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DEVELOPMENT OF NICOSOMES ENTRAPPED WITH  
EXTRACTS FROM NATIVE THAI SILK (*Bombyx mori*)  
AS ANTI-WRINKLE COSMETIC PRODUCTS

SUPANIDA WINTOCHAI

DOCTOR OF PHILOSOPHY  
IN PHARMACY

THE GRADUATE SCHOOL  
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**A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN  
PARTIAL FULFILLMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY  
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THIS THESIS HAS BEEN APPROVED  
TO BE A PARTIAL FULFILLMENT OF THE REQUIREMENTS  
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<b>Thesis Title</b>	Development of Niosomes Entrapped with Extracts from Native Thai Silk ( <i>Bombyx mori</i> ) as Anti-wrinkle Cosmetic Products	
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### ABSTRACT

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The aim of this research was to develop an anti wrinkle serum containing niosomes entrapped with sericin and oil from native Thai silkworms (*Bombyx mori*). Sericin and oil were extracted from five native Thai silkworms, Keaw Sakon, Nang noi Srisaket, Sam Rong, Nang Luang and None Ruesee. The yields of oils from the five native Thai silkworms by Soxhlet and maceration methods were 24-29% and 5-7%, respectively. Oils extracted from None Ruesee by the Soxhlet method, and oils extracted from Nang Leung, Sam Rong, and None Ruesee by the maceration method showed free radical scavenging activity. Oil extracted from None Ruesee by the maceration method gave the highest free radical scavenging activity. Moreover, oil extracted by the Soxhlet extraction from None Ruesee gave the highest tyrosinase inhibition activity, but lower than that of the standard vitamin C and kojic acid. The silkworm pupae oil obtained from Soxhlet extraction had unsaturated fatty acid content in the range of 72-79 %, and alpha - linolenic acid content in the range of 32-44 %, whereas that obtained from the maceration extraction had the unsaturated fatty acid contents in the range of 75-80%, and alpha-linolenic acid contents in the range of

40-46 %. The yields of sericin obtained by alkaline (0.5 N  $\text{Na}_2\text{CO}_3$ ) and autoclave method (at  $121^\circ\text{C}$ , three hours) were in the range of 22.57-28.34 and 28.93-35.20 %, respectively. Sericin extracted from Nangnoi Srisaket by alkaline and autoclave method gave the highest sericin contents. Sericin extracted from None Ruesee and Nang Luang by alkaline gave the highest tyrosinase inhibition activity ( $\text{IC}_{50}$ = 1.20 and 2.22 mg/ml). Sericin extracted from Nang Luang and Sam Rong by autoclave method exhibited the highest free radical scavenging activity ( $\text{SC}_{50}$ = 13.65 and 15.45 mg/ml). The percentages of the protein contents were determined by the Lowry method. The average percentages of protein contents by autoclave and alkaline methods were in the range of 20.10-25.74 and 16.52-20.19 %, respectively. Different silk varieties contain distinct sericin with various amino acid compositions, which were significantly influenced by the extraction method used. Then, oil and sericin of None Ruesee strain silkworm were entrapped in the blank niosomes composed of Tween 61 and cholesterol at 1:1 molar ratio which was prepared by the chloroform film with sonication method. The blank niosomes were physical stable with uniform size and no sedimentation. The maximum loading of the sericin and oil in niosome was 1 and 1 % w/v. The niosomes were stable for 8 weeks. The average particle size of niosomes by zetasizer analyzer was 92-800 nm. The morphology of the prepared niosomes was in the mixture of unilamellar and multilamellar vesicles (MLVs), and large unilamellar vesicles (LUVs). The oil and sericin entrapped in niosomes gave lower free radical scavenging and tyrosinase inhibition activity than the entrapped oil and sericin. The compositions of the developed anti-wrinkle serum were 0.15 Carbopo<sup>®</sup> Ultrez 21 polymer, 1.5  $\text{C}_{14-22}$  alkyl alcohol and  $\text{C}_{12-20}$  alkylglucoside, 1.6 cyclopentasiloxane, dimethiconol, dimethicone crosspolymer (and) blend, 1 sodium polyacrylate (and) dimethicone (and) cyclopentasiloxane (and) trideceth-6 (and) PEG/PPG -18/18 dimethicone, 6 niosome (containing 1%w/w sericin and 1% w/w oil of None Ruesee silkworm), 0.6 Naomi and light yellow color. The characteristics of the serum had the viscosity of 14,500 cP, pH 6.97, light yellow color with  $L^*$   $a^*$   $b^*$  value of 75.57, 0.54, 28.19 with no phase separation. The total plate counts of bacteria and yeast/mold were less than 10 colony/g. The *in vivo* tests in human volunteers showed that this serum gave superior skin hydration determined by transepidermal water loss, and improved the skin elasticity significant after the 8-week treatment. The estimated cost of the



