

สารสกัดว่านชักมดลูกที่กักเก็บในระบบตัวพาไขมันแข็งขนาดนาโนเมตร เพื่อเพิ่มการซึมผ่านผิวหนัง

ดำรงศักดิ์ จินารัตน์¹, บัญชา ยิ่งงาม², วันดี รังสิจิตรประภา^{3*}

¹ นักศึกษาระดับปริญญาตรี สาขาหลักสูตรเภสัชศาสตร์ดุสิตบัณฑิต คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี

² ปร.ด. (เภสัชศาสตร์) ผู้ช่วยศาสตราจารย์ กลุ่มวิชาเภสัชเคมีและเทคโนโลยีเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี

³ Dr.rer.nat. (Pharmaceutical Technology) รองศาสตราจารย์ กลุ่มวิชาเภสัชเคมีและเทคโนโลยีเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี

* ติดต่อผู้พิมพ์: รศ.ดร.วันดี รังสิจิตรประภา กลุ่มวิชาเภสัชเคมีและเทคโนโลยีเภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี อำเภอวารินชำราบ จังหวัดอุบลราชธานี โทร. 045-353630 อีเมลล์: wandeer@ubu.ac.th; Orcid ID: <https://orcid.org/0000-0001-8382-618X>

บทคัดย่อ

สารสกัดว่านชักมดลูกที่กักเก็บในระบบตัวพาไขมันแข็งขนาดนาโนเมตร เพื่อเพิ่มการซึมผ่านผิวหนัง

ดำรงศักดิ์ จินารัตน์¹, บัญชา ยิ่งงาม², วันดี รังสิจิตรประภา^{3*}

ว. เภสัชศาสตร์อีสาน 2562; 15(4) : 96-111

รับบทความ : 12 มิถุนายน 2562

แก้ไขบทความ: 22 กรกฎาคม 2562

ตอบรับ: 2 สิงหาคม 2562

ว่านชักมดลูกประกอบด้วยสารไฟโตเอสโตรเจนที่มีรายงานถึงฤทธิ์ทางชีวภาพหลายประการ แต่การศึกษาเพื่อใช้ทางผิวหนังยังมีอยู่น้อย และยังไม่มียารักษาถึงศักยภาพการเป็นเวชสำอางอย่างเป็นระบบ ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อทดสอบฤทธิ์ทางชีวภาพต่อผิวหนัง รวมถึงพัฒนาตัวพาไขมันแข็งขนาดนาโนเมตร (NLC) เป็นระบบนำส่งสารสกัดว่านชักมดลูกผ่านผิวหนัง วิธีดำเนินการวิจัย: ทำการสกัดผงของว่านชักมดลูกในสารละลายเฮกเซนด้วยเครื่อง Soxhlet ศึกษาฤทธิ์ทางชีวภาพต่อผิวหนังของสารสกัด ได้แก่ ฤทธิ์ต้านออกซิเดชัน ฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนส และ ฤทธิ์ยับยั้งเอนไซม์คอลลาจีเนส แบบนอกกาย พัฒนา NLC โดยศึกษาปัจจัยชนิดไขมันแข็ง น้ำมัน และสารเพิ่มการซึมผ่านผิวหนัง ต่อคุณลักษณะอนุภาค ร้อยละการกักเก็บสารสกัด สมบัติการเปลี่ยนแปลงทางความร้อน การซึมผ่านผิวหนังนอกกาย ด้วย Franz diffusion cell และทดสอบเสถียรภาพของตำรับ ผลการวิจัย: สารสกัดว่านชักมดลูกที่ได้ มีลักษณะเป็นสารกึ่งแข็ง ประกอบด้วยสารไฟโตเอสโตรเจนในปริมาณสูง แสดงฤทธิ์ทางชีวภาพต่อผิวหนังค่อนข้างอ่อนเมื่อเทียบกับกลุ่มควบคุม เมื่อพัฒนาเป็นระบบ NLC มีขนาดอนุภาคอยู่ในช่วง 120 – 170 นาโนเมตร ดัชนีการกระจายขนาดที่ 0.25 - 0.29 ศักย์ไฟฟ้าที่ผิวอนุภาคประมาณ -20 มิลลิโวลต์ กักเก็บสารสำคัญมากกว่าร้อยละ 90 และมีเสถียรภาพที่ดีเมื่อเก็บที่อุณหภูมิ 25 และ 40 องศาเซลเซียส เป็นเวลา 90 วัน หลังจากทำการทดสอบ 24 ชั่วโมง สารเพิ่มการซึมผ่านผิวหนังสามารถช่วยการซึมผ่านของสารสำคัญ DA2 ได้ 30 – 57 ไมโครกรัมต่อตารางเซนติเมตร คุณสมบัติการเป็นสารไม่ละลายน้ำของสารสกัดว่านชักมดลูกเป็นข้อจำกัดของการนำส่งสารสำคัญ ผ่านผิวหนัง และสาร DA2 ส่วนใหญ่จะสะสมที่ชั้นหนังกำพร้าของผิว สรุปผลการวิจัย: ระบบ NLC ที่กักเก็บสารสกัดว่านชักมดลูกมีศักยภาพในการพัฒนาเป็นระบบนำส่งสารไฟโตเอสโตรเจนเพื่อพัฒนาเป็นตำรับนำส่งทางผิวหนังที่ออกฤทธิ์เฉพาะที่ได้

คำสำคัญ: ว่านชักมดลูก, ตัวพาไขมันแข็งขนาดนาโนเมตร, ต้านออกซิเดชัน, ยับยั้งเอนไซม์ไทโรซิเนส, ยับยั้งเอนไซม์คอลลาจีเนส, การซึมผ่านผิวหนัง, สารเพิ่มการซึมผ่าน



Curcuma comosa Roxb Extract loaded Nanostructured Lipid Carriers for Enhancement of Skin Permeation

Damrongsak Jinarat¹, Bancha Yingngam², and Wandee Rungseevijitprapa^{3*}

¹ Graduate student, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University

² Ph.D. (Pharmaceutical Sciences), Assistant Professor, Division of Pharmaceutical Chemistry and Technology.
Faculty of Pharmaceutical Sciences, Ubon Ratchathani University

³ Dr.rer.nat. (Pharmaceutical Technology), Associate Professor, Division of Pharmaceutical Chemistry and Technology.
Faculty of Pharmaceutical Sciences, Ubon Ratchathani University

* **Corresponding author:** Associate Professor Wandee Rungseevijitprapa, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Warinchamrab District, Ubon Ratchathani, Thailand 34190. Tel. 045-353630, Email: wandee.r@ubu.ac.th; Orcid ID: <https://orcid.org/0000-0001-8382-618X>

Abstract

Curcuma comosa Roxb Extract loaded Nanostructured Lipid Carriers for Enhancement of Skin Permeation

Damrongsak Jinarat¹, Bancha Yingngam², and Wandee Rungseevijitprapa^{3*}

IJPS, 2019; 15(4) : 96-111

Received: 12 June 2019

Revised: 22 July 2019

Accepted: 2 August 2019

Curcuma comosa composed of phytoestrogenic compounds that have been reported to have several bioactivities. However, the scientific support for the use of *Curcuma comosa* in dermal applications are still limited. The purposes of this research were to investigate the potential skin bioactivities of *C. comosa* and develop a nanostructure lipid carrier system for transcutaneous delivery. **Methods:** The pulverized of *Curcuma comosa* was extracted in n-hexane in the Soxhlet extraction apparatus. Skin bioactivities of the extract were investigated include antioxidation, tyrosinase inhibitor and in vitro collagenase inhibition. Nanostructure lipid carrier (NLC) systems were developed. Effect of solid lipid, oil as well as chemical skin permeation enhancers on particle characterization, % encapsulation efficiency (EE), thermal property of lipid, in vitro skin permeation ability by Franz diffusion cells and stability of the formulations were evaluated. **Results:** The obtained *C. comosa* extract had concentrated semisolid characteristic rich in phytoestrogen. Skin bioactivities compared with the controls were mild activities. The developed NLC systems had the particle size in the range of 120 – 170 nm, PDI 0.25 - 0.29, the approximate zeta potential was -20 mV, higher %EE than 90, good physical stable when kept at 25 and 40 °C for 90 days. After 24 h, chemical enhancers facilitated 30 – 57 µg/cm² of the active DA2 through the skin. The lipophilic property of *C. comosa* extract limited its skin permeation and the active was mostly accumulated within the epidermis skin layer. **Conclusion:** *Curcuma comosa* extract-loaded NLC has a potential to be further developed as transdermal delivery of phytoestrogenic compound for local action.

