EVALUATION OF MICROWAVE–ASSISTED EXTRACTION OF *NARINGI CRENULATA* LEAVES FOR UTILIZATION IN SKINCARE ESSENCE

KHINE MON YEE HLAING

MASTER OF SCIENCE
IN
CREATIVE INNOVATION IN COSMETIC SCIENCE

SCHOOL OF COSMETIC SCIENCE
MAE FAH LUANG UNIVERSITY

2024

EVALUATION OF MICROWAVE–ASSISTED EXTRACTION OF *NARINGI CRENULATA* LEAVES FOR UTILIZATION IN SKINCARE ESSENCE

KHINE MON YEE HLAING

THIS THESIS IS A PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
CREATIVE INNOVATION IN COSMETIC SCIENCE

SCHOOL OF COSMETIC SCIENCE
MAE FAH LUANG UNIVERSITY
2024

THESIS APPROVAL

MAE FAH LUANG UNIVERSITY

FOR

MASTER OF SCIENCE

IN CREATIVE INNOVATION IN COSMETIC SCIENCE

Thesis Title: Evaluation of Microwave–assisted Extraction of *Naringi crenulata* Leaves for Utilization in Skincare Essence

Author: Khine Mon Yee Hlaing

Examination Committee:

Assistant Professor Puxvadee Chaikul, Ph. D.	Chairperson
Naphatsorn Ditthawutthikul, Ph. D.	Member
Assistant Professor Nuntawat Khat–udomkiri, Ph. D.	Member
Assistant Professor Kasemsiri Chandarajoti, Ph. D.	Member

Advisors:

.....Advisor

(Naphatsorn Ditthawutthikul, Ph. D.)

.....Co-Advisor

(Assistant Professor Nuntawat Khat–udomkiri, Ph. D.)

Dean:

.....

(Associate Professor Wandee Rungseevijitprapa, Ph. D.)

ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my advisors, Dr. Naphatsorn Ditthawutthikul and Asst. Prof. Dr. Nuntawat Khat–udmokiri for their invaluable guidance, patience, kindness and encouragement throughout this journey. Their insightful advice and unwavering support have been instrumental in the completion of my thesis.

I am profoundly thankful to the faculty and staff of the School of Cosmetic Science at Mae Fah Luang University for providing me with the knowledge and resources necessary to pursue my research and my proposal and thesis defense committee for giving me valuable advice for my research.

I extend my heartfelt thanks to my lab colleagues and friends for their friendship, listening ears, camaraderie, and help that made this journey memorable.

To my parents, family and close relatives, I owe my deepest appreciation for their unconditional love and support, understanding, and encouragement. Their belief in me has been my greatest source of strength and their support is what made me able to walk along this path.

Finally, I am grateful to the funding and support of Mae Fah Luang University, and to everyone who directly or indirectly contributed to the realization of this work.

Thank you all for being a part of this long journey.

Khine Mon Yee Hlaing

Thesis Title Evaluation of Microwave–assisted Extraction of *Naringi crenulata* Leaves for Utilization in Skincare Essence

Author Khine Mon Yee Hlaing

Degree Master of Science (Creative Innovation in Cosmetic Science)

Advisor Naphatsorn Ditthawutthikul, Ph. D.

Co-Advisor Assistant Professor Nuntawat Khat-udomkiri, Ph. D.

ABSTRACT

Naringi crenulata, or Thanaka, is a medicinal plant possessing antioxidant, astringent, and anti-inflammation properties, making it a promising ingredient for cosmetic formulations. This study investigated the extraction of bioactive compounds from *N. crenulata* leaves, using microwave-assisted extraction with ethanol at varying concentrations (50%, 70%, and 95%). Bioactive compounds such as total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and astringent activity, antioxidant activity by DPPH radical scavenging, and the ferric reducing antioxidant power (FRAP) assays of the extracts were assessed using colorimetric assays.

Among the tested solvents, 50% ethanol achieved the highest extraction yield (16.81%) and maximized bioactive compound recovery (TPC 11.76 ± 0.38 GAE mg/g dry plant, TFC 19.35 ± 0.32 QE mg/g dry plant, 11.51 ± 0.07 TAE mg/g dry plant), along with maximized antioxidant properties (3.35 ± 0.04 AAE mg/g dry plant by DPPH and 3.57 ± 0.05 AAE mg/g dry plant by FRAP method) with IC_{50} value of 624.6 ± 28.95 µg/mL for DPPH radical scavenging activity. In contrast, 95% ethanol demonstrated the highest astringency 64.94 ± 0.13%. These findings highlight the critical importance of solvent polarity in enhancing the extraction of phenolics, flavonoids, and tannins.

The 50% ethanol extract was selected to further formulate into cosmetic formulation because it has the highest antioxidant potential and its integration into essence cosmetic formulations were assessed through heating-cooling cycles and one month of storage at different temperatures (room temperature, 4°C, and 45°C).

This study emphasized the overlooked potential of *N. crenulata* leaves within the field of cosmetic science. Future research may concentrate on techniques to improve stability, evaluate clinical effectiveness, and expand its cosmetic uses.

Keywords:*Naringi crenulata*, Antioxidant, Microwave-assisted Extraction, Astringent

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

ROS	Reactive oxygen species
TPC	Total phenolic content
TFC	Total flavonoid content
TTC	Total tannin content
OH	Hydroxyl group
RNS	Reactive nitrogen species
XO	Xanthine oxidase
SPF	Sun protection factor
UV	Ultraviolet
MAE	Microwave–assisted extraction
HPLC	High–Performance Liquid Chromatography
DAD	Diode Array Detection
MS	Mass Spectrometry
DPPH	2,2–diphenyl–1–picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
W	Watt
°C	Degree Celsius
mg	Milligram
mL	Milliliter
DI	Deionized
µL	Microliter
N	Normality
w/w	Weight by weight
nm	Nanometer
GAE	Gallic acid equivalents
QE	Quercetin equivalents
TAE	Tannic acid equivalents
µM	Micromolar
TPTZ	2,4,6–tri[2–pyridyl]–s–triazine

ABBREVIATIONS AND SYMBOLS

AAE	Ascorbic acid equivalents
rpm	Revolutions per minute
qs	quantum satis (quantity sufficient)
L*	Lightness
a*	redness–greenness
b*	yellowness–blueness
mPa.s	millipascal–second
SD	Standard deviation
ABTS	2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
UAE	Ultrasonic–assisted extraction
EC_{50}	Efficient concentration 50%
IC_{50}	Inhibitory concentration 50%
RSA%	Radical scavenging activity percentage
™	Trademark

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Recent years have witnessed a notable increase in global demand for natural and plant-based ingredients in cosmetics, influenced by heightened consumer awareness, health consciousness, and environmental concerns. This shift indicates a wider societal trend towards sustainability and wellness, as consumers are progressively pursuing products that correspond with their values. Research suggests that this demand represents a significant and enduring change in consumer behavior. A survey conducted in the Middle East indicated that 56.4% of respondents favored cosmetics with natural ingredients, while 48.1% expressed a willingness to pay a premium for these products (Kaliyadan et al., 2020). The global market for natural cosmetics is projected to grow, especially in the Asia-Pacific region, despite challenges related to perceived efficacy, pricing, and product diversity. This growth is driven by increasing awareness and demand for organic products (Meliniasari, 2024).