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developed serum was 859 baht/bottle (50g). The serum was physically stable during the 8-week storage at room temperature (30 °C), 35 and 45 °C. For the consumer test, it showed that 85.4 % of the volunteers accepted the product and 86.58 % of the volunteers interested in buying this developed product. The overall satisfaction of the volunteers on the product was moderate. The developed anti-wrinkle product from this study can be further continued for commercialization.

**ชื่อเรื่องวิทยานิพนธ์**

การพัฒนาน้ำมันโอโซมที่เก็บกักสารสกัดจาก

ไหมพันธุ์ไทยพื้นบ้าน (*Bombyx mori*) เพื่อเป็น

ผลิตภัณฑ์เครื่องสำอางด้านริ้วรอย

**ผู้เขียน**

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**บทคัดย่อ****E41034**

งานวิจัยนี้มีวัตถุประสงค์ เพื่อพัฒนาน้ำมันโอโซมที่กักเก็บเซริซินและน้ำมันสกัดจากไหมพันธุ์ไทยพื้นบ้านเพื่อเป็นผลิตภัณฑ์เครื่องสำอางด้านริ้วรอย ได้คัดเลือกไหมพันธุ์ไทยพื้นเมือง 5 สายพันธุ์ ซึ่งได้แก่ เขียวสกล นางน้อยศรีสะเกษ ลำโรง นางเหลียง และโนนฤๅษี แล้วนำมาสกัดเซริซินและน้ำมันสกัด ผลผลิตของน้ำมันดักแด้ไหมทั้ง 5 สายพันธุ์ จากการสกัดด้วยวิธี Soxhlet และ maceration อยู่ในช่วงร้อยละ 24-29 และ 4-7 ตามลำดับ น้ำมันดักแด้จากไหมพันธุ์โนนฤๅษี จากการสกัดด้วยวิธี Soxhlet และน้ำมันดักแด้ไหมพันธุ์นางเหลียง ลำโรง และโนนฤๅษี จากการสกัดด้วยวิธี maceration มีฤทธิ์ต้านอนุมูลอิสระ โดยน้ำมันจากดักแด้ไหมพันธุ์โนนฤๅษีจากการสกัดด้วยวิธี maceration มีความสามารถต้านอนุมูลอิสระได้ดีที่สุด เมื่อเปรียบเทียบกับน้ำมันจากดักแด้ไหมพันธุ์อื่นๆ น้ำมัน

