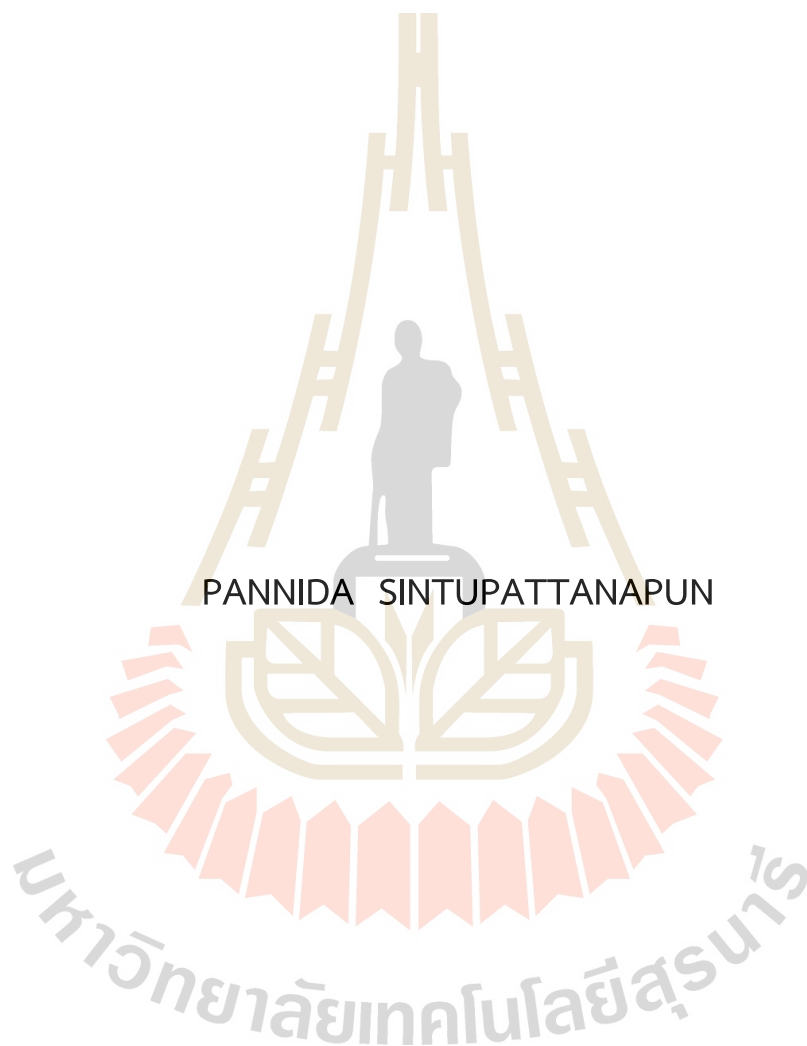


EFFICACY AND SAFETY OF THE PLOOK-FIRE-THATU RECIPE ON
BREAST MILK VOLUME AND BREAST MILK MACRONUTRIENT
COMPOSITION IN POSTPARTUM WOMEN



PANNIDA SINTUPATTANAPUN

A Thesis Submitted in Partial Fulfillment of the Requirements for the
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ประสิทธิผลและความปลอดภัยของตำรับยาปลูกไผ่ธาตุต่อปริมาณน้ำนมและ
สารอาหารหลักในน้ำนมของหญิงหลังคลอด

นางพรรณนิดา ลินธุ์พัฒนพันธุ์

มหาวิทยาลัยเทคโนโลยีสุรนารี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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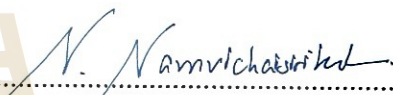
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MILK VOLUME AND BREAST MILK MACRONUTRIENT COMPOSITION IN
POSTPARTUM WOMEN

Suranaree University of Technology has approved this thesis submitted in
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พรรณนิดา สินธุ์พัฒน์พันธุ์: ประสิทธิภาพและความปลอดภัยของตำรับยาปลูกไฟธาตุต่อปริมาณน้ำนมและสารอาหารหลักในน้ำนมของหญิงหลังคลอด (EFFICACY AND SAFETY OF THE PLOOK-FIRE-THATU RECIPE ON BREAST MILK VOLUME AND BREAST MILK MACRONUTRIENT COMPOSITION IN POSTPARTUM WOMEN)

อาจารย์ที่ปรึกษา: อาจารย์ นายแพทย์ ดร.นิวัฒน์ชัย นามวิชัยศิริกุล, 144 หน้า.

คำสำคัญ: ยาปลูกไฟธาตุ/ การแพทย์แผนไทย/ สารอาหารในน้ำนม/ น้ำนมแม่/ เทคนิคฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปกโตรสโกปี

น้ำนมแม่ได้รับการยกย่องให้เป็นอาหารที่ดีที่สุดสำหรับทารก เนื่องจากมีคุณค่าทางโภชนาการครบถ้วนและสามารถเสริมสร้างภูมิคุ้มกันได้อย่างมีประสิทธิภาพ องค์การอนามัยโลกแนะนำให้มารดาเลี้ยงลูกด้วยน้ำนมแม่อย่างเดียวเป็นระยะเวลา 6 เดือน และต่อเนื่องอย่างน้อยจนถึงอายุ 1 ปี การเลี้ยงลูกด้วยนมแม่มีบทบาทสำคัญในการป้องกันโรคที่อาจนำไปสู่ความพิการและการเสียชีวิตในวัยทารก สถานการณ์การเลี้ยงลูกด้วยนมแม่ในประเทศไทยพบว่ามีทารกเพียงร้อยละ 14 เท่านั้นที่ได้รับน้ำนมแม่อย่างเดียวในช่วง 6 เดือนแรกหลังคลอด หนึ่งในอุปสรรคสำคัญที่ส่งผลต่ออัตราการเลี้ยงลูกด้วยนมแม่คือภาวะน้ำนมไม่เพียงพอในช่วงสี่เดือนแรกหลังคลอด ซึ่งสูงถึงร้อยละ 63 ในประเทศไทย ปัจจุบันยังไม่มียาที่ได้รับการรับรองอย่างเป็นทางการสำหรับการกระตุ้นน้ำนมโดยตรง

ยาปลูกไฟธาตุเป็นยาสมุนไพรที่รักษาภาวะน้ำนมไม่เพียงพอ อย่างต่อเนื่องตั้งแต่ ปี พ.ศ. 2450 และได้รับการบรรจุในบัญชียาหลักแห่งชาติเมื่อ ปี พ.ศ. 2555 การอธิบายถึงกลไกการออกฤทธิ์ของตำรับยาดังกล่าวต่อการผลิตน้ำนมยังมีน้อยมากในปัจจุบัน การศึกษานี้มีวัตถุประสงค์เพื่อประเมินประสิทธิผลของตำรับยาปลูกไฟธาตุต่อปริมาณน้ำนม ระดับฮอร์โมนโปรแลคตินในซีรัม และปริมาณสารอาหารในน้ำนม รวมถึงการประเมินความปลอดภัยของตำรับยาผ่านการตรวจค่าทางโลหิตวิทยา ค่าชีวเคมีในเลือด และการติดตามอาการไม่พึงประสงค์ที่ในมารดาและทารก

การวิเคราะห์องค์ประกอบทางเคมีและสารออกฤทธิ์ทางชีวภาพของตำรับยา ใช้เทคนิคโครมาโตกราฟีของเหลวประสิทธิภาพสูง การทดลองทางคลินิกดำเนินการในรูปแบบการทดลองแบบสุ่ม โดยมีระยะเวลาการศึกษา 72 ชั่วโมงหลังคลอด อาสาสมัครเป็นหญิงไทยอายุระหว่าง 20-40 ปี ได้รับการคัดเลือกโดยการสุ่มตัวอย่างตามหลักความน่าจะเป็น กลุ่มทดลองได้รับยาปลูกไฟธาตุขนาด 500 มิลลิกรัม ก่อนอาหาร วันละ 3 ครั้ง ร่วมกับการรักษามาตรฐาน เป็นระยะเวลา 3 วันหลังคลอด กลุ่มควบคุมได้รับการรักษามาตรฐานเพียงอย่างเดียวใน การประเมินผลการทดลองดำเนินการทั้งก่อนและหลังการรับยา โดยมีการเก็บตัวอย่างเลือดในวันที่ 1 และวันที่ 3 หลังคลอด เพื่อวิเคราะห์ระดับฮอร์โมนโปรแลคติน ค่าทางโลหิตวิทยา และค่าชีวเคมีในเลือด ปริมาณน้ำนมถูกวัดในวันที่ 1, 2

และ 3 หลังคลอด บันทึกอาการไม่พึงประสงค์จากการใช้ยาในวันที่ 1, 2, 3, 7 และ 30 หลังคลอด การวิเคราะห์ปริมาณสารอาหารในน้ำนมแม่ ใช้เทคนิคฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปกโตรสโคปี เพื่อประเมินคุณภาพทางโภชนาการของน้ำนมอย่างละเอียด

การวิเคราะห์องค์ประกอบทางเคมีของตำรับยาปลูกไผ่ธาตุพบว่าสารออกฤทธิ์ทางชีวภาพหลัก ได้แก่ piperine และ 8-gingerol ด้านการเปรียบเทียบปริมาณน้ำนมเฉลี่ยระหว่างกลุ่มควบคุมและกลุ่มทดลอง พบว่าในกลุ่มควบคุมมีปริมาณน้ำนมเฉลี่ยในวันที่ 1, 2 และ 3 เท่ากับ 4.12 ± 6.43 , 28.99 ± 30.88 และ 95.69 ± 106.47 มิลลิลิตร ตามลำดับ ในขณะที่กลุ่มทดลองมีปริมาณน้ำนมเฉลี่ยในวันที่ 1, 2 และ 3 เท่ากับ 7.33 ± 10.20 , 56.36 ± 58.06 และ 213.12 ± 111.13 มิลลิลิตร ตามลำดับ การประเมินระดับฮอร์โมนโปรแลคตินในซีรัมภายในกลุ่มทดลองก่อนและหลังการรักษาพบว่า ระดับโปรแลคตินหลังการรักษา (256.36 ± 61.12 นาโนกรัม/มิลลิลิตร) สูงกว่าก่อนการทดลอง (197.93 ± 95.90 นาโนกรัม/มิลลิลิตร) อย่างมีนัยสำคัญ ไม่พบความแตกต่างของค่าทางโลหิตวิทยา รวมถึงการทำงานของตับและไตระหว่างก่อนและหลังการใช้ยา การวิเคราะห์องค์ประกอบสารอาหารหลักของน้ำนมโดยใช้เทคนิคฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปกโตรสโคปี พบว่าน้ำนมแม่ประกอบด้วยสารอาหารสำคัญ ได้แก่ โปรตีน ไขมัน ไตรกลีเซอไรด์ คอเลสเตอรอล คาร์โบไฮเดรต และกรดนิวคลีอิก ซึ่งเป็นสารอาหารที่จำเป็นต่อการเจริญเติบโตและการพัฒนาการของทารก

ผลการศึกษาแสดงให้เห็นว่าสารออกฤทธิ์ทางชีวภาพหลักในตำรับยาปลูกไผ่ธาตุ ได้แก่ piperine ซึ่งสอดคล้องกับข้อมูลจากงานวิจัยก่อนหน้านี้ที่ระบุถึงบทบาทของสารดังกล่าวในการส่งเสริมการหลั่งน้ำนม อาสาสมัครที่ได้รับตำรับยาปลูกไผ่ธาตุมีปริมาณน้ำนมเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติในวันที่ 2 และวันที่ 3 หลังคลอด เมื่อเปรียบเทียบกับกลุ่มควบคุม นอกจากนี้ยังพบว่าอาสาสมัครที่ได้รับยาต่อเนื่องเป็นเวลา 3 วัน มีปริมาณไกลโคเจน และคาร์โบไฮเดรต กรดในน้ำนมสูงกว่าผู้ที่ไม่ได้รับยา การประเมินด้านความปลอดภัยพบยาปลูกไผ่ธาตุ 1,500 มิลลิกรัมต่อวันไม่ก่อให้เกิดอาการไม่พึงประสงค์ที่รุนแรง ผลการศึกษานี้จึงสนับสนุนความเป็นไปได้ในการใช้ตำรับยาปลูกไผ่ธาตุเป็นทางเลือกเสริมในการส่งเสริมการผลิตน้ำนมในมารดาหลังคลอด

สาขาวิชา เวชศาสตร์ปริวรรต
ปีการศึกษา 2568

ลายมือชื่อนักศึกษา..... พวงนันทา สันต์วัฒนพันธ์

ลายมือชื่ออาจารย์ที่ปรึกษา..... ธีรภัทร เกษมทรัพย์

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม..... นิคมพงษ์

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม อ.ดร.นริศลักษณ์ สุวรรณโนบล
(ถึงแก่กรรมเมื่อวันที่ 13 เมษายน 2569)

PANNIDA SINTUPATTANAPUN: EFFICACY AND SAFETY OF THE PLOOK-FIRE-THATU RECIPE ON BREAST MILK VOLUME AND BREAST MILK MACRONUTRIENT COMPOSITION IN POSTPARTUM WOMEN

THESIS ADVISOR: NIWATCHAI NAMVICHASIRIKUL, M.D, Ph.D, 144 PP.

Keyword: Plook-Fire-Thatu/ Thai traditional medicine/ Galactagogues/ Breast milk
Macronutrients/ Fourier Transform Infrared Spectrometer

Breast milk is the best nutrition for babies and highly recommended by the WHO. They advise exclusive breastfeeding for six months and continued breastfeeding for at least one year. However, only 14% of Thai children are exclusively breastfed. Lactation insufficiency affects about 63% of Thai mothers within four months of birth, and currently, no medications are available to increase milk production.

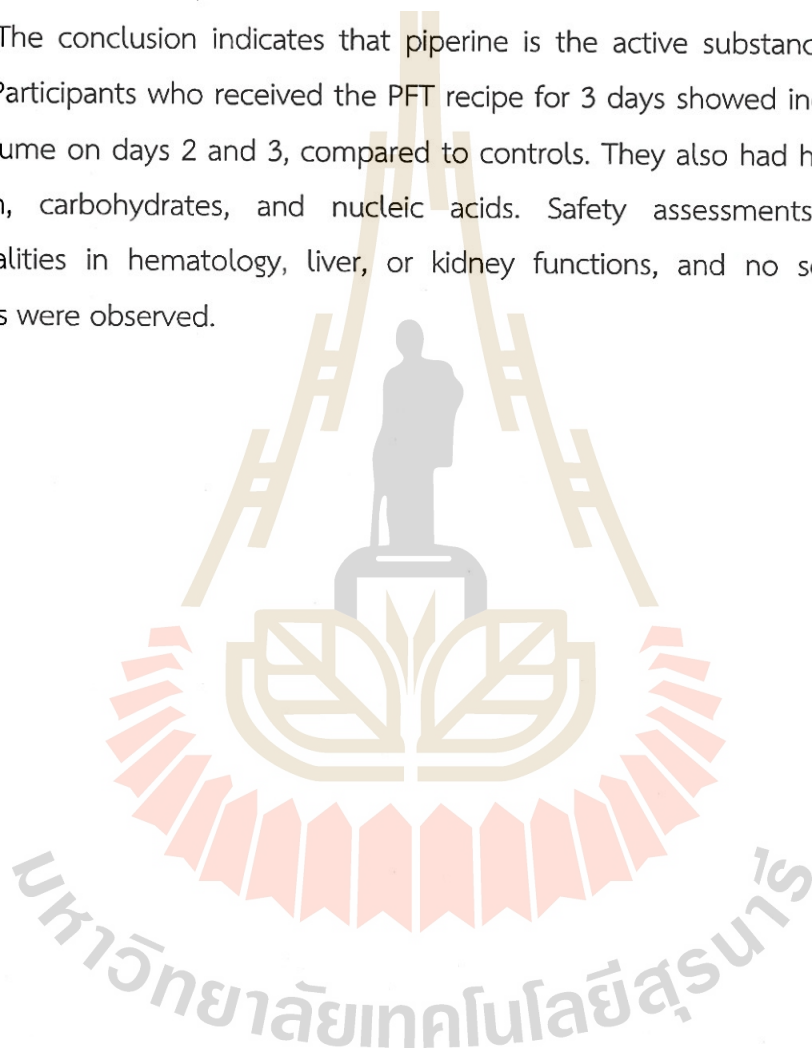
The Plook-Fire-Thatu recipe (PFT) has been used widely since 1907 and has been included in Thailand's NLEM for postpartum women since 2012. However, studies on its mechanism are lacking. This research aims to evaluate PFT's effects on breast milk volume, serum prolactin, nutrient content, safety (as assessed via hematology and biochemistry), and any adverse reactions.

High-performance liquid chromatography (HPLC) was used to analyze the active substance of the recipe. A randomized clinical trial (RCT) was conducted to investigate the effect of the PFT recipe on breast milk production. The labor lasted 72 hours postpartum. Participants in the experimental group ingested 500 mg of the PFT recipe orally three times daily before meals for three days postpartum. The participants were women aged between 20 and 40 years. All participants were selected through random sampling. The study's results were assessed before the PFT recipe. Blood samples were collected on days 1 and 3, respectively. Breast milk production was measured on days 1, 2, and 3 postpartum, and after each feeding. Adverse drug reactions in the mothers were monitored on days 1, 2, 3, 7, and 30 postpartum. Breast milk samples were analyzed for nutritional content using FTIR spectroscopy.

The results show that piperine and 8-gingerol are active substances in the PFT recipe. The mean breast milk volume in the control group on days 1, 2, and 3 was

4.12±6.43, 28.99±30.88, and 95.69±106.47 mL, respectively. In the treatment group, it was 8.31±10.76, then 7.33±10.20, 56.36±58.06, and 213.12±111.13 mL on the same days. Serum prolactin levels increased after treatment in the treatment group, from 197.93±95.90 to 256.36±61.12 ng/mL. No safety issues were found in hematology, liver, or kidney tests. FTIR spectroscopy showed that breast milk mainly contains proteins, saturated lipids, carbohydrates, and nucleic acids.

The conclusion indicates that piperine is the active substances in the PFT recipe. Participants who received the PFT recipe for 3 days showed increased breast milk volume on days 2 and 3, compared to controls. They also had higher levels of glycogen, carbohydrates, and nucleic acids. Safety assessments showed no abnormalities in hematology, liver, or kidney functions, and no severe adverse reactions were observed.



School of Translational Medicine
Academic Year 2025

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Co-advisor's Signature Dr. Nareelux Suwannobol
(Passed away on April 13, 2026)

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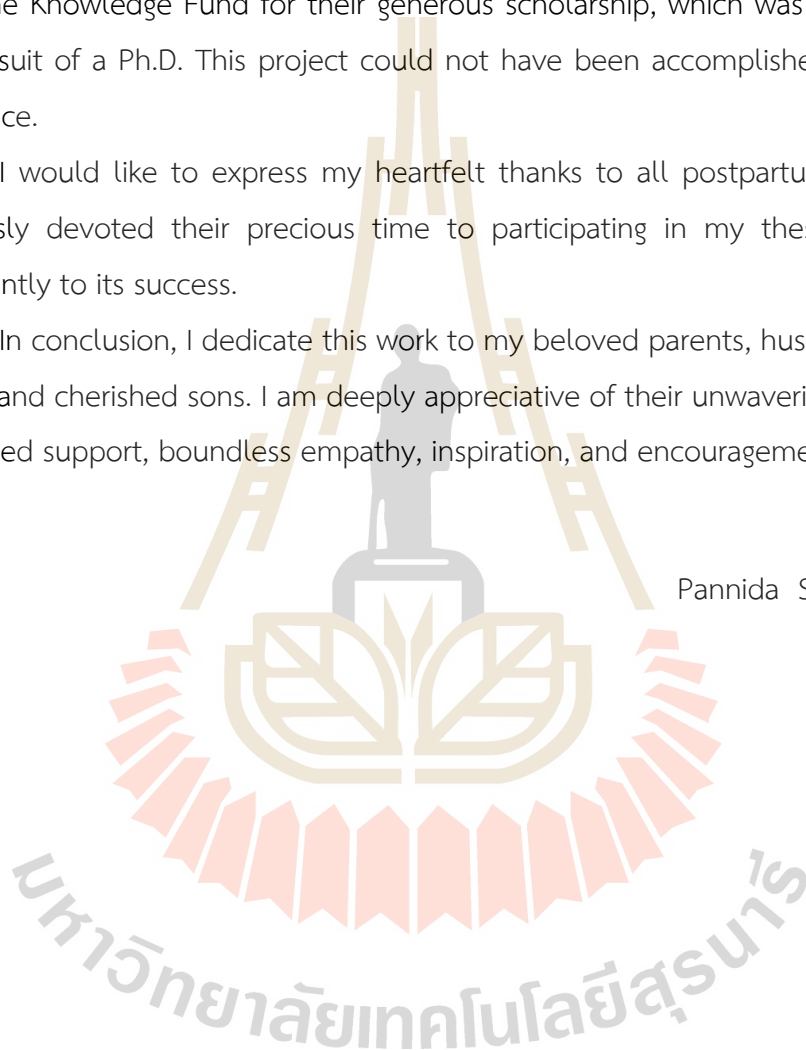


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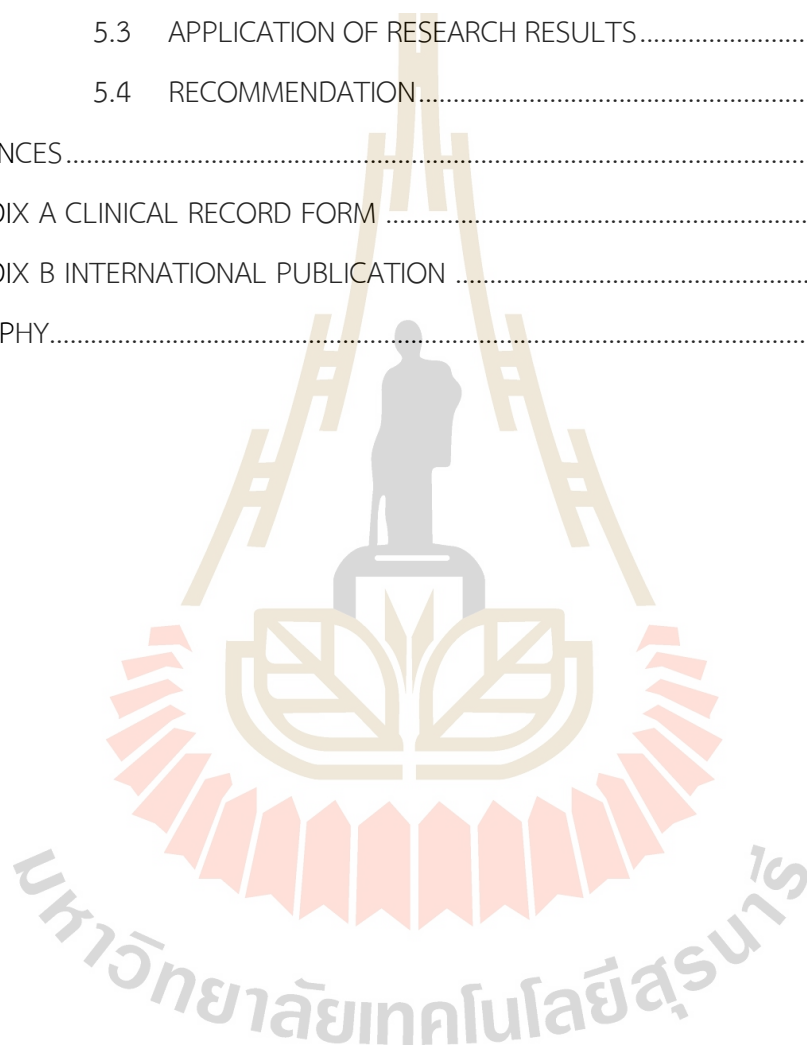
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LIST OF ABBREVIATIONS

WHO	=	World Health Organization
AAP	=	American Academy of Pediatrics
UNICEF	=	United Nations Children's Fund
NLEM	=	National List of Essential Medicines
HPLC	=	High-performance liquid chromatography
RCT	=	Randomized clinical trial
FTIR	=	Fourier transform Infrared spectroscopy
PFT	=	Plook-Fire-Thatu recipe
MCV	=	Mean corpuscular volume
MCH	=	Mean cell hemoglobin
MCHC	=	Mean corpuscular hemoglobin concentration
RDW	=	Red blood cell distribution width
WBC	=	White blood cell count
RBC	=	Red blood cell count
LDLC	=	Low-density lipoprotein-cholesterol
HDLC	=	High-density lipoprotein-cholesterol
AST	=	Aspartate aminotransferase
ALT	=	Alanine aminotransferase
ALP	=	Alkaline phosphatase
BUN	=	Blood urea nitrogen

CHAPTER I

INTRODUCTION

1.1 Thesis title

EFFICACY AND SAFETY OF THE PLOOK-FIRE-THATU RECIPE ON BREAST MILK VOLUME AND BREAST MILK MACRONUTRIENT COMPOSITION IN POSTPARTUM WOMEN

1.2 Background and Problems

Breast milk is the optimal nutrition for all infants. Breastfeeding sets the standard for infant feeding. The neurodevelopmental advantages of breastfeeding highlight the importance of providing human breast milk as a public health priority. The World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) recommend that children begin breastfeeding within the first hour after birth and exclusively breastfeed for the first six months. No other foods or liquids, including water, should be provided. Children should start eating safe and adequate complementary foods at six months, while continuing to breastfeed for up to two years or beyond. WHO aims to boost the exclusive breastfeeding rate for the initial six months to at least 50% by 2025. ("Breastfeeding," 2023) (Meek & Noble, 2022) Breast milk delivers essential nutrients, bioactive compounds, and beneficial bacteria that support the growth of infants and the development of their immune systems. The associated advantages of breastfeeding encompass protection against pathogens, enhanced immune development, comprehensive nutrition, promotion of gut colonization, and a reduction in the incidence of gastrointestinal diseases. (Lyons, Ryan, Dempsey, Ross, & Stanton, 2020).

Globally, breastfeeding rates from 2015 to 2021 show that 47% of newborns-initiated breastfeeding within one hour of birth. During this period, the percentage of

infants under six months who were exclusively breastfed reached 48%, slightly below the target of 50% by 2025 and 70% by 2030.

While 70% of women continue to breastfeed their infants for at least one year, breastfeeding rates decline to 45% by the time their child reaches two years of age. (Organization, 2022). Thailand's breastfeeding rates are even lower than the global average. The low prevalence of breastfeeding among Thai women results in negative health outcomes, which hinders national and socioeconomic development. A study shows that inadequate breastfeeding in Thailand has led to 600 child deaths from diarrhea and pneumonia, along with 1,180 maternal deaths from breast and ovarian cancers. This situation has caused approximately 600 million USD in household spending on infant formula and related products. Moreover, the national health expense for treating child illnesses amounts to almost 6 million USD (about 0.001% of Gross National Income). Between 2006 and 2019, breastfeeding rates in Thailand revealed that 2019% of newborns initiated breastfeeding within one hour of birth, while only 14% were exclusively breastfed for six months. Although 24.6% of women continue to breastfeed their infants for at least one year, breastfeeding rates decline to 15% by two years of age. (Topothai & Tangcharoensathien, 2021).

Lactation failure refers to the necessity of initiating top feedings for a baby within three months of delivery due to insufficient breast milk supply. The synonyms for lactation insufficiency include: lactational inadequacy, breast milk insufficiency, mother's milk insufficiency, perceived insufficient milk, insufficient breast milk, partial lactation failure, neonatal insufficient milk syndrome, and hypogalactia or lactation inadequacy. Total lactation failure indicates a complete absence of milk flow or the secretion of only a few drops of breast milk following suckling for at least seven days (Sultana, Rahman, & Manjula, 2013). Partial lactational failure occurs more frequently at a rate of 94.7% compared to complete lactational failure, which is recorded at 5.3%. The delay in breastfeeding was observed to last from two to five days, primarily due to traditional reasons (77.3%) and the perception among mothers that milk output was inadequate (92%). A comprehensive analysis of various factors reveals the multifaceted causes contributing to lactation failure. Insufficient milk production or the absence of

milk entirely accounts for the most prevalent reason for lactation failure, representing 80% (Mathur, Chitranshi, Mathur, Singh, & Bhalla, 1992).

Handling hypogalactia involves improving breastfeeding techniques and promoting frequent suckling. Galactagogues, or lactogogues, are drugs or substances like metoclopramide, domperidone, and certain herbs that potentially boost milk production. Before using them, assess risks to mother and baby. Domperidone, a peripheral dopamine receptor blocker, increases prolactin to stimulate milk. Its risk of sudden cardiac death is dose-dependent: with an odds ratio of 2.57 (95% confidence interval, 0.79-8.36) for patients utilizing a dosage of 30 mg/day, and an odds ratio of 16 (95% confidence interval, 3.49-73.6) for patients taking more than 30 mg/day (Paul et al., 2015). No adverse effects have been reported in infants whose mothers are administered domperidone. Metoclopramide functions as an antagonist to dopamine release within the central nervous system (CNS), consequently leading to an elevation in prolactin levels. There exist only two documented instances of mild and transient intestinal discomfort in infants who are breastfed by mothers receiving metoclopramide therapy (30 and 45 mg/day). Ten percent of the mothers undergoing treatment reported experiences of nervousness, drowsiness, and fatigue, with a significantly higher prevalence of insomnia, headaches, intestinal disturbances, and extrapyramidal symptoms observed in 1% of cases (Zuppa et al., 2010).

Thai local wisdom related to postpartum healthcare practices includes food consumption, lying by the fire (yuu-fai), warm water and herbal baths, mother and infant-related rituals, warm water and herbal drinks, relaxation and exercise, herbal steam tents, salt pot compression, body massage, sitting over charcoal, herbal ball compression, and herbal remedies (Punyopashtambha, 2019). The herbal medicines of interest that have indications for stimulating lactation in postpartum women. The pluk-fai-tad is a traditional Thai herbal medicine formula found in the Mahachotarat scripture. It was recorded in the Medical Science Book from the Rattanakosin era, composed by Prince Phisanu Prasatwetch (Kong Thawornwetch). The plook-fire-thatu is a recipe for postpartum women who have breast milk insufficiency. It has been used widely in the past to the present. It has been selected to be included in the Thailand

National List of Essential Medicines (NLEM) for treating obstetrics-gynecology syndrome since 2012.

Previous studies found that the Plook-Fire-Thatu recipe, administered at three grams per day for four days, significantly increased breast milk volume compared to the placebo group. This herbal formula is safe; the Plook-Fire-Thatu recipe observed no adverse drug reactions (Nilnetr Virasombat et al., 2020).

Nevertheless, clinical studies of the human body's effectiveness and the mechanism of the human body on breast milk production are lacking. This research aims to examine the effect of the Plook-Fire-Thatu recipe on breast milk volume, serum prolactin level, nutrient content, and adverse effects. The hypothesis is that the Plook-Fire-Thatu recipe would increase breast milk production without adverse drug reaction.

1.3 Research gap

Currently, there are no direct pharmacological treatments for hypogalactia; metoclopramide, oxytocin, and domperidone are used off-label to increase breast milk volume. However, risks such as nervousness, drowsiness, fatigue, or sudden cardiac death in mothers, as well as mild and transient intestinal discomfort in breastfed infants, have been reported.

The Plook-Fire-Thatu recipe is a traditional Thai herbal medicine formulation specifically designed for postpartum women experiencing breast milk insufficiency, and it has been widely used since ancient times. However, there is insufficient scientific data to explain the effects of the Plook-Fire-Thatu recipe on breast milk production.

1.4 Research questions

Can the Plook-Fire-Thatu recipe increase breast milk volume in postpartum women without severe adverse drug reactions? Can the Plook-Fire-Thatu recipe enhance the nutrient content of breast milk?

1.5 Research objectives

1) To clinically test the efficacy of the Plook-Fire-Thatu recipe for increased breast milk production and the safety of this recipe through hematology and blood biochemistry, and adverse drug reactions.

2) To investigate the breast milk macronutrient composition in postpartum women.

1.6 Research hypotheses

Postpartum women who receive the Plook-Fire-Thatu recipe will show a statistically significant increase in serum prolactin levels and breast milk volume compared to the control group (postpartum women who receive the regular standard treatment). The breast milk of the treatment group shows a statistically significant increase in nutritional content (protein, carbohydrate, lipids, and nucleic acids) compared to the control group.

1.7 Scope and limitations of the study

1) This involves studies of bioactive compounds using the High-performance liquid chromatography (HPLC) technique.

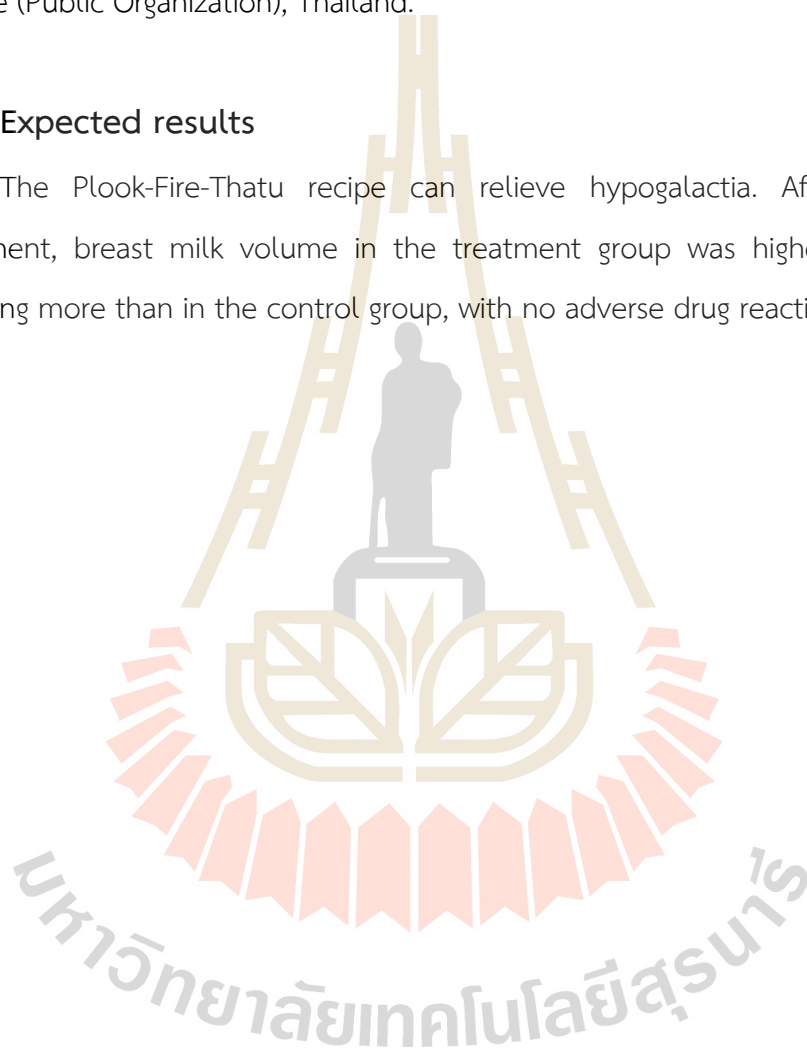
2) The clinical study examines the effects of the Plook-Fire-Thatu recipe on increased breast milk production. The participants will be postpartum women who deliver at Sikhio Hospital. They will be divided into a treatment group and a control group. The treatment group will receive standard treatment (breastfeeding technique) along with the Plook-Fire-Thatu recipe, while the control group will receive only standard treatment. To compare results, Serum prolactin levels will be measured on the first day postpartum (before treatment) and on the third day postpartum (after treatment). And breast milk volume will be measured on the first, second, and third days postpartum.

3) The safety of this recipe study through hematology and blood biochemistry, and adverse reactions. Data on adverse drug reactions (ADR) from the Plook-Fire-Thatu recipe in participants will be collected on days 1, 2, 3, 7, and 30 postpartum.

4) The breast milk macronutrient composition will be analyzed using Fourier transform infrared spectroscopy (FTIR spectroscopy). at the Synchrotron Light Research Institute (Public Organization), Thailand.

1.8 Expected results

The Plook-Fire-Thatu recipe can relieve hypogalactia. After the recipe experiment, breast milk volume in the treatment group was higher than before, increasing more than in the control group, with no adverse drug reactions



CHAPTER II

LITERATURE REVIEWS

The literature reviews for this study are relevant to the research topic. This chapter is divided into six topics, including: 2.1) Anatomy and physiology of lactation, 2.2) Breast milk composition, 2.3) The situation of breastfeeding, 2.4) Thai Traditional Medicine, 2.5) Fourier Transform Infrared Spectrometer (FTIR), and 2.6) Related research.

2.1 Anatomy and physiology of lactation.

2.1.1 Anatomy of Breasts

Breasts are fascinating bilateral structures located on the front of the thoracic wall, particularly in the pectoral area. Though they can be found in both men and women, they typically become more noticeable in females after puberty. In women, breasts house the mammary glands, which are important accessory glands within the female reproductive system. These mammary glands are essential for the wonderful process of lactation.

Surface Anatomy: The breast is located on the front of the thoracic wall, reaching out from the outer edge of the sternum to the middle of the axillary line. It stretches vertically between the second and sixth costal cartilages. This area is positioned just above the pectoralis major and serratus anterior muscles, making it easier to understand its relationship to these important structures.

The breast is composed of two regions: The circular body constitutes the breast's largest and most prominent part. The axillary tail is a smaller section extending along the lower lateral edge of the pectoralis major toward the axillary fossa. Central to the breast is the nipple, mainly made up of smooth muscle fibers.

Surrounding the nipple is a pigmented area called the areola. Within the areola, there are many sebaceous glands that enlarge during pregnancy and secrete an oily substance, acting as a protective lubricant for the nipple.

Anatomical Structure: The breast consists of mammary glands encased in connective tissue stroma. The mammary glands are specialized sweat glands comprising a network of ducts and secretory lobules (15-20). Each lobule contains numerous alveoli, all connected to a single lactiferous duct. These ducts converge at the nipple, resembling the spokes of a wheel. Surrounding the mammary glands is the connective tissue stroma, which includes both fibrous and fatty components. The fibrous stroma condenses to create suspensory ligaments (of Cooper), which serve two primary purposes: anchoring and securing the breast to the dermis and underlying pectoral fascia and separating the secretory lobules within the breast.