The significance of bioactive compounds derived from plant extracts, especially antioxidants, in promoting skin health and mitigating aging is gaining acknowledgement in scientific studies and cosmetic practices. Antioxidants are essential in reducing oxidative stress, which occurs when there is an imbalance between the body's capacity to neutralize reactive oxygen species (ROS) and their buildup. Oxidative stress significantly contributes to skin ageing by accelerating the degradation of collagen and elastin, essential for preserving skin elasticity and firmness (Buranasudja et al., 2023; Grenier et al., 2021). Oxidative stress is associated with cellular damage related to multiple diseases, such as cancer, neurodegenerative disorders, and cardiovascular conditions (Sen & Chakraborty, 2011).

Microwave-assisted extraction (MAE) is a technique that employs microwave energy to improve the efficiency of extracting bioactive compounds from plant materials. This method has become widely popular for its capacity to markedly

decrease extraction time and solvent consumption, while maintaining or enhancing the yield and quality of the extracted compounds (Gallo et al., 2010). Microwave-assisted extraction (MAE) represents a novel approach for the extraction of bioactive and nutraceuticals, demonstrating increased efficiency by necessitating reduced time and energy compared to conventional methods, while preserving the functionality of bioactive compounds throughout the extraction process (Chaari et al., 2024). The reduced processing time and energy efficiency of MAE lead to decreased manufacturing costs, enhanced product yields, and superior, consistent product quality relative to traditional methods (Kanthraj et al., 2024;Chaari et al., 2024)

The Thanaka tree (*N. crenulata*), belonging to the Rutaceae family, is indigenous to Southeast Asia, specifically Myanmar, Thailand, and India. The tree attains a height of up to 10 meters, characterized by gnarled branches, small fragrant white or yellowish flowers, and simple alternate leaves. The fruit is a small, round berry that is green when unripe and yellow upon ripening (Aung et al., 2024; Seiverling et al., 2017). Thanaka is commonly utilized as a natural sunscreen and skin protector, attributed to its cooling properties and efficacy in preventing sunburn, acne, and various skin conditions. *N. crenulata* has applications in traditional medicine beyond its cosmetic uses. The leaves are utilized for the treatment of epilepsy, the roots serve to alleviate pain, vomiting, and dysentery, while the stem powder is employed for anti-acne and anti-aging applications. The bark is utilized for the treatment of puerperal fever, while a decoction of the fruit acts as an antidote for insect toxicity and a remedy for intestinal worms (Aung et al., 2024).

The medicinal properties of *N. crenulata* leaves arise from its variety of bioactive compounds, such as flavonoids, phenolic acids, tannins, and alkaloids, which demonstrate significant antioxidant, anti-inflammatory, and antimicrobial activities (Latha et al., 2005; Pratheeba et al., 2019; Sampathkumar et al., 2012). Quercetin, a prominent flavonoid derived from leaves, exhibits notable antioxidant and antitumor effects by suppressing the proliferation and migration of cancer cells (Singh & Patni, 2018). The leaf extract demonstrates significant antimicrobial efficacy, attributed to the synergistic effects of its bioactive constituents (Latha et al., 2005). The antioxidant properties of *N. crenulata* leaf extract have been validated, demonstrating significance for cosmetic applications by combating oxidative stress (Sarada et al., 2012). The anti-

inflammatory and therapeutic effects, attributed to the presence of alkaloids and tannins, substantiate its traditional medicinal application in addressing various ailments (Pratheeba et al., 2019; Sampathkumar et al., 2012). Topical formulations with leaf extract have demonstrated effective wound healing in experimental models (Bhuvaneswari et al., 2014). The findings indicate that *N. crenulata* leaves may serve as a significant resource for the formulation of cosmetic products designed for skin repair and protection.

The maturation period for *N. crenulata* to achieve readiness for sustainable bark harvesting typically ranges from 5 to 7 years. While the leaves are traditionally used for medicinal purposes, they are often overlooked and discarded, leaving their potential for cosmetic applications largely unexploited. Despite the documented antioxidant, anti-inflammatory, and antimicrobial properties of *N. crenulata*, there is a significant gap in research on its use in cosmetic formulations, particularly concerning formulation development and stability. Most existing studies focus on the plant's bioactive components and their health benefits, but few explore their incorporation into skincare products. Furthermore, there is limited data on the interactions between its bioactive constituents and other cosmetic ingredients, as well as their stability under varying environmental conditions (e.g., heat, light, and pH). This lack of research hinders a comprehensive understanding of *N. crenulata*'s feasibility as a reliable ingredient in commercial skincare products. Addressing these gaps could provide valuable insights for the cosmetic industry and foster the development of innovative products utilizing this underutilized plant resource.

1.2 Objectives of the study

1.2.1 To analyze the bioactive compounds composition (TPC, TFC, TTC) of *N. crenulata* (Thanakha) leaf extract using different concentrations of ethanol solvent by using microwave extraction.

1.2.2 To examine the in vitro antioxidant and astringent characteristics of *N. crenulata* leaf extract.

1.2.3 To examine the stability of *N. crenulata* leaf extract through heating-cooling cycles and short-term stability tests.

1.2.4 Assess the stability and efficacy and compatibility of the leaf extract in a formulated cosmetic product.

1.3 Scope of the Study

This study examines the development and efficacy assessment of *N. crenulata* (Thanakha) leaf extract for cosmetic applications, particularly regarding its bioactive compounds, antioxidant, astringent, and stability characteristics. This research encompasses:

Microwave-Assisted Extraction: The investigation commences with the extraction of bioactive components from sundried *N. crenulata* leaves utilizing microwave-assisted extraction methods. This method is selected to improve the efficiency of extracting phenolic compounds.

Phytochemical Assessment: Following extraction, the total phenol, tannin, and flavonoid contents of the leaf extract are assessed. Furthermore, in vitro studies are performed to evaluate the antioxidant and astringent properties of the extract, enhancing its potential application in skincare products.

Formulation of Cosmetic Essence: The subsequent process entails the creation of cosmetic essence with *N. crenulata* leaf extract.

Stability Testing: The concluding portion of the investigation encompasses stability assessments to analyze the efficacy of the essence formulation under diverse situations. This includes heating-cooling cycles and short-term stability assessments to verify the formulation's effectiveness and shelf-life.

This research seeks to investigate the cosmetic potential of *N. crenulata* leaf extract, providing insights into its viability as a natural component in the skincare sector.