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ดักแค้ใหม่พันธุ์โนนฤๅษีจากการสกัดด้วยวิธี Soxhlet มีฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนส ได้ดีที่สุดแต่ยังต่ำกว่าสารมาตรฐานวิตามินซีและกรดโคจิก มีองค์ประกอบของกรดไขมันไม่อิ่มตัวอยู่ร้อยละ 72-79 และแอลฟาไลโนเลนิกร้อยละ 32-44 จากการสกัดด้วยวิธี Soxhlet ในขณะที่วิธีการสกัด maceration ให้กรดไขมันไม่อิ่มตัวร้อยละ 75-80 และแอลฟาไลโนเลนิกร้อยละ 40-46 ร้อยละผลผลิตของเซริซินใหม่จากการสกัดโดยใช้โซเดียมคาร์บอเนต (0.05 N  $\text{Na}_2\text{CO}_3$ ) และใช้การนึ่งด้วยไอน้ำแรงดันสูง (มิลลิเมตรปรอท) ที่อุณหภูมิ 121 องศาเซลเซียส แรงดันไอน้ำ 15 (ปอนด์ต่อตารางนิ้ว) ระยะเวลา 3 ชั่วโมง อยู่ในช่วงร้อยละ 22.57-28.34 และ 28.93-35.20 ตามลำดับ เซริซินจากใหม่พันธุ์นางน้อยศรีสะเกษจากการสกัดโดยใช้โซเดียมคาร์บอเนตและใช้การนึ่งด้วยไอน้ำแรงดันสูงให้ผลผลิตสูงสุดเมื่อเปรียบเทียบกับเซริซินจากใหม่พันธุ์อื่นๆ เซริซินจากใหม่พันธุ์โนนฤๅษี และนางเหลื่องจากการสกัดด้วยโซเดียมคาร์บอเนต มีฤทธิ์ยับยั้งเอนไซม์ไทโรซิเนส ( $\text{IC}_{50}$ =1.20 และ 2.22 มก./มล) ได้ดีที่สุด เมื่อเปรียบเทียบกับใหม่พันธุ์อื่น ในขณะที่เซริซินจากใหม่พันธุ์นางเหลื่องและสำโรงจากการสกัดโดยใช้การนึ่งด้วยไอน้ำแรงดันสูงมีฤทธิ์ต้านอนุมูลอิสระ( $\text{SC}_{50}$ = 13.65 และ 15.49 มก./มล) ดีที่สุดเมื่อเปรียบเทียบกับใหม่พันธุ์อื่น ในการวิเคราะห์หาปริมาณร้อยละโปรตีนด้วยวิธี Lowry จากการสกัดด้วยการนึ่งด้วยไอน้ำแรงดันสูงและโซเดียมคาร์บอเนตให้ปริมาณโปรตีนอยู่ในช่วงร้อยละ 20.10-25.74 และ 16.52-20.19 ตามลำดับ ปริมาณเซริซินที่เป็นองค์ประกอบในกรดอะมิโนจากใหม่พันธุ์ที่แตกต่างกันมีอิทธิพลอย่างมีนัยสำคัญจากการใช้วิธีการสกัดที่ต่างกัน ได้คัดเลือกน้ำมันดักแค้ใหม่และเซริซินรังใหม่จากพันธุ์โนนฤๅษีมาสกัดด้วยวิธี maceration และด่างโซเดียมคาร์บอเนต ตามลำดับ เพื่อนำมาเก็บกักในนีโอโซม นีโอโซมเปล่าประกอบด้วยทวิน 61 ผสมคอเลสเตอรอล ในอัตราส่วนโมลาร์ 1:1 จากการเตรียมโดยวิธี chloroform film ร่วมกับการใช้คลื่นความถี่สูง พบว่าให้ความคงตัวทางกายภาพ มีขนาดอนุภาคสม่ำเสมอ และไม่ตกตะกอน นีโอโซมสามารถเก็บกักน้ำมันและเซริซินในปริมาณสูงสุดร้อยละ 1 และ 1 % โดยน้ำหนักต่อปริมาตร ตามลำดับ นีโอโซมที่ได้มีความคงตัวดี เมื่อเก็บเป็นเวลา 8 สัปดาห์ มีขนาดอนุภาคที่วัดด้วย Zetasizer analyzer อยู่ในช่วง 92-800 นาโนเมตร นีโอโซมที่ได้มีลักษณะเป็นอนุภาคผนังสองชั้นหุ้ญเดี่ยวจำนวนหลายหุ้ญ ผสมกับอนุภาคผนังสองชั้นหุ้ญเดี่ยวขนาดใหญ่ พบว่าฤทธิ์ต้านอนุมูลอิสระ (DPPH radical scavenging metal chelating และ tyrosinase inhibition) ของนีโอโซมที่เก็บกักน้ำมันดักแค้ใหม่และเซริซินมีค่าน้อยกว่าสารสกัดที่ไม่ได้เก็บกักในนีโอโซม ได้พัฒนาสูตรผลิตภัณฑ์เซรั่มที่มีส่วนผสมของนีโอโซมที่เก็บกักเซริซิน และน้ำมันดักแค้ใหม่ไทยพันธุ์พื้นบ้านที่ประกอบด้วย 0.15 Carbopol® Ultrez 21 polymer, 1.5  $\text{C}_{14-22}$  alkylalcohol and  $\text{C}_{12-20}$  alkylglucoside, 1.6 cyclopentasiloxane, dimethiconol, dimethicone crosspolymer (and) blend, 1 sodium Polyacrylate (and) dimethicone (and) cyclopentasiloxane