Keywords: *Curcuma comosa* Roxb., nanostructured lipid carrier, antioxidation, tyrosinase inhibitor, collagenase inhibitor, skin permeation, permeation enhancer

1. Introduction

Deficiency of estrogen in post-menopausal women results in atrophic skin changes and acceleration of skin aging. Application of estrogen as a hormone replacement therapy has been reported to improve signs of skin aging such as epidermal hydration, skin thickness and reducing skin wrinkles and increase collagen content. (Pritchard, 2001). Although administration of estrogen can delay or prevent skin aging manifestations, estrogen therapy increases the risk of breast cancer by 1-2% per year. Currently, there is an increasing interest to alleviate the menopausal symptom by the use of products containing phytoestrogens as alternatives to steroid hormone. Phytoestrogens have been found to be beneficial to skin aging in postmenopausal women through the binding of estrogen receptors (ER- α and ER- β) that exist in large quantities in the skin (Sator, 2006). Topically applied skin care products containing phytoestrogens can enhance the skin hydration, improve the vascular function, and maintain the skin elasticity while the risk of systemic effects is reduced. (Yingngam and Rungseewijitprapa, 2012).

Curcuma comosa Roxb. from the family of Zingiberaceae, commonly known as Waan Chak Modluk in Thai, has long been traditionally used for the treatment of postpartum uterine bleeding (Piyachaturawat *et al.*, 1995). It contains phytoestrogenic diarylheptanoids composing three major compounds; (3S)-1-(3,4-dihydroxy-phenyl)-7-phenyl-(6E)-6-hepten-3-ol (DA1); (3R)-1,7-diphenyl-(4E,6E)-4,6-heptadiene-3-ol (DA2); and (3S)-1,7-diphenyl-(6E)-6-hepten-3-ol (DA3) in which DA2 performs highest ER binding affinity (Winuthayanon *et al.*, 2009). *C. comosa* diarylheptanoids have been reported to have anti-inflammatory properties by reducing the expression of TNF- β and suppressing the expression of I κ B kinase and activation of NF κ B. The extract was also implicated as promising therapeutic agents to prevent impairment of vascular relaxation in estrogen-deficient animals via the ER-eNOS pathway as well as through its ability to promote an anti-inflammatory response (Sodsai *et al.*, 2007).

Oral bioavailability of *C. comosa* major compounds were reported quite low of DA2 and DA3, phytoestrogenic

compounds at 17.66 and 17.73 %, respectively, with high clearance value (Su *et al.*, 2012). Low bioavailability occurred when the compound undergoes first pass metabolism in the liver and absorbed from the gastrointestinal tract into blood circulation. The hexane extract of *C. comosa* and its major compounds, DA2 and DA3 are very lipophilic with log *P* values of 4.36 and 4.87, respectively. Formulating topical preparation of such compounds with relatively good skin permeation is quite challenging. Su *et al.*, (2013) developed nanoemulsions by *C. comosa* in an attempt to increase *in situ* intestinal absorption. The formulations were composed of 0.83 % extract, and 0.013 and 0.026 % of DA2 and DA3, respectively, with Tween 60, cetomacrogol 1000 and olive oil as ingredients compare to the extract and compounds in an oil solution. The result revealed that the intestinal absorption rate of the developed nanoemulsions were five to ten folds faster than the oily solution (Su *et al.*, 2013).

Transdermally applied nanoparticles are becoming elegant dosage forms than oral or injectable hormone therapy products and provide better patient compliance. Besides, it expands therapeutic duration of its controlled release system associated prolong therapeutic blood level, the transdermal administration avoids first pass hepatic metabolism and acid hydrolysis provides a more steady blood level (Watkins *et al.*, 2015). The transdermal nanoparticles deliver hormone therapy directly to the systemic circulation exhibited higher treatment outcome and lower adverse effects than conventional oral hormone therapy (Kopper *et al.*, 2009). The nanoparticles delivery system is, furthermore, designed to increase cellular uptake or drug accumulation. Aside from ligand-targeted nanoparticles, phagocytosis, macropinocytosis and endocytosis are the routes of cellular uptake nanoformulation into the target cells (Yameen *et al.*, 2014).

Topical film containing *C. comosa* extract has been investigated as a systemic menopausal hormone therapy (Tuntiyasawasdikul *et al.*, 2016). A transdermal delivery system of the extract was also studied of with permeation enhancers. As a result, ethanol/water binary vehicle system

and Transcutol increased DA2 and DA3 permeation. Although the diarylheptanoids nanoemulsions were five to ten times faster absorbed than those in oily solutions, it contained relatively low concentrations of *C. comosa* extract. However, the amounts of DA1 and DA2 retained in the skin were higher than in the receptor compartment after 24 h of applications. When the concentration of *C. comosa* extract was increased from 30% to 50%, the cumulative amount of diarylheptanoids within the skin significantly increased. Topical film using Eudragit® NE and HPMC base also provided high diarylheptanoids accumulation within the skin (Tuntiyasawasdikul *et al.*, 2018).

The lipid nanoparticles delivery system through dermal absorption, thus, is interesting in this research. Because of its highly lipophilic property, lipid nanoparticles would be the most appropriate to entrap and deliver the extract. With the nanometer scale of particle size, it has the capability to increase skin retentions for local effect and skin penetration of bioactive compound into blood circulation. Nanostructured lipid carriers (NLCs) consisting of a lipid matrix with a special nanostructure had been developed. The NLCs are produced using blends of solid lipids (long chain) and liquid lipids (medium and long chain). Compared to SLNs, NLCs possess lower melting point due to their oil content, while maintaining their particulate solid character at body temperature.

The aims of this research were to investigate the dermal activities of *C. comosa* Roxb. Extract, some preformulation properties and develop *C. comosa* extract loaded lipid nanoparticles for the enhancement of skin penetration.

2. Material and methods

2.1 Plant and chemicals

Rhizome part of *C. comosa* Roxb. from Kampaensaen district, Nakornpratom province, Thailand. Standard compound, DA1 was kindly provided by Assoc. Prof. Rawiwan Kaewamatawong, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University; and DA2 and DA3 from Prof. Apichart Suksamrarn, Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Thailand. 2,2-azino-bis-(3-ethylbenzothiazoline-

6-sulfonic acid) diammonium salt (ABTS) was purchased from Fluka Chemie (Buchs, Switzerland). 2,2-diphenyl-2-picrylhydrazyl, mushroom tyrosinase, epigallocatechin gallate (EGCG), oleic acid and Poloxamer®188 were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-3,4-dihydroxyphenylalanine (L-DOPA), d-limonene and N-methyl-2-pyrrolidone, Trolox, Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT) were from Acros Organics (Geel, Belgium). Collagenase/gelatinase assay kits were from Molecular Probe (Eugene, OR, USA). Oliwax® was from (Hallstar, Italy). Precirol® ATO5 and Compritol® ATO888 were purchased from Gattefosse (France). Glyceryl monostearate (GMS) was from Namsiang (Thailand); medium chain triglyceride (MCT) was from BASF (Germany). The tested pig skin (obtained from a local slaughter) of permeation study used the abdominal part of fetal, neonatal porcine skin, removing hair, fat and subcutaneous tissue and stored in -20 °C until experiment.