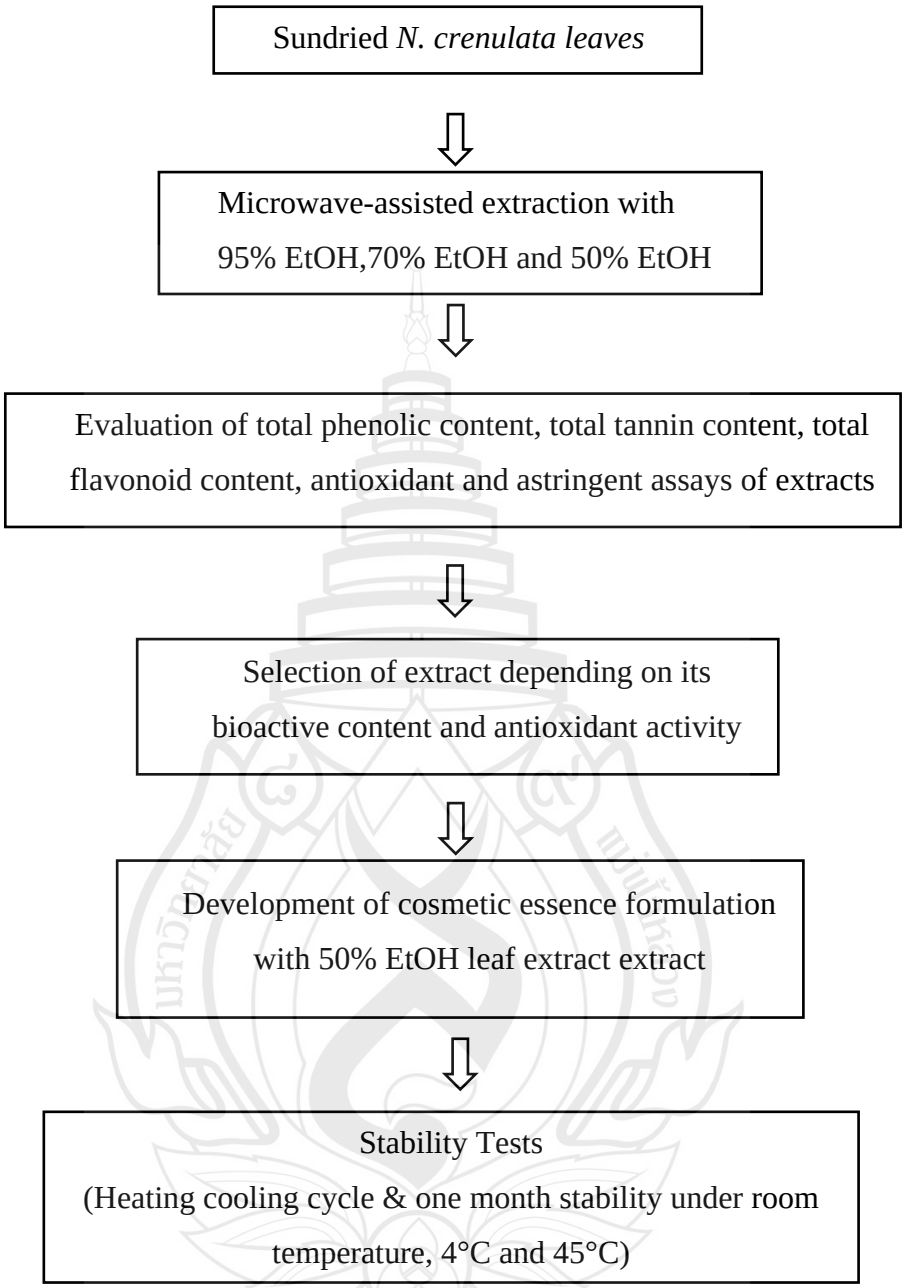


Figure 1.1 Scope of Study

CHAPTER 2

LITERATURE REVIEW

2.1 *Naringi crenulata*

N. crenulata, also known as *Hesperethusa crenulata* (Roxb.) M. Roem or *Limonia crenulata* Roxb, is commonly referred to as "Thanaka" in Myanmar and "belinggai" in Malaysia. It belongs to the Rutaceae family. This species is native to Southeast Asia, specifically in Myanmar, Thailand, India, Malaysia, Sri Lanka, and additional areas such as Java and Pakistan. The tree is spinous and small, can grow up to a height of approximately 8 to 12 meters. The tree features smooth, yellowish-grey bark, winged, imparipinnate leaves, and clusters of white flowers, along with oblong fruits that have a smooth black rind and red or purplish flesh. Thanaka typically grows in dry hilly regions and dry jungles, demonstrating a strong adaptation to these environments (Aung et al., 2024; Lim et al., 2021).

Thanaka powder, obtained by grinding the bark of the tree, possesses significant cultural and historical relevance in Myanmar. The bark powder exhibits a smooth texture and a yellowish hue, which holds both aesthetic appeal and cultural significance for Myanmar people. This natural product has served as a skincare and cosmetic agent for over two millennia, with its popularity historically linked to Queen Phantwar of Peikthano, a city from the Pyu dynasty. Thanaka powder is esteemed for its cosmetic and protective attributes, with a historical tradition among the Burmese as a natural remedy for improving skin health and appearance. Thanaka powder, traditionally used for its cooling and beautifying effects, is thought to exhibit antioxidant, anti-inflammatory, and photoprotective properties, positioning it as an attractive choice for cosmetic applications (Lim et al., 2021).

Source Pratheeba et al. (2019)

Figure 2.1 *N. crenulata* plant

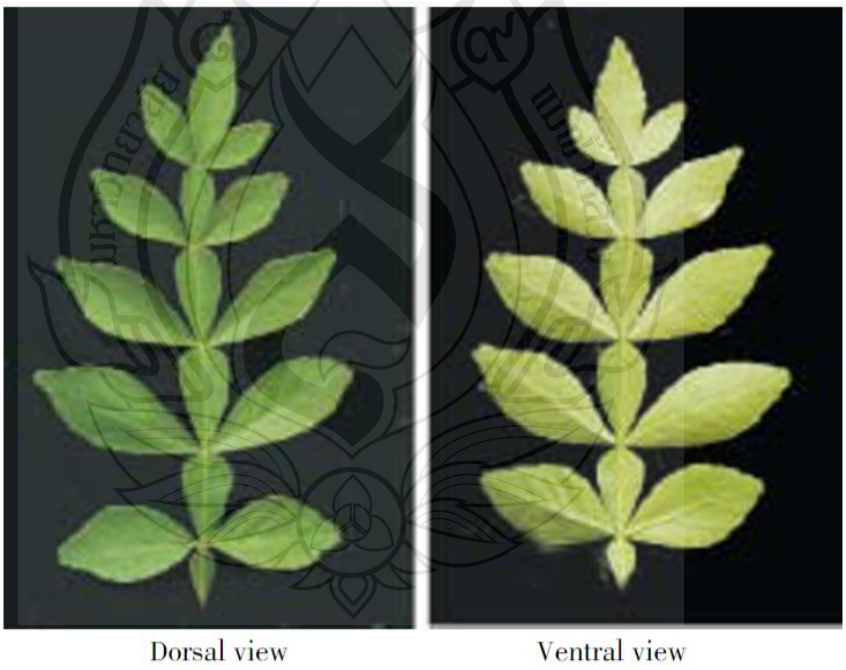
The *N. crenulata* plant is acknowledged in traditional medicine throughout Southeast Asia and has a documented history of therapeutic use for various tropical diseases (Sarada et al., 2012). Each component of the plant—roots, stem, bark, fruit, and leaves—provides distinct health benefits.

1. Roots: Historically employed to relieve symptoms associated with cobra bites, colic, bodily pain, vomiting, and dysentery (Sampathkumar et al., 2011).
2. Stem: Stem powder is utilized for its anti-acne and anti-aging properties, underscoring its potential in dermatological therapies (Sampathkumar et al., 2011).
3. Bark: The bark is recognized for its anti-inflammatory and antipyretic properties, utilized in the treatment of puerperal fever and pita (Sampathkumar et al., 2011). Bark juice is utilized externally for the prompt alleviation of sprains (Pratheeba et al., 2019).
4. Fruit: A decoction derived from the fruit is utilized as an antidote for

insect bites, to address intestinal worms, and for its anthelmintic properties (Sampathkumar et al., 2011).