2.1.2 Mammary gland development

Utero: The human breast begins to develop in utero at four to six weeks of gestation. During this time, paired thickenings known as mammary ridges or milk lines form on the abdominal surface of the embryo. By week seven, the milk lines shorten and thicken into small nodules made up of ectodermal cells. Towards the end of the first trimester, these nodules descend into the embryonic connective tissue to create a mammary bud, regulated by mesenchymal interactions and secretions.

During the second trimester, the mammary bud starts to expand and form branches. Epithelial buds descend into the mesenchyme. These buds evolve into lactiferous ducts. The branching morphogenesis of the secondary bud relies on soluble factors essential for producing hormones and growth factors, which facilitate and control the development of the mammary gland towards the end of the second trimester. Throughout the third trimester, mammary buds persist in branching and canalization.

By the end of gestation, each mammary bud has formed 15 to 20 lobular structures that house lactiferous ducts. The areola surrounding the nipple takes

shape, and by the fifth month of gestation, the epidermis above the inward growth incorporates the mammary pit.

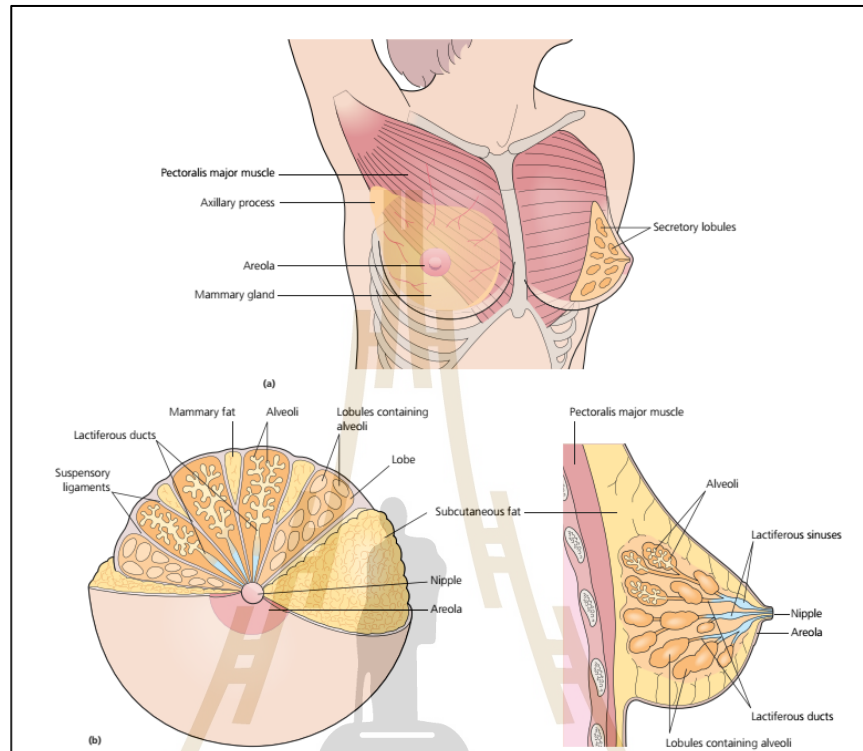


Figure 2.1 Anatomy of Breast (a) Vascular and lymphatic anatomy of the breast region. (b) Section of the breast.

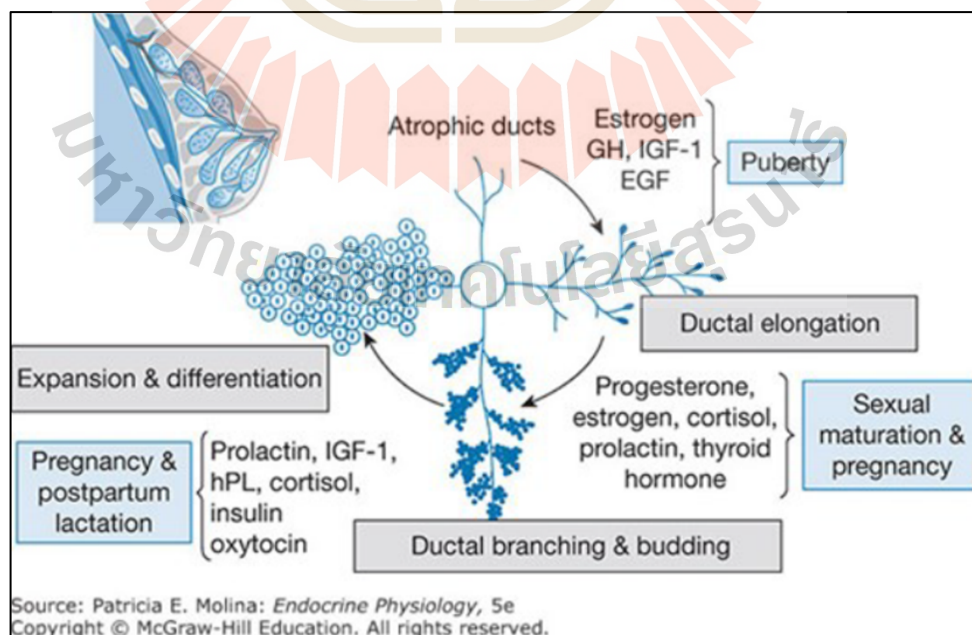


Figure 2.2 Mammary gland development.

Simultaneously, the lactiferous ducts canalize and lead into the retroareolar ampullae, which come together to open at the nipple's tip. At birth, breast and mammary gland development consists of a functional network of mammary lobes and branching lactiferous ducts, all encased in connective tissue.

As maternal hormone levels decline, research indicates that the newborn mammary gland is activated in early infancy by the infant's reproductive hormones. Female infants between two to four months exhibit notably higher estradiol levels compared to males, which is linked to the size of breast tissue. After birth, the areola's pigmentation darkens, and the nipple may become inverted. The development and functionality of the breast from birth to age two occur in three distinct stages of morphological changes that involve the branching of the ductal system, along with four stages of operational changes that pertain to the secretory capabilities of the lining epithelium.

Puberty: Puberty triggers changes in the breasts and mammary glands, mainly driven by estrogen. The growth of epithelial cells results in an increase in both fibrous and fatty tissue within the breast. The epithelium establishes a network of branching duct bundles, leading to the formation of terminal end buds. This branching and ductal elongation occur at the site of the terminal end bud, where alveolar buds and multiple terminal ductules are formed. Surrounding each alveolus is a group of contractile myoepithelial cells. The adult breast's structural unit consists of the terminal duct, while a collection of terminal ductules or acini is known as the Terminal Duct Lobular Unit (TDLU).

Each menstrual cycle leads to the development of more alveoli, while ductal elongation and side branching progress because of circulating estrogen and progesterone. The breast tissue is supported by adipose tissue, blood vessels, fibroblasts, and immune cells. The complete maturation and development of alveolar cells for milk secretion occurs solely during pregnancy under hormonal influence.

Pregnancy: By the twelfth week of pregnancy, lobules undergo rapid growth from cell division, resulting in a larger epithelial surface area. By mid-pregnancy, the expanded lobules encircle the central branching duct, making it impossible to

identify the terminal duct. Continued cell growth and specialization facilitate the emergence of milk-producing cells and progress the gland into the phase of secretory initiation. While mammogenesis starts in puberty, it doesn't reach completion until pregnancy occurs.

Breast growth hormones during pregnancy: Estrogen and placental lactogen stimulate prolactin secretion ten times higher than normal. Prolactin is required to mature the ducts and mammary glands and produce colostrum. Prolactin, in combination with placental lactogen, stimulates glucocorticoids to form protein in milk. Progesterone inhibits prolactin, which enables the production of milk.

The mammary ducts and glands rapidly increase in size and branch in the first to second trimester. The adipose tissue between the milk mass and volume has clearly decreased. The number of white blood cells, lymphocytes, and eosinophils in the intercellular tissue rapidly increases. Fastly increase breast size to feel pain. In the second trimester, visible veins on the skin of the breast and the areola.

By the third trimester, Breasts continue to grow in size and weight from 200 grams before pregnancy increasing to 400-600 grams. Initial nipple formation has begun due to stimulation of the prolactin and placental lactogen at 16 weeks gestation. The size of the mammary glands is widened. The inner epithelial cells are flattened. The walls of the gland are thinner, and the granules of the fat granules are released from the cells.

2.1.3 Breast milk production

The lactogenesis process is divided into three phases. Phase 1 (Lactogenesis I) occurs during pregnancy and creates colostrum. Phase 2 (Lactogenesis II) occurs 32 to 96 hours postpartum and involves the transition from colostrum to mature milk. Phase 3 (Galactogenesis) maintains mature milk production for one to two weeks postpartum.

Lactogenesis I; Lactation initiation occurs mid-pregnancy and is characterized by the secretory differentiation of the alveolar mammary epithelial cells into lactocytes capable of synthesizing milk components. At this stage, the gland can secrete small amounts of protein-rich fluid, which is expelled into the mammary alveoli and discharged from the nipple. This secretion is called colostrum; however,

high levels of progesterone typically inhibit milk secretion before birth. After delivery, the expulsion of the placenta causes an immediate decrease in estrogen and progesterone, accompanied by an increase in insulin, prolactin, and cortisol, which stimulates substantial milk production and signals the onset of lactogenesis II.

Insulin and cortisol produce prolactin to bind to the walls of the mammary cells in the mammary glands. The protein synthesis of amino acids in the mother's blood in the endoplasmic reticulum begins and is exocytosis into the mammary duct of the mammary gland. The gap between the cells allows more minerals, such as sodium and potassium to pass through the cell wall. Some proteins, such as casein and alpha-lactalbumin, are transported into Golgi apparatus to synthesize lactose and then released into the duct of the mammary gland. Lipids directly synthesize from breast fat and mother's blood by transmembrane and drive immunity by transcytosis and paracellular pathway. Mammary epithelial cells quickly develop the ability to synthesize milk. Breast milk volumes reportedly increase from approximately 100 mL to 500 mL by four days postpartum and to 650 mL by eight days postpartum, driven hormonally by the endocrine system.

Lactogenesis II; Lactogenesis postpartum breast milk regulates production through hormones secreted by the pituitary gland, which are controlled by the hypothalamus. After delivery, prolactin stimulates milk-producing cells to adhere to one another. Sucking and pressure signal the hypothalamus to instruct the anterior pituitary gland to release a higher level of prolactin hormone, known as the prolactin reflex.

Prolactin level (ng/ml): The prolactin level average is 200 ng/ml or about 200–500 ng/ml at full-term gestation and increases to 400 ng/ml when sucking breast milk in the first ten days after birth. Prolactin maximizes within 30 minutes of feeding and decreases within 3 hours. Prolactin continues to be secreted after stopping suckling for preparation, produces milk at the next meal, and increases secretion at night.

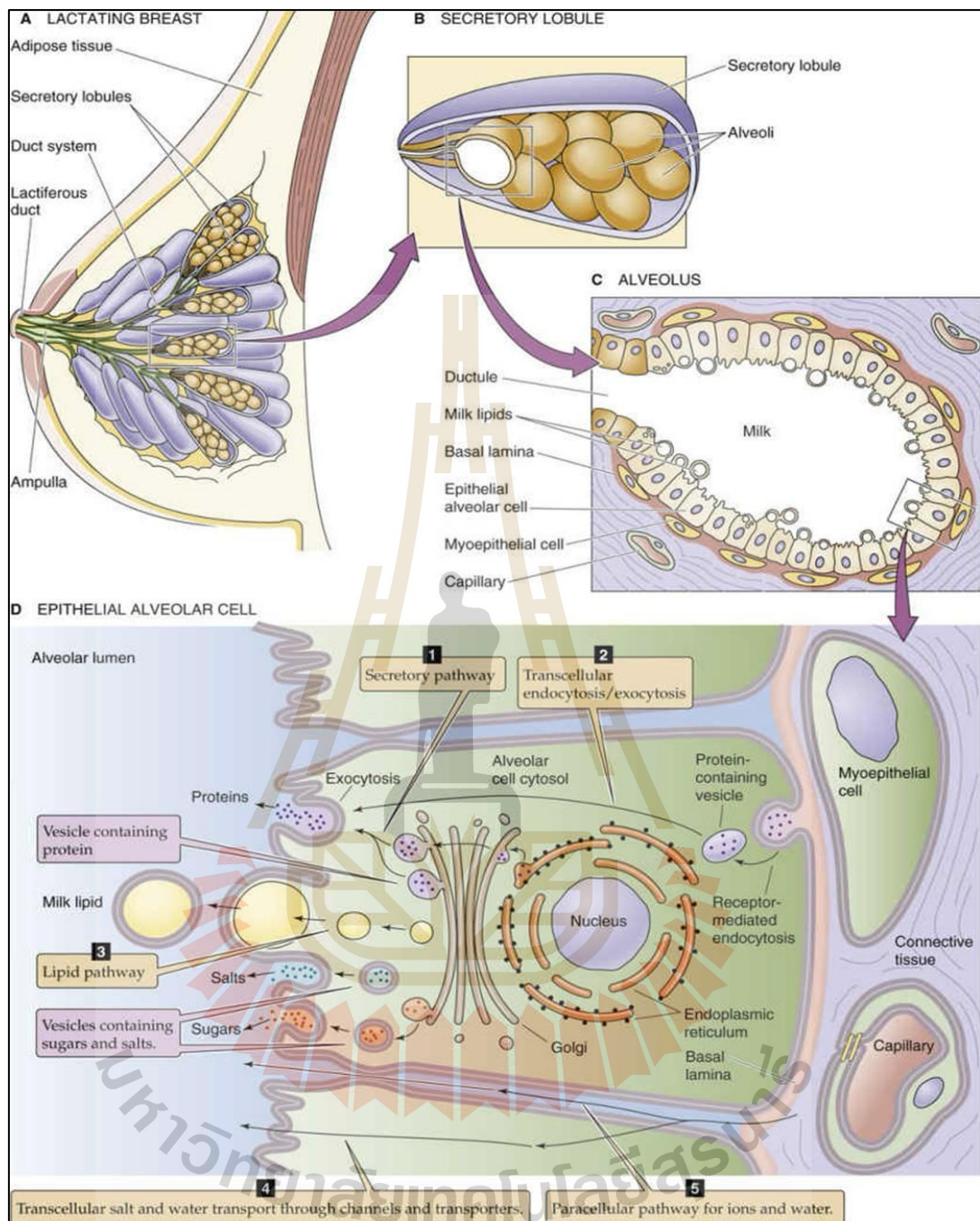


Figure 2.3 Cross section of the breast and milk production.

Prolactin stimulates lactose synthesis on Golgi-apparatus and is released with the protein as water vesicles to increase the amount of milk. The endoplasmic reticulum produces Triglyceride, sediment covering the cell wall, slipped into the milk as fat globules.

Milk secretion during lactogenesis II: Suckling and squeezing stimulate milk secretion. The mammary duct beneath the areola, pressed by the gum and pinched fingers, sends a signal to the hypothalamus, which prompts the release of oxytocin from the posterior pituitary gland, known as the oxytocin reflex.

Galactogenesis; Maintaining milk production after the first and second phases is regulated by various hormones, including prolactin, thyroid hormone, growth hormone, adrenocorticoids, and ovarian steroids. This process occurs approximately two weeks after birth to manage the volume and composition of mature milk. The amount of milk produced depends on the baby's needs, suckling, and compression. There is an increase in protein synthesis, particularly casein. The breasts regulate the amount of milk based on how much is drained. The more milk is expressed from the breast, the more fat granules are released.

Feedback Inhibitor of Lactation (FIL): FIL is a peptide that acts as a chemical regulator to regulate the milk production process, it acts locally on prolactin receptors. Whole breast milk stimulates the elevation of FIL and inhibits prolactin from producing milk, FIL is reduced when milk is excreted, and production continues.

2.1.4 Hormones affecting the mammary gland.

Hormones produced by the mammary gland

Prolactin is a protein composed of amino acids that is produced in the lactotroph cells of the pituitary gland and facilitates lactation. It is essential for the growth and development of mammary glands (mammogenesis), the formation of alveoli (alveologensis), the synthesis of milk proteins, and the ongoing secretion of milk (galactopoiesis).

Parathyroid hormone-related protein is a hormone that shares homology with parathyroid hormone. It plays a significant role in regulating calcium homeostasis during lactation through bone resorption. The mammary gland produces parathyroid hormone-related protein throughout lactation, with both mRNA and

protein synthesis being upregulated. When lactation concludes, the decrease in progesterone and estradiol speeds up the production of parathyroid hormone-related protein. Suckling also stimulates mRNA and protein expression, which is mediated by prolactin. Some researchers suggest that serotonin produced by the mammary gland during lactation may also promote parathyroid hormone-related protein synthesis and release.

Serotonin is produced in several peripheral tissues, such as the intestines, bones, and mammary glands. It is formed by tryptophan hydroxylase-1 from L-tryptophan. In the mammary gland, serotonin serves two roles: it provides negative feedback on milk synthesis and secretion and positively influences parathyroid hormone-related protein synthesis and secretion. The suckling reflex is a crucial mechanism that helps regulate and keep serotonin levels low in breast milk. When the mammary gland fills with milk, serotonin sends negative feedback that inhibits milk production in the mammary epithelium. The expression of β -casein is decreased in mammary epithelial MCF-12A cells and is linked to the expression of the serotonin-7 receptor (5HT7). Furthermore, during lactation, serotonin negatively regulates β -casein expression in the mammary gland through the serotonin-7 receptor (5HT7). It is increased mRNA abundance of calcium transporters Ncx1, Serca2, SPCA2, Pmca2, and SGLT1, but not the calcium-sensing receptor expression in the mammary gland, and increased osteoclasts and resorption of bone.

Hormones in the mammary gland

Glucocorticoid stimulation promotes the differentiation of the secretory epithelium and boosts milk production. Maternal cortisol levels spike sharply in the third trimester of gestation and again during parturition. Furthermore, the increase in circulating glucocorticoids is not the main trigger for lactogenesis; instead, it serves a permissive role in the effects of prolactin on α -lactalbumin and casein synthesis. Glucocorticoid receptors act as survival signals in the mammary gland during lactation and as inducers of milk protein gene expression and secretion. In addition, glucocorticoids and prolactin activate the prolactin-inducible protein (PIP) and have an antiapoptotic effect on the mammary gland during lactation.

Oxytocin, a peptide hormone produced in the hypothalamus, functions as a neurotransmitter. It is secreted into the bloodstream via the posterior pituitary gland. Among its roles, oxytocin serves as a hormone at peripheral sites.

During lactation, suckling triggers an increase in oxytocin levels in maternal circulation. This hormone is associated with milk ejection, facilitating the contraction of myoepithelial cells in mammary tissue.

Insulin: Previous studies revealed that insulin, prolactin, and corticoids are necessary for maintaining the synthesis of milk components. The postpartum days 3 and 4 showed increased insulin levels. In lactating humans and rats, the suckling reflex enhances insulin release.

Calcium-sensing receptor: This receptor is a G-protein-coupled receptor that detects and responds to minor fluctuations in extracellular ionized calcium levels within the mammary gland and bone. The calcium-sensing receptor serves as a feedback regulator and increases its expression during lactation in the mammary gland. In the mammary gland, calcium regulates and manages the secretion of parathyroid hormone-related protein through the calcium-sensing receptor.

2.1.5 Suckling reflex

The neuroendocrine responses that arise when an infant suckles are referred to as suckling reflexes. The reflex's afferent limb is neuronal, whereas the efferent responses are hormonal. Suckling reflexes fulfill three essential roles: Suckling acts as the main trigger for prolactin secretion, operating through neural pathways to suppress dopamine release in the hypothalamus. Prolactin levels remain elevated for the duration of breastfeeding. Each feeding session causes spikes in prolactin concentration, ensuring that milk production aligns with the infant's needs. Prolactin secreted during feeding promotes milk production in anticipation of the next feeding.

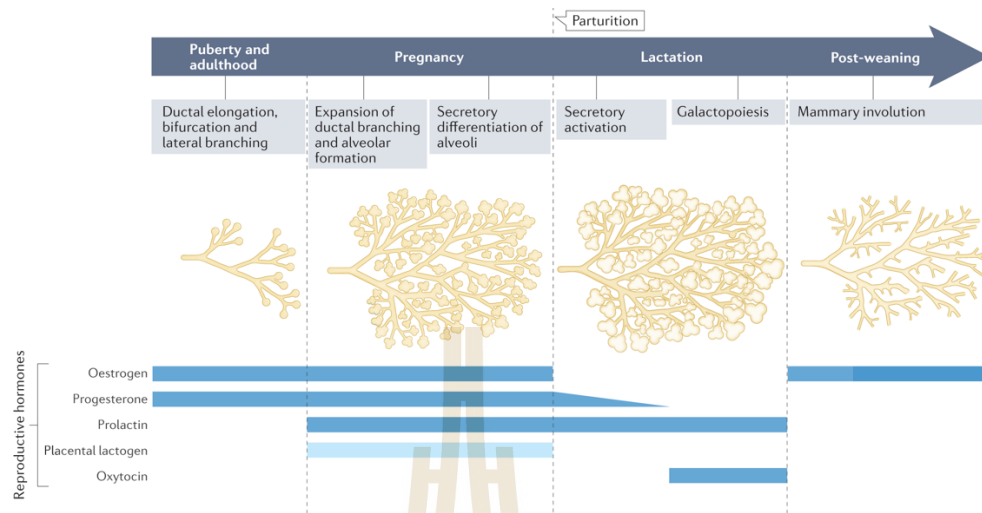


Figure 2.4 Hormonal regulation of mammary gland development and lactation

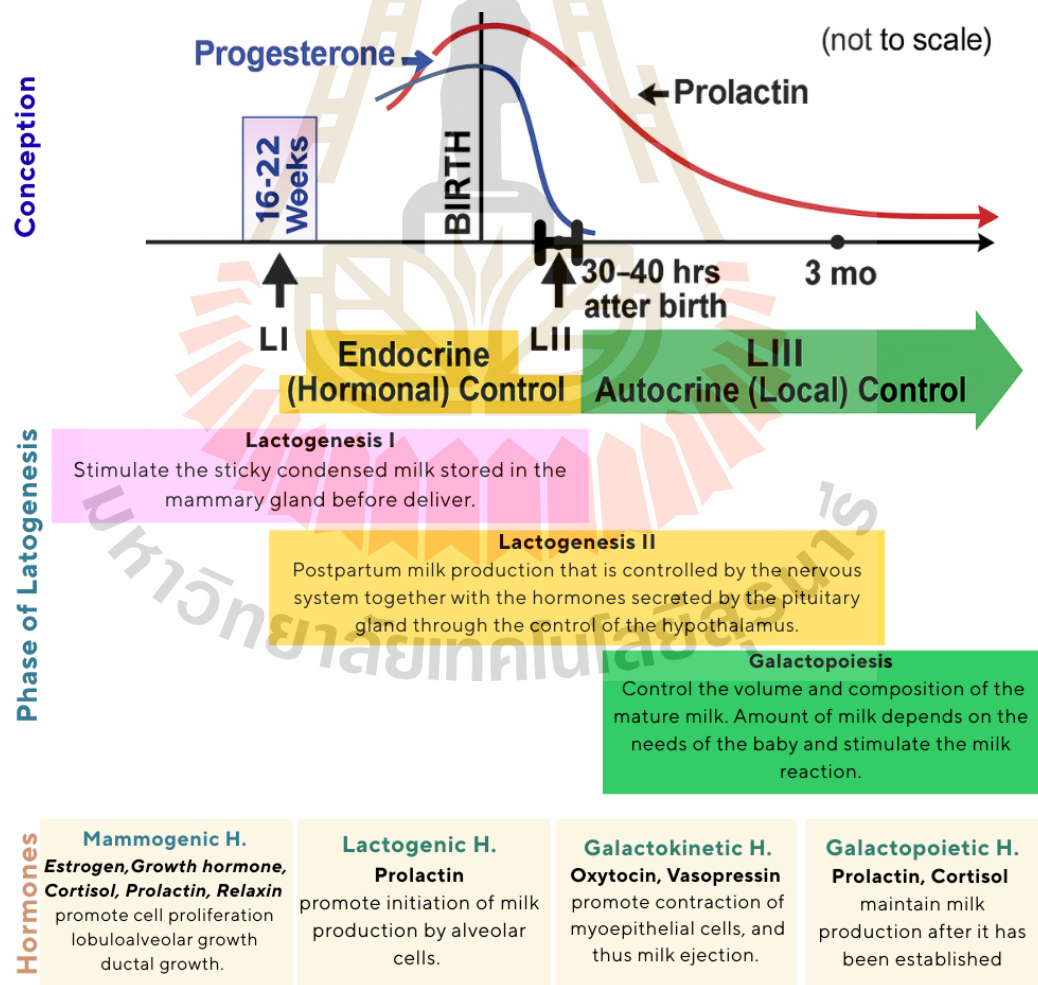


Figure 2.5 The stage of lactation and affecting hormone.

The suckling reflex stimulates oxytocin secretion from neurons in the posterior pituitary. Oxytocin causes the contraction of myoepithelial cells and milk ejection (let-down) from the breast. For instance, the reflex can be conditioned by the sound of an infant crying. The suckling reflex inhibits the GnRH pulse generator, suppressing FSH and LH release, which in turn affects the menstrual cycle in most breastfeeding mothers. The effectiveness of this suppression depends on feeding frequency and can lead to the absence of menses for more than two years. Mothers who do not breastfeed typically resume their menstrual cycles within 2 to 4 months after giving birth.

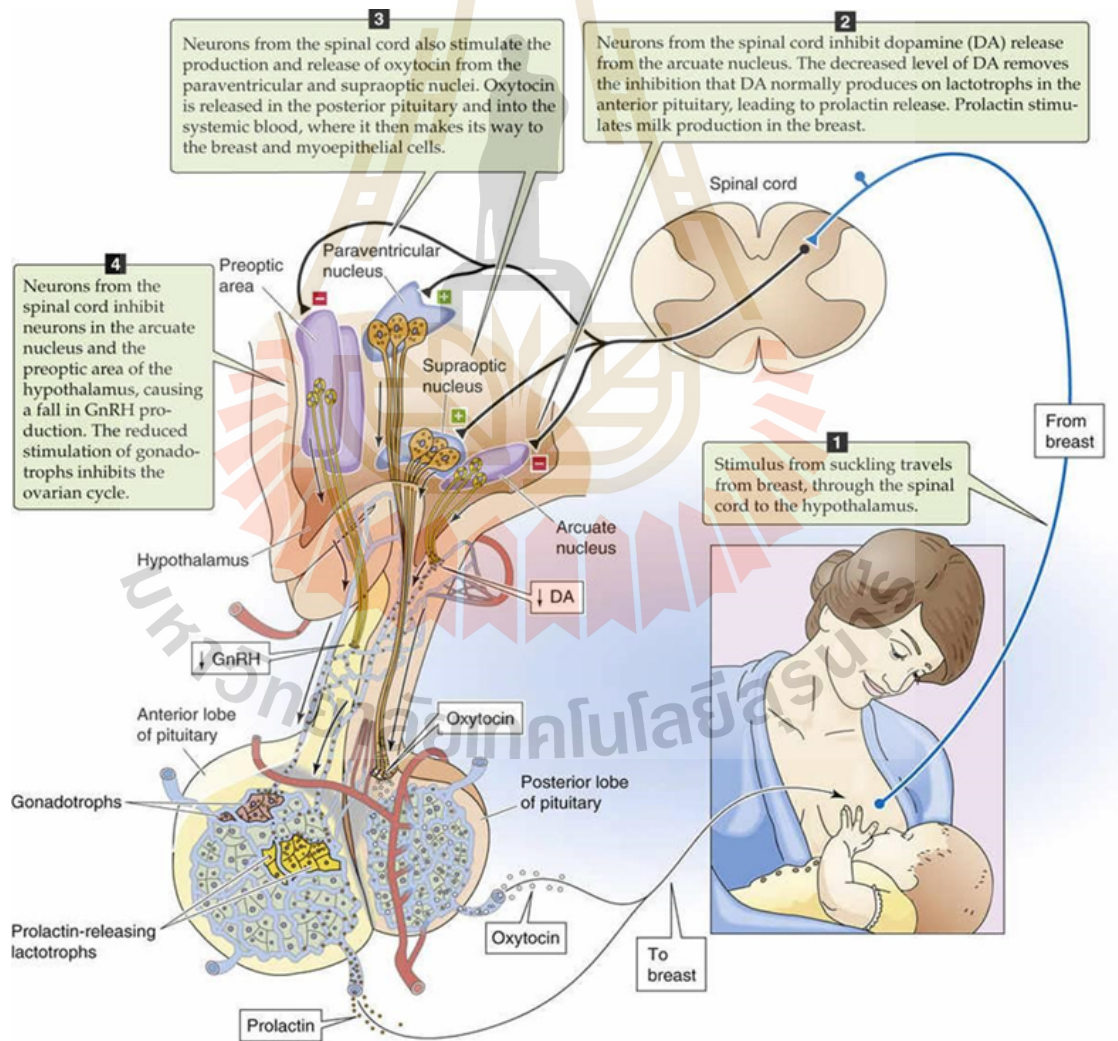


Figure 2.6 Suckling reflexes

2.2 Breast milk composition

Macronutrients: The macronutrient composition of human milk varies among mothers and throughout different stages of lactation. The average macronutrient content of mature, term milk is estimated to be approximately 0.9 to 1.2 g/dL for protein, 3.2 to 3.6 g/dL for fat, and 6.7 to 7.8 g/dL for lactose (Mosca & Gianni, 2017). Energy estimates range from 65 to 70 kcal/dL and are closely related to the fat content of breast milk. There are differences in macronutrient composition between preterm and term milk, with preterm milk generally containing higher levels of fat and protein than term milk.

Proteins Indeed; Proteins are essential for infants' healthy growth. The functions of proteins include serving as carriers for other nutrients (i.e., lactoferrin, haptocorrin, alpha-lactalbumin, folate-binding protein, beta-casein), promoting gut development (growth factors, lactoferrin), aiding nutrient absorption (bile salt-stimulated lipase, amylase, alfa1-antitrypsin), and possessing immune and antimicrobial activity (lactoferrin, secretory IgA, osteopontin, cytokines, lysozyme, etc.). Milk proteins can be categorized into caseins, whey, and mucins. Caseins are assembled in micelles, whereas whey proteins are present in solution. The main whey proteins include lysozyme, alpha-lactalbumin, lactoferrin, and secretory IgA; caseins consist of α -, β -, and κ -casein. Notably, caseins in human milk represent 13% of the total proteins, making it the lowest casein concentration of any species. Mucins are incorporated into the membrane of the milk fat globule. During digestion, most proteins are broken down into free amino acids that are absorbed and utilized as building blocks for synthesizing new proteins in the body. However, it must be considered that some nutritious proteins are also affected. Moreover, it is now well recognized that some bioactive proteins are detected intact in an infant's stool. These data suggest that proteins play a role in the mechanisms responsible for the beneficial health effects associated with breastfeeding, such as the reduced risk of developing morbidities, particularl (Ballard & Morrow, 2013).

Non-protein nitrogen; Including urea, creatinine, nucleotides, free amino acids, and peptides, these components make up 25% of human milk. Nucleotides are considered conditionally essential nutrients. They play a crucial role in various cellular

functions, acting as metabolic modulators, influencing enzymatic activities, and supporting the development and maturation of the gastrointestinal and immune systems.

Carbohydrates; The main sugar in human breast milk is lactose, a disaccharide. Among macronutrients, human breast milk has the lowest lactose concentration. Yet, higher levels of lactose in mothers' milk contribute to increased milk production. Human breast milk also contains important carbohydrates called oligosaccharides, which can measure around 1 g/dL, varying with lactation stage and genetic factors. Moreover, lactose is a key source of galactose, crucial for the central nervous system's development. Oligosaccharides (HMO) in human breast milk range from 1-10 g/L in mature milk and 15-23 g/L in colostrum. In mature milk, 35-50% of HMO is fucosylated, 12-14% sialylated, and 42-55% is unspecified. HMOs act as prebiotics, providing metabolic substrates for bacteria that support the growth of beneficial species like Bifidobacteria and Bacteroides. Additionally, HMOs regulate several mucosal and systemic immune functions in infants.

Human milk oligosaccharides (HMOs) are intricate glycans present at levels of 20–25 ng/L in colostrum, decreasing to 5–15 ng/L in mature milk. Although HMOs cannot be digested by infants, they represent the third most abundant element in human milk, after lactose and lipids. HMOs function as prebiotic agents, acting as metabolic substrates that encourage the growth of beneficial microorganisms within the infant's intestinal microbiome.

Lipids act as the main energy source, accounting for 44% of the total energy in human breast milk. They also provide significant essential nutrients, such as polyunsaturated fatty acids (PUFA), lipid-soluble vitamins, complex lipids, and bioactive compounds. These nutrients are contained within dispersed fat globules, mainly as triacylglycerols produced in the endoplasmic reticulum of mammary epithelial cells. Lipid is released from the endoplasmic reticulum into the cytosol, where the core is first encased by an inner membrane derived from the endoplasmic reticulum of the mammary alveolar cells. Bioactive lipid compounds encompass glycerophospholipids, sphingolipids, sphingomyelin, glycolipids, cholesterol, and glycosylated proteins. Triacylglycerols make up 98% of the fat found in human breast

milk. This milk contains more than 200 fatty acids, each varying in concentration. Reports indicate that milk lipids from European women contain 35-40% saturated fatty acids, 45-50% monounsaturated fatty acids, and 15% polyunsaturated fatty acids. (Mosca & Gianni, 2017).

Micronutrients; Human breast milk sets the standard for infant nutrition. However, many micronutrients in human breast milk, which depend on maternal diet and genetics, include vitamins A, B1, B2, B6, B12, D, and iodine. Vitamin K and vitamin D are very low in human breast milk.

MicroRNA; MicroRNAs (miRNAs) are small, non-coding RNA molecules that influence gene expression after transcription. Human breast milk ranks among the richest sources of microRNAs, and other bioactive compounds in milk are thought to support infant development. These microRNAs in human breast milk are derived from the mammary gland. Furthermore, research has compared the miRNA profiles of mothers nursing full-term versus preterm infants. A recent study revealed that miRNA-148 is present in lower levels in milk from preterm mothers compared to that from full-term mothers. Additionally, the microRNA content in human breast milk is notably higher than in infant formula.

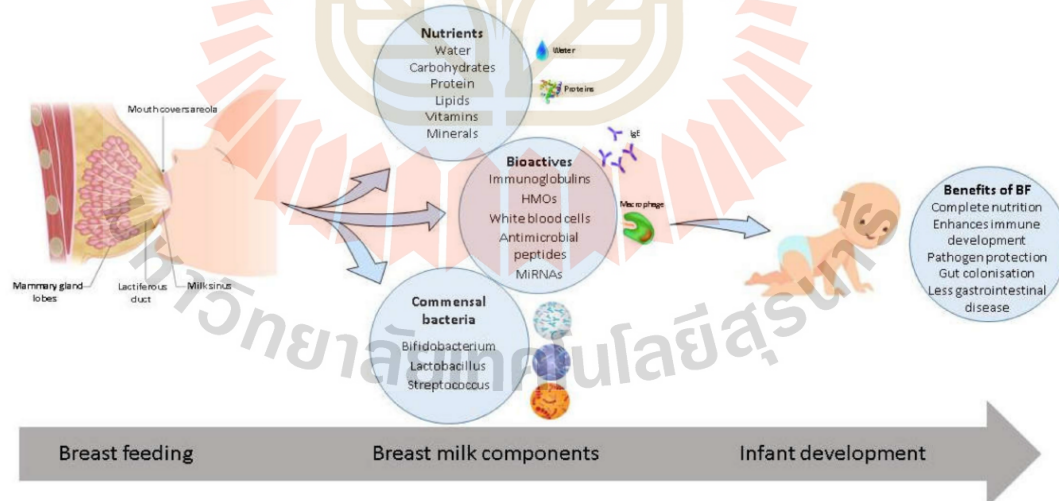


Figure 2.7 Breast milk composition and associated benefits.

Table 2.1 Protein composition of human colostrum and mature breast milk (per liter).

Constituent	Measure	Colostrum (1-5 days)	Mature Milk (>30 days)
Total protein	G	23	9-10.5
Casein	mg	1400	1870
α -Lactalbumin	mg	2180	1610
Lactoferrin	mg	3300	1670
IgA	mg	3640	1420

Ref: Worthington-Roberts B, Williams SR. Nutrition in Pregnancy and Lactation, 5th ed. St. Louis, MO, Times Mirror/Mosby College Publishing, p. 350, 1993.

Table 2.2 Composition of Human Colostrum, Human Milk, and Cow's Milk (per Deciliter of Fluid)

Component	Human colostrum	Human milk	Cow's milk
Total protein (g)	2.7	0.9	3.3
Casein (% of total protein)	44	44	82
Total fat (g)	2.9	4.5	3.7
Lactose (g)	5.7	7.1	4.8
Caloric content (kcal)	54	70	69
Calcium (mg)	31	33	125
Iron (μ g)	10	50	50
Phosphorus (mg)	14	15	96
Cells (macrophages, neutrophils, and lymphocytes)	$7-8 \times 10^6$	$1-2 \times 10^6$	—

Ref: <https://doctorlib.info/physiology/medical/319.html>

2.3 Situation of breastfeeding

The rationale behind breastfeeding recommendations has undergone substantial changes in the last thirty years. This shift is supported by findings from epidemiological studies and a deeper understanding of epigenetics, along with the developmental origins of health and illness. The WHO has established several indicators for assessing the feeding practices of infants and young children:

- 1) Early initiation of breastfeeding (the percentage of infants born in the last 24 months who were breastfed within one hour after birth).
- 2) Exclusive breastfeeding for infants under six months (the percentage of infants aged 0–5 months who receive only breast milk).
- 3) Continued breastfeeding at one year (the percentage of children aged 12–15 months who are still breastfed).
- 4) Continued breastfeeding at two years (the percentage of children aged 20–23 months who are still breastfed).