2.2 Experimental method

2.2.1 Plant collection and extraction

Rhizome of *Curcuma comosa* Roxb. one year old, was harvested, washed, sliced and oven-dried at 55 °C. After that, the dry rhizome part was pulverized through 40-mesh sieve and then kept in a desiccator until use. The extraction process is performed by placing the pulverized herb and n-hexane in the Soxhlet extraction apparatus (Suksamrarn *et al.*, 2008) and the solvent was evaporated under a rotary evaporator (BÜCHI, Switzerland). Subsequently the chemical fingerprint and the biomarker concentrations of the crude *C. comosa* extract was determined by high performance liquid chromatography (HPLC) (Ultimate™ 3000, Thermo Scientific, MA, USA). Pure chemicals of DA1, DA2 and DA3 served as standard biomarkers. Finally, the crude extract was kept at -20 °C for further experiment.

2.2.2 Screening bioactivities *in vitro* experiments

2.2.2.1 Anti-oxidant activities

1) ABTS^{•+}

The analysis protocol was: The radical of ABTS^{•+} solution (7 mM) was induced by 2.5 mM K₂S₂O₈ in water base reaction and incubated at room temperature for 12 h in the dark. After that, the ABTS^{•+} solution was diluted with

an appropriate solvent to obtain initiate absorbance at 0.7. Then various concentrations of *C. comosa* extract was mixed and stored in the dark for 6 min and the absorbance was measured at 475 nm (Re *et al.*, 1999).

2) DPPH[•]

DPPH[•] acts as a radical whereas sample compound scavenges the radical by donating a hydrogen atom. Briefly, the samples were mixed with DPPH solution, incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm (Thaipong *et al.*, 2006).

Trolox solution was used to obtain standard curve for both ABTS and DPPH assays, vitamin E served as positive control and the absorbance of both methods were measured by microplate reader (BioTek Epoch 2, USA). The results were expressed as 50% inhibition concentration (IC₅₀) by the equation;

$$\% \text{ inhibition} = (A_c - A_s) \times 100 / A_c \quad (1)$$

where A_c is the control absorbance, A_s is the sample absorbance

3) Lipid peroxidation

The lipid peroxidation assay was conducted by thiobarbituric acid reactive substance assay (TBARS) to generate malondialdehyde. The method was modified from Adithya *et al.*, (2017). Briefly, 100 μ L of *C. comosa* extract was diluted in appropriate solvent, and mixed with 10 % v/v egg yolk (unsaturated fatty acid-rich source) in DI water. The reaction was induced by 20 μ L of mM FeSO₄, mixed well and allowed for the reaction to occur for 30 min at room temperature. Then, 1.5 ml of 20 % acetic acid, 0.5 ml of 0.8% TBA (in 1.1% SDS) and 0.5 ml of 20% trichloroacetic acid was mixed and boiled in 95 °C for 1 h. Then the reaction was immediate ceased in an ice bath, centrifuged 3000 rpm for 10 min. The supernatant part was determined from the malondialdehyde content at 532 nm. BHT was used as standard reagent.

2.2.2.2 Tyrosinase inhibition activity

Tyrosinase is the enzyme that plays important role in melanin pigment synthesis at melanocytes and the tyrosinase inhibition activity has led to the demonstration of

whitening characteristic of skin. The assay (Jeon *et al.*, 2005) was conducted by pre-incubating mushroom tyrosinase with the test sample at 25 °C for 10 min. Then L-DOPA was added in order to initiate the reaction and the solution was further incubated at 25 °C for 20 min. Inhibitory activity was measured as the 470 nm absorbance (EZ read 400, Biochrom, UK) and IC₅₀ was calculated. Vitamin C was used as a positive control.

2.2.2.3 Anti collagenase and anti gelatinase

Collagenase and gelatinase inhibitions were determined using collagenase/gelatinase kits (Molecular Probes, OR, USA) using gelatin-fluorescein conjugate as the substrate. The substrate and various concentrations of the test samples were dissolved separately in the Tris-HCl buffer pH 7.4. *C. comosa* extract (80 μ L), 20 μ L of substrate, and 100 μ L collagenase were mixed in a 96-well plate, and incubated for 90 min under light protection at room temperature. The fluorescence intensity was measured at 485 nm excitation and 538 nm emission wavelength, using the fluorescence microplate reader (CLARIOstar®, BMG Labtech, Ortengerg, Germany) with 1,10-Phenanthroline and EGCG as a positive inhibitor and a standard, respectively. The collagenase inhibition activity of the extract calculated as:

$$\% \text{ Inhibition} = [(A-B)-(C-D)] \times 100 / (A-B) \quad (2)$$

where A is the control fluorescent intensity (without the extract sample); B is the blank of A intensity (without sample and enzyme), C is the sample with enzyme intensity, and D is the sample without enzyme intensity. The activities were expressed as an IC₅₀ value.

2.2.3 pH stability test

Since the low oral bioavailability of diarylheptanoid biomarkers, pH stability was investigated to provided pH stability data according to the condition compounds exposed including; skin, blood serum and even gastric environments in case of oral formulation. The extract was dissolved in methanol to a concentration of to 0.1 mg/mL and placed in HCl solution pH 1.2, phosphate buffer solution (PBS) pH 5.5 nad 7.4 (Katakam *et al.*, 2011). The samples were taken at 1, 2, 4, 6 and 24 h to analyze biomarker concentrations compared to the initiate concentration (n=3).



Table 1. Solid lipids, oils and chemical penetration enhancers in comosa-NLC composed of 10 %w/w poloxamer and 2 %w/w extract.

Formulation	Solid lipid (%w/w)				Liquid lipid (%w/w)		Penetration enhancer	
	GMS	Compritol [®] ATO888	Precirol [®] ATO5	Oliwax	Oleic acid	MCT	d-limonene	NMP
F1	5.6	-	-	-	2.4	-	-	-
F2	-	5.6	-	-	2.4	-	-	-
F3	-	-	-	5.6	2.4	-	-	-
F4	-	-	5.6	-	2.4	-	-	-
F5	-	-	5.6	-	-	2.4	-	-
F6	-	-	5.6	-	2.4	-	1	-
F7	-	-	5.6	-	2.4	-	-	1

2.2.4 Preparation of NLC

NLC was prepared by the de novo o/w emulsification method modified by Mueller *et al.*, (2002). Briefly, compositions of lipid phase according to Table 1 were melted at 80 - 85 °C. Simultaneously, an aqueous phase containing 10% Poloxamer 188 was heated to the temperature above 5 °C of the lipid phase. After that, the hot aqueous phase was slowly mixed into the lipid phase and immediately homogenized with high speed homogenizer (Ultra turrax T10, IKA, Germany) at the speed of 12,000 rpm for 5 min. Droplet size of NLC was reduced by probe-type sonicator (Vibracell VC50T, USA) for 4 min twice. Finally, the obtained NLC was allowed to cool down at room temperature.

2.2.5 Characterization

2.2.5.1 Particle size and zeta potential (ZP)

Particle size analysis and ZP measurement were conducted using photon correlation spectroscopy (Zetasizer® Nano-ZS, Mavern instrument, UK). To prepare the test sample solution for the optimum density, NLC sample was diluted with DI water in 1:200 (v/v) before the measurement. Average particle size as well as a polydispersity index (PDI) was determined at 25 °C after 2 min time incubation, in triplicate. Electrophoretic mobility was applied to determine the surface charge of the NLC by using the same instrument. The data were analyzed using Zetasizer version 7.02 software.

2.2.5.2 Differential scanning calorimetry (DSC)

Lipid crystallinity, polymorphisms, and thermal behavior studies of lipid, physical mixture and NLC formulations were determined using a Mettler DSC 821e (Mettler Toledo, Gießen, Germany). The test samples (1-2 mg) were accurately weighed into aluminium crucibles. The instrument was calibrated with indium. The experiment was set to start heating from 20 to 90 °C, 10 K /min flushing with 30 ml/min N₂ (Gonullu *et al.* 2015). Melting peaks and enthalpies were calculated using STARe analysis software and the crystallinity index (CI) were calculated by the equation;

$$CI = (M_s / M_p f_L) \times 100 \quad (3)$$

where M_s and M_p are melting enthalpy (J/g) of NLC particle and solid lipid, respectively, and f_L is a percentage of the solid lipid in NLC.