5. Leaves: The aromatic leaves, related to other citrus varieties, serve as a remedy for digestive issues in children when combined with milk. Traditionally, they are utilized for the management of epilepsy (Latha et al., 2005).

The leaves of *N. crenulata* display notable macroscopic features. Initially, they exhibit a dark green hue, transitioning to a lighter green upon drying, accompanied by an aromatic fragrance. The leaves exhibit a pinnate structure, with lengths ranging from 2.5 to 10 cm, and possess sharp spines measuring between 1.2 and 2.5 cm. The petiole and rachis exhibit jointed structures, characterized by a narrowly winged petiole and a rachis with broad wings. Each leaf generally possesses 2–4 pairs of trapezoid-ovate leaflets, which may be obtuse or occasionally acute, featuring a notched (crenulate) tip, smooth (glabrous) surfaces, and cuneate bases. The joints of the rachis are characterized as obovate to oblong with a crenulate edge. (Latha et al., 2005).



Source Sampathkumar et al. (2012)

Figure 2.2 Leaf of *N. crenulata*

Several studies have explored the phytochemical composition of *N. crenulata*, highlighting its potential for therapeutic and cosmetic applications. Latha et al. (2005)

performed a preliminary phytochemical analysis of *N. crenulata* leaves, examining the extractive values across various solvents. Methanol was found to yield the highest extractive value (5.21%–5.65%), followed by ethanol (5.1%–5.25%), acetone (3.42%–3.92%), petroleum ether (3.1%–3.4%), chloroform (1.05%–1.25%), and benzene (0.5%–1.12%). The high extractive values in methanol and ethanol suggest that these solvents are optimal for isolating bioactive compounds, indicating their suitability for cosmetic and therapeutic formulations. The phytochemical composition of the ethanol extract was further investigated, revealing the presence of substantial quantities of alkaloids, phenols, carbohydrates, and proteins, each exhibiting a strong positive reaction (++). Moderate levels of tannins and amino acids were also identified, whereas flavonoids, steroids, saponins, and fixed oils and fats were absent (-). These findings underscore the plant's potential as a rich source of bioactive compounds, particularly alkaloids and phenols, known for their diverse biological properties (Latha et al., 2005).

Pratheeba et al. (2019) expanded on this analysis by examining methanolic extracts of *N. crenulata* and confirmed the presence of key phytoconstituents such as phenols, alkaloids, tannins, saponins, carbohydrates, quinones, and proteins. These findings are consistent with earlier reports, further validating the plant’s phytochemical richness.

Sampathkumar et al. (2012) conducted a qualitative phytochemical analysis of ethanol extracts from *N. crenulata* leaves using Soxhlet extraction. The analysis revealed the presence of several key bioactive compounds, including proteins, lipids, phenols, tannins, flavonoids, saponins, and quinones. Among these, phenols were found in the highest concentration (+++), followed by lipids (++), saponins (++), and moderate levels of tannins, flavonoids, and proteins (+). In contrast, carbohydrates, reducing sugars, triterpenoids, alkaloids, and anthraquinones were not detected. Further quantitative analysis of secondary metabolites showed that the leaf extract was particularly rich in phenols, with a content of 13.12% (w/w). Flavonoids (3.65%) and tannins (2.64%) were also present in significant amounts, along with notable levels of vitamins C (10.17%) and E (0.68%). The high phenolic content suggests potent antioxidant activity, which aligns with the plant’s traditional use in skin protection and anti-aging treatments. These secondary metabolites, especially phenols and flavonoids, are well-known for their roles in preventing oxidative stress and promoting skin health,

reinforcing the cosmetic potential of *N. crenulata* leaf extract (Sampathkumar et al., 2012).

Singh et al. (2017) employed Soxhlet extraction with 80% methanol to extract quercetin from *N. crenulata* leaves and callus. Using High-Performance Thin Layer Chromatography (HPTLC), they quantified the quercetin content and found that the leaf powder contained a significantly higher amount (0.13%) compared to the callus (0.032%). The high quercetin content in the leaves suggests a strong potential for synthesizing this bioactive flavonoid, known for its antioxidant and skin-enhancing properties. This makes *N. crenulata* leaves a promising candidate for inclusion in cosmetic formulations, particularly those targeting skin protection and rejuvenation.

In addition to its phytochemical properties, *N. crenulata* has been investigated for its hepatoprotective and antioxidant effects. Sarada et al. (2012) evaluated the ethanol extracts of *N. crenulata* leaves and bark for their ability to protect against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. The study revealed that the plant extracts improved liver function, as indicated by favorable changes in liver marker enzymes such as SGOT, SGPT, and ALP. Additionally, the extracts enhanced the levels of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRD), while reducing thiobarbituric acid reactive substances (TBARS). These results suggest that *N. crenulata* possesses strong hepatoprotective and antioxidant activities, with a protective efficacy comparable to that of silymarin, a well-known hepatoprotective drug.

In summary, the studies reviewed here collectively demonstrate the significant phytochemical, antioxidant, and hepatoprotective properties of *N. crenulata*. The high extractive values in methanol and ethanol, coupled with the plant's rich content of alkaloids, phenols, flavonoids, and quercetin, underscore its potential for therapeutic and cosmetic applications. The plant's demonstrated hepatoprotective effects and antioxidant activity further support its use in formulations aimed at protecting against oxidative stress and promoting skin health. These findings provide a strong basis for future research and development of *N. crenulata*-based products in cosmetic science.

2.2 Phenolic Compounds

Phenolic compounds are crucial secondary metabolites in plants, classified within the phenylpropanoid group, and are distinguished by their aromatic rings that possess hydroxyl (OH) groups. Compounds can be categorized according to the number and configuration of phenolic rings, as well as the nature of the radicals that connect these rings (Manach et al., 2005; Hussain et al., 2019). These compounds are essential for plant defense against pathogens, UV radiation, and herbivores, and they participate in various physiological processes within the plant (Ruesgas-Ramón et al., 2017). The variation in the distribution of phenolic compounds among plant species and tissues is significantly influenced by genetic, environmental, and developmental factors (Özcan et al., 2014).

Phenolic compounds are categorized into simple phenolics, polyphenols, and other phenolic compounds, as outlined by Al Mamari (2021).

1. Simple Phenolic Compounds:

    1) Simple Phenolics: Examples are catechol, resorcinol, and hydroquinone.

    2) Phenolic Acids: They consist of hydroxybenzoic acids, hydroxycinnamic acids, and coumarins.

2. Polyphenols:

    1) Flavonoids: represent a diverse category comprising six principal subtypes: flavanols, flavones, anthocyanins, flavanols, flavanones, and isoflavones.

    2) Tannins: characterized by their astringency and intricate polymeric structures.

3. Other Phenolic Compounds:

    Stilbenes, Lignans, and Lignins

Despite this diversity, phenolic compounds are generally categorized into two main groups: flavonoids and non-flavonoids. Flavonoids consist of heterocyclic oxygen atoms linked to two aromatic rings, with structural variations influenced by hydrogenation levels. Non-flavonoid phenolics, including cinnamic and benzoic acids,

generally possess an aromatic ring linked to organic acids. Lignins, stilbenes, and tannins are classified within the non-flavonoid category (Gutiérrez-Grijalva et al., 2016).