2.3.1 The prevalence of global breastfeeding

Worldwide, breastfeeding at twelve months is most common in sub-Saharan Africa, South Asia, and Latin America (figure 2-6). In contrast, most high-income countries report rates below 20%. Significant disparities are observed between the UK (<1%) and the USA (27%), as well as between Norway (35%) and Sweden (16%). Nonetheless, early initiation rates were consistently low across all regions, along with exclusive breastfeeding rates (figure 2-7). In low-income and lower-middle-income areas, however, breastfeeding at twelve months is more prevalent.

Among children under six months in LMICs, 36.3 million (63%) were not exclusively breastfed according to the national survey. The breakdown showed 53% in low-income countries, 61% in lower-middle-income countries, and 63% in upper-middle-income countries. In children aged 6 to 23 months in LMICs, 64.8 million (37%) were not receiving any breast milk at the time of the most recent national survey. The corresponding rates were 18% in low-income countries, 34% in lower-middle-income countries, and 55% in upper-middle-income countries. According to international

recommendations, 101.1 million children in LMICs were not breastfed (Victora et al., 2016).

WHO recommends that mothers globally breastfeed their infants for the initial six months and continue doing so for up to two years to ensure optimal growth, development, and health. The economic impact of the adverse effects of not breastfeeding in Thailand found that optimal breastfeeding was estimated to reduce the deaths of 262 babies per year, reducing public health costs. From illness down to 7.65 million US dollars a year. Reduce the household expenditure required to treat illnesses with diarrhea. And pneumonia in children and reduce household expenses from purchasing infant formula by up to 25% (Walters et al., 2016).

Although breastfeeding benefits maternal and infant health, the National Health Security Office, the United Nations Children's Relief Organization (UNICEF), and the Statistical Office conducted a survey about children and women in Thailand. They found that the rate of the scorecard regarding breastfeeding still meets the criteria for advancement. Half of infants are breastfed immediately after birth, but this rate tends to decrease. Exclusive breastfeeding for six months increased by 23.1 percent, while about 40 percent of infants are breastfed with water or other liquids, such as fruit juice. Exclusive breastfeeding rates in Thailand are lower than in other developing countries in the region: Laos 4% (2017), Cambodia 65.2% (2014-5), Nepal 56.9% (2014), and Bangladesh 62.6% (2019), but are close to Vietnam at 24.3% (2014). (Table 2-4)

The survey report in Ubon Ratchathani and Sisaket provinces (1981-1982) found that more than 95-98% of mothers were breastfeeding mothers for two years. Infant feeding in 3 sub-districts of San Pa Tong District Chiang Mai Province (1985-9) Other feeding was also observed in the first week (13%), increasing to 81% in 6 weeks. The average age of the children who started other feeding was four weeks. Mashed and bananas account for 15% of the energy other diets provide at three months of age. They were feeding babies other foods that are lower in nutritional value than breast milk, putting them at risk of being deficient in nutrients.

The situation of breastfeeding in that survey of postpartum women in all of the hospitals in Thailand found that 68.1% exclusively breastfed until three

months, then gradually decreased 48.3 and 38.4 percent at four and five months, respectively and only 32.8 percent left at six months. In conclusion, breastfeeding in Thailand still needs more practicality and quality support to benefit mothers, babies, and families (Winichagoon & Damrongwongsiri, 2020).

2.3.2 Factors Affecting Breastfeeding

Traditional, cultural and Faith factors: In the region of Thailand, it was represent that exclusive breastfeeding was shortly after delivery because it was believed that only breast milk was not sufficient. The baby still cries after breastfeeding, or the baby cries frequently, thus solving the problem by starting powdered feeding or other foods such as mashed bananas and mashed rice from just a few weeks old.

Preparing mothers for breastfeeding is essential and should begin at the gestational stage, including physical care, education and attitudes. Some mothers also understand that milk powder is fortified with nutrients and substances that have benefits close to or better than breast milk. Postnatal women without breastfeeding experience may not be able to position the baby to latch on properly. Empowerment of postpartum women is a crucial factor. Mothers who suffer from insufficient breast milk are often stressed and failing themselves for not being able to breastfeed completely. Therefore, stop breastfeeding quickly and replace it with powdered milk.

Support by family and healthcare professionals: Awareness of and understanding of the roles and duties of family members, especially husbands of lactating women. It also has a typical role, such as caring for, encouraging, helping, taking care of babies, washing clothes, changing diapers, etc., making it successful in breastfeeding and encouraging fathers to be involved in emotional support and appreciation of values. Providing information and resources by learning through behavioral skill practice showed increased fathers' attitudes and supportive behaviors and longer duration of exclusive breastfeeding. Post-delivery support by nurses or relevant staff significantly contributed to the success of breastfeeding and continued breastfeeding after discharge from the hospital.

2.3.3 Insufficient Milk Production

Approximately five percent of mothers might encounter primary lactation insufficiency stemming from insufficient glandular tissue, which can occur due to conditions like hypoplastic breasts or previous surgeries, including mastectomy, breast reduction, or cyst removal.

The predisposing factors include:

1) Baby-related factors like prematurity, low birth weight, birth asphyxia, illnesses, and physical defects.

2) Maternal factors contributing to milk insufficiency, such as maternal health, mammatogenesis issues (insufficient breast tissue or previous breast surgery), lactogenesis concerns (retained placenta or delayed breastfeeding), and galactopoiesis challenges (inadequate breast drainage or infant tongue-tie); low motivation; rarely sticking to scheduled feeds; poor management of local breast problems (like sore, flat, retracted, or overly large nipples); sedation due to anxiety, medications, fatigue, or contraceptives; improper breastfeeding techniques; and a history of chronic psychiatric disorders, including possible postpartum depression.

3) Environmental factors encompass separation of the baby from the mother, painful infections, episiotomies, cesarean sections, lack of adequate facilities for working mothers, insufficient support and guidance from healthcare professionals, and aggressive marketing of baby foods.

2.4 Thai traditional medicine

Thai traditional medicine is the primary traditional medical system widely practiced in Thailand. Historical evidence has been traced from the Dvaravati period to the reign of King Rama III. In 1910, Thai traditional medicine gained popularity as Western medicine started to influence healthcare in Thailand. In 1987, the government integrated Thai traditional medicine into the primary health policy, restoring its prominence. The theory of traditional Thai medicine holds that the human body consists of four elements: earth, water, wind, and fire. It posits that disease occurs when there is an imbalance among these elements. Thai traditional medicine is divided into four branches: Thai Therapeutic Medicine, Thai Therapeutic Pharmacy, Midwifery

in Thai Traditional Medicine, and Thai Therapeutic Massage. It encompasses the diagnosis, treatment, prevention, promotion, and rehabilitation of human health through Thai massage, herbal compression, herbal remedies, and other methods of traditional Thai medicine.

Midwifery in Thai Traditional Medicine involves caring for mothers and babies using traditional practices from the onset of pregnancy to the postpartum period. Thai postpartum health care evolved from local wisdom surrounding puerperal practices, which are categorized into 13 methods. These methods include dietary practices, lying by the fire (Yuu-fai), warm water and herbal baths, rituals related to mother and infant that involve warm water and herbal drinks, relaxation and exercise, herbal steam tents, salt pot compresses, body and herbal massages, sitting over charcoal, herbal ball compresses, managing gynecological tumor incidence, and utilizing herbal plants for women's healthcare. (Punyopashtambha, 2019).

2.4.1 The Plook-Fire-Thatu recipe

The Plook-Fire-Thatu is a traditional Thai herbal medicine formula found in the Mahachotarat scripture, documented in the Medical Science Book of the Rattanakosin era 126, composed by Prince Phisanu Prasatwetch (Kong Thawornwetch). The Plook-Fire-Thatu is a recipe designed for postpartum women with insufficient breast milk. The Plook-Fire-Thatu recipe has been widely used in the past and has been included in the Thailand National List of Essential Medicines (NLEM) for the treatment of obstetric-gynecological syndrome since 2012.

The data in the Thailand National List of Essential Medicines

Indication: Stimulates milk production and improves blood circulation in postpartum women. Administration/ dosage: Take 500 mg - 1.5 g each time, 3 times a day, before meals. Contraindication: Do not use in women with postpartum hemorrhage and pregnant women. Precautions: Caution in combination with phenytoin, propranolol, theophylline, and rifampicin, as this formulation contains high levels of pepper. Side effects: Heartburn, cardialgia

Table 2.3 Situation of breastfeeding by National survey (MICS survey)

Index Prevalence (percent)	Year		
	2548-9	2555-6	2558-9
Breastfeeding within the first hour after delivery	49.6	46.3	39.9
Exclusive breastfeeding for 6 months	5.4	12.3	23.1
Predominant breastfeeding 6 months	-	40.8	42.1
Continue breastfeeding for 1 year	-	32.4	33.3
Continue breastfeeding for 2 year	18.7	17.8	15.6
Average duration of breastfeeding (months)	-	6.5	7.9

Data from National Statistical Office Survey of children and women in Thailand 2005-2006, 2012, 2016.

Table 2.4 The situation of breastfeeding in some countries in Asia compared with Thailand.

Country	Year	Suckling immediately after birth. (%)	EFB 6 m. (%)	BF with water 6. (%)	BF 1 year (%)	BF 2 years (%)	Total BF period (median, month)	Food for ages at 6-8 m. (%)
Thailand	2015-6	39.9	23.1	42.1	33.3	15.6	7.9	84.6
Lao	2017	50.1	44.9	59.5	64.9	27.2	15.7	86.7
Cambodia	2014-5	62.6	65.2	11.3	-	-	19.0	81.7
Indonesia	2011	24.4	34.6	74.5	60.1	42.6	23.1	-
Vietnam	2013-4	26.5	24.3	49.0	65.6	21.8	15.8	90.7
Bangladesh	2019	46.6	62.6	73.0	93.0	84.2	28.6	75.5
Nepal	2014	48.7	56.9	74.9	93.6	86.7	36.0	73.5

Data from the survey of the situation of children and women (Multiple Indicator Cluster Survey, MICS) Last year reported (<https://mics.unicef.org/>) Except Cambodia obtained from Population and health survey (Demographic and Health Survey, DHS)

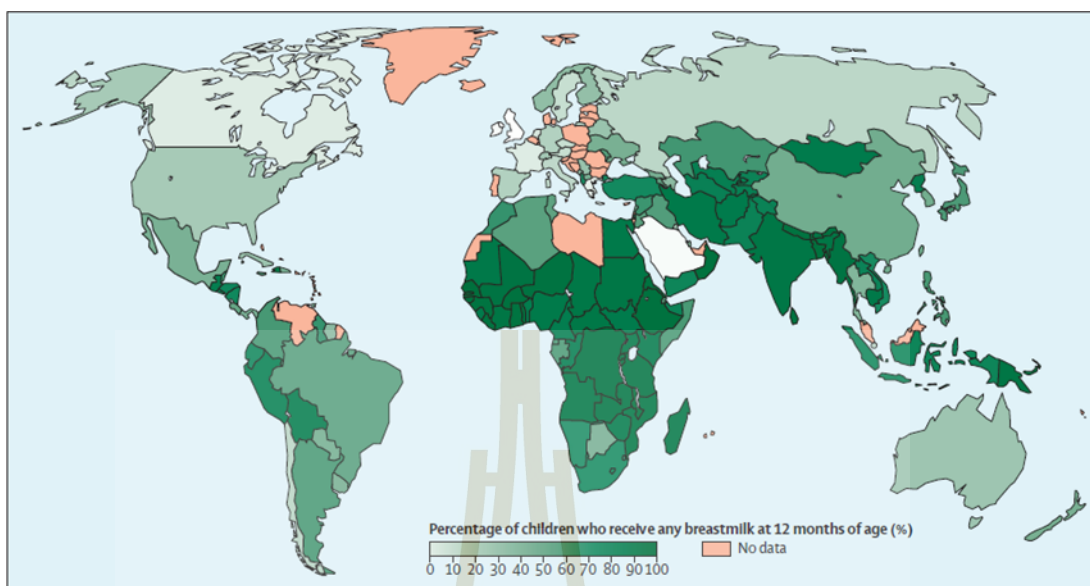


Figure 2.8 Global distribution of breastfeeding at 12 months Data are from 153 countries between 1995 and 2013.

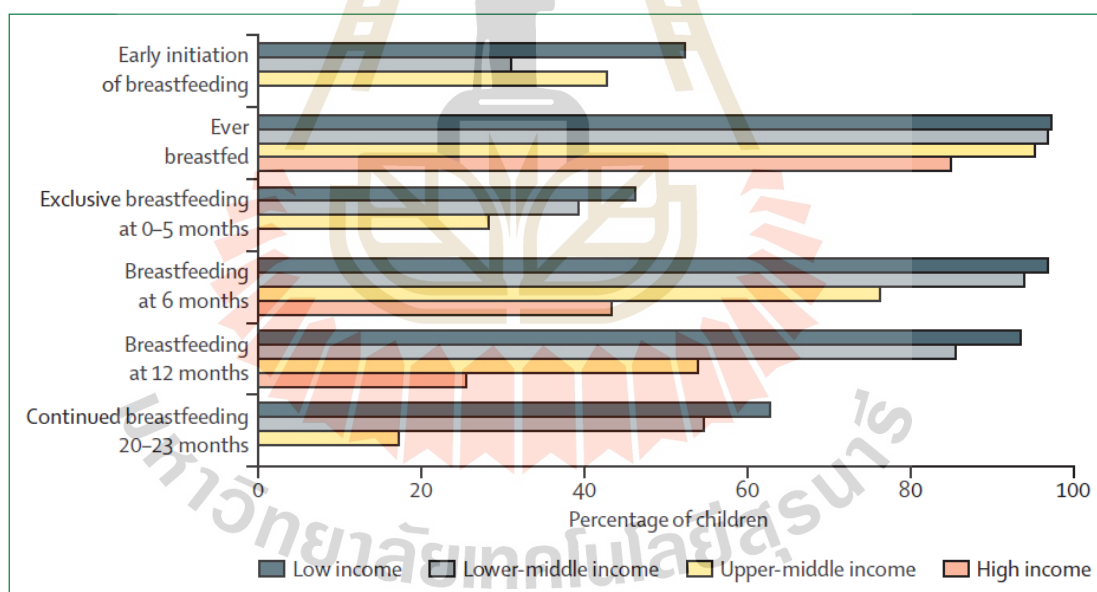


Figure 2.9 Breastfeeding indicators by country income group in 2010 Data are from national surveys that used standard indicators, and were weighted by national populations of children under 2 years. Data for up to 153 countries.

2.4.2 White Pepper

White Pepper is the dried seed of ripe fruit, deprived of the outer part of the pericarp of *Piper nigrum* L.

Family: PIPERACEAE

Common name: White Pepper

Synonyms: Pepper, Pepper Corn

Category: Aromatic, stomachic, carminative

Constituents: White pepper contains volatile oil, alkaloids of which piperine is a significant component.

Description of plant: Woody perennial climber, aromatic; branches stout, trailing, and rooting at the nodes. Leaves simple, highly variable in length from 12.5 to 17.5 cm, long 5-12.5 cm, wide sometimes glaucous underneath, apex acuminate, base acute, rounded, or cordate, equal or unequal; basal nerves 5 to 9 with one pair extending to the apex. Petiole 1.2-3.7 cm long, stout. Flowers are usually dioecious, but often the female bears 2 anthers and the male a pistillode, with 2 anthers. Fruiting spike measures 10-12 cm or more in length. Fruit is a drupe, globose or ovoid, sessile, 1-seeded, 4-6 mm in diameter, orange-red to reddish when ripe. Seed usually globose; testa thin.

Description: Odour aromatic, taste pungent.

2.4.3 Ginger

Zingiberis Officinalis Rhizoma, Ginger is the dried rhizome of *Zingiber officinale* Roscoe.

Family: ZINGIBERACEAE

Category: Carminative, antifatulent

Constituents: Giger contains oleoresin, predominantly consisting of gingerols. Constituents: Giger contains oleoresin, mainly made up of gingerols, shogaols, and volatile oil. Its volatile oil contains zingiberene, borneol, geraniol, and other compounds. It also contains fatty acids, starch, etc.



Figure 2.10 *Piper nigrum* L., 1. habit, 2. inflorescences (a), part of inflorescences (b), 3. Leaves and inflorescences 4. Inflorescence showing different stages of fruits 5. Crude drug

Description of the plant: Perennial herb with a pseudostem up to 2 meters tall, leafy; subterranean, branched, fleshy, aromatic rhizome. Leaves are simple, lanceolate, 10-30 cm long, 1.5-3 cm wide, with an acuminate apex and a sheathing base. Inflorescence from rhizome with a spike 4-9 cm long, 2-3 cm wide, on an erect peduncle up to 25 cm, with 5-9 green sheaths. Bracts are globose, 2-3 cm long, 2-2.5 cm wide, green, incurved, glabrous, with cream to yellow tips. Bracteoles are elliptic, 2.5-2.8 cm long, 1-1.5 cm wide, glabrous. The white corolla tube has three lobes, 2.2-2.5 cm long; the dorsal lobe is about 1.7-1.8 cm long, and the lateral lobes are 1.6-1.7 cm long. The three-lobed labellum is about 1.6 cm long, with a rounded or obovate midlobe, dark red with yellow blotches; side lobes about 7 mm long, dark red dotted. Another is 1 cm long, with a 3 mm filament and a 7-9 mm red appendage. Stylode is 7-8 mm long. The ovary is inferior, glabrous, with three locules. The fruit is an oblong capsule with many blackish, arillate seeds.

Description: Odour, characteristic and aromatic; taste, pungent and aromatic.

Warning: It should not be used in children under 6 years of age or in pregnant women. It should not be used in patients with bile duct obstruction except under medical supervision. Concomitant use with anticoagulants and antiplatelets should be used with caution.

Additional information: Ginger containing not less than 2 per cent v/w of volatile oil can be administered orally as an anti-emetic.

2.4.4 Long Pepper

Long Pepper is the dried red mature infructescence of *Piper retrofractum* Vahl.

Family: PIPERACEAE

Synonyms: Piperis Retrofracti Fructus, Java Long Pepper

Category: Carminative, expectorant, stomachic, anti-emetic, oxytotic.

Constituents: Long Pepper contains piperine, piperlongumine, pyridine alkaloids, sesamin, volatile oil, etc.



Figure 2.11 *Zingiber officinale* Roscoe 1. rhizomes, showing lower part of pseudostems and young spike, 2. pseudostems and leaf blades, 3. spike with flowers, one in full bloom (front view), 4. flower (oblique view), 5. mature rhizome 6. crude drug

Description of the plant: Climber, hairless, somewhat fleshy, supported by adventitious roots, rarely creeping. The stout, cylindrical stem is highly branched and thickens above nodes. Leaves are simple, alternate, 3-20 cm long and 2-13 cm wide; lower ones ovate or lanceolate with a cordate base, upper ones oblong or oval with various bases. All are unequal, taper to a point, entire, hairless, with sunk reticulate venation above and raised beneath. Petioles are 1.5-3 cm; stipules are 1-1.5 cm, membranous, lanceolate, fall off early. The inflorescence is a spike, erect or spreading, with a 0.7-2 cm peduncle; bracts are broadly oval-ovate, 1.5-2 mm. Flowers are unisexual or bisexual. Male spikes are 2.5-8.5 cm with 2-3 short stamens; female spikes are 1.7-3 cm with 2-3 short, obtuse stigmas. Infructescence is cylindro-conic; berries are attached to the bract stalk, broad, bright red. Seeds are 2-2.5 mm.

Description: Odour, aromatic; taste, pungent. Standard piperine solution: Dissolve about 10 mg of piperine, accurately weighed, in sufficient 1,2-dichloroethane to produce 100.0 mL.

2.4.5 Piper Sarmentosum Leaf

Piper Sarmentosum Leaf is the dried leaf of *Piper sarmentosum* Roxb.

Family: PIPERACEAE

Common name: *Piperis Sarmentosifolium*, Piper Sarmentosum Leaf

Synonyms: cha phlu , phak i loet , phluling

Category: Carminative.

Constituents: Piper Sarmentosum Leaf contains phenylpropanoids.

Description of the plant: Herb that erects or creeps, often stoloniferous, with swollen nodes. Leaves are simple, alternate, stipulate; suborbicular, ovate, or ovate-oblong, 7 to 15 cm long and 5 to 10 cm wide, with a surface that may be glabrous or covered in short hairs. The apex is acute to shortly acuminate, and the base is cordate to obliquely obtuse or rounded. The margin is entire and slightly undulate, with veins that are palmately 5- to 7-nerved and prominent on the lower surface. The petiole is 2 to 5 cm long. The inflorescence is a leaf-opposed dense spike, generally unisexual. The male spike is white, measuring 1.5 to 3 cm long, with a

peduncle 0.5 to 1.5 cm long; it has 2 to 3 stamens, with a very short filament and a subglobose anther.



Figure 2.12 *Piper retrofractum* Vahl. 1. habit 2. flowering twig 3. Different stages of infructescence 4. red mature infructescence 5. Crude drug

The female spike is whitish, 2 to 5 cm long, with a peduncle of the same length; it has 3 to 4 stigmas. The fruit is a drupe, obovoid, dark green when ripe. Seeds are small.

Description: Odour, characteristic; taste, slightly pungent.

2.4.6 Piper Wallichii vine

Piper Wallichii vine is the dried stem of *Piper wallichii* (Miq.) Hand.-Mazz.

Family: PIPERACEAE

Common name: Piper Wallichii vine

Category: Carminative, stomachic, anti-inflammatory.

Constituents: Piper Wallichii vine contains amide alkaloids (e.g., pellitorine), lignans, phenylpropanoids, aromatic glycosides, etc.

Description of the plant: Dioecious woody climber with black, ridged, hispidulous stems and climbing roots. Simple, alternate, ovate-lanceolate leaves (5-26 cm long; 4-17.5 cm wide) have an acute to acuminate apex, a cuneate or cordate base, an undulate margin, and a papery blade. The upper surface is dark green; the lower surface is glaucous and greyish when dry. Veins number 5-7, pinnately palmate; petiole measures 0.4-1 cm. Hairy, oblong-lanceolate stipules are present. The pendant cylindrical inflorescence is yellow or green-yellow, with spiral, pubescent, orbicular bracts (~1 mm). Male spikes (5-15 cm long, 3-5 mm wide) feature a puberulous peduncle (~2 cm), sparsely pubescent rachis, orbicular bracts (1-1.2 mm), and 3-4 stamens. Female spikes (5-7 cm long, 2-5 mm wide) have pubescent peduncles (2-4.2 cm), ovate, pointed ovaries, and 3-4 lobed stigmas (1-1.5 mm). The infructescence is 10-30 cm long, 1-2 cm wide, with a 2-4 cm puberulous stipe; the fruit is a sessile, globose, tuberculate drupe (3-4 mm), orange to red when ripe. The stigma and bract are persistent.

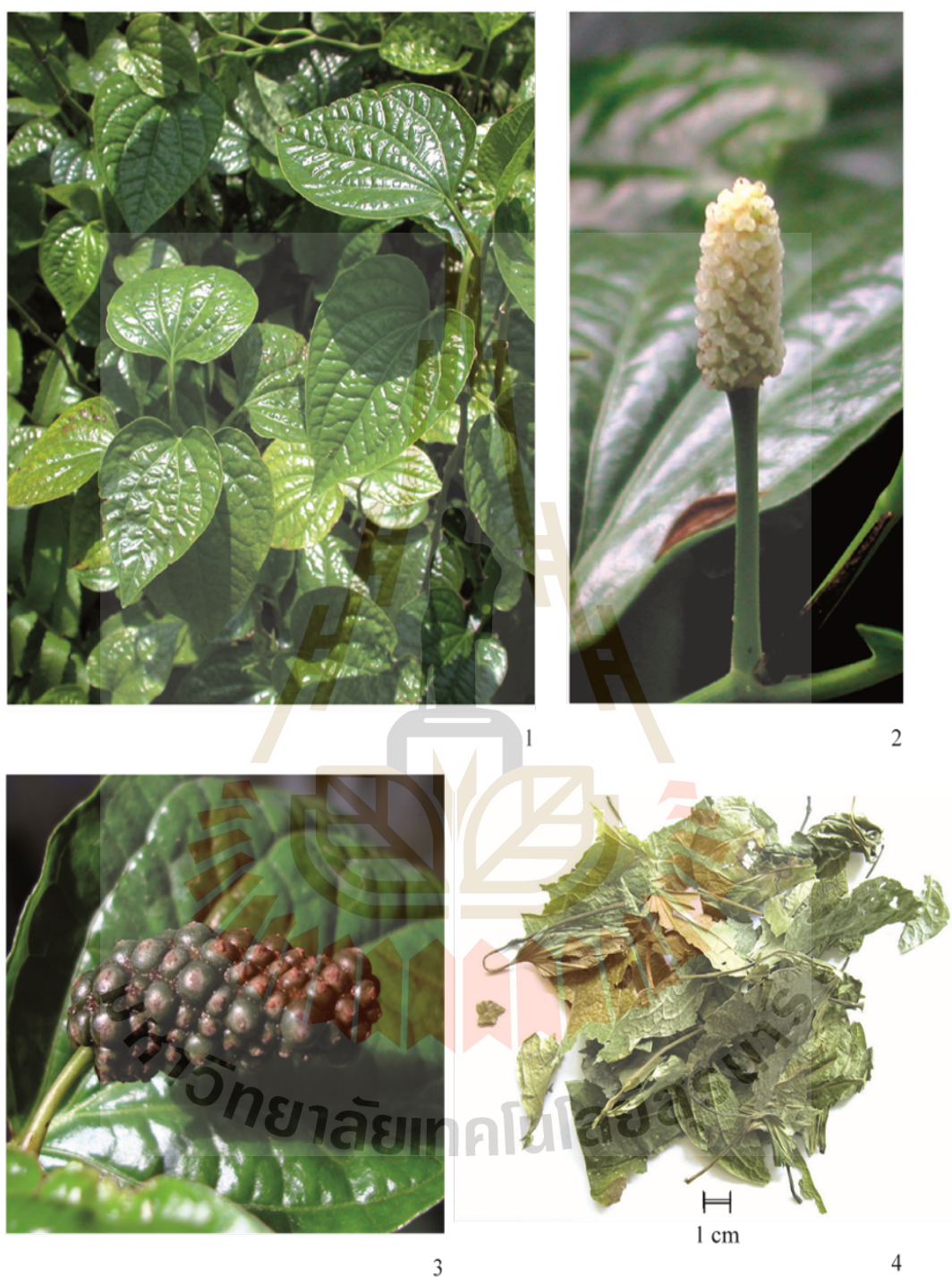


Figure 2.13 *Piper sarmentosum* Roxb. 1. Habit 2. Inflorescence 3. Infructescence
4. Crude drug

Description: Odour, aromatic and pungent; taste, pungent and spicy.

Additional information: The crude drug entitled “Sakhan” sold in herbal markets in Thailand may refer to other *Piper* species. However, *Piper wallichii* (Miq.) Hand.-Mazz. is cultivated and commercially available in the markets throughout northern Thailand. It is commonly used with other herbal drugs in Thai traditional herbal preparations.

2.4.7 Pi lang ka sa

Scientific name: *Ardisia polycephala* Wall.ex A.DC

Family name: MYRSINACEAE

Local name: Thulang kasa, Ram yai (Chumphon); Langphisa (Trat); Pue na (Malay-Narathiwat)

Plant part used: Fruit.

Habit: Pi lang ka sa is a small shrub or tree, reaching heights of 1–4 meters and possibly up to 10 meters. The trunk is erect, with rounded or angular branches, grayish brown in color, and young branches are reddish-brown, branching out around the trunk.

The leaves are simple, alternate, and densely arranged at the branch tips. They are ovate to obovate-oblong, 2.5–5 cm wide, and 6–12 cm long, with a pointed to rounded tip. The base is wedge-shaped, with a smooth surface and margins, and the leaf blades are glandular, with scattered spots. The leaves are thick and shiny, with short, red petioles. The veins are indistinct.

The flowers are borne in terminal or branch-top clusters of 4–8 flowers. The petals are white with a pink tinge. The inflorescence peduncle is 1.5–2.5 cm long, with 8–15 mm long peduncles connected at the base into a short tube, divided into five lobes, each lanceolate and pointed at the tip. The sepals are green. The base is joined together, and the tip is divided into five segments. The fruit is the size of a kapok bean. Young fruits are red and ripe, turning purple black.



Figure 2.14 *Piper wallichii* (Miq.) Hand.-Mazz. 1. Habit(a), stem(b), 2. Male inflorescence 3. Female inflorescence 4. Infructescence 5. Crude drug

The fruit is round and flat with a smooth surface, measuring approximately 6 millimeters in diameter. Young fruits are red and ripe, turning dark purple. It contains a single, round seed.

Description: The leaves have a bitter, hot flavor and are used to treat liver disease, diarrhea, and coughs. The fruit has a hot, astringent flavor and is used to treat fever, diarrhea, hives, and other imbalances.

2.4.8 Citrus Hystrix Peel

Citrus Hystrix Peel is the dried exocarp and mesocarp of green mature fruit of *Citrus hystrix* DC.

Family: RUTACEAE

Synonyms: Kaffir Lime Peel, Leech Lime Peel, Mauritius Papeda Peel, Porcupine Orange Peel

Category: Pharmaceutical aid (flavouring agent), carminative.

Constituents: Citrus Hystrix Peel contains volatile oil, of which β -pinene, limonene, β -phellandrene, and citronellal are its major components. It also contains linalool, borneol, camphor, sabinene, germacrene D, aviprin, umbelliferone, β -sitosterol, etc.

Description of the plant: Shrub or small tree 2 to 12 m tall; branchlets compressed-angular when young; spine solitary, 0.5 to 1.5 cm long. Leaves alternate or spirally arranged, very young leaves light reddish purple; winged petioles obovate to obcordate-oblong, 1 to 8 cm long, 1 to 4.5 cm wide; lamina orbicular-ovate or ovate-oblong, 3 to 15 cm long, 2.5 to 6 cm wide, apex obtuse to obtusely acuminate or slightly emarginate, base cuneate or rounded, margin more or less crenate. Inflorescence axillary or terminal, 1- to 15-flowered, fragrant; pedicel 2 to 5 mm long; calyxcupular, 1 to 1.5 mm long, 4-lobed; petals 4 to 5, oval-oblong, 6 to 10 mm long, 3 to 5 mm wide, obtusely acuminate, yellowish white or suffused with violet, with scattered pellucid dots; stamens 24 to 30, free, filaments 2 to 6 mm long.



Figure 2.15 *Ardisia polycephala* Wall.ex A.DC

Ovary superior, sub globose. Fruit ellipsoid, ovoid or globose, 5 to 7 cm in diameter, irregular bumpy; fruit-pulp yellowish green, very sour, slightly bitter. Seed's ovoid-oblong, 1 to 1.5 cm long, 3 to 5 mm wide.

Description: Odour, characteristic, aromatic; taste, bitter.

2.4.9 Fennel

Scientific name: *Oenanthe javanica* (Blume) DC.

Family name: APIACEAE

Synonym name: *Cyssopetalum javanicum* Turcz., *Dasylooma corticatum* Miq., *Dasylooma japonicum* Miq., *Dasylooma javanicum* (Blume) Miq., *Dasylooma latifolium* Lindl., *Dasylooma subbipinnatum* Miq

Plant part used: Fruit

Habit: It is a small, hollow plant about 1-2 feet tall. Leaves are composed of 2-3 pairs at the ends with a single leaf. The leaves are lanceolate, almost rectangular, with a pungent aroma. Small white flowers like coriander flowers in a bouquet.

2.4.10 Sweet Flag Root

Sweet Flag Root is the dried rhizome of *Acorus calamus* L.

Family: ACORACEAE

Synonyms: Acorus, Calamus, Sweet Flag

Category: Carminative.

Constituents: Sweet Flag Root contains volatile oil comprising β -asarone, cis-methyl isoeugenol, α - and γ -asarone, asarylaldehyde, acorone, acoroxide, acorin, calamene, linalool, calamol, calameone, eugenol, methyl eugenol, azulene, pinene, cineole, camphor, and others. Other constituents include sesquiterpenoids (e.g., acoragermacrone, acolamone and iso-acolamone), acoric acid, tannin, and resin.

Description of the plant: Aquatic perennial herb with long creeping horizontal rhizome, growing in shallow water, marshes, or swamps.



Figure 2.16 *Citrus hystrix* DC. 1. habit 2. fruiting twin 3. Crude drug



Figure 2.17 *Oenanthe javanica* (Blume) DC.

Leaves equitant, grass-like or sword-shaped, up to 2m long, 1 to 3.5 cm wide, long and slender without distinction of petiole, and finely parallel-veined. Inflorescence naked spike-like; spadix up to 10 cm long, projecting out and upwards from the leaf-like scape and spathe. Flower tiny, densely arranged, bisexual with perianth of 6 concave segments; stamens 6; ovary 2- to 4-celled with 2 to several ovules. Fruit baccate.

Description: Odour, sweet and aromatic; taste, bitter and pungent.

Warning: For reasons of optimal drug safety, the use of β -asarone-rich races shall be avoided.

Additional information: It is commonly used with other herbal drugs in Thai traditional herbal preparations.



Figure 2.18 *Acorus calamus* L. 1. Habit 2. Juvenile spadix 3. Flowering spadix
4. fruiting spadix 5. Crude drug

2.4.11 Phak phaeo daeng

Scientific name: *Iresin herbstii* Hook.

Family name: AMARANTHACEAE

Local name: Phak phaeo daeng, Phak phaeo suan (Central), Ee pae (Bangkok); a ong bai dan (Chiang Mai)

Plant part used: All part

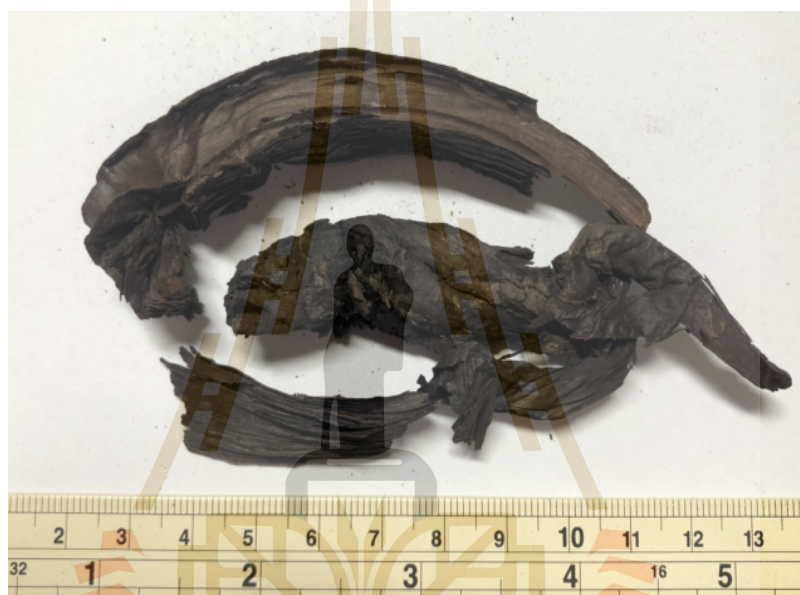


Figure 2.19 *Iresin herbstii* Hook.

2.4.12 Nutgrass

Scientific name: *Cyperus rotundus* L.

Family: CYPERACEAE

Local name: Yaa haeo muu (General); Ya khon mu (Mae Hong Son)

Plant part used: Rhizome

Constituents: *Cyperus Rotundus* Corm contains a volatile oil, of which cyperotundone and cyperene are its major components. It also contains flavonoids, etc.

Description of the plant: Perennial herb, 10 to 60 cm tall, stoloniferous; culm trigonous, glabrous, tuberous at the base. The corm is fusiform, ovoid or ellipsoid, measuring 0.7 to 2.3 cm in length and 0.5 to 1 cm in diameter, with a blackish-brown, woody appearance. Stolons are long and creeping. Leaves are basal, arranged in 3 ranks, with blades that are linear, up to 60 cm long and 2 to 6 mm wide, with an acuminate apex, entire margins, and a greyish-green to dark green color. The leaves are scabrous, flat, and longitudinally grooved in the middle. The leaf sheath is pale brown, sometimes with a reddish tint, and becomes fibrous. The inflorescence is terminal, usually a compound umbel-like spike. Involucral bracts number 2 to 5, are leaf-like, and measure 2 to 35 cm long and 0.5 to 4 mm wide. The primary branch is 2 to 12 cm long, with secondary branches (if present) 0.7 to 2 cm long. The spike is 1.5 to 5 cm long with an axis of 2 to 8 mm. Spikelets range from 3 to 10 per spike, are suberect to patent, narrowly oblong to linear, 1 to 3 cm long and 1.5 to 3 mm wide. They are flattened, with a more or less straight, winged axis. The glumes are 2-ranked, with 9 or more on the longest spikelet, ovate to ovate elliptic, 3 to 3.5 mm long and about 2 mm wide, obtuse and mucronate, with 5 to 7 nerves, reddish or purplish brown with a pale narrow hyaline margin, and a greenish keel.

The stamens are 3, with anthers about 1 mm long. The ovary is superior, unilocular, and contains one ovule. The style measures 1.3 to 3.5 mm long, and there are 3 stigmas that are 1.8 to 3.3 mm long, exerted from the glume. Nutlets are cylindric to obovoid-cylindric, trigonous, measuring 1.3 to 1.5 mm long and 0.5 to 0.7 mm wide, and are brown to blackish brown with minuscule punctations.

Description: Odour, mildly aromatic, spicy; taste, slightly spicy and bitter.



Figure 2.20 *Cyperus rotundus* L. 1. habit 2. inflorescences 3. spikelets
4. Crude drug

มหาวิทยาลัยเทคโนโลยีสุรนารี

Table 2.5 Drug formula of the Plook-Fire-Thatu recipe.