2.2.5.3 Entrapment efficiency and drug loading capacity

Entrapment efficiency (EE) of *C. comosa* in NLC formulations was determined by direct extraction of the compound from the pellet particles as described previously (Su *et al.*, 2013). Briefly, the NLC dispersions was filled in centrifuge tubes with filtration (Amicon, Merck, Germany), the pellet particles were separated from the aqueous medium by centrifugation at 7500 rpm. Then the pellet particles were dissolved by an appropriate solvent and the concentration of entrapped DA2 and DA3 by HPLC. Entrapment efficiency is calculated by equation;

$$\% EE = \frac{\text{entrapped diarylheptanoids} \times 100}{(\text{entrapped} + \text{unentrapped diarylheptanoids})} \quad (4)$$

2.2.5.4 HPLC

HPLC analysis was performed by Ultimate™ 3000 (Thermo Scientific, MA, USA) with reversed phase C18 column (4.6 x 250 mm, 5 µm diameter). An elution using the gradient of methanol and 0.5 % acetic acid (70:30 to 100:0) was run at flow rate 1 ml/min. Chromatogram was plotted with UV 254 nm detector.

2.2.6 Skin penetration study

Since the active compound, DA2 has been very lipophilic, the use of any additive to enhance the drug release under sink condition is necessary. In our preliminary study, 50% ethanol in PBS 7.4 was essential to be used in the receptor medium in order to enhance the solubility of DA2 and the medium was proved to maintain the integrity of the porcine skin. The use of ethanolic solution as the receptor medium has been accepted and applied in several other publications (Sakdiset *et al.*, 2017; Sacha *et al.*, 2019).

The skin at the donor compartment was filled with 0.2 mL NLC and receptor compartment contained 50 % ethanol in PBS 7.4, stirred by magnetic bar and kept at 32 ± 2 °C. During the time interval of 1, 3, 6, 12 and 24 h, the sample medium was collected and the amount of DA2 was determined by HPLC as described in section. Skin permeation of the oily solution containing *C. comosa* extract with the same active concentration was also investigated. The experiments were run at n=4.

The evaluation of drug accumulated in the skin was performed according to tape stripping method with some modifications (Erdal *et al.*, 2016). After the 24 h permeation study, the skin was washed gently with deionized water. The unabsorbed NLC and outer stratum corneum were removed by tape stripping (20-tape). Then the obtained viable epidermis and dermis layer was cut into small pieces and the amount of D2 diarylheptanoids retained within the skin layer was extracted in by methanol in an ultrasonic bath for 60 min. The concentration of DA2 in the supernatant was determined by HPLC.

2.2.8 Stability testing of NLC and encapsulated DA2

The stability of NLCs formulations was investigated following the ICH guideline at storage temperature 25 and 40 °C and 75% relative humidity (ASEAN ICH). Physical stability was investigated by mean of particle size analysis at day 3, 30, 60 and 90. The experiment was done in triplicate.

The concentration of DA2 encapsulated in the storage NLC was determined remaining percentage at day 30, 60 and 90 compared to day 1 after preparation.

2.3 Data analysis

Data of experimental determinations were reported as the mean ± standard deviation (SD). Statistical analyses of mean between two samples were calculated by Student's t-test. The differences among the two groups or more were analyzed by one-way analysis of variance (ANOVA) with Turkey HSD post hoc test. The statistically significant differences were considered as *P* value < 0.05.

3. Results and discussion

3.1 Plant extraction

The dried samples of *C. comosa* were pulverized and subsequently extracted with *n*-hexane using a Soxhlet apparatus. Determination of crude extract revealed the concentrations of DA1, DA2 and DA3 were 1.1 ± 0.1, 180.1 ± 15.3 and 84.1 ± 5.1 µg/mg crude extract, respectively. It's obvious that the amount of DA2 and DA3 were presented in the extract in a much larger extent than the DA1.

Moreover, the DA2 was reported exhibiting the most potent estrogenic activity. Therefore, it confirmed that the *n*-hexane extract of *C. comosa* was suitable for further investigation with DA2 was used as the marker.

3.2 Bioactivities

While the hexane extract of *C. comosa* has been reported to have the strongest estrogenic activity, there is still lack of some information to support the potential benefit of it to be used as a topical skin care. Therefore, the *in vitro* screening bioactivities assays include antioxidation, tyrosinase inhibition and collagenase inhibition activities of the semisolid fraction of *C. comosa* were investigated and the results were shown in Table 2.

**Table 2.** Bioactivity of *C. Comosa* (n=3)

Compounds	IC ₅₀ (µg/mL)				
	ABTS	DPPH	Lipid peroxidation	Tyrosinase inhibition	Collagenase inhibition
Trolox	4.6 ± 0.1	7.0 ± 0.2	-	-	-
Vitamin E	13.9 ± 0.2	17.1 ± 0.8	-	-	-
BHT	-	-	1.4 ± 0.1	-	-
Vitamin C	-	-	-	37.0 ± 3.2	-
EGCG	-	-	-	-	40.2 ± 4.8
Comosa ext	42.1 ± 3.3	84.0 ± 4.3	38.5 ± 2.6	200.0 ± 8.1	> 320

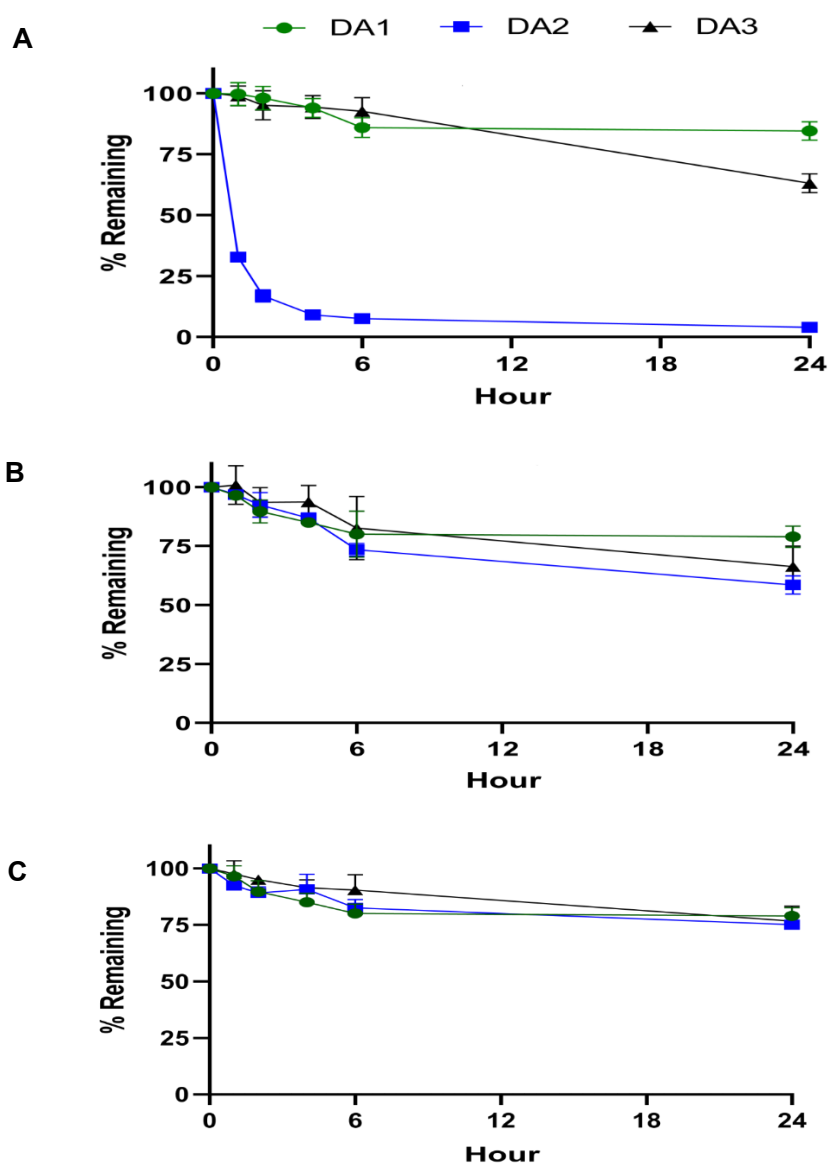
**Figure 1.** The pH stability of DA1, DA2 and DA3 in *C. comosa* extract at (A) pH 1.2, (B) pH 5.5 and (C) pH 7.4 at 37 °C for 24 h (n=3)