The bioactivities of phenolic compounds have attracted considerable research attention owing to their potential health benefits, including antimicrobial, neuroprotective, antioxidant, cardioprotective, anti-inflammatory, and anticancer properties (Hussain et al., 2019). They are crucial for flavor, astringency, and color, which are vital attributes for medicinal and cosmetic uses. Their effectiveness as free radical scavengers, which is crucial for antioxidant activity, is primarily influenced by chemical structure, the position and number of hydroxyl groups, and modifications like glycosylation (Cai et al., 2006).

Phenolic compounds demonstrate anti-inflammatory properties by reducing the production of reactive nitrogen species (RNS) and reactive oxygen species (ROS), both of which are associated with inflammation and activation of proinflammatory enzymes (Mizgier et al., 2016; Bowen-Forbes et al., 2010). These represent significant potential in anti-inflammatory therapies. Natural phenolic derivatives have been utilized in pharmaceuticals as essential compounds for anticancer drugs due to their proven effectiveness in disease prevention and health protection (Rubió et al., 2013; Gordaliza & Marina, 2007). Phenolics function as crucial bioactive agents, applicable in antimicrobial, anti-tumoral, hepatoprotective, and antioxidant therapies.

Phenolic compounds are essential in protecting the skin against UV damage, a major factor in photoaging. Certain phenolic compounds absorb UV radiation due to chromophores in their molecular structure, functioning as natural UV filters (Butkevičiūtė et al., 2022). This property is significant in sunscreen formulations, as phenolic compounds can improve protection against harmful UV rays. Studies demonstrate that phenolic compounds inhibit UV-induced damage and enhance skin repair processes, thereby reinforcing their application in cosmetic formulations designed to minimize photoaging (Hammami et al., 2023).

Phenolic compounds enhance skin moisture and elasticity, contributing to anti-aging effects. Ferulic acid and its derivatives enhance collagen synthesis and improve skin moisture retention, contributing to their popularity as anti-wrinkle agents. Phenolic compounds engage with skin proteins and lipids, facilitating moisturization and

diminishing trans epidermal water loss (Nascimento et al., 2021). Phenolic chemicals are essential in cosmetic formulations aimed at aging skin due to their beneficial properties.

2.3 Flavonoids

Flavonoids represent a significant category of plant-derived phenolic compounds characterized by a structure consisting of two aromatic rings linked by a three-carbon chain. They are extensively found in fruits, vegetables, herbs, cereals, nuts, flowers, and seeds (Santos et al., 2017). This group of plant phenols has been extensively studied and is recognized for its therapeutic benefits, especially its antioxidant, anti-inflammatory, and antimicrobial properties. Flavonoids are important components in multiple domains, particularly in cosmetics, due to their role in enhancing skin health and appearance (Gaweł-Bęben et al., 2015; Cefali et al., 2019).

Flavonoids are classified based on structural modifications, leading to several important subclasses:

Flavonols: Compounds like quercetin, offer strong antioxidant and anti-inflammatory effects.

Flavones: Apigenin and luteolin in this group show anti-aging and skin-protective properties.

Flavanones: Examples like hesperidin are studied for skin-whitening benefits.

Flavan-3-ols: Known for their UV protection and radical-scavenging properties, catechins are prominent in this class.

Anthocyanins: These are responsible for the pigmentation in fruits and flowers, adding antioxidant benefits (Chen et al., 2023).

The stability and arrangement of hydroxyl groups in flavonoids significantly influence their antioxidant properties. The primary mechanisms involve the direct scavenging of reactive oxygen species (ROS), the activation of antioxidant enzymes, and the inhibition of oxidase enzymes responsible for generating harmful free radicals. Flavonoids inhibit xanthine oxidase (XO) and nitric oxide synthase, thereby reducing

oxidative stress on skin cells. This action is crucial for maintaining skin health and preventing premature aging (Engwa & Godwill, 2018; Zeng et al., 2019).

Flavonoids exhibit notable anti-inflammatory properties. Compounds such as apigenin and quercetin decrease inflammation by inhibiting inflammatory molecules and increasing levels of IL-10, a natural anti-inflammatory agent. They protect cells from damage by inhibiting enzymes like lipoxygenase, which are involved in inflammation. Furthermore, specific flavonoids diminish the synthesis of inflammatory molecules and safeguard cellular health, particularly in the eyes during oxidative stress (Chen et al., 2023).

In cosmetic science, flavonoids are noted for their antioxidant and soothing effects. Their applications focus on:

Sun Protection: Flavonoids facilitate the absorption of UV light and mitigate oxidative stress caused by sunlight, thereby offering a natural sunscreen effect. Linarin derived from *Buddleja cardioides* exhibits a sun protection factor (SPF) of 9, whereas quercetin and rutin from *Moringa oleifera* demonstrate an SPF value of 2 (Saewan et al., 2013; Mottaghipisheh et al., 2021; Ali et al., 2013).

Anti-Aging: Flavonoids play a crucial role in safeguarding the skin from signs of aging. A cream containing 3% taxifolin demonstrated enhancements in skin elasticity and penetration rate, while flavonoid-rich extracts from *Nymphaea lotus* are commonly utilized in self-care products for their skin benefits (Bjørklund et al., 2022; Micek et al., 2021; Tran et al., 2022).

Anti-Inflammatory: Flavonoids inhibit inflammatory enzymes such as phospholipase and cyclooxygenase, thereby regulating immune responses and cytokine production, which contributes to their efficacy in reducing skin inflammation (Yahfoufi et al., 2018).

In summary, the multifunctional properties of flavonoids, notably their antioxidant, anti-inflammatory, and sun-protective effects, establish them as essential bioactive compounds in cosmetics designed to improve skin health, appearance, and resilience.

2.4 Tannins

Tannins are naturally occurring polyphenolic compounds in plants, primarily known for their capacity to bind proteins, pigments, and other macromolecules. Significant antioxidant activity is also exhibited (Melone et al., 2013). Tannins are widely distributed across various plants and food sources, including fruits like grapes, blueberries, as well as tea, coffee, leguminous forages such as trefoil, legumes, and specific trees and grasses, including *Acacia* spp., *Sesbania* spp., sorghum, and corn. Tannins, as secondary metabolites, are synthesized by plants in response to stress and serve protective functions, including defense against UV radiation, free radicals, and deterrence of harmful organisms and environmental stressors such as dryness (Fraga-Corral et al., 2021).

The antioxidant properties of tannins are directly associated with their phenolic-rich chemical structure. The phenolic rings enable tannins to bind diverse molecules and function as electron scavengers, facilitating the neutralization of ions and radicals (Fraga-Corral et al., 2021; Vuolo et al., 2019). The capacity of tannins to scavenge various free radicals and inhibit lipid peroxidation is notably augmented under conditions of cellular stress, which frequently leads to elevated concentrations in plants (de Hoyos-Martínez et al., 2019).

Tannins are traditionally categorized into two primary types: hydrolysable tannins and condensed tannins, the latter commonly known as proanthocyanidins. Contemporary classifications have evolved to incorporate structural properties and chemical characteristics, categorizing tannins into five distinct groups:

Gallotannins and ellagitannins: Hydrolysable tannins predominantly located in fruits, berries, legumes, leafy vegetables, and various tree species (Shirmohammadli et al., 2018; Shahidi et al., 2015). Tannins possess antioxidant and antimicrobial properties and are frequently employed in industries like leather production.