Common name	Scientific name	Part of herb	Important substance	pharmacological effects	Weight (100 g.)
White pepper พริกไทย	<i>Piper nigrum</i> L.	Ripe fruit	Piperine Piperettine	-Antioxidant activity (Singh, et al., 2013) -Analgesic effect (Tasleem, et al, 2014). -Anti-inflammatory effect (Intouch et al., 2014)	50
Ginger ขิง	<i>Zingiber officinale</i> Rosc.	Rhizome	Gingerols Shogaol	-Antioxidant activity -Inhibitory effect on lung cancer cells (Phuaklee, et al., 2010)	5
Long Pepper ดีปลี	<i>Piper retrofractum</i> Vahl.	Fruit	Piperine	-Anti-inflammatory effect -Analgesic effect (Sireeratawong, et al., 2012). -Anti-ulcer activity (Morikawa, et al., 2004).	5
Piper Sarmenosum ชาพลู	<i>Piper sarmentosum</i> Roxb.	All part	hydrocinnamic acid β -sitosterol	The hypoglycemic effect (Vongthoung, et al., 2016) Anti-inflammatory effect (Zakaria, et al., 2010).	5

Table 2.5 Drug formula of the Plook-Fire-Thatu recipe. (Continued)

Common name	Scientific name	Part of herb	Important substance	pharmacological effects	Weight (100 g.)
Piper Wallichii สะค้าน	<i>Piper wallichii</i> (Miq.) Hand.-Mazz	vine	Aristololac tam β -sitosterol	-Anti-inflammatory effect -Analgesic effect (Sireeratawong, et al., 2012).	5
Pi lang ka sa พิลังกาสา	<i>Ardisia polycephala</i> Wall.ex A.DC.	Fruit	embelin	-Antibacterial -Platelet activating factor receptor binding	5
<i>Citrus hystrix</i> มะกรูด	<i>Citrus hystrix</i> DC.	Peel of fruit	Linalool sabinene	-Anti-inflammatory effect(Tangyuenyongwatanaand Gritsanapan, 2014)	5
Fennel ผักชีล้อม	<i>Oenanthe javanica</i> (Blume) DC.	Seed	apigenin	-Antifungal effect -Antioxidant -Antibacterial effect -Anti-infective effect	5
Sweet Flag Root ว่านน้ำ	<i>Acorus calamus</i> Linn.	Rhizome	β -asarone α -ararone	-Anti-inflammatory effect on nerve pain (Muthuraman, et al, 2011). -Diuretic effect (Ghelani, et al, 2016).	5

Table 2.5 Drug formula of the Plook-Fire-Thatu recipe. (Continued)

Common name	Scientific name	Part of herb	Important substance	pharmacological effects	Weight (100 g.)
Phak phaeo Daeng ผักแพวแดง	<i>Iresin herbstii</i> Hook.	All part	Alkaloid	-Antibacterial	5
Nutgrass แห้วหมู	<i>Cyperus rotundus</i> L.	Rhizome	Alkaloid cardiac glycosides flavonoids	-Antidiarrheal effect (Uddin, et al., 2006). -Antibacterial activity against pathogenic bacteria -Antioxidant activity (Kilani, et al., 2008).	5

2.5 Fourier Transform Infrared Spectrometer

The Fourier transform infrared spectrum (FTIR) of any biological system, including cells or tissues, acts as a unique fingerprint of phenotypic expression in relation to environmental influences. It provides vital chemical information that primarily signifies the presence of key biocomponents like proteins, lipids, carbohydrates, and nucleic acids. It is crucial to note that FTIR spectroscopy does not allow for the identification of individual chemical species that may arise as metabolic byproducts or stress indicators; instead, it functions as a tool for assessing the overall biochemical condition of biological processes. Variations in spectral biomarkers associated with specific biomolecule groups can reveal the existence of certain diseases, environmental stressors, or genetic alterations. This biochemical “fingerprint” can offer initial insights into biological or pathological processes, but a thorough interpretation of FTIR spectra requires sophisticated analytical techniques, such as multivariate analysis

2.5.1 Principle of FTIR

Infrared (IR) spectroscopy is a chemical analysis method that leverages the interaction between materials and infrared light. This type of light is part of the electromagnetic spectrum, positioned between visible light and microwaves, with wavelengths that vary from 780 nm to 1 mm.

Infrared light is divided into three categories: Near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). NIR possesses the shortest wavelength and highest wavenumbers, whereas FIR has the longest wavelength and lowest wavenumbers. Generally, in the context of IR spectroscopy, MIR is the most commonly used type of IR light. This particular IR range is significant because it corresponds to an essential characteristic of chemical compounds: their vibrations.

Atoms within chemical compounds are in constant motion and exhibit various vibrations. Each vibration takes place at a distinct frequency that corresponds to the specific chemical bond and compound. As previously noted, these frequencies resonate with the frequencies of light found in the MIR region of the electromagnetic spectrum. Since these frequencies align with infrared (IR) light, chemical compounds absorb IR light, which excites the vibrations within the molecules.

While IR spectroscopy broadly refers to the chemical analysis technique that detects molecular vibrations via IR light, Fourier Transform IR Spectroscopy (FT-IR) is the most widely used method. In the past, IR spectroscopy involved testing each individual frequency of IR light to see if the sample absorbed it, making this process quite time-consuming. FT-IR has since replaced this method by simultaneously analyzing all IR light wavelengths through an interferometer. This results in significantly faster and more accurate measurements, providing a superior signal-to-noise ratio. However, FT-IR generates a different data set than traditional IR spectroscopy.

To address this, a mathematical process called a Fourier transform is utilized to convert the data into the familiar IR spectrum associated with IR spectroscopy.

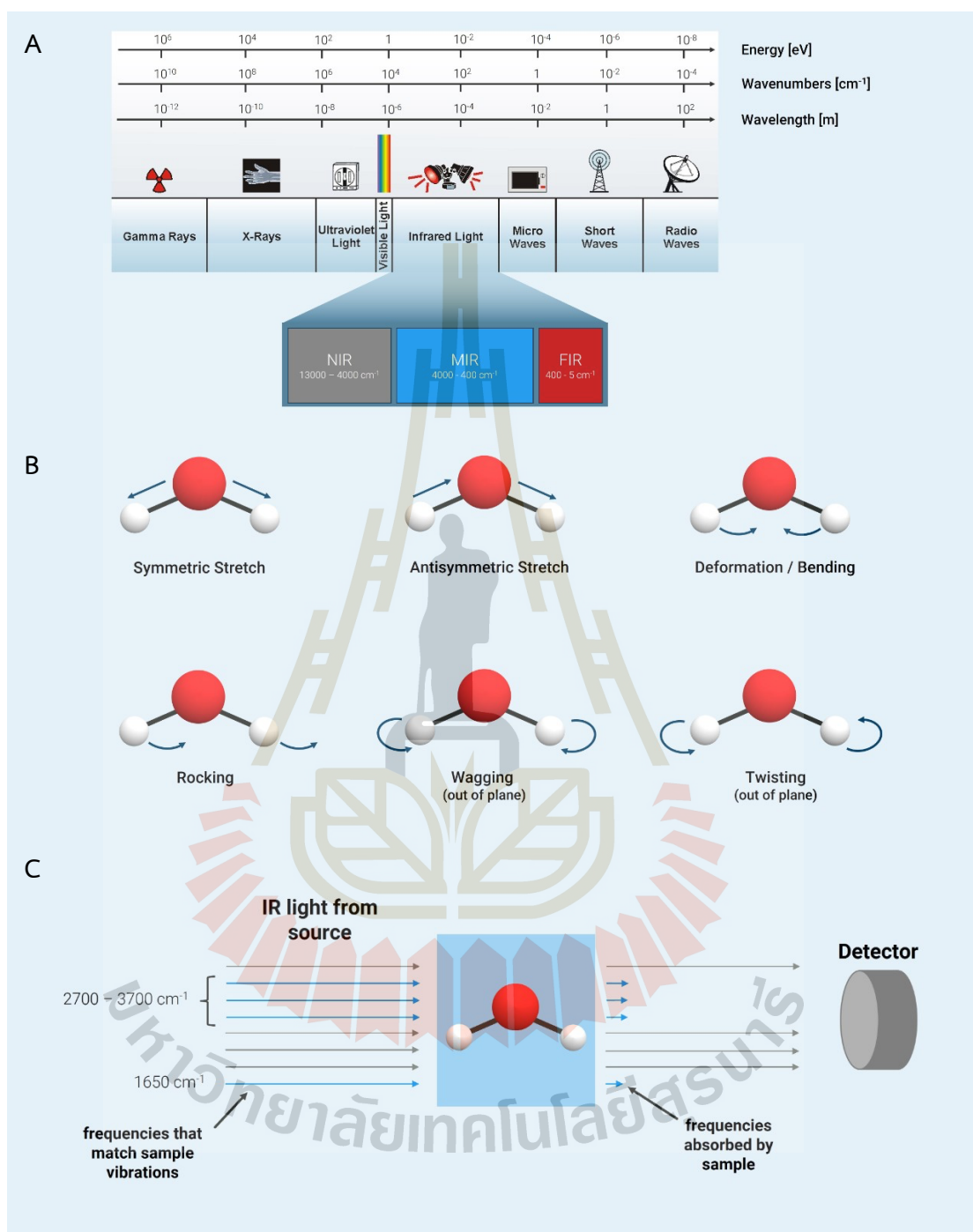


Figure 2.21 Infrared light categories: Near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR) (A), Types of vibrations in IR spectroscopy (B), Detecting IR absorption (C)

FT-IR revolutionized IR spectroscopy by simplifying the technique considerably. Dispersive IR spectroscopy is time-consuming because each wavelength of IR light must be analyzed individually. In contrast, FT-IR quickly evaluates all light wavelengths, accelerating the process. FT-IR achieves this through interferometry, utilizing the properties of waves and their interactions to extract details from a sample.

In the FT-IR spectrometer, once the light is recombined, it is aimed at the sample for detection. The mirror then shifts to change the phase difference between the beams, leading to a varied interference pattern upon recombination. Each unique interference pattern corresponds to different wavelengths of IR light in the mixed beam. The mirror's rapid movement through various positions facilitates quick detection of IR absorbance across the entire range of IR wavelengths.

Once all data is gathered, it can be transformed into an interferogram. This interferogram illustrates the sample's absorption of IR light, yet it differs significantly from the IR spectrum produced by dispersive IR spectrometry. While the IR spectrum shows how intensely the sample absorbs each frequency of IR light, the interferogram demonstrates this absorption as it relates to the position of the movable mirror within the interferometer. Therefore, data from an FT-IR experiment needs to be processed to generate a corresponding IR spectrum, requiring the extraction of absorption data for each wavelength of IR light from the interferogram's information.

Three main measurement techniques can be used in FT-IR spectroscopy: Transmission, Attenuated Total Reflection (ATR), and Reflection. Although all approaches rely on the core principles of FT-IR spectroscopy, each method utilizes a distinct process for analyzing samples and is appropriate for different applications.

Transmission IR spectroscopy: Transmission represents the foundational technique utilized in IR spectroscopy. In this method, infrared light passes through the sample and is absorbed. For effective transmission, the IR light needs to pass entirely through the sample, often necessitating careful sample preparation. If a sample is excessively thick, the infrared light cannot fully pass, resulting in a substantial amount of IR light being absorbed, a condition referred to as total absorbance. This results in diminished spectral quality and complicates the identification of peaks.

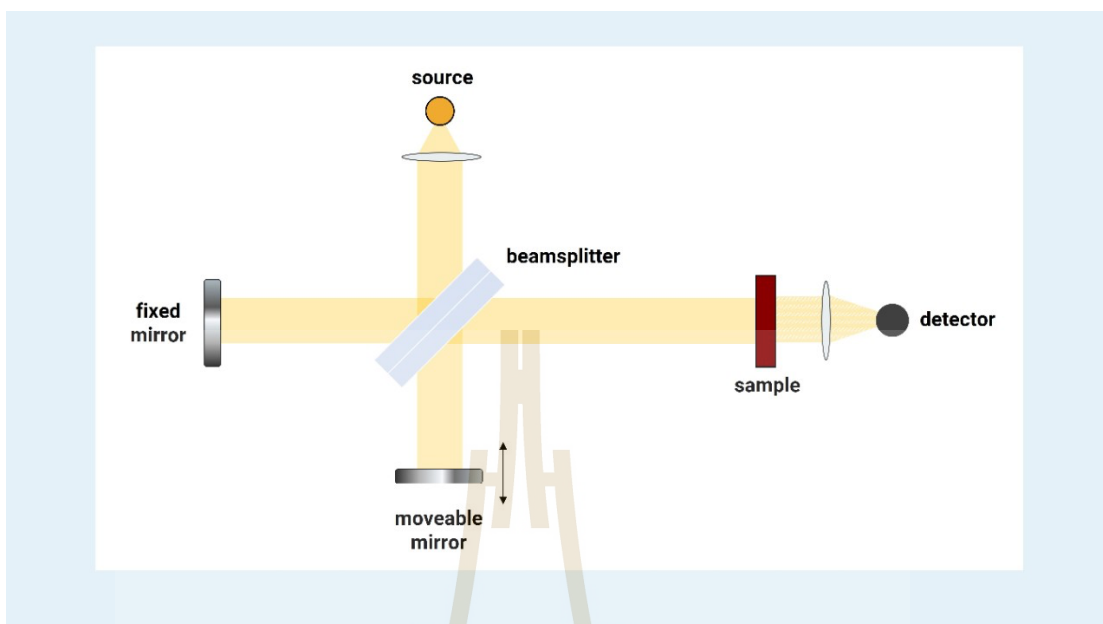


Figure 2.22 Inside the FT-IR spectrometer

To prevent total absorbance, the sample is diluted, which enables the IR light to pass through with reduced absorption. The diluting substance must not absorb IR light in the same range as the sample; otherwise, it would also show up on the resulting spectrum.

To analyze a liquid sample, it is typically diluted with a solvent, often carbon tetrachloride (CCl_4). For solid samples, the material must be ground and combined with another solid, usually potassium bromide (KBr), which does not absorb light in the MIR range. This mixture is then pressed into a pellet for analysis. Alternatively, the sample can be thinly sliced and positioned on a KBr window. This preparation step may be omitted if the sample is delicate (less than $15\ \mu\text{m}$).

The sample preparation process for transmission detection is quite time-consuming and requires significant effort. Moreover, this method of preparing samples destroys the original material. Consequently, transmission detection is primarily utilized for specific spectroscopic applications, such as analyzing polymer films, proteins, and oil-in-water samples. However, transmission is extensively used in FT-IR microscopy in forensics and while examining tissue samples and microplastics.

ATR IR spectroscopy: ATR has primarily outperformed transmission and has become the leading measurement technique due to its minimal sample

preparation and non-destructive nature. In ATR, the sample is positioned on a crystal, generally composed of diamond, germanium, or zinc selenide. The IR light is introduced through the crystal, where it is partially absorbed by the sample. Afterward, the IR light traverses the crystal once more for detection.

In this technique, light interacts solely with the top few microns of the sample. Since the IR light fully penetrates the sample, ATR requires minimal to no sample preparation to generate an IR spectrum. This results in a method that is not only easy to execute but also reliably yields high-quality spectra, irrespective of the analyzed sample.

It is important to note that ATR and transmission produce slightly different spectra due to the inherent differences between the two techniques. These spectral discrepancies arise from how various wavelengths of IR light interact with the sample when the light is only partially absorbed during ATR. However, the spectra generated by ATR and transmission remain quite similar. The differences can be easily corrected using computer software that directly compares spectra obtained from different measurement techniques.

Reflectance IR spectroscopy: The final measurement method is reflection, which detects the IR light bounced off the sample's surface instead of the IR light that penetrates the sample. This characteristic makes Reflection IR Spectroscopy essential for analyzing solid samples that are hard or impossible to assess using transmission or ATR methods. Different techniques can be employed for Reflection IR Spectroscopy, tailored to the specific sample under analysis.

Types of reflectance IR measurements can be performed: reflection-absorption, specular reflection, and diffuse reflection.

Reflection-absorption: Referred to as "transflectance," this technique is analogous to measurement methods that are based on absorption. In the course of this process, infrared (IR) light traverses the sample and subsequently reflects off a reflective substrate. It is essential for the sample to possess a minimal thickness in order to facilitate the proper transmission of IR light without absorption occurring.

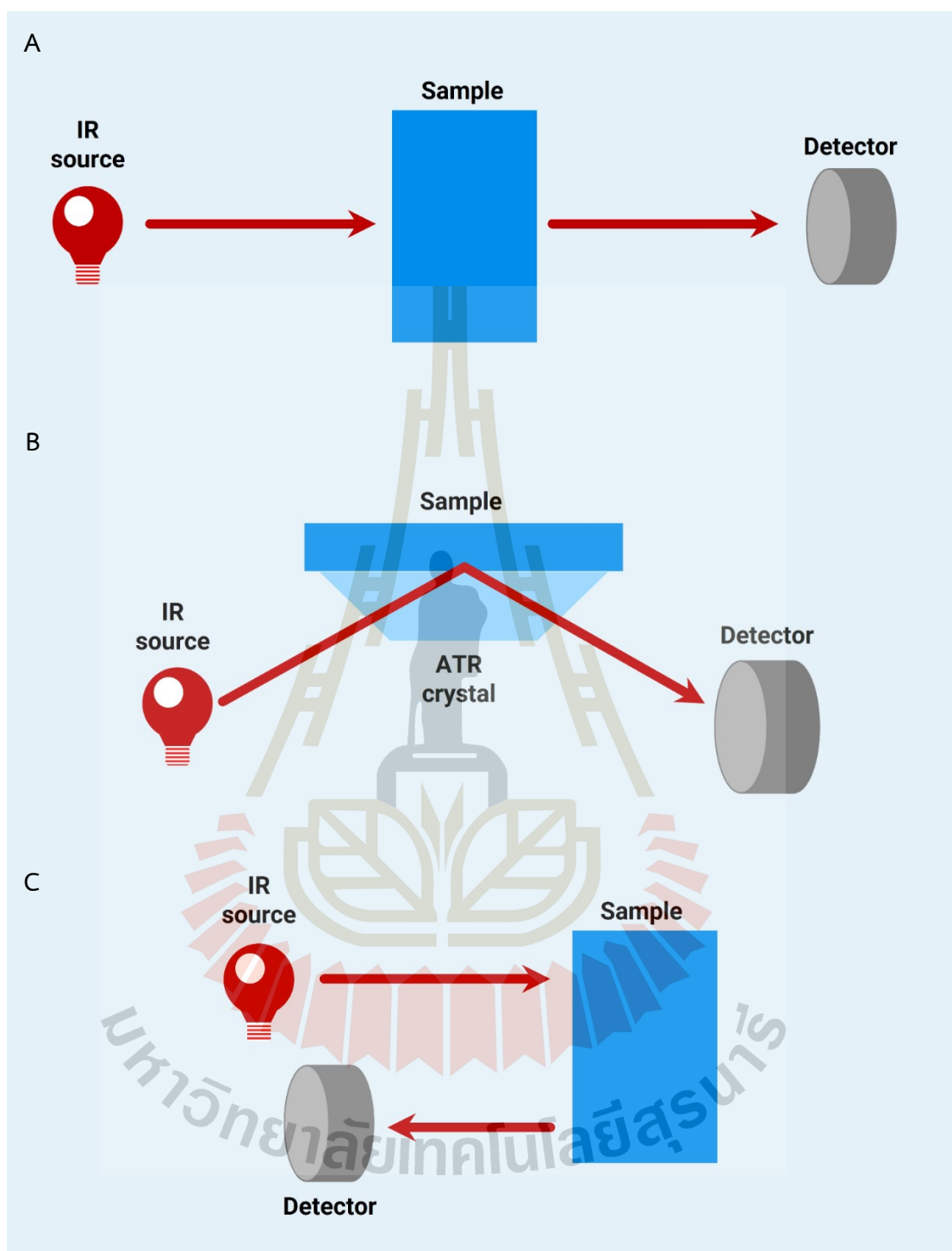


Figure 2.23 Three main measurement techniques: Transmission (A), Attenuated Total Reflection (ATR) (B), and Reflection (C).

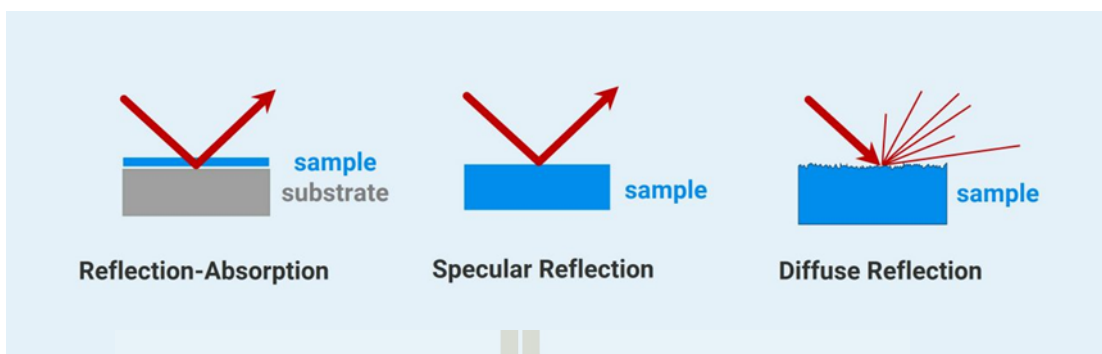


Figure 2.24 Types of reflectance IR measurements

The angle of the infrared (IR) light beam, commonly referred to as the incident angle, can be adjusted to modify the trajectory of the IR light as it traverses the sample. An increase in the incident angle extends the travel distance of the IR light within the sample, thereby enhancing the quantity of IR light absorbed. This adjustment facilitates the analysis of exceptionally thin samples via reflection absorption, including samples with a thickness as minimal as a single molecule, provided that the incident angle is sufficiently generous. Consequently, reflection absorption proves to be particularly effective for the examination of very thin specimens, such as biological tissues and various coatings. Furthermore, reflection absorption is capable of determining the thickness of a layer or coating and characterizing the surface properties of a material.

Because reflection absorption mirrors absorption techniques, the resulting spectra are notably similar. These spectra do not need additional data processing or conversion for direct comparison with spectra obtained from transmission measurements.

Specular reflection: In specular reflection, infrared (IR) light reflects off the surface of the sample, necessitating that the sample be both smooth and reflective for effective use of this technique. This method is effective in analyzing various reflective materials, including metallic coatings, plastics, glass, minerals, and gemstones. Furthermore, it is a non-contact analysis method, making it particularly suitable for quick material identification and the examination of artwork.

Nonetheless, a challenge occurs with specular reflection, as the resulting spectra show substantial differences compared to those obtained via absorption measurements. This discrepancy mainly arises from the interaction of various IR light wavelengths with the surface of the sample. These spectral differences can be adjusted using a mathematical method called the Kramers-Kronig Transformation (KKT), allowing for a comparison between spectra obtained from specular reflection and those collected from transmission.

Diffuse reflectance and DRIFTS: Specular reflectance measurements require the removal of diffuse reflection when infrared (IR) light is directed at the sample, yet the method primarily assesses diffuse reflection. This approach is known as Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS). Throughout the process, the IR light interacts with the sample, scattering off the particles in multiple directions. The scattered light is then collected using gold mirrors and detected afterward.

The amount of diffusely reflected light is significantly affected by the characteristics of the sample, such as the shape, size, and density of its particles, as well as the sample's refractive index. The quality of the DRIFTS spectrum depends greatly on the quantity of diffusely reflected light that is captured. Therefore, to obtain a high-quality spectrum, it is essential for the IR light to penetrate deeply into the sample, enhancing the diffusely reflected light. Adequate sample preparation is crucial for achieving a high-quality DRIFTS spectrum.

For proper preparation of a sample for DRIFTS analysis, it is crucial that the particles are small and uniform. Thus, the sample needs to be ground and mixed thoroughly. Additionally, it should be diluted with a non-reactive solid like KBr that does not interact with IR light. By following these steps, the IR light can penetrate deeper into the sample, leading to more diffusely reflected light and a high-quality DRIFTS spectrum.

Creating the Spectrum from the Data: The Fourier Transform, a mathematical function, can efficiently execute this task utilizing computer software. It conducts an analysis of the data produced by wave interference across varying frequencies and identifies the original frequencies of those waves. When the Fourier

Transform is employed, the resulting output consists of two signals that represent the frequencies of the initial waves, one at 3 cm^{-1} and the other at 5 cm^{-1} . In the context of a Fourier Transform Infrared Spectroscopy (FT-IR) experiment, the interferogram encodes information regarding the various frequencies of infrared light that reach the detector subsequent to their interaction with the sample. By applying the Fourier Transform to the interferogram, one can extract the frequencies of the infrared light absorbed by the sample, resulting in the widely recognized infrared spectrum.

To ensure a clean and high-quality spectrum, an additional step is taken. This is essential because the IR light passing through the sample can interact with environmental compounds, like carbon dioxide or water, causing their absorbed IR light to appear in the spectrum. To avoid this, a reference interferogram is created to measure the IR light absorbed in the absence of the sample. The Fourier transform is then applied to this reference interferogram to produce a reference spectrum. Finally, the sample's IR spectrum is divided by the reference spectrum to yield the final IR spectrum.

2.5.2 Spectral Features of Maccromolecules in FTIR

The Fourier transform infrared spectrum (FTIR) of any biological entity, like a cell or tissue, acts as a unique identifier of phenotypic expression in response to environmental factors. It provides comprehensive chemical information, primarily indicating the presence of key biomolecules such as proteins, lipids, carbohydrates, and nucleic acids. It's crucial to note that FTIR spectroscopy is not intended for detecting specific chemical species, which may manifest as metabolic byproducts or stress indicators; instead, it evaluates the overall biochemical state of biological processes. Variations in spectral biomarkers associated with particular biomolecule groups can signal the emergence of specific diseases, environmental stressors, or genetic alterations. This biochemical “fingerprint” serves as an initial indication of observed biological or pathological events; however, a thorough interpretation of FTIR spectra necessitates advanced analytical techniques, such as multivariate analysis.

Figure 2.13 displays the FTIR spectra of individual macromolecules found in a biological sample, alongside a typical spectrum for a cell or tissue. The comparison of

band positions and shapes across all spectra clearly indicates the presence of bands from the most abundant biocomponents in the cell or tissue spectrum.

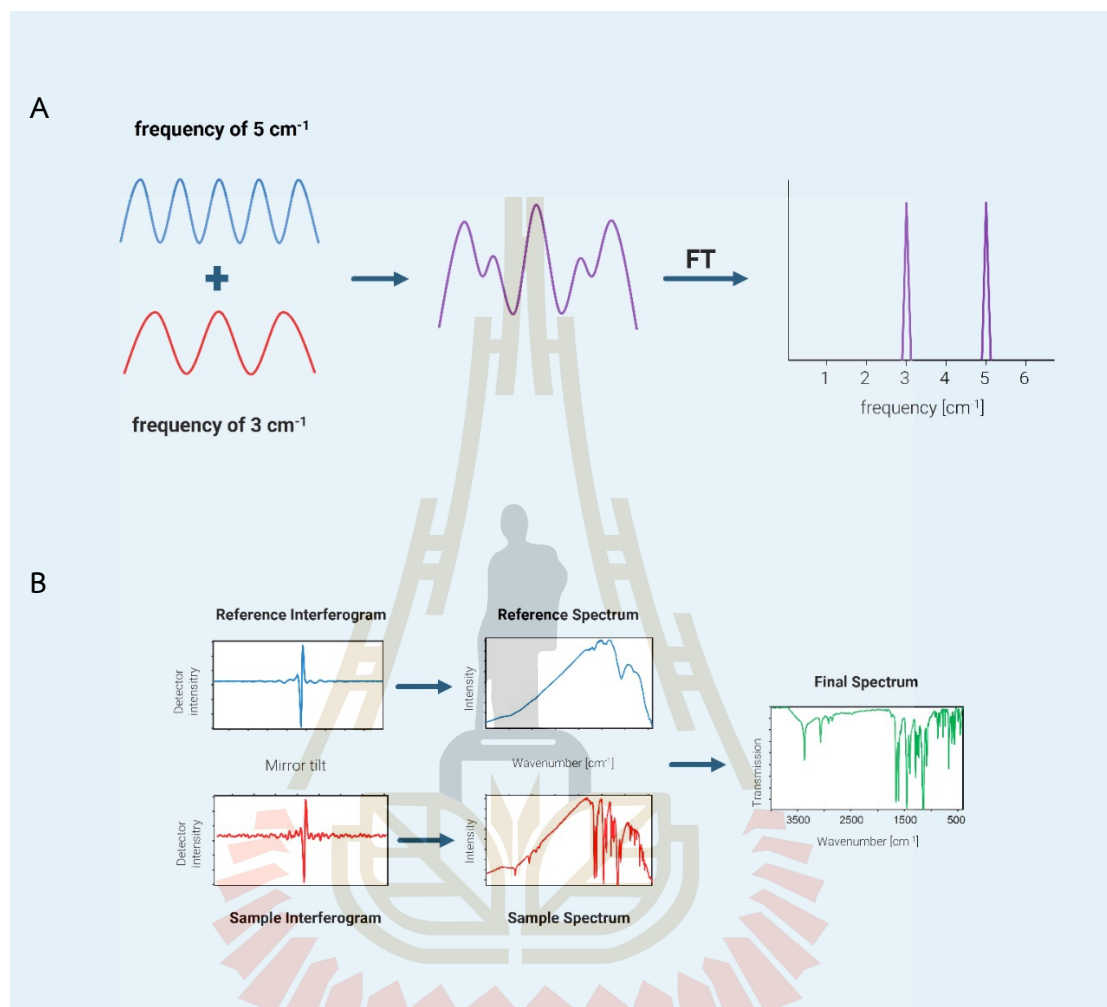


Figure 2.25 The Fourier Transform: The frequencies of the two original waves (A), Fourier transform to the interferogram (B)

Figure 2.14 shows the second derivative IR spectrum, which reveals subtle differences in the shapes of spectral bands and emphasizes the primary absorption features associated with proteins, lipids, carbohydrates, and nucleic acids. The IR bands detected in the FTIR spectra of cells and tissues are assigned to specific vibrations and macromolecules.

Proteins: Proteins serve as the fundamental components of all cells and tissues, allowing their vibrational fingerprint to be easily detected using infrared microspectroscopy. The infra-red spectrum of proteins exhibits two key features: the

amide I and amide II bands. These features are frequently utilized in studies of protein secondary structure due to their sensitivity to structural configuration.

The vibrations mainly linked to C=O stretching and N-H bending modes occur in the frequency ranges of 1670–1620 and 1560–1530 cm^{-1} . The amide I method is the most common approach for quantifying α -helical, β -sheet, turn, and unordered protein structures. Typically, infrared spectra of cells and tissues show amide bands at 1665 and 1540 cm^{-1} , which indicate the presence of protein α -helical chains. Shoulders found at 1695–1670 and 1640–1620 cm^{-1} suggest alterations in protein secondary conformation toward a β -sheet structure.

Carbohydrates: The spectral range from 1150 to 1000 cm^{-1} is typical of carbohydrates. Peaks at 1085 and 1050 cm^{-1} , along with a shoulder near 1030 cm^{-1} , are linked to mono- and polysaccharides as well as proteoglycans. The identification of a particular carbohydrate is often connected to existing data on biochemical processes. Hydration affects the spectral profile; however, some studies indicate that glycogen in cells is semi-hydrated, causing its characteristic bands to appear at 1150, 1080, and 1028 cm^{-1} . The glycogen bands at 1080 and 1028 cm^{-1} can often overshadow the nucleic acid bands. Infrared maxima at 1150 and 1050 cm^{-1} observed in the spectra from oral mucosa, cervical cells, and glandular cell cytoplasm are attributed to mucin, composed of glycosylated proteins. Mucus containing mucin coats cell surfaces, whereas glandular cells secrete mucin. The 1050 cm^{-1} band from mucin can obscure the 1038 cm^{-1} band associated with DNA and RNA.

Lipids: Lipids are predominantly concentrated in plasma membranes and microsomal pellets, which include endosomes and secretory vesicles, while only a small amount can be found in the cytoplasm. The most prominent spectral characteristics of lipids or phospholipids arise from the stretching modes of CH_2 and $=\text{CH}$ groups (2800–3050 cm^{-1}), the ester C=O bond (1736 cm^{-1}), and the scissoring vibrations of long-chain CH_2 groups (1468 cm^{-1}). Key biochemical processes related to lipid metabolism are mainly identified by examining the high-frequency region.

Usually, just four bands are detected from lipids, relating to the asymmetric and symmetric stretching vibrations of the methyl and methylene groups at around 2960 [$\text{V}_{\text{as}}(\text{CH}_3)$], 2923 [$\text{V}_{\text{as}}(\text{CH}_2)$], 2873 [$\text{V}_{\text{s}}(\text{CH}_3)$], and 2850 cm^{-1} [$\text{V}_{\text{s}}(\text{CH}_2)$].

Furthermore, a weak band in the range of 3010–3030 cm^{-1} is connected to the CH stretching vibration of unsaturated fatty acids [$\nu(\text{=CH})$].

The bands associated with the methylene group mainly originate from saturated lipid chains, whereas the $\nu(\text{CH}_3)$ bands come from the vibrations of methyl groups found in proteins, lipids, and nucleic acids. The most notable spectral changes that reflect modifications in lipid structure and composition are evident in the methylene vibrations.

Analyzing $\nu(\text{C=O})$ absorbances distinguishes the structure and composition of mitochondrial and plasma membranes in cells. The C=O stretching mode observed at 1740 cm^{-1} correlates with triglycerides and cholesterol esters, whereas the band at 1713 cm^{-1} indicates the presence of free fatty acids, although it usually overlaps with the amide I band. Variations in the intensity of the 1470–1460 cm^{-1} band suggest changes in ester-containing macromolecule content, corroborated by similar intensity shifts in the 1343 cm^{-1} (CH_2 wagging mode) and 1170 cm^{-1} (CO-O-C asymmetric stretches) bands associated with triglycerides and cholesterol esters, respectively. Additionally, the C-O vibrations of cholesterol found in atherosclerotic aortas lead to a band at 1055 cm^{-1} . The differentiation between phospholipids and lipids is further highlighted by the presence of phosphate group bands, especially at 1088 cm^{-1} ; however, this band is frequently masked by sub-bands from phosphodiester groups in various biomolecules.

Nucleic Acid: FTIR spectra for nucleic acids usually show several unique bands. These bands mainly result from the molecular vibrations of acid components, including C=O and ring CC stretching of purine and pyrimidine bases (which appear in the 1720–1600 cm^{-1} range), along with the asymmetric and symmetric stretching of the phosphate group and C-O bonds in the phosphodiester and sugar units (found between 1300–900 cm^{-1}). In dry cell or tissue samples, the most noticeable bands are prevalent throughout the fingerprint region. However, in animal or human samples, the IR bands corresponding to nucleic acids in the 1720–1600 cm^{-1} range are often obscured by protein bands that are consistently present. As a result, nucleic acids are mainly indicated by IR bands found in the 1300–900 cm^{-1} range, which are heavily affected by environmental factors and are detectable only in the absence of glycogen.

The spectral profiles of DNA and RNA exhibit notable differences, making FTIR analysis an effective technique for monitoring the structural and compositional variations of these vital cellular components.

The IR bands observed at 1160, 1120, 1036, 996, and 916 cm^{-1} , along with their corresponding bandwidths, uniquely identify RNA. Notably, the significant 1120 and 996 cm^{-1} bands, linked to the vibrations of ribose and uracil, act as distinct indicators for RNA detection. While there are vibrational modes for the carbohydrate CH_2OH and C-O groups in this spectral range, their intensity is comparatively lower than that of the nucleic acid bands. Additionally, a rise in RNA concentration notably impacts the prominent DNA band located at 1240 cm^{-1} .

The main DNA vibrations occur at roughly 1225 cm^{-1} and 1080 cm^{-1} , which correspond to the asymmetric and symmetric stretching vibrations of the phosphodiester, respectively. The location of the asymmetric phosphodiester stretching vibration in DNA varies with its conformation. In low ionic strength solutions, both native DNA and most synthetic double-stranded poly- and oligonucleotides adopt a B-form conformation. Conversely, under the same conditions, double-stranded RNAs take on an A-type conformation.

2.6 Related research

The previous research indicated that the galactogenic effect of the Plook-Fire-Thatu recipe was not significantly different from that of placebo; however, the proportion of nursing mothers who had an increased breast milk volume of 110 ml and higher in the Plook-Fire-Thatu recipe was significantly higher than that in the placebo group. This herbal formula is safe, as no adverse drug reaction was observed (Nilnetr Virasombat et al., 2020).

Study of active substances showed that Plook-Fire-Thatu crude drugs were extracted with 50% alcohol, evaporated with Rotary Evaporator, and dried with a Freeze Dryer. It was found that the percent yield was 5.98. Analysis of the standard sample with High-performance liquid chromatography (HPLC).

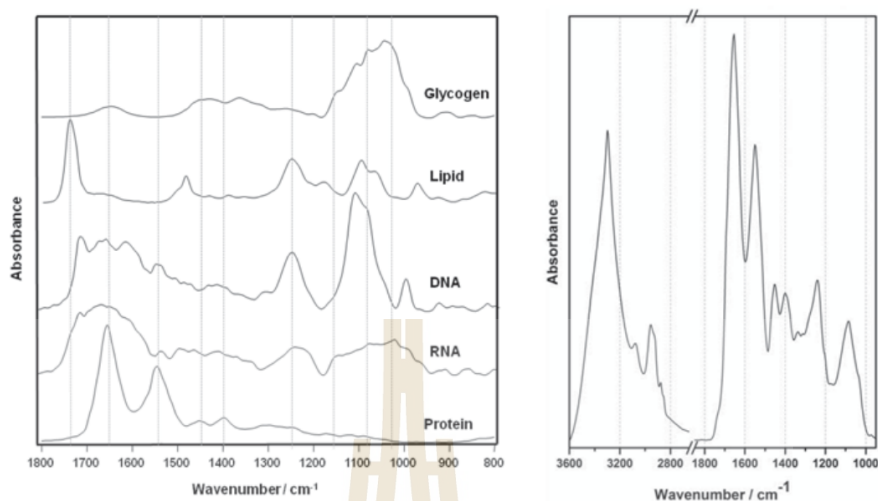


Figure 2.26 FTIR spectra of major biological components revealing the characteristic spectral regions for proteins, nucleic acids, lipids, and carbohydrates (glycogen) (left); a typical FTIR spectrum of a sample of a biological origin (right)

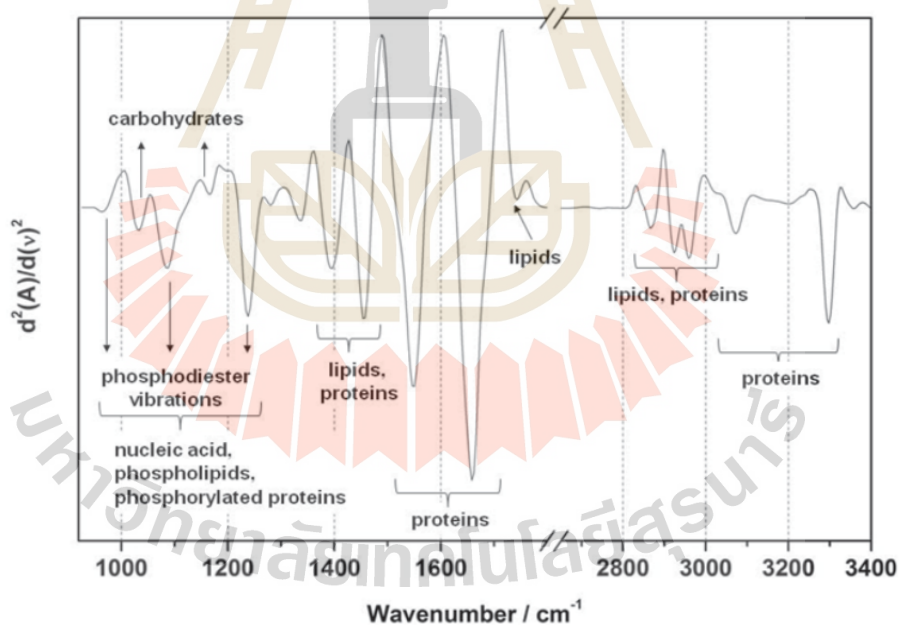


Figure 2.27 Second derivative IR spectrum of a sample of a biological origin with labelled spectral regions specific for macromolecules.