Table 3. Particle size, PDI, zeta potential and % EE of comosa-NLC (n=3)

NLC	Size (nm)	PDI	zp (mV)	% EE DA2
F1	216.2 ± 6.6	0.460 ± 0.013	-20.6 ± 1.0	N/A
F2	149.0 ± 0.8	0.267 ± 0.008	-19.8 ± 1.1	98.8 ± 5.6
F3	148.4 ± 4.9	0.250 ± 0.018	-34.9 ± 1.4	95.8 ± 11.7
F4	131.2 ± 0.9	0.291 ± 0.016	-23.8 ± 0.2	95.3 ± 2.9
F5	173.5 ± 1.4	0.270 ± 0.004	-37.6 ± 1.9	93.9 ± 2.3
F6	119.7 ± 0.5	0.267 ± 0.011	-25.1 ± 1.2	83.5 ± 4.7
F7	115.7 ± 0.3	0.381 ± 0.008	-25.5 ± 4.1	92.8 ± 3.6

N/A formulation **was not stable**

The ABTS and DPPH IC₅₀ data of *C. comosa* were relatively much lower than that of the Trolox for 9 and 12 folds, respectively. The lipid peroxidation IC₅₀, the melanin pigment inhibition of the extract demonstrated mild activity. Finally, the IC₅₀ of the collagenase inhibition assay could not be determined due to solubility limitation of the solvent used for the assay.

3.3 pH stability of *C. comosa* markers

All markers were stable at pH 5.5 and 7.4 represented normal skin and blood circulation pH, respectively (Figure 1). Their remaining concentrations were between 80 – 90 % concentrations at 6-hour determination comparing to the initiate concentration and degraded to 60 % for DA2 at pH 1.2 of gastric condition, while DA1 and DA3 concentrations were slowly decrease similar in pH 5.5 and 7.4, DA2 showed degradation rapidly. The concentration of DA2 decreased to 32.8 ± 1.3 % and remained only 9.1 ± 2.1 % at 4-hour of gastric pH exposure. Two double-bond skeletal chain of DA2 is and easy addition reacted by protic acid such as HCl explained by the Markownikoff's rule reaction (Markownikoff, 1870). The result revealed that the high estrogenic compound, DA2, should be avoided oral administration without effective coating delivery system or alternative routes of drug administration should be considered.

3.4 Particle size of *C. comosa* loaded NLC

Particle size of NLCs composed Compritol® ATO888 (F2), Oliwax® (F3) and Precirol® ATO5 (F4) were

149.0 ± 0.8, 131.2 ± 0.9 and 148.4 ± 4.9 nm, respectively. Apart from the unstable F1, all NLC formulations, exhibited narrow PDIs of less than 0.3, zeta potentials from -19.8 to -37.6 mV with %EE of DA2 higher than 90% (Table 3). Therefore, the results indicated that these formulations are appropriate to prepare transdermal delivery of NLC.

Since the F4 formulation demonstrated the smallest particle size range, so it was chosen for further investigation the effect of another liquid lipids (MCT) and the skin permeation enhancer (d-limonene and NMP). Results showed that the NLC composed of Precirol® ATO5 with MCT as liquid lipids, exhibited larger particle size than that of the F4 containing oleic acid. The addition of penetration enhancer, d-limonene or NMP into the NLC formulation reduced the particle size to 119.7 ± 0.5 and 115.7 ± 0.3 nm, respectively. Limonene and NMP chemical structures compose of both polar and non-polar molecules similar to surfactant. Hence, they may act as a co-surfactant contributes to their interfacial arrangement to facilitate the efficiency in the size reduction of NLC.

3.5 Thermal properties of lipids and NLC

DSC was used as a tool to investigate the thermal behavior of materials in lipid nanoparticles. DSC thermograms of solid lipid bulk material and the NLC were shown in Figure 2. The thermograms of the pure solid lipids of Compritol® ATO888 and GMS exhibited the melting endotherm of 74.8 and 73.6 °C, respectively. Interestingly, Precirol® ATO5 showed the melting point at 58.4 °C which

was the lowest melting point compared to those of other investigated lipids (Figure 2. a-d). The melting point of the solid lipid is considered the main factor that affect the particle size as we could clearly see that F4 exhibited the smallest particle size range (Gonullu *et al.*, 2015). Melting points and the percentage crystallinity of all NLC formulations were decreased from the melting point of the bulk solid lipid, (Figure 2.e-h). The *C. comosa* extract exhibited no DSC peak, therefore the depression of crystallinity was influenced by the liquid lipid level to show

an amorphous form or imperfection of the lipid matrix (Almeida *et al.*, 2012).

DSC thermograms of F5 which contained triglyceride as liquid lipid showed slightly decrease of CI. On the other hand, oleic with penetration enhancer exhibited drastically decreased CI from those of F4, F6 and F7, respectively. This indicated that oleic acid, limonene and NMP contribute better NLC characterization for developing transdermal lipid nanoparticles.

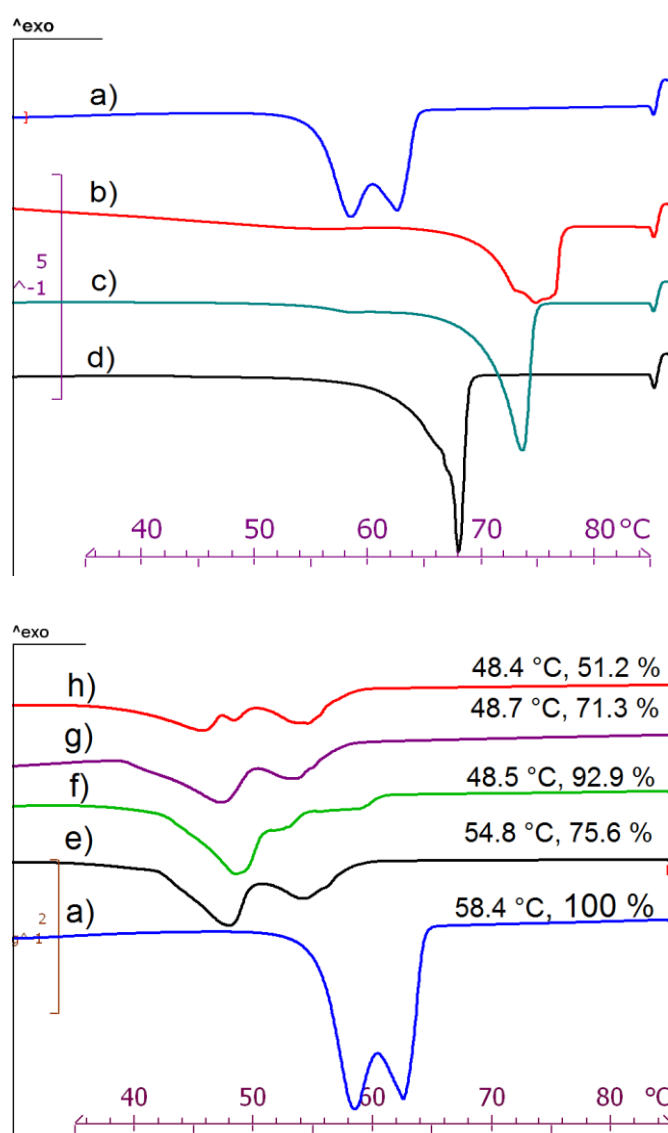


Figure 2. DSC thermogram of solid lipid bulk material and NLC: a) Precirol® ATO5, b) GMS, c) Compritol® ATO888 and d) Oliwax®; and thermograms, melting points and crystallinity indexes of comosa-NLC: e) F4, f) F5, g) F6 and h) F7.