Condensed tannins: also known as proanthocyanidins, constitute more than 90% of global tannin production and are generally characterized as polymeric or oligomeric flavan-3-ols. Upon heating in acidic ethanol, they decompose into anthocyanidins (Macáková et al., 2014).

Complex tannins: High molecular weight compounds formed through the bonding of flavan-3-ols with either gallotannins or ellagitannins. Sources of these tannins include tree species such as *Quercus* spp. and *Castanea sativa* (Okuda et al., 2011).

Phlorotannins: present solely in brown marine algae, can constitute up to 30% of the algae's dry weight and are recognized for their antimicrobial, photoprotective, and antioxidant properties (Erpel et al., 2020; Salminen et al., 2011).

Tannins have garnered considerable interest due to their various biological effects, such as antioxidants, anti-inflammatory, antimicrobial, anti-diabetic, and cardioprotective activities (Fraga-Corral et al., 2020; Serrano et al., 2009). Their properties have facilitated applications in the food, nutraceutical, and pharmaceutical industries, where they enhance product safety, extend shelf life, and function as natural preservatives or clarification agents in beverages (Huang et al., 2018; Sharma et al., 2021). Furthermore, certain tannins, including tannic acid, have shown significant anti-aging properties by mitigating UV-induced oxidative damage in fibroblast cell models, indicating their potential utility in skin care applications (Fraga-Corral et al., 2021).

Tannins possess beneficial properties that include antioxidant, antimicrobial, and anti-inflammatory effects, astringency leading to their expanded application across multiple industries. Their use as food additives or clarifying agents demonstrates potential for improving product safety and quality. Their toxic effects have been assessed and continue to be a subject of research, particularly regarding the determination of safe levels for human consumption (Barbehenn et al., 2011). Tannins and tannin-rich extracts demonstrate efficacy in in vitro studies, animal models, and human clinical trials, underscoring their versatility and therapeutic potential (Smeriglio et al., 2017).

2.5 Microwave Extraction

Microwave-assisted extraction (MAE) is a technique that utilizes microwave energy to improve the extraction efficiency of bioactive compounds from plant materials. The method has become popular for its effectiveness in decreasing extraction

time and solvent usage, while preserving or enhancing the yield and quality of extracted compounds (Gallo et al., 2010). The principle of MAE involves the swift heating of both the solvent and the plant matrix, thereby promoting the extraction of target compounds into the solvent. The localized temperature and pressure produced by microwaves facilitate the rapid migration of target compounds, surpassing the efficiency of traditional extraction methods like Soxhlet extraction or maceration, which require several hours or days (Li & Jiang, 2010; Sampaopan & Suksaeree, 2022).

MAE offers multiple advantages. The primary benefits include:

Reduction in Extraction Time: In contrast to conventional techniques that often necessitate prolonged processing durations, MAE can attain similar or superior yields in a matter of minutes, thereby markedly improving laboratory efficiency (Sampaopan & Suksaeree, 2022).

Lower Solvent and Energy Consumption: MAE allows the utilization of reduced solvent volumes, rendering it a more sustainable approach. The decrease in solvent utilization minimizes waste, simplifies post-extraction procedures, and lessens environmental impact (Périno-Issartier et al., 2010).

Higher Extraction Yield: MAE has shown superior yields of bioactive compounds relative to traditional methods, especially for compounds exhibiting antioxidant properties. MAE has demonstrated superior extraction of antioxidant activity from specific plant extracts compared to ultrasound-assisted extraction, enhancing the recovery of valuable constituents including flavonoids, tannins, and essential oils (Villa et al., 2022; Gallo et al., 2010).

Suitability for Thermolabile Compounds: MAE offers a significant advantage through its capacity to quickly heat plant material and solvent, thereby reducing the duration that sensitive compounds undergo exposure to elevated temperatures. This is particularly advantageous for thermolabile compounds, including flavonoids and essential oils, which are susceptible to degradation when exposed to prolonged heat in conventional methods. MAE maintains the structural integrity and biological activity of these compounds, resulting in a higher quality extract appropriate for health and cosmetic applications (Álvarez et al., 2017).

Scalability and Reproducibility: MAE provides reproducibility and enables the simultaneous extraction of multiple samples, rendering it suitable for high-throughput

applications. The rapid process and user-friendliness enhance efficiency and yield high purity in the final product, which is especially beneficial for commercial and cosmetic applications (Li & Jiang, 2010).

Although MAE demonstrates significant effectiveness, it is not without limitations. Elevated microwave intensity can lead to excessively high extraction temperatures, potentially resulting in the oxidation or degradation of specific compounds and a reduction in extract quality. It is crucial to optimize microwave intensity and exposure duration to prevent these problems, particularly when dealing with heat-sensitive compounds (Sampaopan & Suksaeree, 2022).

MAE demonstrates efficiency and environmental advantages, including decreased solvent and energy usage, establishing it as a sustainable alternative to traditional extraction methods. The environmentally friendly extraction process, along with minimized waste and enhanced safety, renders MAE an effective technique for generating bioactive plant extracts, especially for applications in cosmetics and pharmaceuticals where the stability and purity of compounds are essential (Gallo et al., 2010; Li & Jiang, 2010).

2.6 Analysis of Phenolic Compounds

The analysis of phenolic compounds is crucial for comprehending the efficacy and stability of cosmetic formulations. Standard methods for analyzing phenolic compounds include spectrophotometric assays, which yield an overview of total phenolic content, and advanced chromatographic techniques such as High-Performance Liquid Chromatography (HPLC), which facilitate accurate identification and quantification of individual phenolic constituents. Analytical techniques are essential for the formulation and evaluation of cosmetic products, guaranteeing their efficacy and safety.

2.6.1 Determination of Total Flavonoid Content

Quantitative analysis of flavonoids is essential for understanding their biological activities and potential health benefits. Various methods have been developed for this purpose.

The Aluminum Chloride Colorimetric Method is a popular technique for flavonoid quantification, noted for its simplicity, cost-effectiveness, and rapid results. This method utilizes the formation of a complex between aluminum chloride ($AlCl_3$) and flavonoid compounds in an acidic environment. Aluminum ions interact with the adjacent hydroxyl groups of flavonoids, resulting in a bathochromic shift (shift to longer wavelengths) and a hyperchromic effect (increased absorbance) in the UV-Vis spectrum, which can be quantified spectrophotometrically at a designated wavelength (Fernandes et al., 2012). The color intensity of the complex is directly related to flavonoid concentration, making this method particularly effective for quantifying flavonols and flavones because of their structural compatibility with $AlCl_3$ (Hudz et al., 2023). This technique is commonly used for routine analysis; however, it is constrained by possible interference from other phenolic compounds that may interact with $AlCl_3$. High-Performance Liquid Chromatography (HPLC) is a well-established technique for flavonoid analysis, recognized for its specificity and sensitivity, particularly in the analysis of complex mixtures. High-Performance Liquid Chromatography (HPLC) effectively separates individual flavonoid compounds according to their chemical properties, facilitating precise profiling and quantification. The integration of detectors like Diode Array Detection (DAD) or Mass Spectrometry (MS) with HPLC significantly improves the identification and quantification of flavonoids (Zhang et al., 2017; Guo et al., 2016). Zhang et al. (2017) demonstrated the effectiveness of HPLC-DAD in profiling flavonoids in food matrices, highlighting its significance in quality control and standardization. Despite its advantages, HPLC is resource-intensive, necessitating considerable time for method development, calibration, and validation. Co-extracted impurities may hinder the detection of minor flavonoid compounds; however, this issue can frequently be addressed through pre-treatment or the optimization of chromatographic conditions (Nolvachai & Marriott, 2012; Kalili & Villiers, 2011).