Vibrational mode	Wavenumber (cm ⁻¹)	Assignment
NH stretch (amide A)	ca. 3290	Proteins
NH stretch (amide B)	3060	Proteins
=CH olefinic stretch	3010–3030	Unsaturated lipids
CH ₃ asymmetric stretch	2954–2962	Lipids and protein
CH ₂ asymmetric stretch	2915–2924	Saturated lipids and side chain of proteins
CH ₃ symmetric stretch	2870	Lipids and protein
CH ₂ symmetric stretch	2847–2855	Saturated lipids and side chain of proteins
SH stretch	2550	Cysteine thiol group
C=O stretch (ester)	1740–1730	Lipids, phospholipids
C=O stretch	1705–1690	Bases of nucleic acids
C=O stretch + NH bend (amide I)	1695–1670	Proteins—anti-parallel β -sheet and β -turn structures
	1670–1660	Proteins— 3_{10} -helix structure
	1660–1648	Proteins— α -helix structure
	1637–1623	Proteins— β -sheet structure
ring CC stretch of Tyr and Phe residues, and nucleotides	1620	Proteins, nucleic acids
NH bend + C-N stretch (amide II)	1580–1480	Proteins
ring CC stretch of Tyr residues	1520	Tyrosine proteins
CH ₂ scissoring mode	1473–1462	Lipids proteins,
CH ₂ , CH ₃ deformation modes	1455	Proteins
COO ⁻ symmetric stretch	1400	Fatty acids, amino acids
CH ₃ deformation of aliphatic side groups of amino acid residues	1379	Proteins
CH ₂ wagging	1343	Phospholipids, fatty acids, triglycerides, amino acid side chains
Amide III	1340–1240	Proteins
PO ₂ ⁻ asymmetric stretch	1238	DNA
CO-O-C asymmetric stretch	1171	Cholesteryl esters
C-OH stretch	1171	Serine, threonine, threonine
C-O stretch	1151	Glycogen, mucin
C-O stretch of ribose ring	1120	RNA
PO ₂ ⁻ symmetric stretch	1083–1086	Nucleic acids, phospholipids
C-C stretch	1078	Glycogen
-CO-O-C stretches	1063	Cholesterol esters, phospholipids
COH deformation	1050–1056	Mucin, carbohydrates
	1030	Nucleic acids
	1022–1028	Glycogen, carbohydrates
Dianionic phosphate monoester	970	Phosphorylated proteins
C-N-C stretch of ribose-phosphate skeletal vibrations	965	Nucleic acids
	933	Z type DNA

Figure 2.28 Assignment of FTIR bands of cells and tissues

The analysis and the correlation were established between the area under the graph and the concentrations of piperine, 8-gingerol, 6-shogaol, and 10-gingerol standards. The chromatograms are obtained as shown in Figure 2-47. The location of piperine, 8-gingerol, 6-shogaol and 10-gingerol were at 19.92, 31.63, 34.01 and 41.47 minutes, respectively. Alcohol extraction with 50% had the highest content of piperine, followed by 8-gingerol, 10-gingerol, 6-shogaol and 6-gingerol. The active substance of piperine, 8-gingerol, 10-gingerol, 6-shogaol, 6-gingerol were 292.3 ± 17.1 , 49.2 ± 12.4 , 32.6 ± 0.3 , 30.2 ± 2.0 and 4.9 ± 1.1 $\mu\text{g}/\text{mg}$ respectively. (Table 2.6)

Table 2.6 The content of important substances in the Plook-Fire-Thatu recipe and extracts from the medicinal plant formulas

Sample	The content of important substances ($\mu\text{g}/\text{mg}$)				
	piperine	8-gingerol	10-gingerol	6-shogaol	6-gingerol
Plook-Fire-Thatu recipe	292.3 ± 17.1	49.2 ± 12.4	32.6 ± 0.3	30.2 ± 2.0	4.9 ± 1.1

A study examined the safety and sub-chronic toxicity of PFT extract in an animal model. Results indicated that both the control group and the treatment groups (male and female) displayed comparable behaviors, showing no abnormalities like convulsions, loss of appetite, vomiting, ataxia, drowsiness, or any irregularities in urination or defecation. Body weight increase and food intake remained consistent among all animals. Blood biochemical parameters, hematological data, urine biochemical parameters, and histological appearances in all PFT-treated rats were similar to those observed in control rats. Administration of PFT extract at doses of 125 mg, 250 mg, and 500 mg/kg BW over 90 days, with observations extending to 120 days, revealed no toxic effects on either male or female rats.

Table 2.7 Comparison of Herbal Galactagogue Clinical Trials^a

Reference	Regimen	Patients	Infant Age (FT/PT)	Milk Attainment	Effect on Milk Production	Infant Weight
Turkyilmaz 2011	Fenugreek tea, ³ 3 cups daily	Fenugreek tea (n=22) Control (n=22) Placebo (n=22)	1 day (FT)	Nursing, except day 3 postdelivery by mechanical breast pump	Day 3 postdelivery: fenugreek 73.2 ± 53.5 mL; control 38.8 ± 16.3 mL; placebo 31.1 ± 12.9 mL (<i>p</i> = 0.004) 7.3 ± 2.7 mL; placebo 9.9 ± 3.5 mL	Lactation consultant for all groups
Swafford 2000	Fenugreek 3 capsules daily	n=10		Breast pump	Daily average increase from 207 to 464 mL (<i>p</i> =0.004)	
Di Pierro 2008	Micronized milk thistle 420 mg/day for 63 days	Milk thistle (n= 25) Control (n=25)		Nursing, then pumping to void the gland	Milk thistle 601.92 mL (day 0), 989.76 mL (day 30), and 1119.24 mL (day 60); an 85.95% increase Placebo 530.36 mL (day 0), 649.76 mL (day 30), 700.56 mL (day 60); a 32.09% increase	

FT = full-term delivery; PT = preterm delivery. ^aNo data have been reported on maternal or infant adverse events.

Table 2.8 Comparison of Oxytocin Nasal Spray (within 5 minutes prior to each pump) Clinical Trials.

Reference	Regimen	Patients	Infant Age (FT/PT)	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Huntingford 1961	Oxytocin before feeding for up to 10 days	Oxytocin (n=24) Placebo (n=24)	Day 1		Milk production day 7: oxytocin, mean 3.4 oz; placebo, mean 2.3 oz ($p<0.001$)	Day 4: Oxytocin, -147g, placebo - 207 g ($p=0.01$)	Minor epistaxis (n=1)		Oxytocin 4-5 IU Randomized, double-blind, Average length of use: oxytocin 70 hrs. placebo 78 hrs. No significant difference in primary outcome of level of breast engorgement

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

Table 2.8 Comparison of Oxytocin Nasal Spray (within 5 minutes prior to each pump) Clinical Trials. (Continued)

Reference	Regimen	Patients	Infant Age (FT/PT)	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Ruis 1981	Oxytocin before pumping for 5 days	Oxytocin (n=8) Placebo (n=4)	Day 1 (PT)	Pump	Primiparous: 3-to 5-fold increase, Multiparous: 2- fold increase, Oxytocin: 1964±308mL Placebo: 510±142mL (p=0.0002)				Oxytocin 3 IU 1 spray, pump 10 minutes, instill 2 sprays, pump 10 minutes, repeat for another breast, RCT, double-blind GA <38week
Fewtrell 2006	Oxytocin prior to pumping for 5 days	Oxytocin (n=27) Placebo (n=24)	(PT)	Pump every 3 hours	Day 5: Oxytocin median 667 g Placebo median 530 g (p=0.9)		None	None	Oxytocin 100µL (- 4 IU/dose) RCT, double-blind, intention-to-treat, maintained 80% power, Mean GA 29 wks

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

Table 2.9 Comparison of Domperidone Clinical Trials.

Reference	Regimen	Patients	Infant Age FT/PT	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Campbell-Yeo 2010	10 mg 3 times/day placebo for 2 wks	Domperidone one (n=22) Placebo (n=24)	3-4 weeks (PT)	Pump	Baseline to 14-day milk volume: Domperidone 184.4-380.0 mL (266.8%) Placebo 217.7-250.8 mL (18.5%) P<0.005		None	None	Gestation age <31 weeks Milk volume increased within 48 hrs
Wan 2008	10 mg 3 times/day or 20 mg 3 times/day for 2 weeks Crossover	Domperidone one (n=6)	16-117 days (mean 53) (PT)	Pump	Baseline: 30 mg mean 8.7 g/h ⁻¹ 60 mg mean 23.6g/h ⁻¹ (p=0.0217) 60 mg mean 29.4g/h ⁻¹ (p=0.0047)		Abdominal cramps (30mg:1;60 mg:2) Constipation(60mg:1) Dry mouth (30mg:3;60mg:5) Depressed mood (60mg:1) Headache (30mg:1;60mg:3)		Randomized, double blind, Gestational age, mean 26.5 weeks (24-29.4) 2 non responders

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

Table 2.9 Comparison of Domperidone Clinical Trials. (Continued)

Reference	Regimen	Patients	Infant Age FT/PT	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Da Silva 2001	Domperidone 10 mg 3 times/day for 1 week	Domperidone (n=11) Placebo (n=9)	Mean 31- 33 days (PT)	Pump	Domperidone: 112.8-162.2 mL (45.5%; P<0.05) Placebo 48.2-56.1 mL (16.6%)		None	None	Conducted in Australia Randomized, double-blind, Gestational age, mean 29.1 weeks

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

Table 2.9 Comparison of Domperidone Clinical Trials. (Continued)

Reference	Regimen	Patients	Infant Age FT/PT	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Petraglia 1985	Domperidone 10 mg 3 times/day for 3-10 days	Group A: Domperidone one (n=8) Placebo (n=7) Group B: Domperidone one (n=9) Placebo (n=8)	Group A: 2 days Group B: 2 weeks	Nursing	Domperidone: day2, 347±36mL Placebo: day2, 335±30mL Domperidone: day10, 673±44mL, Placebo: day10, 398±45mL (p<0.01) Group A: Domperidone: day2, 105±35mL Domperidone: day5, 475±51mL (p<0.05) Group B: Domperidone: day2, 371-417mL; day5, 631-708 mL	Infants weighed pre-and post-feeding to determine milk amount.	None	None	Group A: history of lactation problem Group B: Primiparous with insufficient lactation after 2 wks, Feeding 6-7 times/day Milk supply significantly higher with domperidone vs placebo at days 4,6,8, and 10

Table 2.9 Comparison of Domperidone Clinical Trials. (Continued)

Reference	Regimen	Patients	Infant Age FT/PT	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Ehrenkranz 1986	10 mg 3 times/day for 7 days, then tapered over 2 days	Metoclopramide (n=17)	32±3.7 days (PT)	Electrical or hand breast pump	Metoclopramide 93.3±18 mL (day1) to 197.4±23mL (day7) (p<0.001)		Diarrhea (1) Nervousness (1) Tiredness (1)	None	All babies premature; mean gestational age 30.4±0.7weeks
Kaupila 1981	5, 10 or 15 mg 3 times/day for 2 weeks	5 mg (n=10) 10 mg (n=13) 15 mg (n=14)	8-62 days (FT)	Nursing	5mg:12.1mL(92.4vs104.5), Placebo:4.4mL (96.8vs 92.4). 10mg: 42.5mL (96.3 vs 138.8) (p<0.001), Placebo: 10.5mL (96.3 vs 106.8). 15mg: 50mL (80.3vs130.3) (p<0.05) Placebo: -0.3mL (80.3 vs 80).	5 mg: 380±153g Placebo:331±144g 10 mg: 439±143g Placebo:385±132g 15 mg: 480±138g Placebo:449±162g	Tiredness (1), headache (1), anxiety (1), hair loss (2), intestinal disorder (2)		Crossover vs placebo for each strength of metoclopramide Lactational response measured by weighing infant -8 dropout

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

Table 2.9 Comparison of Domperidone Clinical Trials. (Continued)

Reference	Regimen	Patients	Infant Age (FT/PT)	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Kaupila 1981	10 mg 3 times/day for 3 weeks, off 1 week, repeat for 2 weeks	(n=17)	18-141 days (mean 40.8) (unknown whether FT/PT)	Nursing	Baseline vs end of treatment First treatment: 194 mL (433±55mL vs 626±76mL; p<0.001). Second treatment :214 mL (390±73mLvs606±56mL; p<0.01)	Baseline, mean 4653 g Gain, mean 5%/week	Tired (5), nausea Gas (1), headache (1), vertigo (1), Gas (1)	Gas (1)	
Kaupila 1985	10 mg 3 times/day for 3 weeks	(n=11) Placebo (n=14)	4-20 weeks (unknown whether FT/PT)	Nursing	285±75mL to 530±162 mL/day (p<0.01) Placebo: no change		Tired (4), tired plus headache (1), tired plus nausea (1)	None	Randomized controlled conducted in Finland

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

Table 2.9 Comparison of Domperidone Clinical Trials. (Continued)

Reference	Regimen	Patients	Infant Age (FT/PT)	Milk Attainment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Hansen 2005	10 mg 3 times/day for 10 days	(n=28) vs placebo (n=29)	3 days (PT)	Electric breast pump	Metoclopramide not associated with significant increase in milk volume compared with placebo on each of 17 study days		Facial rash and itching (1)	1 death at 10, not related to study drug	Randomized controlled All received education from lactation nurse
Sakha 2008	10 mg 3 times/day for 15 days	(n=10) vs control (n=10)	Unknown (FT)	Nursing	Data not provided	No difference between groups over 15 days Metoclopramide 351.1 g vs control 328.5 g			

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

Table 2.9 Comparison of Domperidone Clinical Trials. (Continued)

Reference	Regimen	Patients	Infant Age (FT/PT)	Milk Attain ment	Effect on Milk Production	Infant Weight	Maternal ADRs	Infant ADRs	Notes
Fife 2011	10 mg 3 times/day for 8 days	(n=13) vs placebo (n=13)	Within 6 hours (PT)	Pumpin g every 3 hours	All increased significantly from baseline No differences between groups		Moderate to very severe reports Fatigue (10), nervous (3), constipation (2), depression (2), headache (1), diarrhea (1) No difference compared with placebo	None	RCT 3 metoclopramide and 4 placebo dropouts Did not maintain power (10 pts per group) Preterm infants delivered ² 34 weeks gestation All pts. received lactation education Milk volume exact data not presented (only represented in graph)

ADRs = adverse drug reactions; FT = full-term delivery; PT = preterm delivery

CHAPTER III

RESEARCH METHODOLOGY

3.1 Study design

This study is a randomized clinical trial (RCT) type of clinical superiority trial. The Plook-Fire-Thatu recipe (PFT) was analyzed for bioactive compounds by High-Performance Liquid Chromatography (HPLC). Clinical studies were conducted to investigate the effects of PFT on breast milk production. The duration of clinical studies was 72 hours postpartum. The participants in the experimental group consumed the Plook-Fire-Thatu recipe 500 mg orally three times per day before meals for three days postpartum. The participants were women aged between 20 and 40, with the inclusion criteria for the study and consent to participate in research based on informed consent. All participants were read and signed the informed consent before participating in the study. All participants were selected by random sampling based on probability. The study's results were evaluated before and after taking the PFT. Blood samples were collected on day 1 and day 3 postpartum, respectively. The breast milk production and adverse effects at various time points were determined. Breast milk samples were investigated for nutritional content by Fourier transform Infrared spectroscopy (FTIR).

3.2 Study area

The active substances of the recipe were studied by the HPLC technique at the phytochemical laboratory of Thai Traditional Medicine, Rajamangala University of Technology Isan, Sakon Nakhon Province.

The clinical tests were performed on postpartum women who delivered and recovered at the postpartum ward in Sikhio Hospital, Nakhon Ratchasima Province.

Milk samples were tested by FTIR at Synchrotron Light Research Institute (Public Organization), Thailand.

3.3 Population and Sample size

Under the approval of the Human Research Ethics Committee of the Suranaree University of Technology, 60 women whose babies were delivered from November 2021 to November 2022 at Sikhio Hospital were recruited for the study. All participants received study information during their third trimester antenatal care visit and provided written informed consent prior to enrolment. It was considered inappropriate to seek consent during labour or immediately postpartum, as this could impair decision-making capacity.

3.3.1 The inclusion criteria

- 1) Postpartum women who had term vaginal delivery (≥ 37 weeks gestation age).
- 2) Age 20-40 years.
- 3) With out abnormalities of the breast or nipple.

3.3.2 The exclusion criteria

- 1) Major complications after the delivery, such as endometritis, sepsis, or hemorrhage.
- 2) Allergy essential of herbs in the PFT, including white pepper, ginger, piper sarmentosum leaf, java long pepper, citrus hystrix peel, sweet flag root, piper wallichii stem, phak phaeo daeng, pi lang ka sa, fennel, and nutgrass.
- 3) Used drugs that may interact with the PFT, including phenytoin, propranolol, theophylline, and rifampicin.
- 4) Received anticoagulant or antiplatelet or clinical bleeding.
- 5) There were abnormalities in liver or kidney function.
- 6) Congenital diseases, including epilepsy, asthma, chronic gastritis, GERD, hypertension or underlying diseases that cause low milk production (e.g. GDM)

3.3.3 Discontinuation criteria

1) Serious adverse events following administration include stomach irritation, rash, itching, swelling, chest pain, and bleeding. Other symptoms that doctors deem unsafe for the participants.

2) Taking PFT not continuously follow the plan.

3) Received another galactagogue that affects milk stimulation.

4) Do not consent to continue participating in research.

3.3.4 Sample size

Calculation of the sample size to compare the mean of two independent groups was performed on the formula as follows:

$$n/gr = \frac{2(Z_{\alpha} + Z_{\beta})^2 \sigma^2}{(\mu_1 - \mu_2)^2}$$

Previous studies have shown that the standard deviation of Fenugreek Herbal Tea intervention with breast milk volume is 18.8 ml. The researchers stipulated that the average breast milk volume of the experimental group and the control group are 50.8 and 35.5 ml, respectively, which will be considered clinically significant (El Sakka, Salama, & Salama, 2014)

The value was replaced in the formula as follows:

$$n/gr = \frac{2(Z_{\alpha} + Z_{\beta})^2 \sigma^2}{(\mu_1 - \mu_2)^2}$$

$$n/gr = \frac{2(1.96 + 0.84)^2 18.8^2}{(50.8 - 35.5)^2}$$

$$n/gr = \frac{5,541.94}{234.09}$$

$$n/gr = 23.67$$

$$n/gr \approx 24$$

Then, the dropout rate was calculated as 20% = 30 samples per group. Therefore, the sample sizes used in this clinical test were 60 samples, divided into a treatment group and a control group each of 30 samples.

3.4 Sample sampling

Participants were recruited after delivery using simple random sampling. Randomisation was performed immediately following birth by a registered nurse, prior to the participants' transfer to the postpartum ward. Participants were randomly allocated to either the control or treatment group in a 1:1 ratio, using sequential numbering from 1 to 60. Odd numbers (1, 3, 5, etc.) were assigned to the control group, while even numbers (2, 4, 6, etc.) were assigned to the treatment group. Participant numbers were not displayed on medical charts or beds to maintain allocation concealment.

3.5 Materials and methods

3.5.1 Materials

This PFT formulation is a bolus preparation composed of 11 medicinal herbs. Each 100 g of the mixture contains 50 g of pepper fruit (*Piper nigrum*) and 5 g each of the following: ginger rhizome (*Zingiber officinale*), long pepper fruit (*Piper longum*), all parts of wild betel leaf (*Piper sarmentosum*), Wallach's pepper vine (*Piper wallichii*), Ardisia fruit (*Ardisia elliptica*), peel of kaffir lime fruit (*Citrus hystrix*), Oenanthe seed (*Oenanthe javanica*), sweet flag root (*Acorus calamus*), pink Arnebia (*Arnebia euchroma*) and nut grass rhizome (*Cyperus rotundus*).

The herbs were dried, powdered, and thoroughly mixed before being formed into bolus units with honey. Each bolus contained 100 g of the herbal powder. The formulation was produced in accordance with the standards outlined in the Thailand NLEM and met the Good Manufacturing Practice certification requirements. The lot number of the product used in this study was 126411, with an expiry date of 23 December 2023.



Figure 3.1 Plook-Fire-Thatu recipe; Package (A) Bolus (B)

3.5.2 Methods

3.5.2.1 High-Performance Liquid Chromatography (HPLC)

The bioactive compounds study of the Plook-Fire-Thatu recipe used High-Performance Liquid Chromatography (HPLC) for analysis. Piperine and 8-gingerol are the main active substances of pepper and ginger, which are the main components of this recipe.

Assay for active compounds in PFT extract.

Weigh 5 mg of the PFT extract into a 10 mL volumetric flask. Subsequently, add 5 mL of methanol. Sonicate the mixture in an ultrasonic bath for 15 minutes. Then, adjust the volume to 10 mL with methanol. Filter the sample using a 0.45 μm syringe filter set. The sample preparation should be conducted in triplicate (three replicates).

Analytical conditions.

The standard compounds employed for the quality control of the Ya Pluk Fai That herbal extract are Piperine and 8-Gingerol. The analysis is conducted using high-performance liquid chromatography (HPLC) with a Zorbax Eclipse Plus C18 column (3.5 μm , 4.6 $\times$ 100 mm). A gradient mobile phase is utilized, comprising 0.5% acetic acid in acetonitrile and 0.5% acetic acid in water. The ratio varies from 30:70 to 100:0 (acetonitrile to water phase). The mobile phase flow rate is set at 1.5 mL/min. A photodiode-array detector is used to measure absorbance at 280 nm.

Standard curve preparation.

Standard solutions of piperine and 8-gingerol were prepared by dissolving them in methanol at the following concentrations: 10, 20, 40, 80, and 160 µg/mL for piperine, and 25, 50, 100, 200, and 400 µg/mL for 8-gingerol. Each standard concentration was subsequently analyzed using High-Performance Liquid Chromatography (HPLC). The peak area and corresponding concentrations were utilized to construct the standard calibration curve.

3.5.2.2 Randomized clinical trial (RCT)

The RCT was used to determine if the treatment group treated with the PFT combined with standard treatment was more effective in treating hypogalactia than the control group that received standard treatment. A double-blind study with allocation concealment, and simple randomisation was used.

All participants received standard postpartum care and breastfeeding support. This included guidance on breastfeeding techniques: early initiation (breastfeeding within 1 h of birth), frequent feeding (every 2–3 h, day and night) and proper positioning to ensure effective latching.

In addition to standard care, the treatment group received 500 mg of PFT orally three times daily, administered before meals for three consecutive days postpartum. The control group received standard care only and did not receive PFT or a placebo. The dosage and duration were selected based on the NLEM recommendation.

The intervention commenced with the first meal after delivery, within 12 h postpartum. The first day of the study was defined as the day participants began receiving the intervention. Participants were discharged 72 h after delivery.

Medication preparation was carried out by the hospital pharmacy. Interventions were distributed by a registered nurse, who remained blinded to the contents. The medication was sealed and only opened by the nurse immediately prior to administration.

The uterus recovery was measured by the distance from the fundus of the uterus to pubic symphysis at different postpartum time points by a soft

tape measure. It was assessed on days 1, 2 and 3 postpartum (Lu, Ye, Qiu, Wang, & Zheng, 2019).

Breast milk volume measurement was Breast Pumping Method using an electronic pump three times daily—30 min after each meal (breakfast, lunch and dinner) on days 1, 2 and 3 postpartum. Each measurement was conducted at least one hour after the last breastfeeding session. The total daily breast milk volume was calculated as the sum of all expressions regardless of mealtimes. Each pumping session to empty both breasts lasted approximately 30 min, after which the expressed milk was fed to the infant. No participants' babies received formula supplementation during the day.

The participant's blood was collected in 10 ml and divided into two tubes. The first tube had a volume of 6 ml. Put in a test tube containing Ethylene diamine tetra acetate (EDTA) (Tube EDTA) and shake well. To analyse hematological values, which consist of hemoglobin (Hb), hematocrit (Hct), and blood glucose. The second tube, 4 ml. was put in a tube without anticoagulant (Tube Clotted Blood), take the blood sample and spun with a centrifuge at 3,000 rpm. for 5 minutes and sucked out only the serum part. Microcentrifuge tubes were stored in a refrigerator at 4 degrees Celsius to analyse blood biochemistry values. These include blood sugar, serum prolactin level (PRL), total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDLC), high-density lipoprotein-cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and blood urea nitrogen (BUN), creatinine, and albumin. The serum prolactin (PRL) levels were measured by the enzyme-linked immunosorbent assay (ELISA). Blood (10 mL) samples were collected from all participants two hours after delivery to establish baseline measurements. The same volumes of blood were subsequently collected on day 3 postpartum following treatment. (El Sakka et al., 2014) .

Adverse effects in mothers and infants were assessed at days 1, 2, 3, 7 and 30 postpartum.

To minimize nutritional confounding during the three-day intervention period, dietary intake was standardized. A registered dietitian provided participants with meals averaging 1800 kcal per day, comprising approximately 20 %

protein, 30 % fat, and 50 % carbohydrates as follows: Table 3.1. Participants were asked about their habitual diet and lifestyle, and dietary intake was recorded for 3 days. Dietary assessment will be collected by food record and calculated using the Thai Nutri-Survey Program. Menus were carefully designed to exclude ingredients known to influence lactation (such as ginger or other galactagogue herbs) that could confound the study outcomes. If such ingredients were present, the nutritionist had discretion to exclude affected participants. A standardized menu plan was followed, and participants' dietary intake was retrospectively recorded and monitored for compliance and nutritional analysis.

3.5.2.3 Fourier transform Infrared spectroscopy (FTIR)

The nutritional content of breast milk was determined to show that the treatment group treated with the PFT combined with standard treatment had more nutritional content than the control group that received standard treatment by FTIR.

Breast milk (1.5 mL) samples were collected from all participants two hours after delivery to establish a baseline. The same volumes of breast milk samples were subsequently collected on day 3 postpartum following treatment. Breast milk samples were stored in sterile tubes and frozen until nutritional analysis was performed.

Breast milk nutritional content was analysed using FTIR spectroscopy. Spectra were acquired using a Bruker Hyperion 3000 spectrometer equipped with a focal plane array detector. Measurements were performed in reflection mode across the spectral range of 3000–900 cm^{-1} using OPUS 7.5 software. A 15x objective lens was employed with 64 scans per sample and 64 background scans, at a resolution of 4 cm^{-1} . Principal component analysis (PCA) was applied to visualise patterns and differences within the spectral dataset by reducing its dimensionality. PCA transforms the original variables into principal components that capture the greatest variance, thereby simplifying interpretation of complex data. The analysis was conducted using Unscramble X 10.1.

3.6 Data collection

Data collection was structured into seven key components: (1) general characteristics, including age, weight, body mass index and gravida number; (2) haematology (haemoglobin (Hb), haematocrit (Hct)) and blood biochemistry (blood glucose, serum prolactin level (PRL), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine and albumin); (3) uterus recovery; (4) dietary assessment; (5) breast milk volume; (6) adverse drug reactions; and (7) breast milk nutritional content.

3.7 Data analysis

I interpreted the breast milk volume, hematological and blood chemistry of the treatment and control groups into a mean score. Compare the difference values within the group before and after the experiment and between groups on the first and third day postpartum.

The statistical analysis used in the study was classified according to the following variables, shown in Table 3.2.

3.8 Ethical statement

All procedures performed in human participant studies follow the Ethical Standards of the Suranaree University of Technology. The study was conducted by the Declaration of Helsinki and approved by the Human Research Ethics Committee of Suranaree University of Technology (protocol code EC-64-135, date of approval: January 12, 2022). There is no conflict of interest in this study

Table 3.1 Nutrients distributed for one day.

Food Groups	Breakfast	Lunch	Dinner
Rice / Flour (g.)	110	215	200
Meat / Eggs (g.)	65	110	110
Vegetable (serving)	1	2	2
Fruit (serving)	-	1	1
Fat (serving)	1	2.5	2.5

Table 3.2 Statistical analysis in this study.

Variables	Levels of measurement	Statistical analysis
age, body weight, body mass index, blood pressure, and heart rate	Ratio	Chi-square test
education, systemic disease, medication or food supplementary	Ordinal	Chi-square test
breast milk volume, hematological and blood chemistry <i>within the group</i>	Ratio	Paired Sample t-test
breast milk volume, hematological and blood chemistry <i>between the groups</i>	Ratio	Independent Sample t-test
Comparison of breast milk volume first day to third day postpartum	Ratio	Repeated measures ANOVA

3.9 Budget

The estimated budget for this study is 600,000 baht, as shown in **Table 3.3** below.

Table 3.3 Budget details in this study

Items	Amount (Baht)
1. Personnel category	
1.1 Researcher's compensation	50,000
1.2 Wages for research assistants	140,000
1.3 Participants compensation	30,000
2. Laboratory analysis category	
2.1 Active substance by HPLC	80,000
2.2 Breast milk nutrition analysis by FTIR	60,000
2.3 Hematological and blood chemical analysis	153,500
3. Other expenses	
3.1 The Plook-Fire-Thatu recipe	1,500
3.2 Payment for research participation	80,000
3.3 clinical record form	5,000
Total cost (Bath)	600,000

CHAPTER IV

RESULTS AND DISCUSSION

According to the research purposes, the results and discussions were divided into four parts: 4.1 Bioactive compound of the Plook-Fire-Thatu recipe, 4.2 The efficacy for increased breast milk production, 4.3 The safety of the Plook-Fire-Thatu recipe, and 4.4 Breast milk macronutrient composition.

4.1 Bioactive compound of the recipe.

Results: Report on the results of extracting the PFT with 50% ethanol and 99% ethanol. The extract appeared to be brown flakes. Figure 4.1. The extracted drug formula had a yield percentage (% yield), as shown in Table 4.1

Table 4.1 The results of extracted the Plook-Fire-Thatu recipe

Extract	Color	%yield
PFT bolus extract by 50% ethanol	Dark brown	37.79%
PFT powder extract by 99% ethanol	Brown	4.19%



Figure 4.1 Characteristics of the drug powder extract with 50% ethanol (A), extract by 99% ethanol (B), and evaporated with a Rotary Evaporator (C).

HPLC was used to analysis of standard substances. Piperine and 8-gingerol are the main active substances in pepper and ginger, which are the main components of the PFT, so they were analyzed this time.

The analysis of **piperine** standard substances by HPLC revealed retention time values of 6.14. When standard substances at various concentrations were analyzed, and the area under the graph was used to create a standard graph, the linear equation obtained from the standard graph of piperine $R^2 = 0.9991$. as shown in Figure 4.2 (A)

They were then analyzed for the essential substances in the PFT samples: bolus extract and powder extract. After taking the area under the graph of the substance, whose retention time matches the standard sense, analyze and compare it with standard substances. It was found that the critical substance, piperine, was detected in each sample chromatogram of the standard, compared with the HPLC fingerprint of the bolus extract and the powder extract for the PFT. As shown in Figure 4.3. The average amount of **piperine** found in the bolus extract and the powder extract by water of the PFT was 0.028 ± 0.03 (mg $\pm$ S.D.) and 0.423 ± 0.411 (mg $\pm$ S.D.), respectively.

Analysis of **8-gingerol** standard solutions by HPLC revealed retention times of 11.71. The standard substances at various concentrations were analyzed, and the areas under the curves were used to create a standard graph. The linear equation obtained from the standard graph of 8-gingerol $R^2 = 0.994$. The standard graph was not validated, which caused it to appear in the curve, as shown in Figure 4.2 (B)

Analyzed for the essential substances of the powder extract of the PFT. After taking the area under the graph of the substance, whose retention time matches the standard, analyze and compare it with the standard substances. It was found that the critical substance, 8-gingerol, was detected in each standard sample chromatogram, compared with the HPLC fingerprint of the PFT powder extract. As shown in Figure 4.4. The PFT extracts with 50% Eth and 95% Eth contained 8-gingerol 404.25 ± 7 ($\mu\text{g} \pm$ S.D.) and 715.19 ± 34.44 ($\mu\text{g} \pm$ S.D.) per milligram extract, respectively.

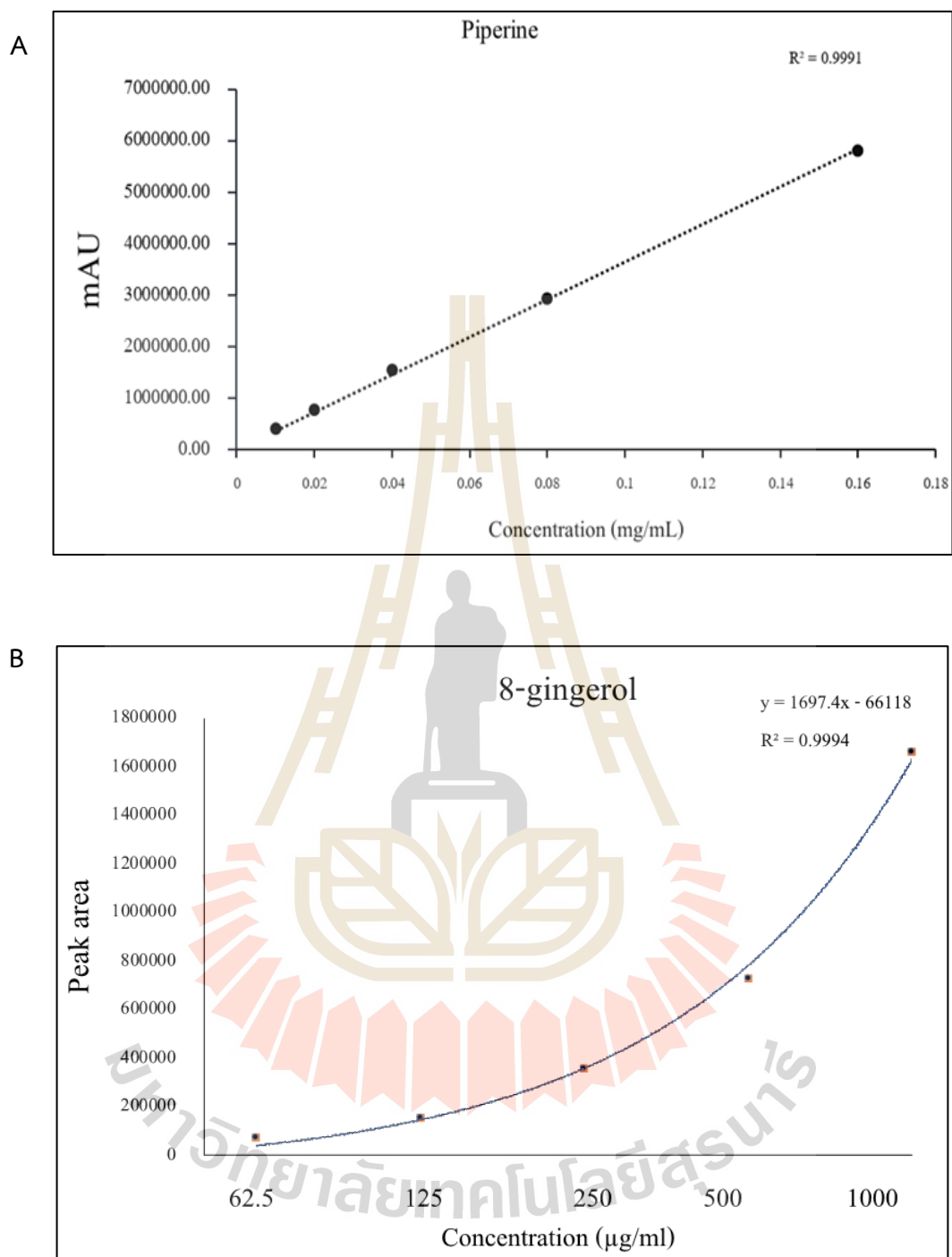


Figure 4.2 The standard graph of piperine (A) and 8-gingerol (B).