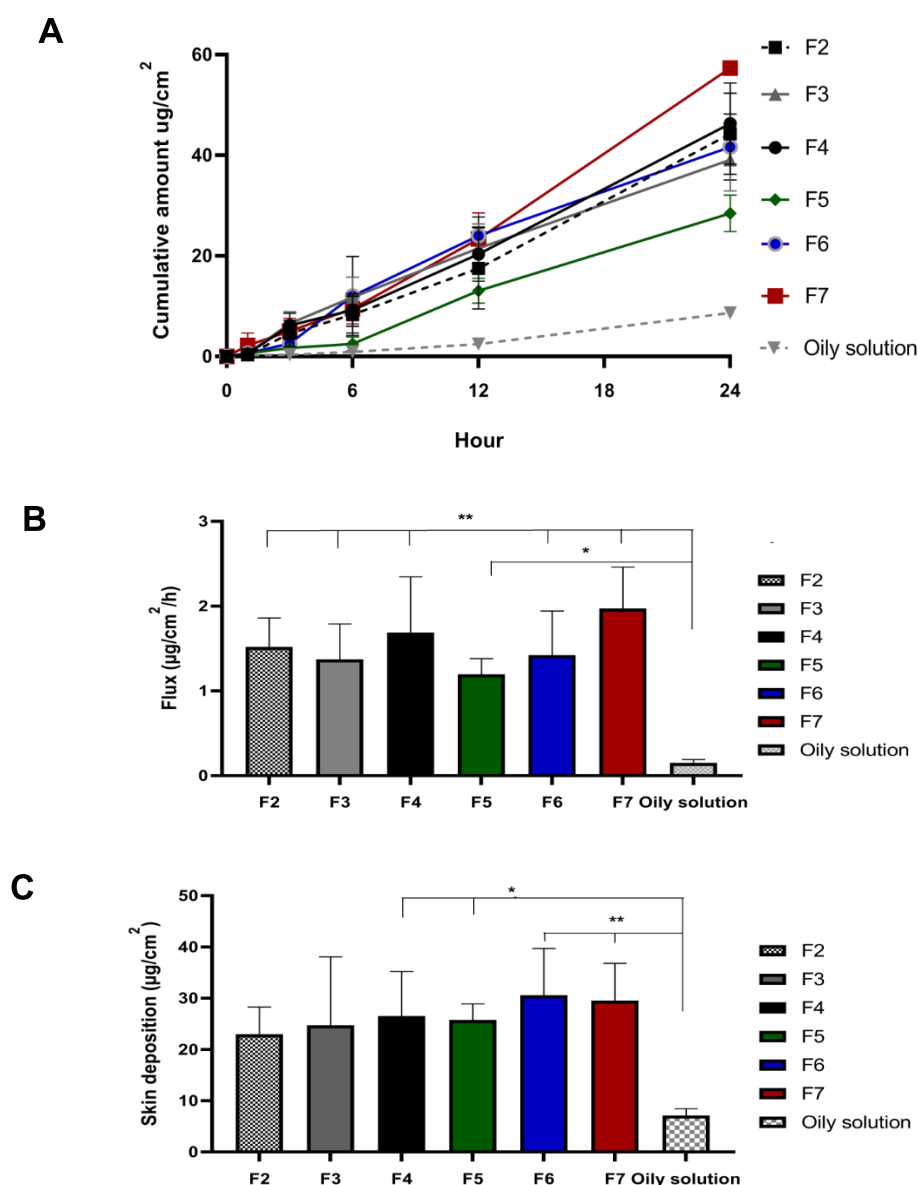


Figure 3. Percentage skin permeation of comosa-NLC A) Type of solid lipids, and B) type of liquid lipids, type of penetration enhancers (n=4)

* $p < 0.05$ compared to oily solution

** $p < 0.01$ compared to oily solution

3.6 Skin penetration

Investigation of the solubility showed the active compounds exhibited very low solubility in PBS pH 7.4. The solubility was slightly increased in 30 % ethanol in PBS (data not showed). Therefore, 50 % ethanol: PBS 7.4 was used as the receptor medium in order to achieve the sink condition environment for skin permeation study. The skin barrier function has been attributed to the stratum corneum

and represents a major challenge to administer the active compound across the cutaneous route. The skin penetration of *C. comosa* loaded NLC composing with different solid lipid formulations was determined using the Franz diffusion method. Lipid nanoparticles formulated in this study are expected to display an occlusive effect and reduce the transepidermal water loss. This resulted in an enhancement



in the amount of drug penetrated into the skin layers. DA2 amount was measured as $1050 \pm 50 \mu\text{g}$ in 0.2 ml initial loaded at the donor part of each formulation. The cumulative amount of diarylheptanoids DA2 permeated through the stillborn porcine skin was assessed at 24 h and was presented in Figure 3. All formulations delivered DA2 compound through porcine skins in the range of 30 – 57 $\mu\text{g}/\text{cm}^2$ (Figure 3 A) gave the fluxes 1.20 – 1.66 $\mu\text{g}/\text{cm}^2/\text{h}$ (Figure 3 B) whereas free DA2 in oily solution penetrated through skin of $5.4 \pm 1.8 \mu\text{g}/\text{cm}^2$ with flux $0.50 \pm 0.15 \mu\text{g}/\text{cm}^2/\text{h}$ (Table 4). The cumulative permeation independent of solid lipid composition used in the NLC formulations. The DA2 delivered by NLC accumulated in skin about 25 – 30 $\mu\text{g}/\text{cm}^2$ (Figure 3 C). However, the NLC system showed a

penetration capacity higher than the oily solution application of the extract.

3.7 Stability of *C. comosa* loaded NLC

Aggregation of unstable nanoparticles limits the application of lipid nanoparticles despite the capability to encapsulate high loading of lipophilic bioactive. Therefore, the effect of storage time and temperature of *C. comosa* loaded NLC composed by Precirol®ATO5 as solid lipid were investigated at 25 and 40 °C for 90 days. Physical stability of the NLC formulations was accessed in term of particle size, whereas the chemical stability of DA2 encapsulated in the NLC formulations were presented in Table 4.

Table 4. Evaluation physical stability of the NLC represented by particle size and chemical stability of encapsulated DA2 at 25 ± 2 and 40 ± 2 °C storage for 90 days

Formulation /storage temp (°C)	Particle size (nm)				% DA2 remaining		
	Day 1	Day 30	Day 60	Day 90	Day 30	Day 60	Day 90
F4 at 25°C	131.3 ± 0.9	131.9 ± 1.7	135.0 ± 1.7	137.8 ± 1.0	95.0 ± 3.8	90.3 ± 1.8	87.7 ± 5.3
at 40°C	131.3 ± 0.9	139.9 ± 4.8	150.7 ± 3.9 *	144.8 ± 2.3 *	91.1 ± 3.1	89.7 ± 2.6	78.5 ± 0.6 *
F5 at 25°C	173.5 ± 1.4	171.2 ± 2.1	171.7 ± 1.6	171.2 ± 2.3	95.6 ± 2.8	94.2 ± 1.7	87.3 ± 5.7
at 40°C	173.5 ± 1.4	176.6 ± 3.9	177.1 ± 3.5	175.1 ± 4.3	93.0 ± 0.8	91.8 ± 2.0	72.3 ± 8.0 *
F6 at 25°C	119.7 ± 0.5	118.8 ± 1.5	117.8 ± 2.0	119.9 ± 2.3	95.3 ± 2.7	92.8 ± 1.4	82.7 ± 1.6
at 40°C	119.7 ± 0.5	121.4 ± 1.9	119.3 ± 2.4	118.4 ± 2.8	94.5 ± 1.4	87.5 ± 2.8	76.1 ± 3.9 *
F7 at 25°C	115.7 ± 0.3	114.1 ± 1.8	115.8 ± 1.2	114.2 ± 0.3	92.9 ± 1.7	88.4 ± 3.2	71.7 ± 6.0
at 40°C	115.7 ± 0.3	117.8 ± 1.5	117.7 ± 1.4	117.5 ± 0.7	90.7 ± 1.6	78.5 ± 3.5	69.2 ± 4.5
Oily at 25°C	-	-	-	-	89.1 ± 1.2	71.8 ± 1.4	55.9 ± 6.0
at 40°C	-	-	-	-	82.7 ± 2.5	68.4 ± 1.2	46.0 ± 4.6