Both the aluminum chloride colorimetric method and HPLC are effective techniques for flavonoid quantification, each appropriate for different analytical requirements. The aluminum chloride method provides an efficient and economical option for the routine analysis of particular flavonoid classes. In contrast, HPLC

delivers enhanced specificity and a detailed profile for more complex samples, though it incurs higher costs and requires more preparation time.

2.6.2 Determination of Total Tannin Content

Measuring tannins in plant extracts is crucial for assessing their effectiveness in diverse applications, such as cosmetics, owing to their antioxidant, anti-inflammatory, and astringent characteristics. Various analytical techniques are employed, each possessing distinct advantages and limitations in the measurement of tannin concentration and structure.

The Folin-Ciocalteau Colorimetric Method is a prevalent technique for quantifying total tannins, encompassing both monomeric and polymeric forms, owing to its simplicity, speed, and cost-effectiveness (Adegbusi et al., 2022; Lima et al., 2012). This method, approved by the Association of Official Analytical Chemists (AOAC), utilizes redox reactions between tannins and the Folin-Ciocalteau reagent, resulting in a quantifiable color change measured through UV-Vis spectrophotometry (Hung et al., 2010). This method effectively captures overall tannin content; however, it is susceptible to interference from other reducing agents, potentially resulting in overestimated values (Hung et al., 2010).

High-Performance Liquid Chromatography (HPLC) is an advanced method utilized for the separation, identification, and quantification of individual tannin compounds. This method is particularly effective for the analysis of complex tannin mixtures, as it separates compounds according to their molecular properties, including polarity and size (Wigley et al., 2020; Sanz et al., 2010). HPLC can be integrated with mass spectrometry (HPLC-MS), improving sensitivity and specificity in the profiling of tannins. HPLC is recognized as the gold standard for tannin analysis due to its capacity to deliver detailed compositional information, thereby providing insights into tannin structure and concentration (Wigley et al., 2020; Sanz et al., 2010).

The Protein Precipitation Assay utilizes the capacity of tannins to bind and precipitate proteins, rendering it effective for the detection of both condensed and hydrolysable tannins. This assay involves mixing a tannin solution with a protein solution, such as bovine serum albumin, to estimate tannin levels based on the degree of precipitate formation (Wigley et al., 2020). A variation of this assay, the radial diffusion method, quantifies tannin concentration by assessing clear zones in agarose

gels resulting from the precipitation of proteins by tannins (Siqueira et al., 2012; Graça & Bärlocher, 2020). This method is especially effective for identifying tannins that exhibit a strong affinity for protein binding.

The Vanillin Assay is a colorimetric technique used to quantify condensed tannins, including proanthocyanidins. In this method, tannins react with vanillin and hydrochloric acid, resulting in the formation of a measurable color complex (Amr et al., 2020). The vanillin method, while historically popular, may result in an overestimation of tannin concentration due to interference from non-tannin phenolic compounds and related flavonoids, impacting reproducibility (Dentinho et al., 2018). This method has been largely supplanted by more reliable techniques, such as the acid-butanol assay, to mitigate these limitations.

The Acid-Butanol Assay is a favored technique for the precise quantification of condensed tannins, particularly proanthocyanidins, owing to its specificity. This method employs an acid-catalyzed reaction in butanol to generate colored anthocyanidins, which are subsequently quantified using spectrophotometry. The acid-butanol assay offers enhanced precision in quantifying condensed tannins compared to the vanillin assay, particularly in the analysis of tannins within complex plant matrices (Shay et al., 2017; Grabber et al., 2013).

In conclusion, the selection of a method for tannin quantification is based upon the sample matrix, required specificity, and possible interference. Colorimetric methods such as Folin-Ciocalteau and acid-butanol assays are effective for general tannin quantification; however, HPLC is preferable for detailed compositional analysis.

2.7 Evaluation of *in vitro* Antioxidant Activity

Assessing the antioxidant potential of natural compounds is essential for determining their health and cosmetic benefits, especially when sourced from botanical origins. In vitro methods are commonly utilized in this process, enabling controlled and reproducible analysis of the antioxidant activity of different compounds. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and the Ferric Reducing

Antioxidant Power (FRAP) assay are among the most employed methods, noted for their efficacy and ease of use.

The DPPH assay is favored for its simplicity and effectiveness in measuring the radical scavenging capacity of antioxidants. This assay is based on the principle that antioxidants can donate hydrogen atoms to the DPPH radical, resulting in a color change from deep purple to yellow. The DPPH radical reduction is quantifiable spectrophotometrically at 517 nm, enabling a clear assessment of radical-scavenging capacity (Salah et al., 2015). The DPPH assay is favored in diverse research settings due to its simplicity and minimal equipment needs, facilitating quick evaluations of antioxidant activity (Wei & Shibamoto, 2010). Nonetheless, the DPPH assay has limitations; colored compounds in samples may interfere with results, and there is a risk of overestimating antioxidant capacity due to low molecular weight compounds that can react with DPPH without providing genuine antioxidant protection (Dhanira et al., 2020).

The FRAP assay, alongside DPPH, is commonly employed to evaluate antioxidant capacity by measuring a compound's ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The reduction produces a colored complex with 2,4,6-tripyridyl-s-triazine (TPTZ), measurable spectrophotometrically at 593 nm (Henderson et al., 2015). The FRAP assay quantifies electron-donating capacity, which is essential for antioxidant function (Rothe et al., 2023). This assay is especially significant for its use in samples containing water-soluble antioxidants, although it may not comprehensively assess the activity of lipid-soluble antioxidants.

To achieve a thorough assessment of antioxidant capacity, researchers frequently employ the FRAP assay alongside DPPH. Each assay provides distinct insights into antioxidant behavior, with DPPH measuring free radical scavenging activity and FRAP evaluating the capacity to reduce metals (Mo et al., 2012). The integration of these methods facilitates a comprehensive assessment of a sample's antioxidant potential, addressing the strengths and limitations associated with each assay type.

2.8 Analysis of *in vitro* Astringent Activity

The analysis of *in vitro* astringent activity is essential for understanding the mechanisms behind astringency and its implications in various industries, particularly in food and cosmetics.

The perception of astringency is closely associated with the interactions of tannins, especially with proteins and polysaccharides. Numerous chemical methods employed in the wine industry for measuring astringency are based on the interactions between tannins and proteins or polysaccharides, resulting precipitation of complexes serving as a correlation for sensory perception of astringency.

The protein precipitation assay is a widely utilized method for assessing astringency. This method measures the interaction between tannins and proteins by assessing the capacity of tannins to denature proteins, leading to the formation of protein-tannin precipitates. This method accurately represents perceived astringency, as the degree of precipitation is associated with the intensity of the astringent sensation (Watrelot, 2021).