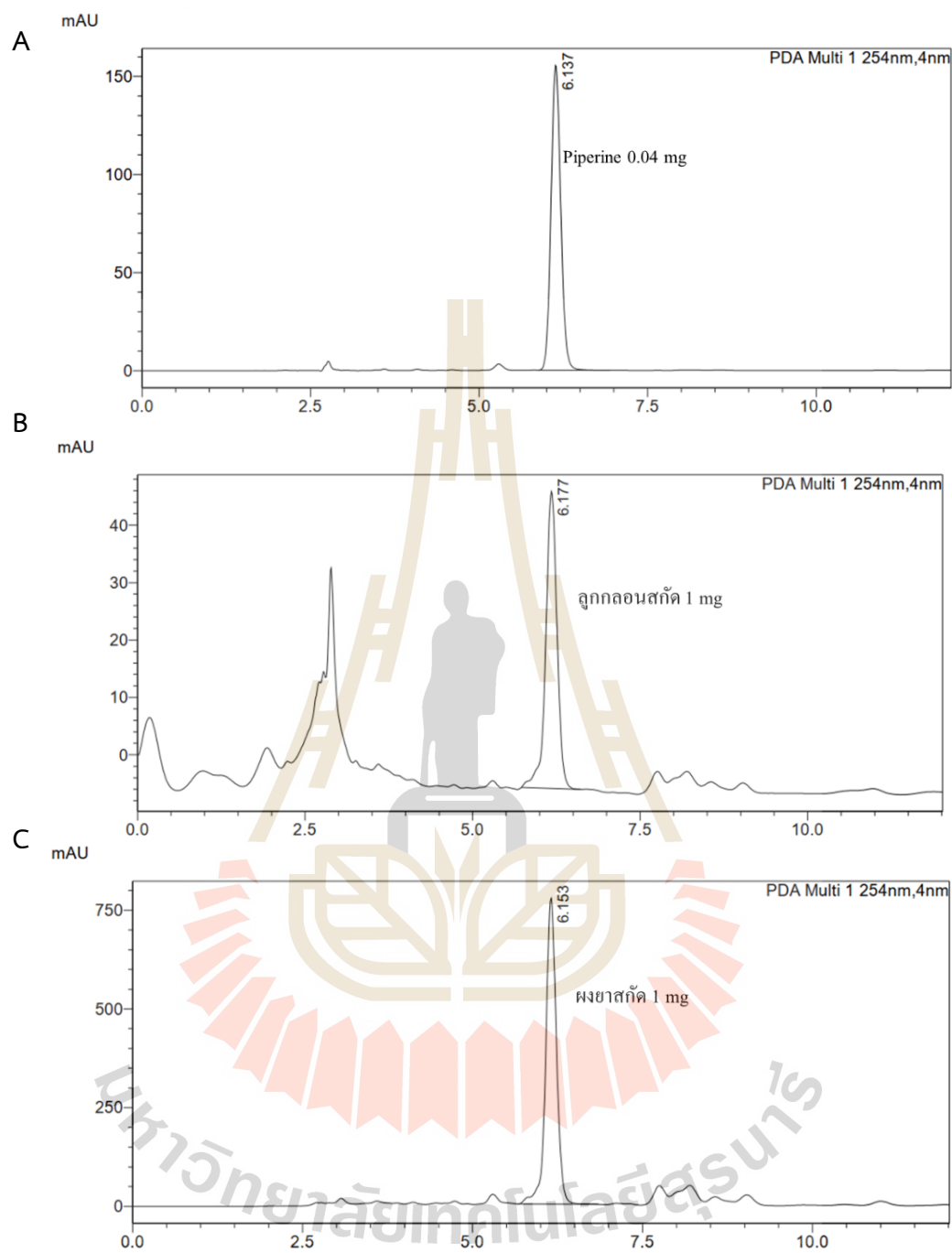


Figure 4.3 Chromatogram of standard solution piperine (A), bolus extract (B), and powder extract (C).

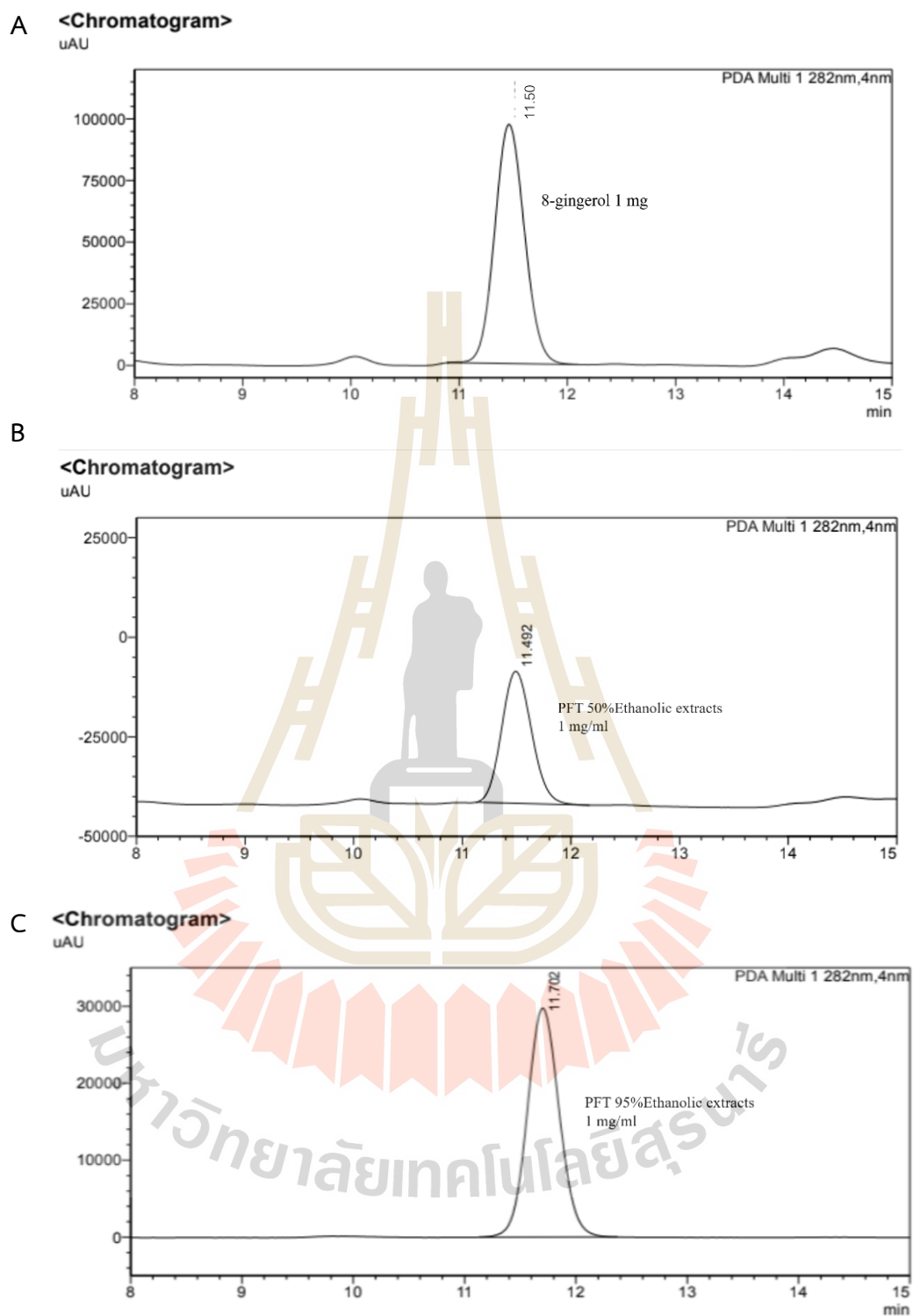


Figure 4.4 Chromatogram of standard solution 8-gingerol (A), PFT 50% Ethanolic extracts 1 mg/ml (B), PFT 95% Ethanolic extracts 1 mg/ml (C).

Discussions: Previous research by Bang-orn et al. analyzed the number of essential substances in the PFT. The amount of the primary vital substances was found to be piperine, 8-gingerol, 10-gingerol, 6-shogaol, and 6-gingerol, respectively. In that order, the highest amount was piperine in the formula, corresponding to the PFT recipe's main component, white pepper.

This research studied the content of essential substances. It represents that the piperine content of the powder formula was 0.423 ± 0.411 (mg $\pm$ S.D.) and the bolus formula (0.028 ± 0.03 (mg $\pm$ S.D.)). In addition, 8-gingerol PFT extracts with 50% Eth and 95% Eth contained the 8-gingerol 404.25 ± 7 ($\mu\text{g} \pm$ S.D.) and 715.19 ± 34.44 ($\mu\text{g} \pm$ S.D.) per milligram extract. It represented that PFT in this research was composed of the piperine and 8-gingerol related to the previous study.

4.2 The efficacy for increased breast milk production.

Result: Seventy-six postpartum women met the eligibility criteria for the study; however, four withdrew for personal reasons. Consequently, 72 women were enrolled and randomly assigned to the control and treatment groups, with 36 participants in each group. During the follow-up period, three participants from the control group and four from the treatment group were lost. Additionally, three participants (two from the control group and one from the treatment group) were excluded for consuming other galactagogues, such as mixed herbs and ginger tea provided by caregivers. Four participants (one from the control group and three from the treatment group) did not adhere to the breast milk volume measurement protocol. These participants were excluded from the final analysis, which was conducted using a per-protocol approach. Ultimately, data from 30 participants in each group were available for comparison. The participant flow throughout the study is illustrated in the Randomly enrolled studies of the efficacy of the PFT in postpartum women at the postpartum ward in Sikhio Hospital, participants in 60 cases, and divided into a control group of 30 cases and a treatment group of 30 cases. Figure 4.5

Table 4.2 Descriptive and differences data in participants between the control and treatment groups. (n=60)

Variables	Groups		<i>p</i> -Value
	Control group (n=30)	Treatment group (n=30)	
Age (years)	29.59±5.64	25.84±4.16	0.500
Body weight (kg.)	66.40±10.62	67.21±14.45	0.763
Height (m.)	1.58±0.05	1.59±0.05	0.828
BMI (kg/m ²)	26.37±4.26	26.33±5.10	0.403
Gravida number, n (%)			0.192
1	8 (26.67)	16 (53.33)	
≥2	22 (73.33)	14 (46.67)	
Education, n (%)			0.211
Elementary School	9 (30.00)	9 (30.00)	
High School	20 (66.67)	16 (53.33)	
Bachelor's degree	1 (3.33)	5 (16.67)	
Systemic diseases, n (%)			. ^a
None	30 (100.00)	30 (100.00)	
Dietary supplements consumption, n (%)			. ^a
None	30 (100.00)	30 (100.00)	

Values are mean ± SD

^a No statistics are computed because, the systemic disease and dietary supplements consumption are a constant.

CONSORT 2010 Flow Diagram

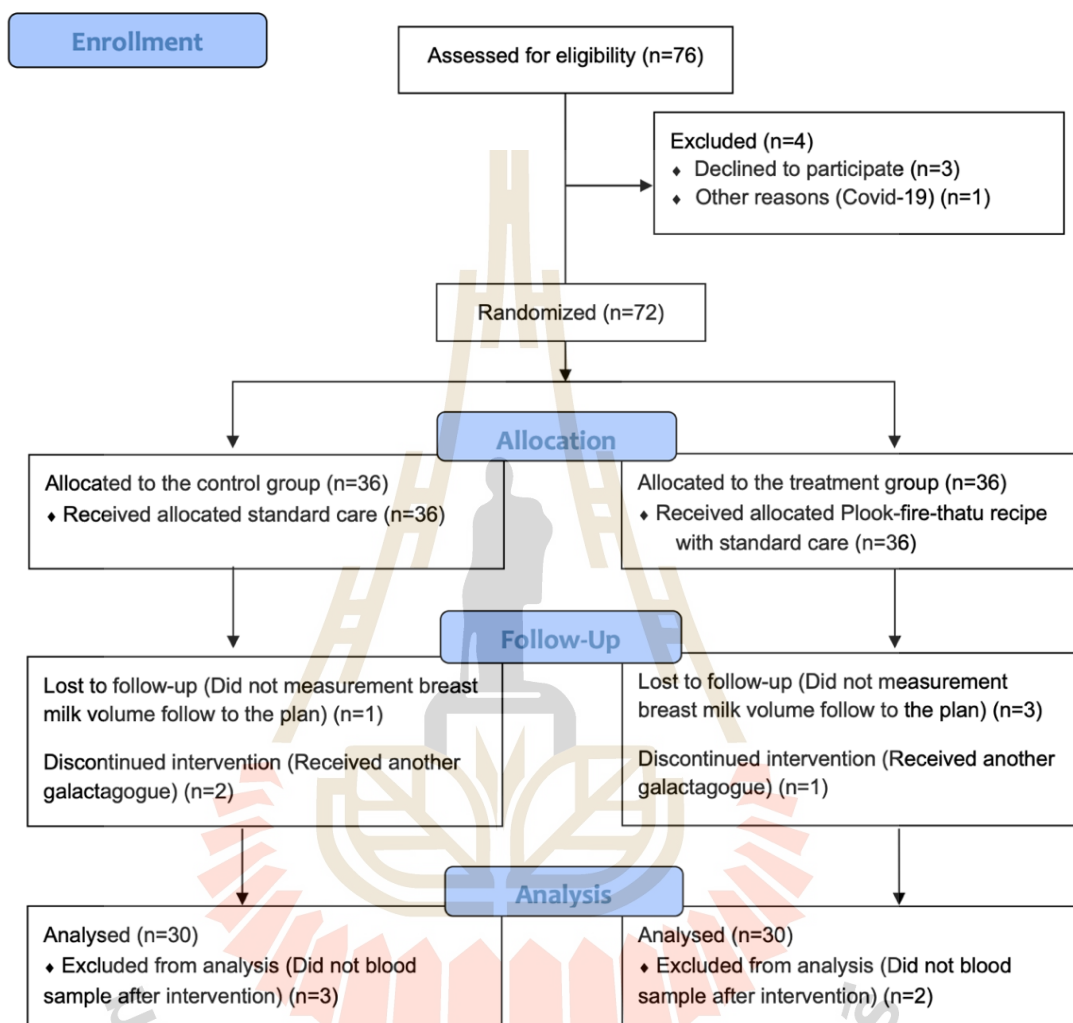


Figure 4.5 CONSORT flow chart for the study

Table 4.3 Descriptive data of Systolic Blood Pressure on day 1, day 2, and day 3 postpartum. (n=60)

Day	Systolic Blood Pressure (mmHg)								<i>p</i> -Value
	Control group				Treatment group				
	Min	Max	Mean	S.D.	Min	Max	Mean	S.D.	
1	105	150	123.53	13.48	96	150	124.83	14.11	0.71
2	90	128	112.10	11.05	98	148	114.57	10.84	0.36
3	95	146	114.40	14.26	90	139	112.97	9.07	0.64

S.D. = Standard Deviation

Table 4.4 Descriptive data of Diastolic Blood Pressure on day 1, day 2, and day 3 postpartum. (n=60)

Day	Diastolic Blood Pressure (mmHg)								<i>p</i> -Value
	Control group				Treatment group				
	Min	Max	Mean	S.D.	Min	Max	Mean	S.D.	
1	56	90	73.67	9.65	56	93	73.77	7.38	0.96
2	52	88	69.20	8.84	51	89	67.70	9.14	0.52
3	50	88	69.63	10.12	57	92	70.60	8.35	0.68

S.D. = Standard Deviation

Table 4.5 Descriptive data of Heart Rate at day 1, day 2, and day 3 postpartum. (n=60)

Day	Heart Rate (beats/min)								<i>p</i> -Value
	Control group				Treatment group				
	Min	Max	Mean	S.D.	Min	Max	Mean	S.D.	
1	60	110	87.80	11.28	64	104	82.70	11.89	0.09
2	62	120	84.40	12.13	66	108	82.27	11.48	0.48
3	68	98	80.93	9.13	66	106	83.43	11.20	0.34

S.D. = Standard Deviation

The control group was 29.59 ± 5.64 years old, and the treatment group was 25.84 ± 4.16 years old, with no statistically significant difference at $p\text{-Value} = 0.50$. The body weight of the control group was 66.40 ± 10.62 kg, and the treatment group was 67.21 ± 14.45 kg, with no statistically significant difference at $p\text{-Value} = 0.763$. The average height of the control group was 1.58 ± 0.05 , and the treatment group was 1.59 ± 0.05 ($p\text{-Value} = 0.828$).

The average BMI of the control group was 26.37 ± 4.26 kg/m², and the treatment group was 26.33 ± 5.10 kg/m², with no statistically significant difference at $p\text{-Value} = 0.403$. The gravida number of both groups no statistically significant difference at $p\text{-Value} = 0.192$. Most participants were educated in high school, followed by elementary school and a bachelor's degree. Regarding comorbidities and medications, I didn't find that the participants had any comorbidities or were taking dietary supplements that might affect milk production.

The differentiation data in participants between the control and treatment groups was compared to identify the differences between groups before the experiment. Found that eight of the eight variables were not different statistically significantly at the 0.05 level, consisting of age, body weight, height, BMI, gravida number, education, systemic diseases, and dietary supplement. These results are shown in Table 4.2

The descriptive data of participants, the average **Systolic Blood Pressure** on day 1, day 2, and day 3 postpartum, in the control group, were 123.53 ± 13.48 mmHg, 112.10 ± 11.05 mmHg, and 114.40 ± 14.26 , respectively. The treatment group were 124.83 ± 14.11 mmHg, 114.67 ± 10.84 mmHg, and 112.97 ± 9.07 mmHg as shown in Table 4.3

In terms of **Diastolic Blood Pressure** on day 1, day 2, and day 3 postpartum, in the control group, were 73.67 ± 9.65 mmHg, 69.20 ± 8.84 mmHg, and 69.63 ± 10.12 mmHg, respectively. The treatment group were 73.77 ± 7.38 mmHg, 67.70 ± 9.14 mmHg, and 70.60 ± 8.35 mmHg as shown in Table 4.4

In addition, these showed the average **Heart Rate** at day 1, day 2, and day 3 postpartum in Table 4.5. The control group was 87.80 ± 11.28 beats/min, 84.40 ± 12.13

beats/min, and 80.93 ± 9.13 beats/min. The treatment group was 82.70 ± 11.81 beats/min, 82.27 ± 11.48 beats/min, and 83.43 ± 11.20 beats/min, respectively.

The high uterine fundus of participants on day 1, day 2, and day 3 are shown in Table 4.7. On the first day postpartum, the mean high of the uterine fundus in the control group was 12.55 ± 1.66 cm., 11.68 ± 1.73 cm, and 10.82 ± 1.95 cm. respectively. The data of mean of high uterine fundus in the treatment group were 13.38 ± 2.10 cm., followed by 12.49 ± 1.95 cm., and 11.86 ± 2.03 cm. The average of the high uterine fundus of both groups is not different statistically significantly at the 0.05 level.

Regarding **serum prolactin levels**, on the first day postpartum (before treatment), there were 60 women in a control group (standard nurse care), 30 cases, and 30 women in a treatment group (Plook-Fire-Thatu recipe intake). The mean serum prolactin level in the control group was 253.25 (ng/mL) (S.D.=154.66), treatment group 197.93 (ng/mL) (S.D.=95.90). In addition, on the third day postpartum (after treatment) the average serum prolactin level in the control group was 273.32 (ng/mL) (S.D.=164.92), treatment group 256.36 (ng/mL) (S.D.= 64.12). They were not different statistically significant at the 0.05 level. Table 4.9

However, a comparison of the mean serum prolactin level within the group before and after treatment found that serum prolactin level in the treatment group after treatment was higher than before treatment (256.36 ± 64.12 ng/mL vs 197.93 ± 95.90 ng/mL, $p\text{-Value} < 0.002$), which was significantly different as shown in Table 4.9

Breast milk volumes were collected on day 1 (before treatment), day 2, and day 3, three times a day. Collecting breast milk using an electronic breast pump for 30 minutes, each collected 3 hours apart and at least 1 hour from the last breastfeeding. A comparison of the difference in the average breast milk of the two groups is shown in Table 4.10. The mean breast milk volume in the control group day 1, day 2 and day 3 were 4.12 ± 6.43 mL, 28.99 ± 30.88 mL, and 95.69 ± 106.47 mL, respectively. The mean of breast milk volume in treatment group day 1, day 2 and day 3 were 7.33 ± 10.20 mL, followed by 56.36 ± 56.08 mL and 213.12 ± 162.76 mL, respectively.

In addition, a comparison of breast milk volume between day 1, day 2 and day 3 postpartum in the control and treatment groups found that the mean in day 2 in

the treatment group was higher than the control group ($\bar{d} = -27.37$), which was significantly different ($p\text{-Value} = 0.028$). Also, the mean breast milk volume on day 3 in the treatment group was higher than the control group ($\bar{d} = -117.43$), which was significantly different ($p\text{-Value} < 0.002$)

When the results of breast milk volume within the group day 1, day 2, and day 3, the control group, there were a statistical difference; the means of the breast milk volume (mL) on day 1, day 2, and day 3 were 4.12, 28.99, and 95.69, respectively. (Table 4.11) The treatment group was statistically different ($p\text{-Value} < 0.001$) at all tests, collected with a tendency to increase the mean of the breast milk volume for both the day 1, day 2, and day 3, which were 7.33, 56.36, and 213.12, respectively. (Table 4.12) The results of the comparative analysis of breast milk volume in both the control group and the treatment group.

Discussion: Description of the data of participants of the control and treatment groups. Seven variables were not significantly different. Both groups were within the normal range for systolic, diastolic, and heart rate. The height of the uterine fundus decreased from day 1 to day 3 postpartum, but there was no statistically significant difference within and between groups.

This investigation determined that PFT administered at a dosage of 1.5 g per day for three consecutive days significantly enhanced breast milk volume on the second and third days in comparison to the control group. Although participants within the treatment cohort appeared to have a higher baseline milk production, the difference did not reach statistical significance. Notably, the intervention group demonstrated a marginally higher mean breast milk volume on day 1 relative to the control group (7.33 ± 10.20 mL vs. 4.12 ± 6.43 mL; $p=0.152$), indicating comparable baseline milk production across groups. Furthermore, participants were randomized without prior selection based on breastfeeding capacity or hypogalactia, thereby suggesting that the observed differences are not attributable to differences in hypogalactia prevalence.

Prior research has shown that a PFT dose of 3 g/day taken over three days significantly increases average breast milk volumes within that period ($p < 0.001$), with 25% of participants experiencing a mean increase of 110 mL or more—a result notably better than that observed in the placebo group ($p < 0.05$). (Virasombat et al., 2020)

Krungkraipetch and Kwanchainon (2023) found that administering PFT at 3 g per day for seven days resulted in 60.4% of postpartum women achieving an adequate level of milk ejection, which rose to 96.6% by the end of the period ($p < 0.05$). Additionally, PFT administered at 90 mg three times daily for three days showed a statistically significant increase in breast milk production compared to both the domperidone and placebo groups ($p \leq 0.01$). Therefore, it can be concluded that using PFT at 270 mg for three days, 1.5 g per day for three days, or 3 g per day for either three or seven days—according to the recommendations outlined in the NLEM—may help improve breast milk production.

4.3 The safety of the Plook-Fire-Thatu recipe.

Results: The hematological values within the control group found that Hemoglobin, MCV, MCH, MCHC, RDW, Basophil, and atypical lymphocytes were not different before and after the experiment. The hematological values that decreased significantly after the experiment included WBC, RBC, hemoglobin, hematocrit, Neutrophil, Monocyte, and Band form. In addition, in the control group, there was a statistically significant increase in platelet counts, Eosinophil, and Lymphocytes after treatment. The result of hematology values within the treatment group before and after treatment found that MCV, MCH, MCHC, RDW, lymphocyte, monocyte, basophil, and atypical lymphocyte were not statistically significant at the 0.05 level. On the other hand, WBC, RBC, hemoglobin, hematocrit, neutrophils, and band form decreased and were statistically significant at $p\text{-Value} < 0.001$. In the part of platelet count, eosinophils and lymphocytes are increased statistically substantially. The comparison of hematological values after treatment between the groups showed that WBC and neutrophils increased. In terms of lymphocytes, there was a statistically significant decrease at the 0.05 level. (Table 4.6)

Blood glucose values before and after treatment (112.66 ± 40.22 vs 75.82 ± 8.45 mg/dL, $< 0.001^{**}$) within the control group were different and statistically significant. At the same time, blood glucose before and after treatment within the treatment group (95.95 ± 24.81 vs 74.03 ± 7.40 mg/dL, $< 0.001^{**}$) was also statistically significant, as shown in Table 4.6. Blood glucose after treatment were decreased but not statistically significant between the groups ($p\text{-Value} = 0.399$).

Lipid profile before and after treatment within the control group showed that Total cholesterol, Triglyceride, and LDLC did not differ statistically. Whereas HDLC, decreased differently and was statistically significant. Within the treatment group, it found that Total cholesterol, Triglyceride, and LDLC were not different statistically significant at the 0.05 level. HDLC decreased differently and was statistically significant. Before and after treatment between the groups, total cholesterol, Triglyceride, LDLC, and HDLC were not different statistically significant at the 0.05 level, as shown in Table 4.6.

Liver function test values before and after treatment, within the control group showed variables not different statistically significantly at the 0.05 level, consisting of AST, ALT, and Albumin. In contrast, ALP was decreased different and statistically significant $p\text{-Value} < 0.001$. Within the treatment group, AST and Albumin were not different statistically significant at the 0.05 level. At the same time, ALT increased after treatment ($p\text{-Value} = 0.002$), and ALP decreased ($p\text{-Value} < 0.001$), which were different and statistically significant. Before and after treatment between the groups, AST, ALT, Albumin, and ALP were not different statistically significant at the 0.05 level. As shown in the result in Table 4.6.

Kidney function test values within the control group, BUN ($p\text{-Value} = 0.047$) and Creatinine ($p\text{-Value} = 0.001$) increased, statistically significantly. The BUN ($p\text{-Value} = 0.001$) within the treatment group found that it tended to increase but was not statistically significant at the 0.05 level, whereas Creatinine ($p\text{-Value} = 0.001$) had a statistically significant increase. The comparison between the control and treatment group in BUN and Creatinine was not statistically significant at the 0.05 level, as shown in the results in Table 4.6.

Adverse drug reactions were monitored through participant interviews. Two participants (6.67 %) reported mild epigastric pain on the first postpartum day. According to Naranjo's algorithm, this was classified as a possible adverse drug reaction. No severe adverse reactions were reported, and no adverse events were observed in the infants. As shown in Table 4.8

Discussions: The safety results of this study showed that participants exhibited no abnormalities in hematology, liver, or kidney function tests. Consistent with the research of Sripanichkulchai and Pannangrong (2020), Wistar rats administered daily doses of 125, 250, and 500 mg/kg for 90 days and observed for 120 days showed normal hematology and blood biochemistry, with no abnormalities detected in internal organs.

Adverse drug reactions potentially associated with PFT include heartburn, stomach pain and nausea. (National Drug System Development Committee, 2008) Previous studies reported that postpartum women who took 1000 mg three times daily before meals experienced heartburn in two cases and hot flashes in one case on the first day. (Kaewnaluang et al., 2021) In this study, adverse drug reactions were monitored on days 1, 2, 3, 7 and 30 postpartum; only two participants reported mild epigastric pain on the first day, which did not persist thereafter.

4.4 Breast milk macronutrient composition.

Result: The primary FTIR spectra of milk samples have similar peak profiles. All data analyses were performed in the spectral range from 3000 to 900 cm^{-1} . The integral areas of lipid from 3000 to 2800 cm^{-1} , ester 1765 to 1730 cm^{-1} , protein 1700 to 1500 cm^{-1} , nucleic acid 1300 to 1050 cm^{-1} , and carbohydrate 1250 to 920 cm^{-1} , determined to range respectively, as shown in Figure 4.6

The FTIR second derivative spectrum of biological compounds of milk samples was conducted to resolve the overlapped peaks and to find different scopes.

Table 4.6 The comparison of the mean hematological and blood biochemical values between the control and treatment groups.

Parameters	Control group (n=30)			Treatment group (n=30)				
	Before	After	P^1	Before	After	P^1	P^2	P^3
WBC ($10^3/\text{mm}^3$) (Normal range $4.00-10.00 \times 10^3/\text{mm}^3$)	16.65±4.78	13.39±2.92	0.001*	17.83±5.18	11.61±2.82	<0.001**	0.367	0.025*
RBC ($10^6/\text{mm}^3$) (Normal range $4.50-5.90 \times 10^6/\text{mm}^3$)	4.56±0.57	4.23±0.69	<0.001**	4.49±0.61	4.24±0.52	<0.001**	0.636	0.927
Hemoglobin (g/dL) (Normal range 11.50-16.50 g/dL)	11.85±1.34	11.77±5.14	0.935	11.83±1.29	11.15±0.92	<0.001**	0.953	0.513
Hematocrit (%) (Normal range 36-47%)	36.87±3.96	33.78±4.66	<0.001**	36.82±4.02	34.67±3.04	<0.001**	0.963	0.400
MCV (fl) (Normal range 80-100 fl)	81.37±6.32	81.25±6.34	0.271	82.70±9.66	82.39±9.23	0.405	0.532	0.578
MCH (pg) (Normal range 26-34 pg)	26.20±2.30	26.12±2.32	0.223	26.60±3.14	26.67±3.13	0.380	0.583	0.444

* The level of significance was $p < 0.05$, ** $p\text{-Value} < 0.001$

P^1 Comparison within the group, P^2 Comparison between two groups before treatment, P^3 Comparison between two groups after treatment

Table 4.6 The comparison of the mean hematological and blood biochemical values between the control and treatment groups.

(Continued)

Parameters	Control group (n=30)			Treatment group (n=30)				
	Before	After	P^1	Before	After	P^1	P^2	P^3
MCHC (g/dL) (Normal range 32-36 g/dL)	32.25±0.60	32.16±0.67	0.432	32.20±0.61	32.03±1.09	0.393	0.750	0.577
RDW (%) (Normal range 11-16%)	15.77±1.78	15.85±1.94	0.584	15.76±1.67	15.71±1.83	0.676	0.982	0.783
Platelet count (*10³cell/mm³) (Normal range 150-500*10 ³ /mm ³)	256.52±66.21	277.96±67.04	0.001*	264.13±52.90	276.37±58.81	0.032*	0.632	0.924
Neutrophil (%) (Normal range 38-80%)	77.12±6.95	72.19±6.20	0.007*	79.44±9.78	67.93±5.42	<0.001**	0.294	0.006*
Eosinophil (%) (Normal range 0-8%)	1.11±0.93	2.31±1.37	<0.001**	0.61±0.39	2.01±0.94	<0.001**	0.010*	0.329
Lymphocyte (%) (Normal range 15-40%)	14.25±5.37	17.96±5.50	0.008*	11.90±6.26	22.48±4.66	<0.001**	0.124	0.001*

* The level of significance was $p < 0.05$, ** p -Value < 0.001 P^1 Comparison within the group,

P^2 Comparison between two groups before treatment, P^3 Comparison between two groups after treatment

Table 4.6 The comparison of the mean hematological and blood biochemical values between the control and treatment groups.

(Continued)

Parameters	Control group (n=30)			Treatment group (n=30)				
	Before	After	P^1	Before	After	P^1	P^2	P^3
Monocyte (%) (Normal range 0-13%)	7.38±1.45	6.75±1.19	0.015*	6.49±2.02	6.83±1.38	0.356	0.055	0.819
Basophil (%) (Normal range 0-2%)	0.15±0.43	0.08±0.20	0.451	0.03±0.13	0.03±0.08	0.901	0.167	0.221
Atypical Lymphocyte (%) (Normal range 0-0%)	0.75±0.31	0.72±0.29	0.636	0.59±0.25	0.67±0.23	0.108	0.033*	0.499
Band form (%) (Normal range 0-0%)	0.74±1.06	0.31±0.19	0.023*	0.41±0.26	0.26±0.11	0.003*	0.109	0.176
Blood glucose (mg/dL) (Normal range 74-106 mg/dL)	112.96±40.22	75.82±8.45	<0.001**	95.90±24.81	74.03±7.40	<0.001**	0.060	0.399
Total cholesterol (mg/dL) (Normal range 0-200 mg/dL)	238.96±46.03	229.48±40.16	0.079	238.66±53.19	236.70±45.47	0.629	0.982	0.530

* The level of significance was $p < 0.05$, ** p -Value < 0.001

P^1 Comparison within the group, P^2 Comparison between two groups before treatment, P^3 Comparison between two groups after treatment

Table 4.6 The comparison of the mean hematological and blood biochemical values between the control and treatment groups.

(Continued)

Parameters	Control group (n=30)			Treatment group (n=30)				
	Before	After	P^1	Before	After	P^1	P^2	P^3
Triglyceride (mg/dL) (Normal range 0-150 mg/dL)	229.70±68.65	233.03±53.83	0.796	198.76±75.64	214.58±65.69	0.144	0.113	0.257
LDLC (mg/dL) (Normal range 0-130 mg/dL)	151.76±35.07	145.80±30.28	0.244	149.83±39.80	148.93±34.20	0.973	0.849	0.723
HDLC (mg/dL) (Normal range 40-60 mg/dL)	73.88±17.26	68.44±16.24	0.003*	72.83±13.97	70.13±12.64	0.017*	0.802	0.661
AST (U/L) (Normal range 0-35 U/L)	30.96±20.51	31.29±24.51	0.883	29.53±9.79	28.40±12.13	0.385	0.745	0.568
ALT (U/L) (Normal range 0-35 U/L)	15.22±13.21	16.88±10.87	0.106	14.10±9.94	16.90±9.58	0.002*	0.725	0.996
ALP (U/L) (Normal range 30-120 U/L)	159.07±46.24	125.84±35.67	<0.001**	157.92±38.35	124.85±30.99	<0.001**	0.923	0.914

* The level of significance was $p < 0.05$, ** p -Value < 0.001

P^1 Comparison within the group, P^2 Comparison between two groups before treatment, P^3 Comparison between two groups after treatment

Table 4.6 The comparison of the mean hematological and blood biochemical values between the control and treatment groups.

(Continued)

Parameters	Control group (n=30)			Treatment group (n=30)				
	Before	After	P^1	Before	After	P^1	P^2	P^3
BUN (mg/dL) (Normal range 17-43 mg/dL)	7.18±2.39	8.14±3.35	0.047*	7.04±1.60	8.95±2.06	0.001*	0.801	0.279
Creatinine (mg/dL) (Normal range 0.55-1.02 mg/dL)	0.54±0.11	0.60±0.08	0.001*	0.55±0.09	0.59±0.09	0.001*	0.797	0.822
Albumin (g/dL) (Normal range 3.5-5.2 g/dL)	3.17±0.26	3.09±0.24	0.074	3.19±0.22	3.21±0.19	0.311	0.737	0.053

* The level of significance was $p < 0.05$, ** p -Value < 0.001

P^1 Comparison within the group, P^2 Comparison between two groups before treatment, P^3 Comparison between two groups after treatment

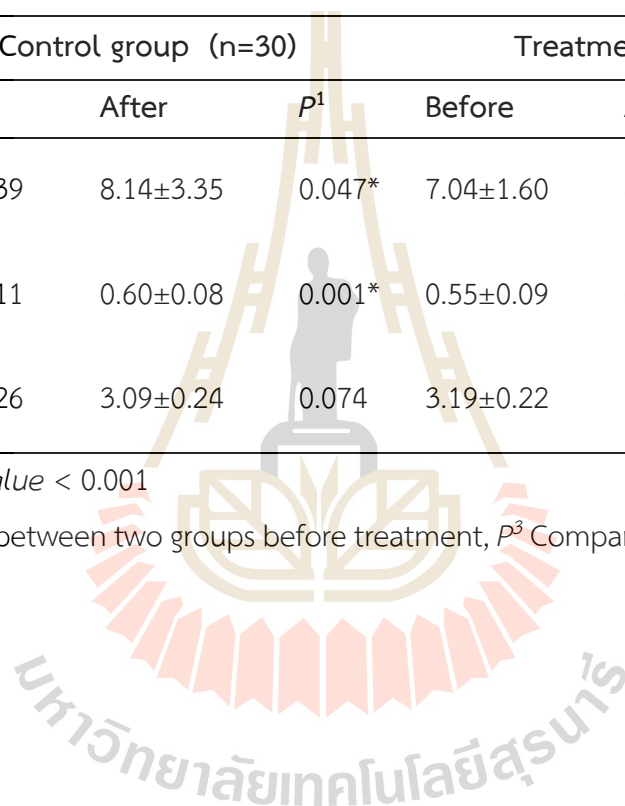


Table 4.7 Comparison of the differences of high of uterine fundus (cm.) between the groups by independent sample t-test. (n=60)

	Control		Treatment		$\bar{d}$	95% CI		t	p-Value
	Mean	S.D.	Mean	S.D.		Lower	Upper		
Day1	12.55	1.66	13.38	2.10	-0.83	-1.81	0.15	-1.69	0.096
Day2	11.68	1.73	12.49	1.95	-0.81	-1.77	0.14	-1.70	0.094
Day3	10.82	1.95	11.86	2.03	-1.03	-2.07	0.00	-2.01	0.049

S.D. = Standard Deviation, $\bar{d}$ = mean difference, 95%CI = 95% confidence interval

t = The test statistic for an Independent Samples t-Test

Table 4.8 Data of adverse drug reaction (ADR) from Plook-Fire-Thatu recipe in participants (n=30).

Day	Adverse drug reaction (ADR) n (%)				No	Total
	Hot flash	Epigastric burn	Stomach pain	Nausea	n (%)	n (%)
1	-	2 (6.67)	-	-	28 (93.33)	30 (100)
2	-	-	-	-	30 (100)	30 (100)
3	-	-	-	-	30 (100)	30 (100)
7	-	-	-	-	30 (100)	30 (100)
30	-	-	-	-	30 (100)	30 (100)

Table 4.9 Comparison of the differences of Serum prolactin level (ng/mL) between the groups by independent sample t-test. (n=60)

Parameters	Control group (n=30)			Treatment group (n=30)				
	Before	After	P^1	Before	After	P^1	P^2	P^3
Serum prolactin level (ng/mL)	253.25±154.66	273.32±164.92	0.413	197.93±95.90	256.36±64.12	<0.002*	0.122	0.620

* The level of significance was $p < 0.05$, ** $p\text{-Value} < 0.001$

P^1 Comparison within the group, P^2 Comparison between two groups before treatment, P^3 Comparison between two groups after treatment

Table 4.10 Differences of breast milk volume between Day1, Day2 and Day3 postpartum in the control group and treatment group by Independent Samples t-Test (n=60).

	Control		Treatment		$\bar{d}$	95% CI		t	p-Value
	Mean	S.D.	Mean	S.D.		Lower	Upper		
Day1	4.12	6.43	7.33	10.20	-3.20	-7.62	1.20	-1.45	0.152
Day2	28.99	30.88	56.36	58.06	-27.37	-51.40	-3.33	-2.27	0.028*
Day3	95.69	106.47	213.12	162.76	-117.43	-188.75	-46.10	3.30	0.002*

Table 4.11 Comparison breast milk volume in the control group by repeated measures ANOVA at day1, day2, and day3 postpartum.

