addition, coconut oil is proven to increase expression of cornified envelope (CE) components which could contribute in improving skin barrier function. Therefore, with the listed advantages, VCO based NLC loaded FD extract is highly potential to enhance skin barrier function.
1.3 Objective

The objective of this research is to formulate and characterize physicochemical and biological properties of Virgin Coconut Oil based Nanostructured Lipid Carrier loaded with *Ficus Deltoidea* extract to improve skin barrier function.

1.4 Scope of Research

In order to achieve the objective of the study, the following scopes have been identified as below:

1. Preparation of Nanostructured lipid carrier based on VCO as co-carrier system using melt emulsification homogenization technique.
2. Encapsulation of FD extract into VCO based NLC.
4. Study on the penetration ability of the formulation of VCO based NLC loaded with FD extract using Franz type diffusion cell.
5. Evaluation of stability of VCO based NLC loaded with FD extract at three different temperatures (4, 25 and 40°C) in term of size and PDI.
6. Analysis of topical cream incorporated with VCO based NLC loaded FD extract on skin barrier function, stratum corneum hydration, erythema, elasticity, pH and melanin index using multifunctional skin physiology monitor.
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