* $p < 0.05$ compared to the same column of the formulation

DA2 remaining of all NLC were significantly higher than oily solution since day 30

All of NLC formulations were stable when storage at 25 °C for the investigated time of 90 days. However, the F4 system displayed significantly increased in the particle size of at the storage temperature of 40 °C for 60 days. The NLC composed of poloxamer 188 as a surfactant that possess steric repulsion that maintains the average particle size. The addition of d-limonene and NMP as a penetration enhancer in F6 and F7 formulations, did not affect the particle size change.

NLC demonstrated as chemical protective delivery system represented by reducing DA2 degradation when storage at 25 and 40 °C for 90 days compared to free DA2 in oily solution. Lipid particles protected internal compound from oxidation and hydrolysis reaction (Gunessekaran *et al.*, 2014).

4. Discussion and conclusion

This study demonstrated the bioactivities of the hexane extract of *C. comosa* for the first time. The extracted contains very high lipophilic diarylheptanoids. Therefore, its molecule always lacks of radical scavenging chemical side chain such as OH-phenol ring or O-diphenoxyl group (Ichikawa *et al.*, 2019). Moreover, free radicals of the assays are initiated in water or alcohol that is polar medium whereas the extract is non-polar which limited sample solubility in the assay protocols. Although the overall bioactivities of the extract exhibited mild activities to be used as active compound for skincare product, there have been previously reported the benefit of the extract to be used topically for the protection of skin aging in peri and post-menopausal women (Tuntiyasawasdikul *et al.*, 2018) and anti-inflammation (Sodsai *et al.*, 2007). Also skin potential benefit of *C. comosa* extract may be from other mechanisms. Therefore, the study of the bioactivities of the extract via dermal estrogen receptor or *in vivo* should be further investigated to propose local and systemic benefit effects simultaneously.

NLC demonstrated as an appropriate delivery system encapsulated *C. comosa* extract with nanoscale of

particle size, high entrapment efficiency as well as physical stability. 50 % ethanol in PBS pH 7.4 was used as permeation medium as reported previously (Sakdiset *et al.*, 2017). Among the investigated NLC formulation, preparation of NLC with Precirol® and MCT (F5) showed significantly lower cumulative permeation than other NLC formulations. This is probably due to the larger particle size of the F5 system that plays the major role in the slowest skin permeation rate. On the contrary, the small particle sizes ensure the closer contact with the stratum corneum, thus promoting the penetration of the active compound into the skin.

Apart from the oils and fatty acids composition of the NLC formulation, d-limonene and NMP were added as the skin penetration enhancer. Results showed that skin penetration enhanced only more slightly cumulative active released into the receptor medium. This imply that NLC and the application of skin penetration enhancer promotes the penetration of drug through the stratum corneum layer but remain accumulated within the skin. Küchler *et al.*, (2010) showed that the interaction rate of the SLNs with the skin lipids and sebum is dependent on the lipophilicity of the incorporated molecule.

D-limonene, lipophilic essential terpene has been used as chemical penetration enhancer because it increase drug solubility within skin subsequence to increase drug partitioning into stratum corneum (Charoenputtakun *et al.*, 2014). On one hand, pyrrolidones including n-mentyl-2-pyrrolidone (NMP) is hydrophilic chemical which increased skin penetration by increase drug diffusivity (Wongpayapkul *et al.*, 2006). Nonetheless, both terpene and pyrrolidone chemical in this study did not significantly increase DA2 diarylheptanoid permeated through skin. Rungsimawong *et al.* (2017) also reported d-limonene did not intact lipid nanoparticles penetrated into the skin as a result of it increased solubility and strongly coat lipophilic active compound inside the particle prevent leakage and release into the skin. Nevertheless, the occlusive property of the NLC can result in the reduction of corneocyte packing and



opening of inter-corneocyte gaps and thus facilitates the active penetration into deeper layers of skin

The attempt to increase the higher skin absorption of diarylheptanoids than previous studies is the challenge of formulation development for the therapeutic level treatment of menopausal symptoms. (Frasor *et al.*, 2003). The skin accumulation of diarylheptanoids was useful to improve aging skin related to estrogen depletion via estrogen receptor located on skin or other pathways. One factor limited drug absorption is the very high lipophilic molecule of the major compounds with n-octanol-water partition coefficient ($\log P$) values of DA2 and DA3 of 4.36 and 4.87, respectively (Su *et al.*, 2013) whereas the appropriate $\log P$ for susceptible passing of chemical through stratum corneum is in 1 – 3 range (N'Da, 2014). The intact rigid NLC tends to follow the transappendageal pathway as high lipophilicity promotes the follicular deposition (Wissing and Muller, 2003). The beneficial effects of phytoestrogens on anti-skin aging due to estrogen depletion have been proved since a few decades (Yingngam and Rungseevijitprapa, 2012), simultaneously, the misused of *P. mirifica* breast cream exerted risk of systemic adverse effect, including breast cancer (Kakehashi *et al.*, 2016). This study indicates that NLC is useful in the delivery of therapeutic compound having poor skin penetration. NLC was reported to exhibit a high adhesion to the stratum corneum enhancing the amount of drug which penetrated into the viable skin (Souto and Muller, 2008). NLC also displayed an occlusive effect, thus enhance the skin hydration and flexibility.

In conclusion, the developed lipid nanoparticles containing *C.comosa* extract can potentially be used topically as an alternative phytoestrogenic therapy for anti-aging cosmeceutical products. The formulation exhibited particle size in the range of 120 - 170 nm with PDI of 0.25 - 0.29. Zeta potential and results from the stability studies confirmed the shelf life of the formulation of at least 90 days of the investigated time. Lipid nanoparticles provided enhanced skin penetration and a high accumulation of diarylheptanoids within skin, indicating the

feasibility of a skin delivery of the *C.comosa* extract. The developed lipid nanoparticles can be applied topically as an alternative therapy for protection of skin aging in pre and post-menopausal women provide localized action on the skin layers with low systemic effects.

Acknowledgement

This study was partially supported by the funding grant from Ubon Ratchathani University, Thailand under the project "Support the potential of Thai researchers for the competitiveness ability" (UBU-Ageing Society research grant /2019).

References

- Adithya ES, Lakshmi MS, Christabel PH, Sasikumar JM. *In vitro* antioxidant, anti-lipid peroxidation activities and HPLC analysis of methanol extracts from bark and stem of *Mahonia leschenaultia* takeda. *Asian J Plant Sci Res* 2013; 3(2): 116-126.
- Almeida ED, Costa AA, Serafini MR, Rossetti FC, *et al.* Preparation and characterization of chloroaluminum
- Chun KS, Sohn Y, Kim HS, Kim OH, *et al.* Anti-tumor promoting potential of naturally occurring diarylheptanoids structurally related to curcumin. *Mutat Res* 1999; 428(1-2): 49-57.
- Frasor J, Barnett DH, Danes JM, Hess R, Parlow AF, Katzenellenbogen BS. Response-specific and ligand dose-dependent modulation of estrogen receptor (ER) alpha activity by ERbeta in the uterus. *Endocrinology* 2003; 144(7): 3159–3166.
- Golden GM, McKie JE, Potts RO. Role of stratum corneum lipid fluidity in transdermal drug flux. *J Pharm Sci* 1987; 76(1), 25–28.
- Gunasekaran T, Haile T, Nigusse T, Dhanaraju MD. Nanotechnology: an effective tool for enhancing bioavailability and bioactivity of phytomedicine. *Asian Pac J Trop Biomed* 2014; 4(Suppl 1): S1–S7.