Breast milk volume	n	Mean	S.D.	<i>p-Value</i> 1	<i>p-Value</i> 2
Day1	30	4.12	6.43	-	-
Day2	30	28.99	30.88	<0.001**	-
Day3	30	95.69	106.47	<0.001**	0.001*

p-Value 1 is the *p-Value* of comparing after treatment (Day2 and Day3) with before treatment (Day1).

p-Value 2 is the *p-Value* of comparing after treatment Day2 with after treatment Day3.

* The level of significance was $p < 0.05$, ** $p\text{-Value} < 0.001$

Table 4.12 Comparison breast milk volume in the treatment group by repeated measures ANOVA at day1, day2, and day3 postpartum.

Breast milk volume	n	Mean	S.D.	<i>p-Value</i> 1	<i>p-Value</i> 2
Day1	30	7.33	10.20	-	-
Day2	30	56.36	58.06	<0.001**	-
Day3	30	213.12	162.76	<0.001**	<0.001**

p-Value 1 is the *p-Value* of comparing after treatment (Day2 and Day3) with before treatment (Day1).

p-Value 2 is the *p-Value* of comparing after treatment Day2 with after treatment Day3.

* The level of significance was ** $p\text{-Value} < 0.001$

It was found that there were similar characteristic spectra with differences in the peak intensity. The prominent regions at $3000\text{--}2800\text{ cm}^{-1}$ (~ 2961 , ~ 2923 , and $\sim 2852\text{ cm}^{-1}$) corresponded to the stretching mode of CH_2 and CH_3 in lipids. The ester group (C=O) was assigned around 1745 cm^{-1} of wavenumber. The protein region was given amide I (1643 cm^{-1}) and amide II (1548 cm^{-1}). Regions at 1039 and 1023 cm^{-1} represented carbohydrates around 1155 cm^{-1} , corresponding to the CO stretching mode in glycogen and mucin. Nucleic acid (PO_2) was obtained at ~ 1233 , ~ 1119 and $\sim 1075\text{ cm}^{-1}$ of wavenumber as shown in Figure 4.

Figure 4.8 A, B, C, D, and E display the average second derivative spectra of the control group before treatment (C1), the control group after treatment (C2), the treatment group before treatment (P1) and the treatment group after treatment (P2). It was found that there were similar characteristic spectra with differences in peak intensity.

The most discernible spectral features of lipids or phospholipids originate from the stretching modes of the chain CH_2 , and $=\text{CH}$ groups ($3000\text{--}2080\text{ cm}^{-1}$); the ester C=O bond was assigned around $1740\text{--}1730\text{ cm}^{-1}$. Only four bands are usually observed from lipids, which are attributed to the asymmetric and symmetric stretching vibrations of the methyl and methylene groups. Thus, this research's regions at 2961 , 2923 , and 2852 cm^{-1} represent saturated lipids. The regions of 1745 cm^{-1} correlate with the C=O stretching mode, attributed to triglycerides and cholesterol esters, as seen in Figure 4.8 A and B

The derivative spectra peaks in Figure 4.8 C and D were 1643 cm^{-1} , and 1548 cm^{-1} regions represent the C=O stretching and NH bending modes of amide I and II, respectively. The amide I mode is most considerably used to quantify α -helical, β -sheet, turn, and unordered structures of proteins. IR spectra display amide bands at 1548 cm^{-1} that signify protein α -helical chains. The appearance at 1643 cm^{-1} indicates, in turn, changes in protein secondary conformation toward a β -sheet structure.

The spectral range of $1150\text{--}1000\text{ cm}^{-1}$ is typical for carbohydrates. The milk sample represents regions at 1039 cm^{-1} assigned to monosaccharide, polysaccharides, and proteoglycans. The glycogen bands occur at 1023 cm^{-1} , around 1155 cm^{-1} ,

corresponding to the CO stretching mode in glycogen and mucin, as shown in Figure 4.8 D

Therefore, the presence of nucleic acids is predominantly revealed by IR bands in the 1300–900 cm^{-1} region. The spectral profiles of DNA and RNA are significantly distinct, making FTIR analysis a helpful tool for monitoring structural and content modifications of these principal constituents of cells and tissues. In turn, IR bands at 1155, 1119, cm^{-1} with their bandwidth are specific for RNA. Among them, the pronounced 1119 cm^{-1} bands assigned to vibrations of the ribose and uracyl moieties provide unique markers to detect RNA. The primary DNA vibrations appear at 1223 cm^{-1} and 1075 cm^{-1} and are assigned to the phosphodiester asymmetric and symmetric stretching vibrations, respectively. As seen in Figure 4.8 E

The biomolecular breast milk samples were analyzed by the integral areas of specific regions of the second derivative spectrum. As seen in Figure 4.9 B

The lipid regions were described by asymmetric and symmetric stretching vibrations of CH_2 and CH_3 groups. A histogram representing the mean integrated areas of the original spectra for the C-H lipid region is shown at 2961, 2923, and 2852 cm^{-1} , caused by increased saturated lipid content after treatment in the control and treatment groups. In the case of the ester group, both increased triglyceride and cholesterol ester content after treatment.

The derivative spectra peaks were 1643 cm^{-1} and 1548 cm^{-1} regions representing amide I and II. FTIR spectra display amide bands at 1548 cm^{-1} that signify protein α -helical chains. The appearance at 1643 cm^{-1} indicates, in turn, changes in protein secondary conformation toward a β -sheet structure. Analysis of the results of this integral area demonstrated the reduction of protein content in both groups after treatment.

The milk sample represents regions at 1039 cm^{-1} assigned to monosaccharides, polysaccharides, and proteoglycans. The glycogen bands occur at 1023 cm^{-1} , around 1155 cm^{-1} , corresponding to glycogen and mucin. The carbohydrate content analysis found that the group that received the PFT had a significantly higher amount of carbohydrates after the experiment than the control group. For the glycogen

observation, the integral area of glycogen shows that content in the treatment group after experimenting was significantly higher than in the control group.

FTIR bands at 1119 cm^{-1} were assigned to the ribose and uracyl. The primary DNA vibrations appear at 1223 cm^{-1} and 1075 cm^{-1} and are given to the phosphodiester asymmetric and symmetric stretching vibrations, respectively. The integral analysis found that after treatment with the PFT, breast milk samples had higher nucleic acid content than the control group. The PFT induced the increment of nucleic acid content.

The PCA score plot clearly shows that the clusters of P2 were separated from those of C1, C2, and P1 along PC1 by 41% (y-axis). In addition, the P2 was separated from C2 along PC2 by 21% (x-axis) (Figure 4.10 A). The analysis of PCA loading plots was used to determine the regions of the FTIR spectrum mainly provided clustering.

The high positive of PC1 loading at 1643 , 1547 , and 1392 cm^{-1} assigned as amide I and amide II peptide of protein and symmetric stretch of fatty lipid (Figure 4.10 B) might be due to the higher protein and fatty acid in P2.

The high negative peaks of PC2 loading at 1644 , 1180 , 1066 , 1039 , and 1002 cm^{-1} were assigned as amide I peptide of protein, CO-O-C stretching of ester peak, COH stretching of nucleic acid, and glycogen carbohydrate.

However, there is an overlap of many peaks from milk samples from P2. The results indicate that P2 was obtained owing to the higher amount of protein, fatty acid, cholesterol ester, nucleic acid, and carbohydrate than C1, C2, and P1.

Discussion: The results suggest that the herbs in PFT may stimulate the secretion of prolactin, a hormone crucial for milk production, in a manner comparable to standard care. Increased prolactin levels can lead to greater milk volume and potentially a higher fat content in the milk produced. Accordingly, the PFT recipe is designed to promote lactation. Enhanced milk production is often associated with elevated carbohydrate levels, particularly lactose, which is the primary carbohydrate in human milk.

FTIR analysis indicated that participants who received the PFT recipe for three days had breast milk containing the primary macronutrients—protein, fat and

carbohydrate. The FTIR integral area for macronutrient content revealed a slightly higher lipid level in the treatment group, although this difference was not statistically significant.

Nevertheless, after determining the biomolecular of breast milk under FTIR spectroscopy, I learned that the primary biological components of breast milk samples are composed of lipids specific to saturated lipids, triglycerides and cholesterol esters. FTIR spectra display amides that signify protein α -helical chains, in turn, changes in protein secondary conformation toward a β -sheet structure. The typical carbohydrates represent monosaccharide, polysaccharides, and proteoglycans. The presence of glycogen and mucin were found. Therefore, the presence of nucleic acids is predominantly revealed in the spectral profiles of DNA and RNA. Among them the pronounced bands assigned to vibrations of the ribose and uracil. The primary DNA are assigned to the phosphodiester asymmetric and symmetric stretching.

Comparing quantities from the integral area and the principal component analysis (PCA) indicated that breast milk from participants who received the PFT recipe for three days was obtained owing to the higher amount of glycogen, carbohydrate, and nucleic acid than those without. On the other hand, the protein content of all groups was reduced after treatment. According to the theory of breast milk, there will be a higher amount of protein, such as casein, in the colostrum than in mature milk. In addition, the amount of carbohydrates will increase.

As milk volume increases due to improved lactation, the total carbohydrate content may also rise, as indicated by the FTIR findings. The observed reduction in protein content following treatment with the PFT recipe may be attributed to its specific nutritional profile, which could influence protein synthesis pathways in lactating mothers. Furthermore, some herbs in the formulation may promote lipid production more strongly than protein synthesis, resulting in a relative decrease in protein content compared to other macronutrients. (Krungraipecth & Kwanchainon, 2023) (National Institute of Child Health and Human Development, 2024)

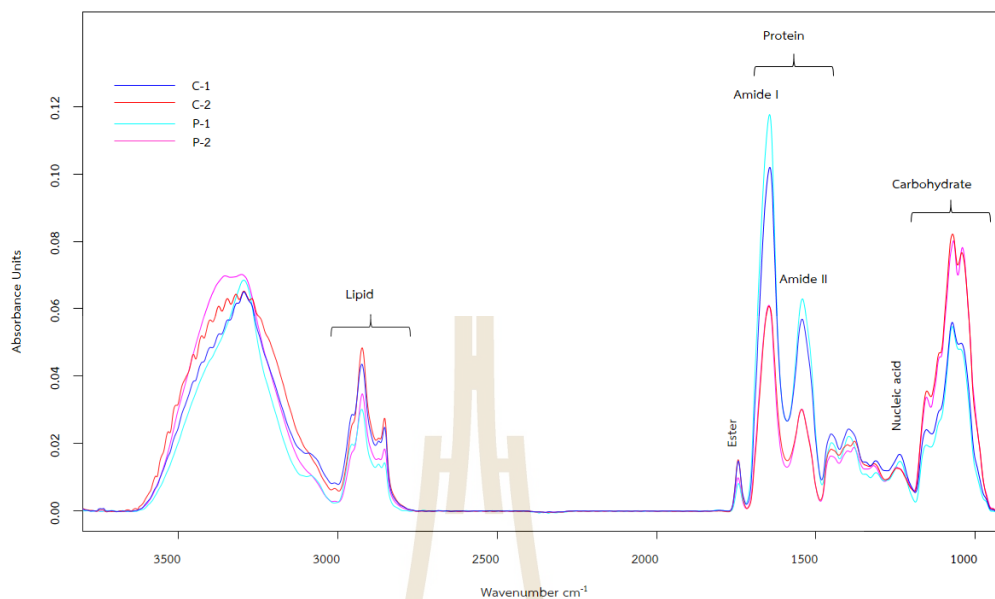


Figure 4.6 Primary spectrum of micronutrition components of breast milk samples, obtained from four different conditions: control group before treatment (C1), control group after treatment (C2), treatment group before treatment (P1), and treatment group after treatment (P2).

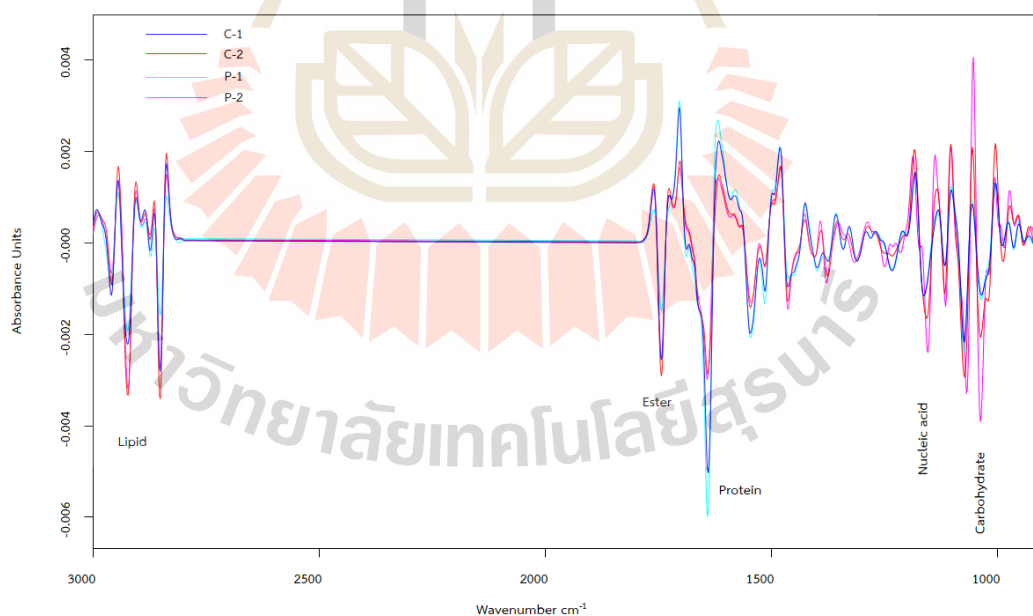


Figure 4.7 Second derivative micronutrition components of breast milk samples, obtained from four different conditions: control group before treatment (C1), control group after treatment (C2), treatment group before treatment (P1), and treatment group after treatment (P2).

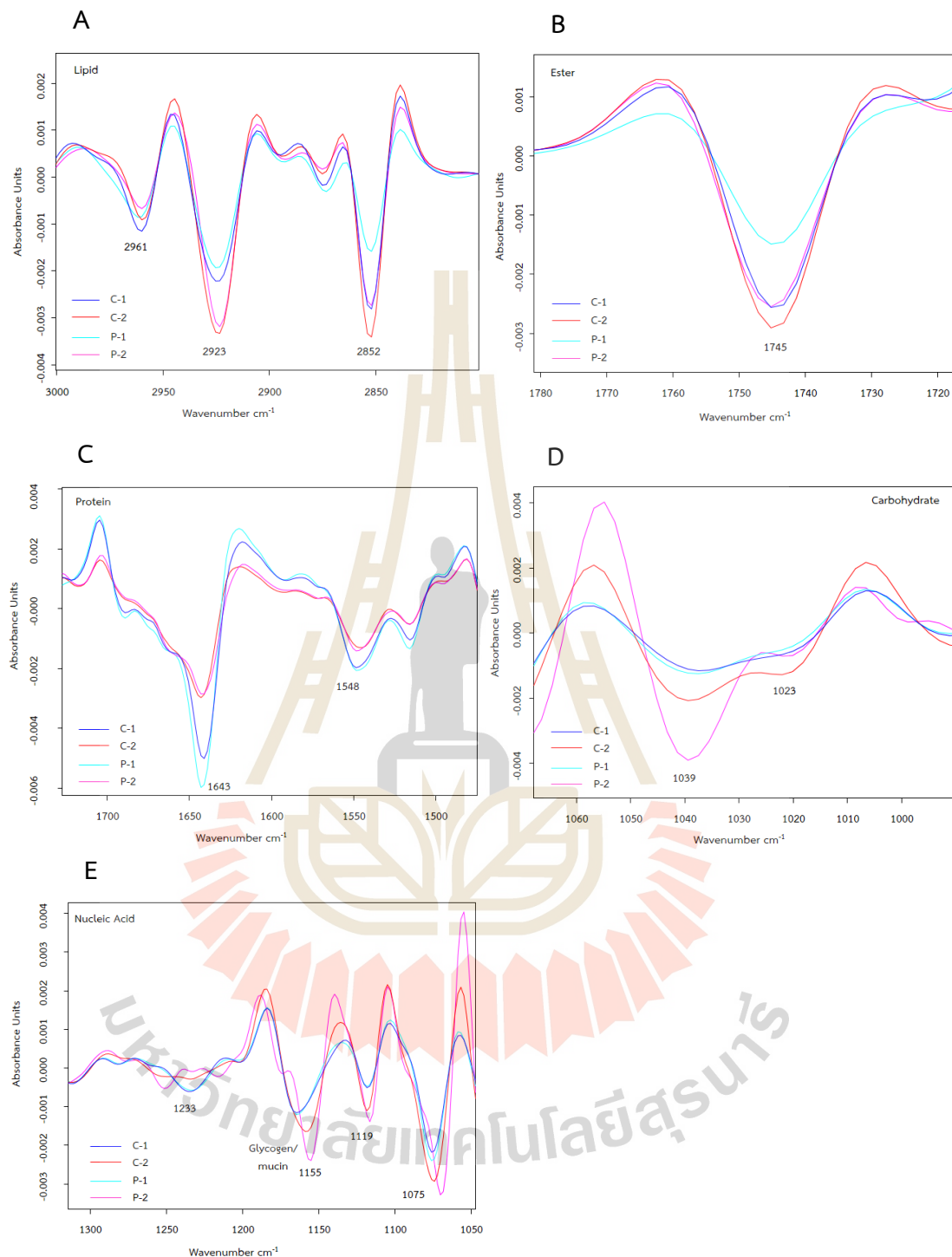


Figure 4.8 Average second derivative spectrum (3000-900 cm^{-1}) of four different conditions: control group before treatment (C1), control group after treatment (C2), treatment group before treatment (P1), and treatment group after treatment (P2).

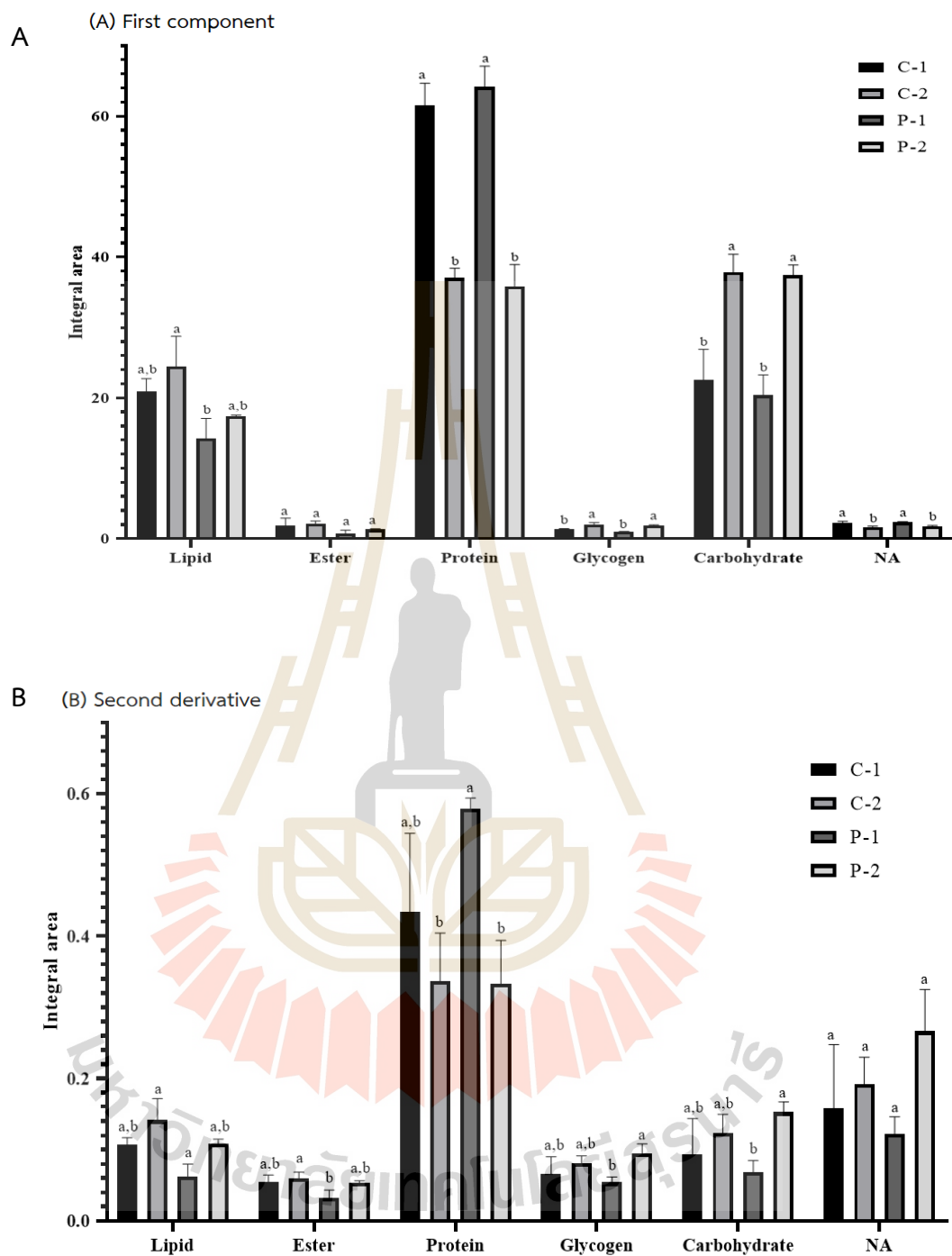


Figure 4.9 Integral area from primary spectrum (A) and Integral area from second derivative spectrum (B). Tests were analyzed by one-way analysis of variance (ANOVA). Means with different lowercase letters (a and b) are significantly different. ($P < 0.05$, error bars indicate standard errors of means).

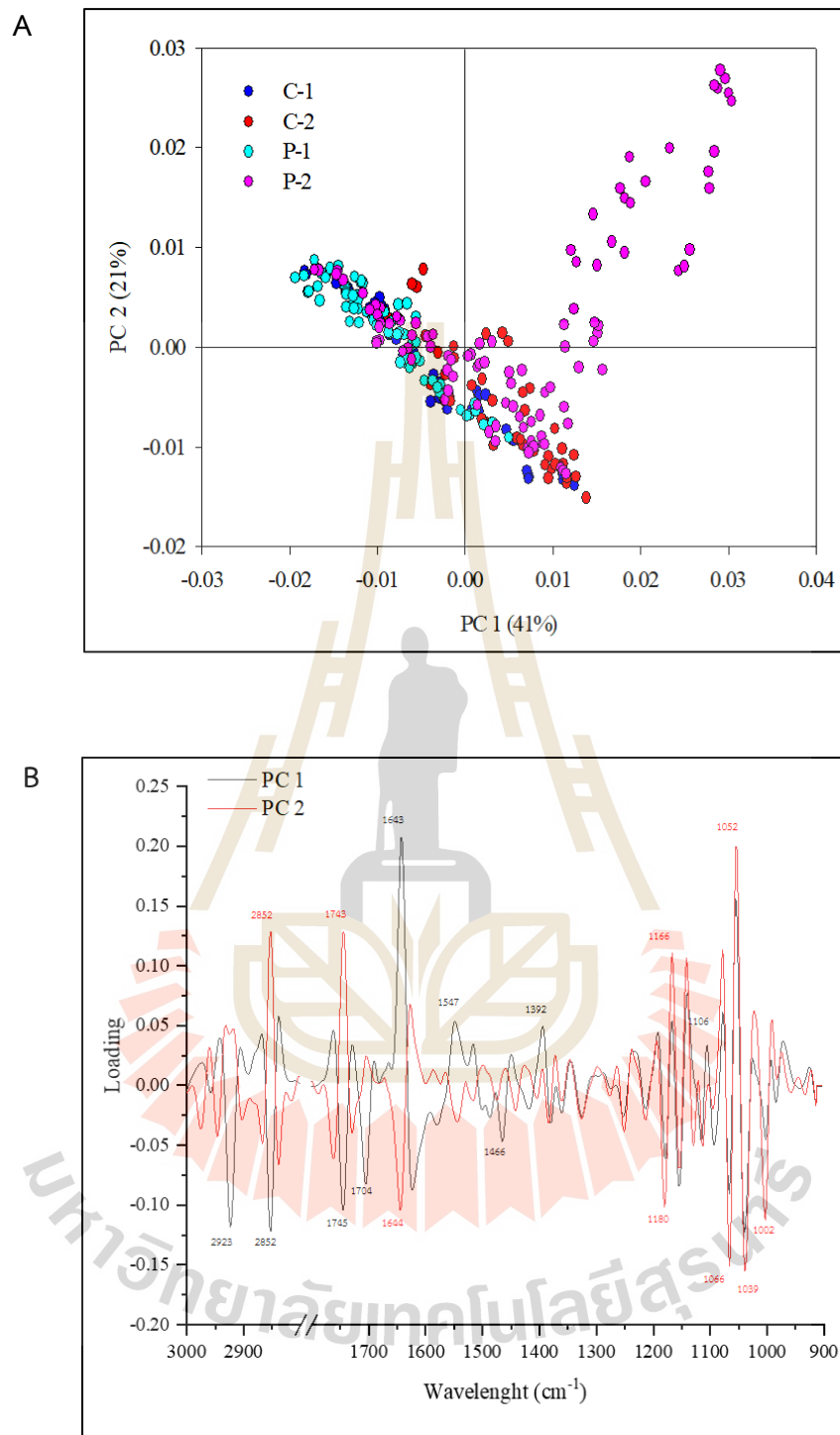


Figure 4.10 Principal component analysis (PCA) score plot (A) and principal loading component (B) result of milk samples. Obtained from four different conditions: control group before treatment (C1), control group after treatment (C2), treatment group before treatment (P1), and treatment group after treatment (P2).

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The findings from this study indicate that the essential bioactive substance in the PFT in the bolus formula is piperine.

This research provides evidence that the PFT recipe enhances breast milk volume by the third day postpartum. Serum prolactin levels within the treatment group post-treatment were significantly higher than baseline. It can be inferred that PFT may stimulate the secretion of prolactin, a hormone crucial for milk production, in a manner comparable to standard care. Increased prolactin levels can lead to greater milk volume. The PFT recipe increased breast milk production without causing adverse drug reactions in mothers or infants. The treatment dosage did not lead to abnormalities in hematology, liver function, or kidney function.

The efficacy of the PFT recipe increases breast milk volume, consistent with the ethnopharmacological analysis based on Thai Traditional Medicine theory. This recipe is the hot test, and all herbal formulas are the principal component. The PFT recipe could stimulate the fire element, and several enhanced the wind element. So, this recipe may increase breast milk production by increasing the fire and wind elements with fiery herbs. And balance the Earth and water elements with astringent and sweet test herbs.

Furthermore, it increased the glycogen, carbohydrate, and lipid content of breast milk. The observed rise in carbohydrate and lipid levels following PFT treatment is likely due to a combination of enhanced lactation dynamics, specific nutritional influences from the herbal components, physiological adaptations to increased feeding demands, and possible alterations in maternal dietary habits.

5.2 Limitation

The limitation of this study was the relatively short, three-day period used to evaluate the effectiveness of PFT. The researchers sought to control for confounding factors such as breastfeeding techniques, diet, medication, and environmental conditions by restricting the study to the first three days postpartum during hospitalization. This approach was intended to isolate changes in milk volume during this critical period. Further research would be valuable to determine whether the increase in milk production persists beyond this initial timeframe.

Additional important limitations include maternal and infant health status, infant feeding behaviours (e.g. latch difficulties, tongue-tie), maternal stress or sleep quality, breast morphology and the precise timing of PFT administration following delivery. Future studies should incorporate these variables to better address confounding factors. These findings present significant opportunities for further research to strengthen the evidence base relating to breast milk production.

The measurement of breast milk volume is subject to confounding factors such as fluid intake, nutrition and medication. To mitigate these influences, all participants were provided with a standardised diet averaging 1800 kcal per day, and nutritional intake was monitored via 24-hour food recall records.

Furthermore, due to a lack of access to specialised food laboratory analysis, the researcher utilised FTIR techniques to analyse breast milk nutrition. FTIR is a valid and accurate method, requiring only a minimal sample volume (approximately 1 mL) and is non-destructive to the sample. However, the mechanistic basis of this recipe requires further elucidation. Additionally, studies comparing various dosages and durations of PFT administration on milk production and safety would provide a valuable foundation for future research.

5.3 Application of research results

The data obtained was used to promote the PFT recipe with specific indications for stimulating milk production. The results of the data gathered will support using the PFT recipe as a first-line drug to breast milk production in Nakhon Ratchasima Province, Health District 9, and at the national level.

Promote the PFT recipe, with specific instructions for stimulating breast milk production, which is included in the National List of Essential Medicines. Postpartum women in Thailand are breastfeeding exclusively for at least 6 months, according to the World Health Organization criteria.

5.4 Recommendation

PFT consists of eleven different herbs containing many important substances. Bioactive compound analysis requires substantial resources to analyze all essential substances using standard methods. Suggestions for future studies should focus on key information for the pharmacopeia of this recipe.

Analysis of 8-gingerol standard solutions by HPLC revealed retention times. The standard substances of 8-gingerol at various concentrations were analyzed, and the areas under the curves were used to create a standard graph. The linear equation obtained from the standard graph was not validated, which caused it to appear in the curve. Further studies should analyze various concentrations to validate and determine retention times.

In addition, breastmilk volume three days postpartum is deficient for the nutritional standard analysis. Therefore, this research chose the FTIR method as a replacement for accurately analyzing the composition of breast milk.

The baseline values of milk volume and serum prolactin before the experiment of both the control and experimental groups were found to be different, although not statistically significant. A relatively wide range of mean differences was also found. Future studies should be the criterion for the prevalence of hypogalactia.

Further studies are warranted to elucidate these mechanisms more clearly and to confirm their effects on human milk composition. The assessment and monitoring

of trends in breastmilk production, liver function, and kidney function, as well as the evaluation of potential adverse effects from continuous use of this recipe for an extended period, may require additional long-term studies lasting at least 3 months.



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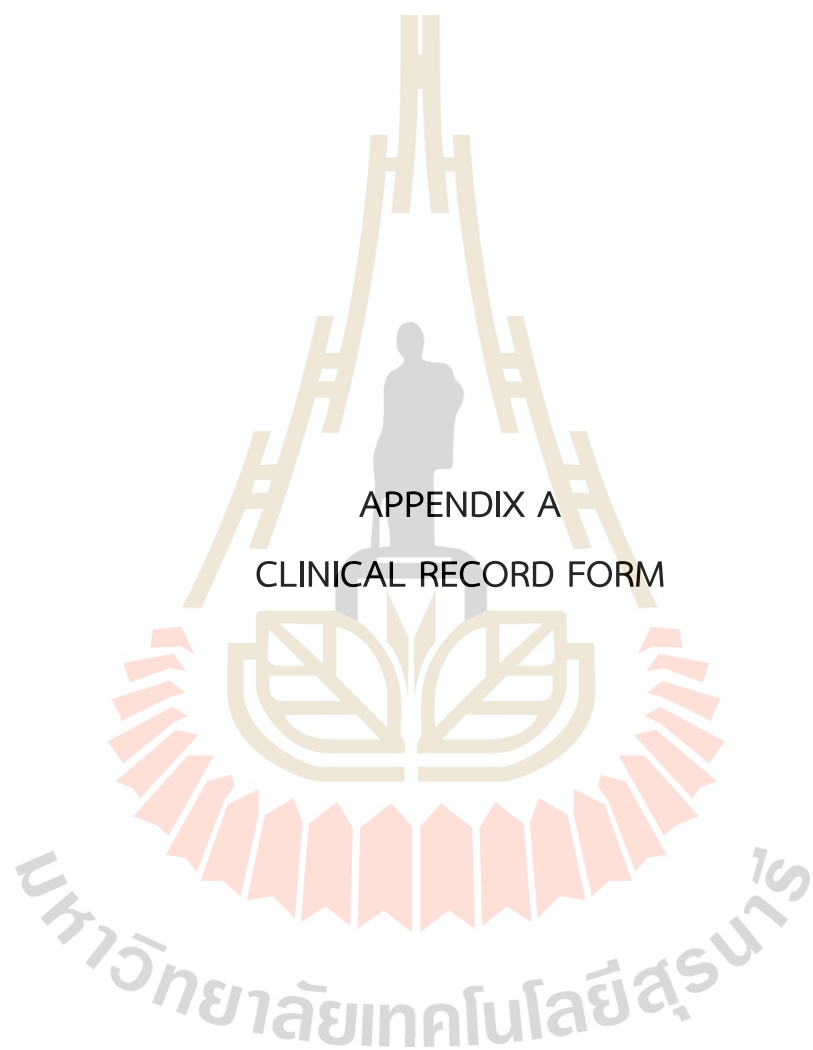
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APPENDIX A
CLINICAL RECORD FORM

ID ☐ ☐ ☐

Date...../..... /.....

Recorder.....

Clinical Record Form

Clinical Record Form is a part of the Ph.D. Thesis in title: *The efficacy of the Plook-Fire-Thatu recipe on breast milk production and nutrient content of postpartum women.*

Directions: Please respond to the following questions by provide your information as truthful as possible and place an "☒" mark in the box of your answer or providing the requested information.

Part 1: General characteristics

1. Age..... years old	3. The systemic disease
2. Gravida number.....	☐ No
3. Education	☐ Yes, (please specify)
☐ None	4. The medication or food supplementary
☐ Elementary School	☐ No
☐ High School	☐ Yes, (please specify)
☐ Bachelor's degree	5. Height.....m. Body weightkg.
☐ Higher than the Bachelor's degree	Body mass index.....kg/m ²

Part 2: The participations criteria

The inclusion criteria	The exclusion criteria	Discontinuation criteria
☐ Postpartum women who had term vaginal delivery (≥ 37 weeks gestation age). ☐ Age 20-40 years. ☐ No abnormalities of the breast and nipple.	☐ Major complications after the delivery such as endometritis sepsis or bleeding ☐ Allergy essential of herbs in the Plook-Fire-Thatu recipe. ☐ Using drugs that may interaction with the Plook-Fire-Thatu recipe including phenytoin, propranolol, theophylline, and rifampicin.	☐ Serious adverse events following administration include stomach irritation, rash, itching, swelling, chest pain, and bleeding. Other symptoms that doctors deem unsafe for the participants. ☐ Taking the Plook-Fire-Thatu recipe not continuously according to plan. ☐ Received another galactagogue that affects milk stimulation. ☐ Do not consent to continue participating in research.

Part 3: The parameters collection

Parameters		Day1	Day2	Day3
Vital sign	Weight (kg)			
	Systolic (mmHg)			
	Diastolic (mmHg)			
	Heart rate (beat/minute)			
Height of the fundus of the uterus (cm.)				
Blood chemistry	Hemoglobin (Hb) (g/dL)			
	Hematocrit (Hct) (%)			
	Blood glucose (mg/dL)			
	Total cholesterol (TC) (mg/dL)			
	Triglyceride (TG) (mg/dL)			
	Low-density lipoprotein-cholesterol (LDLC) (mg/dL)			
	High-density lipoprotein-cholesterol (HDL-C) (mg/dL)			
	Aspartate aminotransferase (AST) (U/L)			
	Alanine aminotransferase (ALT) (U/L)			
	Alkaline phosphatase (ALP) (U/L)			
	Blood urea nitrogen (BUN) (mg/dL)			
	Creatinine (mg/dL)			
	Albumin (mg/dL)			
	Serum prolactin level (PRL) (mg/dL)			

Part 3: The parameters collection (cont.)

Parameters		Day1	Day2	Day3
Breast milk volume (mL/day)				
Dietary	Energy (kcal/day)			
	Carbohydrate (% of energy)			
	Protein (% of energy)			
	Fat (% of energy)			

	Fiber (g/day)			
	Water (mL/day)			

Part 4: Record of adverse effects in mothers and newborns

Questionnaires	Day1	Day2	Day3
Take the Plook-Fire-Thatu recipe in the study strictly as prescribed by your doctor.			
Forgot to take the Plook-Fire-Thatu recipe in this study? If you forget, how many times/day do you forget?			
Take the supplement or herbal that may be increase milk production.			
There is an adverse event after taking Pluk-Fai-Tat (please specify)			

Part 5: Dietary assessment

24-hour food recall for researcher (24-hour food recall is an interview or answer questionnaires of food eaten in the last 24 hours or food that you usually eat in a day)

Meal	Menu	Ingredients	Amount
<i>Example</i>			
เช้า	ข้าวผัดหมู	ข้าวสวย	1.5 ทัพพี
		หมูติดมัน	1 ช้อนกินข้าว
		ผักคะน้า	¼ ทัพพี
		มะเขือเทศ	1 ลูก
		ไข่ไก่	1 ฟอง
		น้ำมันพืช	2 ช้อนชา
	เติมเครื่องปรุงรส	น้ำปลา	1 ช้อนกินข้าว
เที่ยง			

Recorder.....

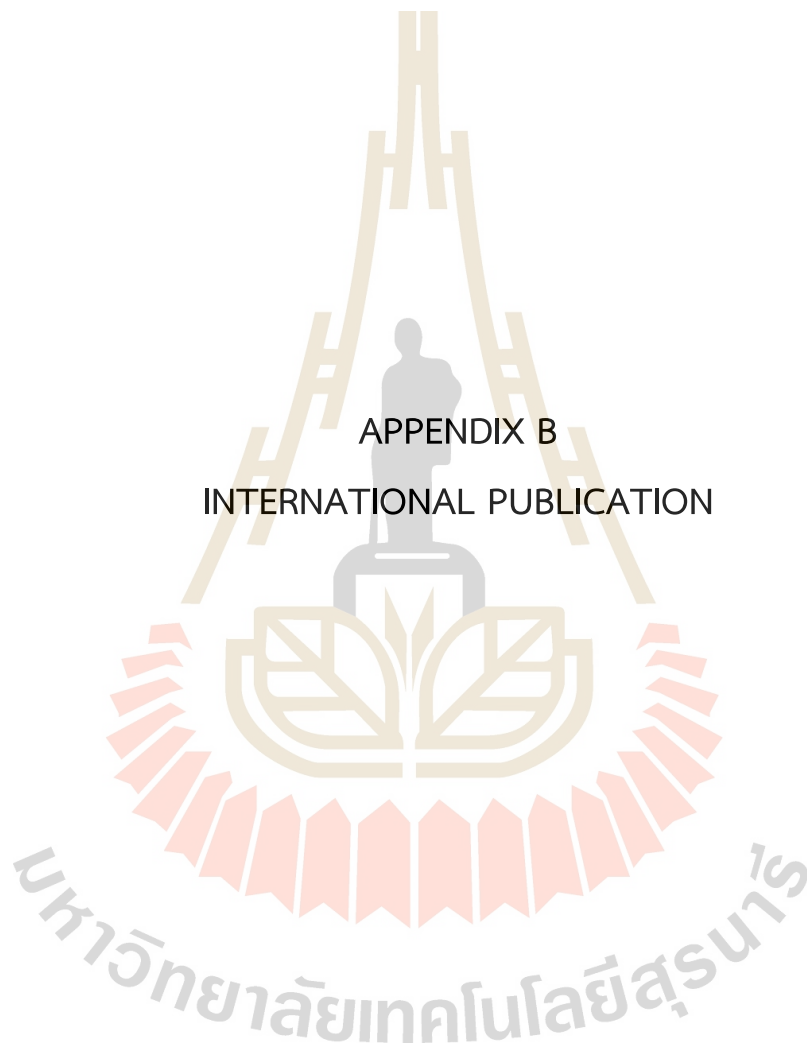
Food consumption record form (For participants)

Food consumption record form is a part of the Ph.D. Thesis in title: *The efficacy of the Plook-Fire-Thatu recipe on breast milk production and nutrient content of postpartum women*

Directions: Please fill in dietary intakes of breakfast, lunch and dinner for 3 days.