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(and) trideceth-6 (and) PEG/PPG -18/18 dimethicone, 6 niosome, (เซริซิน 1 น้ำมันคัสแคเดียม 1% w/v จากไหมพันธุ์โนนถายี), 0.6 กลิ่น Naomi และให้สีเหลืองนวล คุณลักษณะของเซรัมลดริ้วรอย ที่พัฒนาได้มีค่าความหนืดเท่ากับ 14,500 cP ค่าความเป็นกรดต่างเท่ากับ 6.97 มีสีเหลืองเข้มออกน้ำตาลในระบบ  $L^* a^* b^*$  เท่ากับ 75.57, 0.54 และ 28.19 ตามลำดับ มีความคงตัวดีไม่แยกชั้น มีจำนวนแบคทีเรีย ยีสต์ และ ราทั้งหมดน้อยกว่า 10 โคโลนีต่อกรัม เมื่อทดสอบในอาสาสมัคร พบว่าเซรัมที่พัฒนาได้มีความสามารถในการเก็บกักน้ำไว้ที่ผิว และช่วยปรับความยืดหยุ่นของผิวหนังให้ดีขึ้นอย่างมีนัยสำคัญหลังจากการใช้ 8 สัปดาห์ เมื่อเปรียบเทียบกับก่อนใช้ ต้นทุนการพัฒนาผลิตภัณฑ์เซรัมลดริ้วรอย ต่อ 1 หน่วย (50 กรัม / 1 หลอด) มีราคาาคะเนประมาณ 2963.23 บาท เซรัมลดริ้วรอยที่ได้มีความคงตัวในระหว่างการเก็บนาน 8 สัปดาห์ ที่สภาวะอุณหภูมิห้อง (30 °C) ในสภาวะอุณหภูมิ 35 และ 45 °C สำหรับการทดสอบการยอมรับของผู้บริโภคพบว่าร้อยละ 85.40 ของอาสาสมัครยอมรับผลิตภัณฑ์ และร้อยละ 86.58 ของอาสาสมัครสนใจที่จะซื้อผลิตภัณฑ์ คะแนนความชอบของอาสาสมัครที่มีต่อผลิตภัณฑ์อยู่ในระดับปานกลาง จะสามารถนำผลิตภัณฑ์ที่พัฒนาได้จากการศึกษานี้ไปต่อยอดในเชิงพาณิชย์ได้ต่อไป

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**ABBREVIATIONS AND SYMBOLS**

CFS	Chloroform film method with sonication
CFU	Colony forming unit
$\text{CHCl}_3$	Chloroform
DPPH	1, 1-Diphenyl-2-picrylhydrazyl
TEM	transmission electron microscopy
FT-IR	Fourier transforms infrared spectroscopy
HPLC	High performance liquid chromatography
$\text{IC}_{50}$	Concentration providing 50% of tyrosinase inhibition activity
LPO	Lipid peroxide
LUV	Large unilamellar vesicle
mg	Milligram
mL	Milliliter
MLV	Multilamellar vesicle
mM	Millimolar
nm	Nanometer
ppm	Parts per million
$\text{SC}_{50}$	Concentration providing 50% free radical scavenging activity
SUV	Small unilamellar vesicle
TEA	Triethanolamine
TEWL	Transepidermal water loss
Tween 61	Polyoxyethylene sorbitan monostearate
$\mu\text{g}$	Microgram



$\mu\text{L}$	Microliter
$^{\circ}\text{C}$	Celcius degree
Psi	Pound-force per square inch
mmHg	Millimeter of mercury
g.	Gram
kg.	Kiligram
cP	Centric point