- Gunullu U, Uner M, Yener G, *et al.* Formulation and characterization of solid lipid nanoparticles, nanostructured lipid carriers and nanoemulsion of lornoxicam for transdermal delivery. *Acta Pharm* 2015; 65: 1-13.
- Ichikawa K, Sasada R, Chiba K, Gotoh H. Effect of side chain functional groups on the DPPH radical scavenging activity of bisabolane-type phenols. *Antioxidants* 2019; 8(65).
- Jantaratnotai N, Utaisincharoen P, Piyachaturawat P, *et al.* Inhibitory effect of *Curcuma comosa* on NO production and cytokine expression in LPS-activated microglia. *Life Sci* 2006; 78: 571-577.
- Jeon SH, Kim KH, Koh JU, Kong KH. Inhibitory effects on L-Dopa oxidation of tyrosinase by skin-whitening agents, *Bull Korean Chem Soc* 2005; 26(7): 1135-1137.
- Jinarat D, Yingngam B, Suksamrarn A, Rungseewijitprapa W. Development and characterization of *Curcuma comosa* extract loaded nanostructured lipid carriers. *IJPS* 2017;13(Supplement):163-172.
- Katakam P, Kumari AP, Hwisa N, Chandu BR. Development of stability indicating media for in-vitro dissolution testing of didanosine in pharmaceutical dosage forms. *Res Sci Tech.* 2011; 3: 41-45.
- Kopper NW, Gudeman J, Thompson DJ. Transdermal hormone therapy in postmenopausal women: A review of metabolic effects and drug delivery technologies. *Drug Des Devel Ther* 2009; 2: 193–202.
- Küchler S, Herrmann W, Panek-Minkin G, Blaschke T, *et al.* SLN for topical application in skin diseases-characterization of drug-carrier and carrier-target interactions. *Int J Pharm.* 2010; 390(2):225-33.
- Kundu S, Wang Y, Xia W, Mathur M. Thermal stability and reducibility of oxygen-containing functional groups on multiwalled carbon nanotube surfaces: A quantitative high-resolution XPS and TPD/TPR study. *J. Phys. Chem.* 2008;1129(43):16869-16878.
- Markownikoff W. Ueber die Abhängigkeit der verschiedenen Vertretbarkeit des Radicalwasserstoffs in den isomeren Buttersäuren. *Annalen der Pharmacie.* 1870; 153 (1): 228–59.
- N'Da D. Prodrug Strategies for enhancing the percutaneous absorption of drugs. *Molecules* 2014; 19(12): 20780–20807.
- phthalocyanine-loaded solid lipid nanoparticles by thermal analysis and powder X-ray diffraction techniques. *J Therm Anal Calorim.* 2012; 108: 191–196.
- Piyachaturawat P, Ercharuporn S and Suksamrarn A. Estrogenic activity of *Curcuma comosa* extract in rat, *Asia Pac J Pharm* 1995;10:121-126.
- Prasanthi D, Lakshmi P K. Terpenes: Effect of lipophilicity in enhancing transdermal delivery of alfuzosin hydrochloride. *J Adv Pharm Technol Res* 2012;3(4):216–223.
- Pritchard KI. Breast cancer prevention with selective estrogen receptor modulators: a perspective. *Ann NY Ace sci* 2001;949:89-98.
- Rangsimawong W, Tansathien K, Nuntharatanapon N, Jaewjira S, Opanasopit P. Development of Pueraria mirifica extract-loaded lipid nanoparticles for hair spray. *Thai Bull Pharm Sci* 2017; 12(2):13-20.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, & Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; 26(9–10):1231–1237.
- Sakdiset P, Kitao Y, Todo H, Sugibayashi K. High-throughput screening of potential skin penetration-enhancers using stratum corneum lipid liposomes: Preliminary evaluation for different concentrations of ethanol. *J Pharm* 2017;7409420.

- Sator PG. Skin treatments and dermatological procedures to promote youthful skin. *Clin Interv Aging* 2006; 1(1): 51-6.
- Sodsai A, Piyachaturawat P, Sophasan S, Suksamrarn A, Vongsakul M. Suppression by *Curcuma comosa* Roxb. of pro-inflammatory cytokine secretion in phorbol-12-myristate-13-acetate stimulated human mononuclear cells. *Int Immunopharmacol* 2007; 7: 524–531.
- Souto EB, Müller RH. Cosmetic features and applications of lipid nanoparticles (SLN, NLC). *Int J Cosmet Sci.* 2008; 30(3): 157-65.
- Su J, Sripanidkulchai K., Hu Y, Chaiittianan R, Sripanidkulchai, B. Increased In Situ Intestinal Absorption of Phytoestrogenic Diarylheptanoids from *Curcuma comosa* in Nanoemulsions. *AAPS PharmSciTech* 2013; 14(3): 1055–1062.
- Su J, Sripanidkulchai K., Suksamrarn A., Hu Y, Piyachaturawat P, Sripanidkulchai B. Pharmacokinetics and organ distribution of diarylheptanoid phytoestrogens from *Curcuma comosa* in rats. *J Nat Med* 2012; 66(3): 468-475.
- Suksamrarn A, Ponglikitmongkol M, Wongkrajang K, Chindaduang A, et al. Diarylheptanoids, new phytoestrogens from the rhizomes of *Curcuma comosa*: Isolation, chemical modification and estrogenic activity evaluation. *Bioorg Med Chem* 2008; 16(14): 6891-902.
- Tantikanlayaporn D, Wichit P, Weerachayaphorn J, Chairoungdua A, et al. Bone Sparing Effect of a Novel Phytoestrogen Diarylheptanoid from *Curcuma comosa* Roxb. in Ovariectomized Rats. *PLoS ONE* 2013; 8(11): e78739
- Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, *J Food Comp Anal* 2006; 19(6-7): 669-675.
- Tuntiyasawasdikul S, Limpongsa E, Jaipakdee N, Sripanidkulchai B Effects of Vehicles and Enhancers on the Skin Permeation of Phytoestrogenic Diarylheptanoids from *Curcuma comosa*. *AAPS PharmSciTech* 2017; 18(3): 895-903.
- Tuntiyasawasdikul S, Limpongsa E, Jaipakdee N, Sripanidkulchai B. Development and evaluation of topical films containing phytoestrogenic diarylheptanoids from *Curcuma comosa* extract. *Drug Dev Ind Pharm* 2018; 44(8): 1385–1394.
- Watkins R, Wu L, Zhang C, Davis R, Xu B. Natural product-based nanomedicine: recent advances and issues. *Int J Nanomed* 2015: 6055-6074.
- Winuthayanon W, Piyachaturawat P, Suksamrarn A, Ponglikitmongkol M, et al. Diarylheptanoid phytoestrogens isolated from medicinal plant *Curcuma comosa*: Biologic actions *in vitro* and *in vivo* indicate estrogen receptor-dependent mechanisms, *Environ Health Perspec* 2009; 11(7): 1155-1161.
- Wissing SA, Müller RH. Cosmetic applications for solid lipid nanoparticles (SLN). *Int J Pharm.* 2003; 254: 65–68.
- Yameen B, Choi WI, Vilos C, Swami A, et al. Insight into nanoparticle cellular uptake and intracellular targeting. *J Control Release* 2014; 190: 485-499.
- Yingngam B, Brantner AH, Jinarat D, Kaewamatawong R, et al. Determination of the marker diarylheptanoid phytoestrogens in *Curcuma comosa* rhizomes and selected herbal medicinal products by HPLC-DAD. *Chem Pharm Bull* 2018; 66(1): 65-70.
- Yingngam B, Rungseewijitprapa W. Molecular and clinical role of phytoestrogens as anti-skin-ageing agents: A critical overview. *Phytopharmacology* 2012; 3(2): 227-244.