[illegible]

APPENDIX B
INTERNATIONAL PUBLICATION



INTERNATIONAL PUBLICATION

Sintupattanapun, P., Namvichaisirikul, N., Tongdee, P., Suwannobol, N., Thueng-In, K., Chueadet, A., & Tastub, S. (2025). Efficacy and safety of the Plook-Fire-Thatu recipe on breast milk volume and breast milk macronutrient composition in postpartum women: A randomised controlled trial. *Complementary Therapies in Medicine*, 103255.





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Efficacy and safety of the Plook-Fire-Thatu recipe on breast milk volume and breast milk macronutrient composition in postpartum women: A randomised controlled trial

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ABSTRACT

Background: Plook-Fire-Thatu (PFT) is an ancient Thai herbal formulation traditionally believed to increase breast milk volume. However, clinical studies on the PFT's effectiveness for breast milk production are lacking.

Objective: This study aimed to evaluate the effectiveness and safety of PFT in increasing breast milk volume.

Materials and methods: A randomised controlled trial was conducted involving 60 postpartum women. The treatment group received standard care alongside 500 mg of PFT orally, three times daily for three days, while the control group received standard care alone. Serum prolactin levels, haematological parameters and blood chemistry were assessed on days 1 and 3. Breast milk volume was measured using an electronic pump on days 1, 2 and 3. Adverse drug reactions in mothers and infants were monitored on days 1, 2, 3, 7 and 30. Breast milk samples were analysed for macronutrient content using Fourier-transform infrared spectroscopy.

Results: Baseline characteristics were comparable between groups. The treatment group exhibited significantly greater breast milk volume on days 2 ($p = 0.028$) and 3 ($p = 0.002$) compared with the control group. Serum prolactin levels increased significantly in the treatment group ($p = 0.002$). No significant differences were observed in haematological or blood chemistry between groups. Glycogen and carbohydrate levels in breast milk were significantly higher in the treatment group. Two participants reported mild epigastric discomfort on the first day, with no further adverse events reported.

Conclusion: By the third day, PFT significantly increased breast milk volume and serum prolactin levels. Increases in glycogen and carbohydrate content in breast milk were also observed. The formulation was well tolerated, with no significant adverse effects in mothers or infants.

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1. Introduction

Breast milk is recognised by the World Health Organization (WHO) and the American Academy of Pediatrics as the optimal source of nutrition for infants. The WHO recommends exclusive breastfeeding for the first six months of life, with continued breastfeeding for one year or longer.¹ In Thailand, however, only 14 % of infants are exclusively breastfed. Lactation insufficiency affects approximately 63 % of mothers during the first four months postpartum.² Hypogalactia refers to the inadequate production of breast milk to meet an infant's nutritional needs.³ Pharmacological agents such as metoclopramide, domperidone and oxytocin have been used to increase breast milk supply. However, these medications are considered off-label for this purpose, as they have not been approved by the Food and Drug Administration as galactagogues, due to reported adverse effects in mothers receiving them.⁴

According to Thai traditional medicine, health is viewed as a balance among four fundamental elements: earth, fire, wind and water. These elements correspond to different physiological and psychological functions within the body, and maintaining their balance is considered vital for overall well-being. Postpartum, the loss of amniotic fluid and blood is believed to disrupt this elemental balance—particularly the fire element—leading to illness and reduced breast milk production. Thai cultural practices for postpartum care aim to restore this balance and include methods such as lying by the fire (*yuu-fai*), herbal baths, traditional rituals, herbal drinks, steam therapy, salt pot compression, massage, sitting over charcoal, herbal ball compression and the use of herbal formulations.^{5,6}

The *Plook-Fire-Thatu* (PFT) recipe is a traditional Thai herbal medicine documented in the *Mahachotarat* scripture, specifically formulated to promote lactation in postpartum women. It is recorded in the *Medical Science Book* from the Rattanakosin era, section 126, and has been recommended for use in postpartum care. Since 2012, PFT has been included in the Thailand National List of Essential Medicines (NLEM).⁷ According to Thai traditional medicine, the efficacy of herbal formulations is explained through the concept of taste. PFT is believed to simulate the fire element in postpartum women through the use of herbs with a fiery taste, thereby enhancing breast milk production.⁸

The recipe comprises 11 herbal components: pepper,⁹ ginger,^{10–12} long pepper,¹³ wild betel leaf, peel of kaffir lime fruit, nut grass,¹⁴ *Ardisia* fruit, sweet flag root,¹⁵ *Oenanthe* seeds, pink *Arnebia* and Wallich's pepper vine. Among these, pepper and ginger are recognised for their galactagogue properties. The remaining ingredients are traditionally used to promote comfort, vitality and overall well-being, which may indirectly support lactation.

Previous studies have shown that administering PFT at a dosage of 3 g/day for either three or seven days significantly increases mean breast milk volume without causing serious adverse drug reactions.^{16,17} Furthermore, a regimen of 90 mg of PFT taken three times daily for three days has been found to significantly enhance breast milk production compared to both domperidone and placebo groups.¹⁷ Toxicological studies in rats have also reported the safety of PFT. In one study, rats received daily oral doses of PFT extract at 125, 250 and 500 mg/kg body weight for 90 days, with observations continuing up to 120 days. No toxic effects were observed in either male or female rats, indicating that the PFT extract is safe for use.¹⁰ Given the variation in effective doses reported in earlier studies, this researcher adopted the minimum recommended dose as specified in the NLEM to ensure both efficacy and safety.

At present, there is limited direct scientific evidence linking the individual herbs in the PFT formulation to their purported lactogenic effects. Nevertheless, many of these herbs are recognised for their medicinal properties, which may contribute to the overall efficacy of the formulation in addressing hypogalactia. Clinical studies specifically investigating the effectiveness of PFT remain scarce. This study aims to evaluate and compare breast milk volume, haematology, blood biochemistry and adverse drug reactions within 72 h postpartum

between participants who received PFT and those who received standard postpartum care. Additionally, Fourier-transform infrared (FTIR) spectroscopy was employed to analyse changes in biomolecule composition in breast milk between the groups. The central hypothesis is that PFT administration would result in increased breast milk production without including severe adverse drug reactions.

2. Materials and methods

2.1. Study design and participants

The study was designed as a randomised controlled trial. Ethical approval was granted by the Human Research Ethics Committee of Suranaree University of Technology, which reviewed and approved the study protocol, participant information sheet and informed consent form (COA No. 133/2564). The trial was registered with the Thai Clinical Trials Registry (Registration No TCTR20240325002). The study was conducted from November 2021 to November 2022 at Sikhio Hospital in Nakhon Ratchasima Province, Thailand.

A total of 60 postpartum women were enrolled according to the following inclusion criteria: aged between 20 and 40 years, having undergone vaginal delivery at a gestational age of 37 weeks or more, and without any abnormalities of the breast or nipple. Participants were excluded if they had major postpartum complications such as endometritis, sepsis or haemorrhage. Additional exclusion criteria included known allergy to any of the key herbs in the PFT formulation; concurrent use of medications that may interact with PFT (e.g. phenytoin, propranolol, theophylline and rifampicin); use of anticoagulant or antiplatelet therapy; clinical bleeding; abnormal liver or renal function; and the presence of congenital or chronic conditions such as epilepsy, asthma, chronic gastritis, GERD, hypertension or other underlying diseases that cause low milk production (e.g., gestational diabetes, GDM). Discontinuation criteria included the occurrence of serious adverse events such as gastric irritation, rash, pruritus, oedema, chest pain or bleeding. Any other symptoms deemed unsafe by the attending physicians were also considered grounds for withdrawal. Participants who failed to adhere to the study protocol or who consumed other galactagogues that could affect milk production were excluded from the analysis.

The sample size was calculated to compare the mean breast milk volume between two independent groups. Based on prior studies involving Fenugreek herbal tea, the standard deviation of breast milk volume was estimated at 18.8 mL. The mean breast milk volumes for the intervention and control groups were reported as 50.8 and 35.5 mL, respectively.¹⁸ Using these parameters, it was calculated that 24 participants per group would be required to detect a statistically significant difference. To allow for a potential dropout of 20 %, the sample size was increased to 30 participants per group, resulting in a total of 60 participants.

All participants received study information during their third-trimester antenatal care visit and provided written informed consent prior to enrolment. It was considered inappropriate to seek consent during labour or immediately postpartum, as this could impair decision-making capacity.

Participants were recruited after delivery using simple random sampling. Randomisation was performed immediately following birth by a registered nurse, prior to the participants' transfer to the postpartum ward. Participants were randomly allocated to either the control or treatment group in a 1:1 ratio, using sequential numbering from 1 to 60. Odd numbers (1, 3, 5, etc.) were assigned to the control group, while even numbers (2, 4, 6, etc.) were assigned to the treatment group. Participant numbers were not displayed on medical charts or beds to maintain allocation concealment.

Clinical outcomes were assessed by the research team, who were responsible for data collection, measurement of breast milk volume and documentation of adverse drug reactions. Neither the nurses

administering care nor the researchers collecting data were aware of group allocations.

2.2. Interventions

This PFT formulation is a bolus preparation composed of 11 medicinal herbs. Each 100 g of the mixture contains 50 g of pepper fruit (*Piper nigrum*) and 5 g each of the following: ginger rhizome (*Zingiber officinale*), long pepper fruit (*Piper longum*), all parts of wild betel leaf (*Piper sarmentosum*), Wallach's pepper vine (*Piper wallichii*), *Ardisia* fruit (*Ardisia elliptica*), peel of kaffir lime fruit (*Citrus hystrix*), *Oenanthe* seed (*Oenanthe javanica*), sweet flag root (*Acorus calamus*), pink *Arnebia* (*Arnebia euchroma*) and nut grass rhizome (*Cyperus rotundus*).^{19,20} The herbs were dried, powdered and thoroughly mixed before being formed into bolus units. Each bolus contained 100 g of the herbal powder. The formulation was produced in accordance with the standards outlined in the Thailand NLEM and met the Good Manufacturing Practice certification requirements. The lot number of the product used in this study was 126411, with an expiry date of 23 December 2023.

All participants received standard postpartum care and breastfeeding support. This included guidance on breastfeeding techniques: early initiation (breastfeeding within 1 h of birth), frequent feeding (every 2–3 h, day and night) and proper positioning to ensure effective latching.

In addition to standard care, the treatment group received 500 mg of PFT orally three times daily, administered before meals for three consecutive days postpartum. The control group received standard care only and did not receive PFT or a placebo. The dosage and duration were selected based on NLEM recommendation.

The intervention commenced with the first meal after delivery, within 12 h postpartum. The first day of the study was defined as the day participants began receiving the intervention. Participants were discharged 72 h after delivery.

Medication preparation was carried out by the hospital pharmacy. Interventions were distributed by a registered nurse, who remained blinded to the contents. The medication was sealed and only opened by the nurse immediately prior to administration.

To minimise nutritional confounding during the three-day intervention period, dietary intake was standardised. A registered dietitian provided participants with meals averaging 1800 kcal per day, comprising approximately 20 % protein, 30 % fat and 50 % carbohydrates. Menus were carefully designed to exclude ingredients known to influence lactation—such as ginger or other galactagogue herbs—that could confound the study outcomes. If such ingredients were present, the nutritionist had discretion to exclude affected participants. A standardised menu plan was followed, and participants' dietary intake was retrospectively recorded and monitored for compliance and nutritional analysis.

2.3. Data collection

Data collection was structured into six key components: (1) general characteristics, including age, weight, body mass index and gravida number; (2) haematology (haemoglobin (Hb), haematocrit (Hct)) and blood biochemistry (blood glucose, serum prolactin level (PRL), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine and albumin); (3) dietary assessment; (4) breast milk volume; (5) adverse drug reactions; and (6) breast milk nutritional content.

2.4. Measurements

Blood (10 mL) and breast milk (1.5 mL) samples were collected from all participants two hours after delivery to establish baseline

measurements. The same volumes of blood and breast milk samples were subsequently collected on day 3 postpartum following treatment. Breast milk samples were stored in sterile tubes and frozen until nutritional analysis was performed.

Breast milk volume measurement was Breast Pumping Method,¹⁶ using an electronic pump three times daily—30 min after each meal (breakfast, lunch and dinner) on days 1, 2 and 3 postpartum. Each measurement was conducted at least one hour after the last breastfeeding session. The total daily breast milk volume was calculated as the sum of all expressions regardless of mealtimes. Each pumping session to empty both breasts lasted approximately 30 min, after which the expressed milk was fed to the infant. No participants' babies received formula supplementation during the day.

Participants' dietary intake was recorded daily from days 1–3 postpartum. Adverse drug reactions in both mothers and infants were monitored and documented on days 1, 2, 3, 7 and 30 postpartum.

Breast milk nutritional content was analysed using FTIR spectroscopy. Spectra were acquired using a Bruker Hyperion 3000 spectrometer equipped with a focal plane array detector. Measurements were performed in reflection mode across the spectral range of 3000–900 cm⁻¹ using OPUS 7.5 software. A 15× objective lens was employed with 64 scans per sample and 64 background scans, at a resolution of 4 cm⁻¹.

Principal component analysis (PCA) was applied to visualise patterns and differences within the spectral dataset by reducing its dimensionality. PCA transforms the original variables into principal components that capture the greatest variance, thereby simplifying interpretation of complex data. The analysis was conducted using Unscramble X 10.1.

2.5. Data analysis

All data were analysed using SPSS 26.0. The Kolmogorov–Smirnov test was employed to assess the normality of the data distribution. Missing data were addressed using mean imputation. Categorical variables were analysed using the Chi-square test. Paired sample *t*-tests were conducted to evaluate within-group differences in breast milk volume, haematological parameters and blood chemistry. Between-group comparisons were performed using independent sample *t*-tests. Changes in breast milk volume from days 1–3 postpartum were analysed using repeated measures ANOVA.

3. Results

Seventy-six postpartum women met the eligibility criteria for the study; however, four withdrew for personal reasons. Consequently, 72 women were enrolled and randomly assigned equally to the control and treatment groups, with 36 participants in each. During the follow-up period, three participants from the control group and four from the treatment group were lost. Additionally, three participants (two from the control group and one from the treatment group) were excluded for consuming other galactagogues, such as mixed herbs and ginger tea provided by caregivers. Four participants (one from the control group and three from the treatment group) did not adhere to the breast milk volume measurement protocol. These participants were excluded from the final analysis, which was conducted using a per-protocol approach. Ultimately, data from 30 participants in each group were available for comparison. The participant flow throughout the study is illustrated in Fig. 1.

Demographic data indicated no statistically significant differences between the groups in terms of height, body weight or age range. The number of pregnancies varied across participants in both groups (Table 1). No participants had systemic conditions; the underlying causes of low milk production included gestational diabetes and related factors.

Table 2 presents the measured breast milk volumes for each day, alongside 95 % confidence intervals, *t*-values, *p*-values and mean differences (*d*) between the groups. A statistically significant increase in

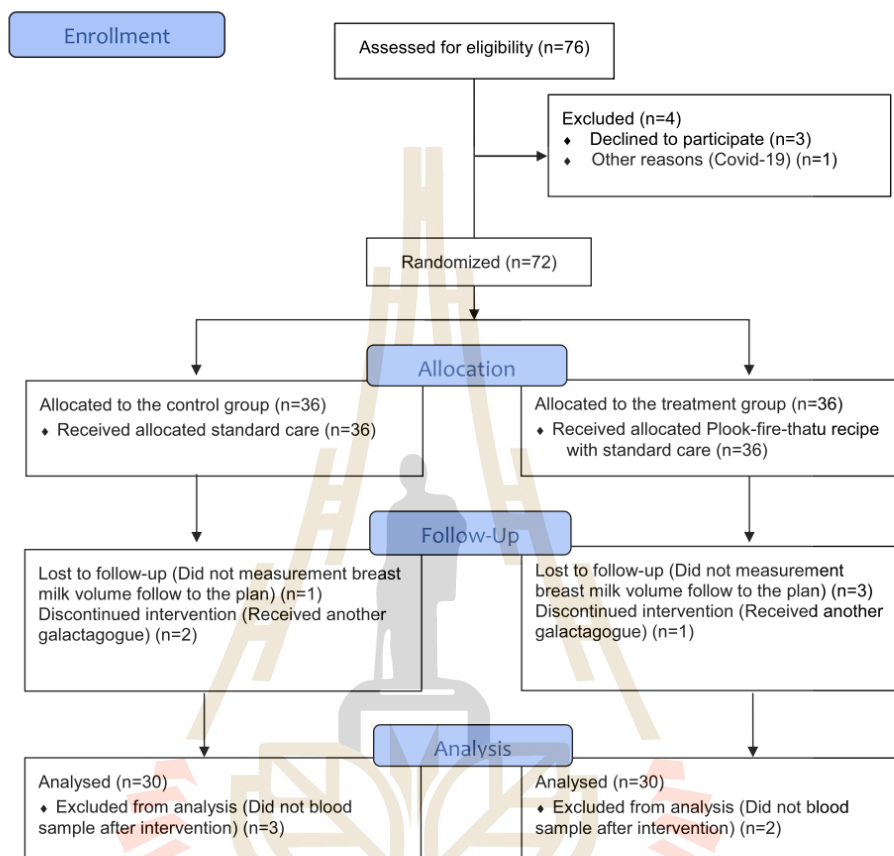


Fig. 1. CONSORT flow chart for the study.

Table 1
General characteristics data for the control and treatment groups.

Parameters	Control group (n = 30)	Treatment group (n = 30)	p
Age (years)	29.59 ± 5.64	25.84 ± 4.16	0.500
Body weight (kg)	66.40 ± 10.62	67.21 ± 14.45	0.763
Height (m)	1.58 ± 0.05	1.59 ± 0.05	0.828
BMI (kg/m ²)	26.37 ± 4.26	26.33 ± 5.10	0.403
Gravida number, n (%)			0.192
1	8 (26.67)	16 (53.33)	
≥ 2	22 (73.33)	14 (46.67)	
Education, n (%)			0.211
Elementary School	9 (30.00)	9 (30.00)	
High School	20 (66.67)	16 (53.33)	
Bachelor's degree	1 (3.33)	5 (16.67)	

Values are mean ± SD

milk production was observed in the treatment group on days 2 and 3 postpartum compared to the control group. The mean differences in breast milk volume between the treatment and control groups were −27.37 mL ($p = 0.028$) on day 2 and −117.43 mL ($p = 0.002$) on day 3, respectively.

Serum prolactin levels did not differ significantly between groups.

However, within the treatment group, the mean serum prolactin level post-treatment (256.36 ± 64.12 ng/mL) was significantly higher than the baseline measurement (197.93 ± 95.90 ng/mL, $p = 0.002$). (Table 3)

Haematological parameters (Hb, Hct), lipid profiles (TC, TG, LDL-C, HDL-C), liver function tests (AST, ALT, ALP) and renal function markers (BUN, creatinine, albumin) were compared between groups, with no statistical significant differences observed ($p > 0.05$). Furthermore, intra-group comparisons indicated that changes in these parameters followed similar trends in both groups (Table 3).

Adverse drug reactions were monitored through participant interviews. Two participants (6.67 %) reported mild epigastric pain on the first postpartum day. According to Naranjo's algorithm, this was classified as a possible adverse drug reaction. No severe adverse reactions were reported, and no adverse events were observed in the infants (Table 4).

The milk samples primarily comprised proteins, carbohydrates and lipids. FTIR spectra of the milk samples were analysed within the spectral range of $3000\text{--}900\text{ cm}^{-1}$. Prominent absorption bands corresponded to lipids, while the protein region exhibited characteristic amide I and amide II bands (Fig. 2A). Carbohydrate-related bands corresponding to glycogen and mucin were also observed, alongside nucleic acid (PO_2) absorption bands (Fig. 2B). The integral areas derived from

Table 2

Breastmilk volume between Day 1, Day 2, and Day 3 postpartum in the control and treatment groups.

Time	Control group	Treatment group	d ⁻	95 % CI		t	p
				Lower	Upper		
Day1	4.12 ± 6.43	7.33 ± 10.20	-3.20	-7.62	1.20	-1.45	0.152
Day2	28.99 ± 30.88	56.36 ± 58.06	-27.37	-51.40	-3.33	-2.27	0.028*
Day3	95.69 ± 106.47	213.12 ± 162.76	-117.43	-188.75	-46.10	3.30	0.002*

d⁻ = Mean difference

CI = Confidence interval

Values are mean ± SD

*Statistical significance set at $p < 0.05$; values significantly different compared to control group**Table 3**

Serum prolactin levels, hematology, and blood biochemical values between the control and treatment groups.

Parameters	Control group (n = 30)			Treatment group (n = 30)				
	Before	After	P ¹	Before	After	P ¹	P ²	P ³
PRL (ng/mL)	253.25 ± 154.66	273.32 ± 164.92	0.413	197.93 ± 95.90	256.36 ± 64.12	0.002*	0.122	0.620
Hemoglobin (g/dL)	11.85 ± 1.34	11.77 ± 5.14	0.935	11.83 ± 1.29	11.15 ± 0.92	<0.001**	0.935	0.513
Hematocrit (%)	36.87 ± 3.96	33.78 ± 4.66	0.271	36.82 ± 4.02	34.67 ± 3.04	<0.001**	0.963	0.400
Blood glucose (mg/dL)	112.96 ± 40.22	75.82 ± 8.45	<0.001**	95.90 ± 24.81	74.03 ± 7.40	<0.001**	0.060	0.399
TC (mg/dL)	238.96 ± 46.03	229.48 ± 40.16	0.079	238.66 ± 53.19	236.70 ± 45.47	0.629	0.982	0.530
TG (mg/dL)	229.70 ± 68.65	233.03 ± 53.83	0.796	198.76 ± 75.64	214.58 ± 65.69	0.144	0.113	0.257
LDLC (mg/dL)	151.76 ± 35.07	145.80 ± 30.28	0.244	149.83 ± 39.80	148.93 ± 34.20	0.973	0.849	0.723
HDLc (mg/dL)	73.88 ± 17.26	68.44 ± 16.24	0.003*	72.83 ± 13.97	70.13 ± 12.64	0.017*	0.802	0.661
AST (U/L)	30.96 ± 20.51	31.29 ± 24.51	0.883	29.53 ± 9.79	28.40 ± 12.13	0.385	0.745	0.568
ALT (U/L)	15.22 ± 13.21	16.88 ± 10.87	0.106	14.10 ± 9.94	16.90 ± 9.58	0.002*	0.725	0.996
ALP (U/L)	159.07 ± 46.24	125.84 ± 35.67	<0.001**	157.92 ± 38.35	124.85 ± 30.99	<0.001**	0.923	0.914
BUN (mg/dL)	7.18 ± 2.39	8.14 ± 3.35	0.047*	7.04 ± 1.60	8.95 ± 2.06	0.001*	0.801	0.279
Creatinine (mg/dL)	0.54 ± 0.11	0.60 ± 0.08	0.001*	0.55 ± 0.09	0.59 ± 0.09	0.001*	0.797	0.822
Albumin (g/dL)	3.17 ± 0.26	3.09 ± 0.24	0.074	3.19 ± 0.22	3.21 ± 0.19	0.311	0.737	0.053

P¹ Comparison within the group,P² Comparison between two groups before treatment,P³ Comparison between two groups after treatment* Statistically significant $p < 0.05$, ** $p < 0.001$

PRL; Serum prolactin level, Hemoglobin, Hb; Hematocrit, Hct; Total cholesterol, TC; Triglyceride, TG; Low-density lipoprotein-cholesterol, LDLc; High-density lipoprotein-cholesterol, HDLC; Aspartate aminotransferase, AST; Alanine aminotransferase, ALT; Alkaline phosphatase, ALP; Blood urea nitrogen, BUN.

Table 4

Data of adverse drug reaction (ADR) from PFT in participants (n = 30).

Day	Adverse drug reactions n (%)				No n (%)	Total n (%)
	Hot flash	Epigastric burn	Stomach pain	Nausea		
Day 1	-	2 (6.67)	-	-	28 (93.33)	30 (100)
Day 2	-	-	-	-	30 (100)	30 (100)
Day 3	-	-	-	-	30 (100)	30 (100)
Day 7	-	-	-	-	30 (100)	30 (100)
Day 30	-	-	-	-	30 (100)	30 (100)

the FTIR second derivative spectra (Fig. 3) indicated no significant differences in lipid and nucleic acid content between groups. However, protein content decreased post-treatment in both groups, whereas carbohydrate and glycogen levels increased significantly in the treatment group following PFT administration.

PCA score plots revealed that clusters representing the control and treatment groups post-PFT administration were distinctly separated from those before administration along PC1, which accounted for 41 % of the variance (y-axis). Likewise, the treatment group's pre- and post-administration samples were separated from the control group's samples along PC2, accounting for 21 % of the variance (x-axis) (Fig. 4A). PCA loading plots further identified the FTIR spectral regions contributing most to the clustering, revealing that while all samples were

generally similar, some variances in the post-treatment control and treatment groups displayed higher lipid peaks and lower protein peaks (Fig. 4B).

4. Discussion

This study found that PFT at 1.5 g/day for three days significantly increased breast milk volume on the second and third days compared to the control group. Although participants in the treatment group appeared to have a higher baseline milk production, the difference was not statistically significant. Specifically, the intervention group exhibited a slightly higher mean breast milk volume on day 1 compared to the control group (7.33 ± 10.20 mL vs. 4.12 ± 6.43 mL; $p = 0.152$), suggesting comparable baseline milk production between groups. Furthermore, participants were randomised without prior selection based on breastfeeding capacity or hypogalactia, indicating that the observed difference cannot be attributed to differing prevalence of hypogalactia.

Previous studies have demonstrated that a PFT dose of 3 g/day for three days significantly increases mean breast milk volumes within the same period ($p < 0.001$), with 25 % of participants experiencing a mean increase of 110 mL or more—significantly higher than the placebo group ($p < 0.05$).¹⁶ Additionally, administration of PFT at 3 g/day for seven days resulted in 60.4 % of postpartum women achieving milk ejection at a reasonable level, reaching 96.6 % by the end of the period ($p < 0.05$).¹⁷ Moreover, PFT at 90 mg taken three times daily for three days showed a significant increase in breast milk production compared to domperidone and placebo groups ($p \leq 0.01$).¹⁷ Therefore, it can be concluded that taking the PFT 270 mg for three days, 1.5 g/day for three days, or 3 g/day for three or seven days, based on NLEM

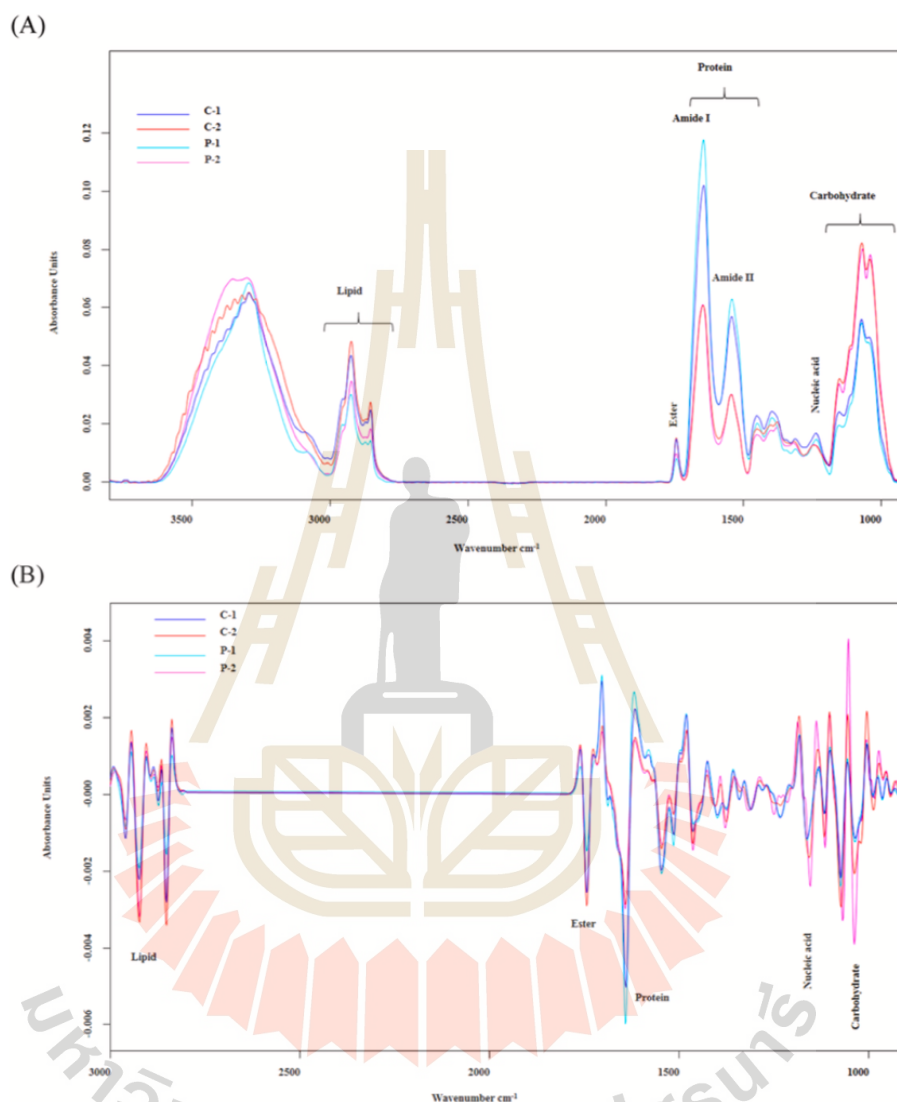


Fig. 2. Primary spectrum of micronutrition (A), Secondary derivative spectrum (B) of breast milk samples. The results were obtained from four different conditions: the control group before (C1) and after (C2) experiments and the treatment group before (P1) and after (P2) experiments.

recommendations, can increase breast milk production.

The safety results of this study showed that participants exhibited no abnormalities in haematology, liver or kidney function tests. Consistent with previous research, Wistar rats administered daily doses of 125, 250 and 500 mg/kg for 90 days and observed for 120 days showed normal haematology and blood biochemistry, with no abnormalities detected in internal organs.¹⁰

Adverse drug reactions potentially associated with PFT include heartburn, stomach pain and nausea.⁷ Previous studies reported that postpartum women who took 1000 mg three times daily before meals experienced heartburn in two cases and hot flashes in one case on the first day.²¹ In this study, adverse drug reactions were monitored on days

1, 2, 3, 7 and 30 postpartum; only two participants reported mild epigastric pain on the first day, which did not persist thereafter.

FTIR analysis indicated that participants who received the PFT recipe for three days had breast milk containing the primary macronutrients—protein, fat and carbohydrate. The FTIR integral area for macronutrient content (Fig. 3) revealed a slightly higher lipid level in the treatment group, although this difference was not statistically significant.

The results suggest that the herbs in PFT may stimulate the secretion of prolactin, a hormone crucial for milk production, in a manner comparable to standard care. Increased prolactin levels can lead to greater milk volume and potentially a higher fat content in the milk produced.

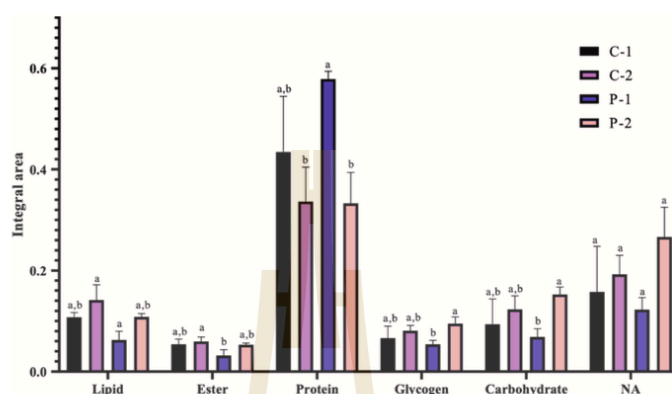


Fig. 3. Integral area from FTIR second derivative spectrum. Tests were analyzed by one-way analysis of variance (ANOVA). Means with different lowercase letters (a and b) are significantly different. ($P < 0.05$, error bars indicate standard errors of means). Obtained from the control group before (C1) and after (C2) experiments and the treatment group before (P1) and after (P2) experiments.

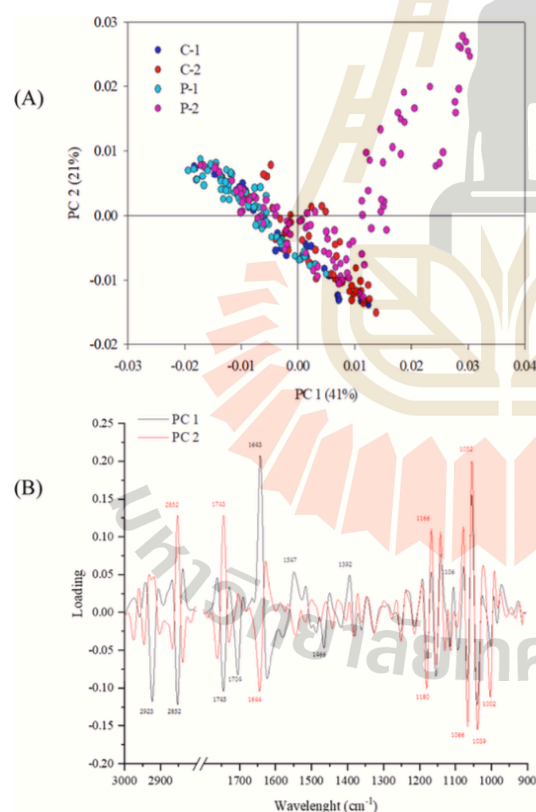


Fig. 4. Principal component analysis (PCA) score plot (A) and principal loading component (B) results of milk samples. Obtained from the control group before (C1) and after (C2) experiments and the treatment group before (P1) and after (P2) experiments.

Accordingly, the PFT recipe is designed to promote lactation. Enhanced milk production is often associated with elevated carbohydrate levels, particularly lactose, which is the primary carbohydrate in human milk.¹⁷ As milk volume increases due to improved lactation, the total carbohydrate content may also rise, as indicated by the FTIR findings. The observed reduction in protein content following treatment with the PFT recipe may be attributed to its specific nutritional profile, which could influence protein synthesis pathways in lactating mothers. Furthermore, some herbs in the formulation may promote lipid production more strongly than protein synthesis, resulting in a relative decrease in protein content compared to other macronutrients.^{17,22}

A limitation of this study was the relatively short, three-day period used to evaluate the effectiveness of PFT. The researchers sought to control for confounding factors such as breastfeeding techniques, diet, medication and environmental conditions by restricting the study to the first three days postpartum during hospitalisation. This approach was intended to isolate changes in milk volume with this critical period. Further research would be valuable to determine whether the increase in milk production persists beyond this initial timeframe.

Additional important limitations include maternal and infant health status, infant feeding behaviours (e.g. latch difficulties, tongue-tie), maternal stress or sleep quality, breast morphology and the precise timing of PFT administration following delivery. Future studies should incorporate these variables to better address confounding factors. These findings present significant opportunities for further research to strengthen the evidence base relating to breast milk production.

The measurement of breast milk volume is subject to confounding factors such as fluid intake, nutrition and medication. To mitigate these influences, all participants were provided with a standardised diet averaging 1800 kcal per day, and nutritional intake was monitored via 24-hour food recall records.

Furthermore, due to a lack of access to specialised food laboratory analysis, the researcher utilised FTIR techniques to analyse breast milk nutrition. FTIR is a valid and accurate method, requiring only a minimal sample volume (approximately 1 mL) and is non-destructive to the sample. However, the mechanistic basis of this recipe requires further elucidation. Additionally, studies comparing various dosages and durations of PFT administration on milk production and safety would provide a valuable foundation for future research.

5. Conclusion

This research provides evidence that the PFT recipe enhances breast

milk volume by the third day postpartum without causing adverse drug reactions in mothers or infants. The treatment dosage did not lead to abnormalities in haematology, liver function or kidney function. Furthermore, it increased the glycogen, carbohydrate and lipid content of breast milk. The observed rise in carbohydrate and lipid levels following PFT treatment is likely due to a combination of enhanced lactation dynamics, specific nutritional influences from the herbal components, physiological adaptations to increased feeding demands, and possible alterations in maternal dietary habits. Further studies are warranted to elucidate these mechanisms more clearly and to confirm their effects on human milk composition.

CRediT authorship contribution statement

Niwattchai Namvichaisirikul: Writing – review & editing, Methodology, Conceptualization. **Pattama Tongdee:** Writing – review & editing, Formal analysis, Conceptualization. **Pannida Sintupattapanun:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Sukanya Tastub:** Writing – review & editing, Resources, Methodology, Investigation. **Kanyarat Thueng-in:** Investigation. **Aree Chueadet:** Writing – review & editing, Resources. **Nareelux Suwannabol:** Methodology, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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BIOGRAPHY

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มหาวิทยาลัยเทคโนโลยีสุรนารี