Salivary protein assays have been invented to evaluate individual differences in astringency perception. The salivary protein difference value (SP D-value) quantifies the replenishment of salivary proteins following exposure to astringent compounds, offering insights into the effects of astringency on oral lubrication (Fleming et al., 2016). This method provides a physiological perspective on astringency perception; however, its practicality for routine analysis is limited by individual variations in salivary protein composition and output.

Another commonly employed method is the turbidity assay, which quantifies the cloudiness of a solution resulting from the interaction between astringent compounds, such as tannins, and proteins or mucins. Tannins bind to proteins, resulting in the formation of aggregates that scatter light and increase turbidity. Research indicates a strong correlation between turbidity and sensory perceptions of astringency, with increased turbidity typically signifying more pronounced astringent interactions. It is also demonstrated that turbidity from polyphenol and mucin

interactions correlates with the perceived astringency of tannic acid, with peak turbidity levels coinciding with maximum astringency perception. (Kelly et al., 2010)

The correlation between antioxidant activity and astringency is significantly affected by the presence of polyphenolic compounds, especially tannins. Tannins serve a dual function, influencing both characteristics. Research indicates a significant correlation between astringency and antioxidant activity in various plant-based sources, including food and herbal extracts. Studies on tannin-rich food items, including nuts and seeds, have shown a statistically significant relationship between the Sensation of Astringency Index (SAI) and Total Antioxidant Activity (TAA) (Amarowicz et al., 2008). A positive correlation was also noted in legume seeds between antioxidant activity and both total phenolic and tannin content, which were also closely associated with astringency levels (Amarowicz et al., 2004).

CHAPTER 3

METHODOLOGY

3.1 Chemicals

Table 3.1 Chemicals

Chemicals	Company
2,2-diphenyl–1–picrylhydrazyl (DPPH)	Sigma, USA
2,4,6–Tri(2–pyridyl)–s–triazine (TPTZ)	Sigma, USA
Absolute ethanol	RCI Labscan, Thailand
Aluminum Chloride	Loba Chemie, India
Folin-Ciocalteu Reagent	Sigma-Aldrich, USA
Gallic acid	Sigma, USA
Hydrochloric acid	Fisher Che, USA
Iron (III)chloride	Ajax Finechem, Australia
Sodium nitrite	Loba Chemie, India
Tannic acid	Himedia, India
Quercetin	Sigma, USA
Sodium acetate	Ajax Finechem, Australia
Sodium carbonate	Loba Chemie, India
Sodium hydroxide	Ajax Finechem, Australia
Swine hemoglobin	Sigma-Aldrich, USA
Ascorbic acid	Ajax Finechem, Australia
Glycerin	S. Tong Chemicals Co., Ltd., Thailand
Butylene Glycol	KH Neochem Co., Ltd., Japan
Emogel Oil™	Suzhou Eleco Chemical Industrial, China
Liquid germall™ plus	S. Tong Chemicals Co., Ltd., Thailand
Hydroxy ethyl cellulose	Alfa Chemical, China
Colem TT™	Suzhou Eleco Chemical Industrial, China

3.2 Instruments

Table 3.2 Instruments

Instruments	Model	Company
2–Digital balance	Pioneer	Ohaus, USA
4–Digital balance	SI–234	Denver, USA
Automatic pipette	Biopett	Labnet, USA
Blender	EM–ICE	Sharp, Japan
Colorimeter	Ultra scan VIS	Hunter Lab, USA
Hot air oven	UM 500, UFE600	Memmert, Germany
Microplate reader	SPECTROstar Nano	BMG–LABTECH, Germany
Microcentrifuge	Spectrafuge 16M	Labnet, USA
Moisture analyzer	Ohaus	MB45, USA
Microwave Extraction	ETHOS™ X	Milestone, Italy
pH meter	pH–200L	Istek, South Korea
Refrigerator	SJ–D24N–SLG	Eutech. Singapore
Rheometer	HAAKE RotoVisco 1	ThermoFisher Scientific, USA
Rotary Evaporator	Hei-VAP Expert	Heidolph, Germany
Vortex Mixer	Vortex-Genie 2	Scientific Industries, USA
Programmable Temperature and Humidity Tester	GT-7005-A	Gotech Testing Machines Inc., Taiwan

3.3 *N. crenulata* Leaf Preparation

The *N. crenulata* leaves were obtained from Mae Sai District, Chiang Rai during May 2024. The leaves were sun-dried, and their moisture content was carefully monitored to maintain minimal residual moisture before storage. Specimen identification was verified by a botanist at Mahidol University Plant Museum (Voucher

number PBM 006410 – 006412). The dried leaves were then grinded into powder and stored in a cool and dry environment until further use.

3.4 Microwave Extraction

The bioactive compounds from the dried *N. crenulata* leaves were extracted using a microwave-assisted method in accordance with the technique outlined by Gunalan (2023). In an ETHOS™ X microwave extraction system, the leaf powder was extracted using ethanol at variable concentrations (50%, 70%, and 95%). The leaf powder-to-alcohol ratio was 1:10 weight by weight, and the extraction was conducted at 600W power and 40°C for 30 minutes. To ensure consistency, a magnetic stirrer was put in each chamber. The extract solution was filtered through Whatman® No.1 filter paper following extraction. The filtrates were subsequently concentrated using a rotary evaporator under controlled temperature and pressure (50°C and 75mbar). The concentrated extracts were maintained at 4°C in sealed vessels until they were subjected to analysis.

3.5 Determination of Total Phenolic Content

The colorimetric assay was implemented on a microplate reader in accordance with Johnson's (2022) methodology to determine the total phenolic content. The extracts were thoroughly mixed using a vortex mixer after being diluted to a concentration of 1 mg/mL. Gallic acid was the preferred standard, which was diluted with deionized (DI) water. A 96-well microplate was used, 20 µL of the sample was put in each well, followed by 100 µL of 0.2 N Folin-Ciocalteu reagent, for the assay. The mixtures were incubated at room temperature for a duration of 10 minutes. After that, 100 µL of 7.5% (w/w) sodium carbonate solution was added, and the plate was incubated in the dark for another 10 minutes. The phenolic content was subsequently determined by measuring the absorbance at 750 nm, and the results were expressed in gallic acid equivalents (GAE).

3.6 Determination of Total Flavonoid Content

The aluminum chloride colorimetric method, as described by Naphatsorn and Chaiyavat (2017), was employed to evaluate the total flavonoid content (TFC) of the *N. crenulata* leaf extracts. 150 µL of a 15% (w/w) sodium nitrite solution was added to 1 mL of the plant extract, which had already been diluted to a concentration of 1 mg/mL, in this procedure. The mixture was incubated for a total of six minutes. Subsequently, 150 µL of a 15% (w/w) aluminum chloride solution was added and the reaction was incubated for an additional six minutes. A 700 µL solution of 8% (w/w) sodium hydroxide was added to the reaction to complete it. The mixture was incubated in the dark chamber at room temperature for 15 minutes to allow for full color development. A UV-visible spectrophotometer was employed to obtain absorbance readings at 510 nm. The total flavonoid content was determined using a quercetin standard curve and expressed in quercetin equivalents (QE).

3.7 Determination of Total Tannin Content

The absorbance of *N. crenulata* leaf extracts was measured using a microplate reader, and the total tannin content was determined using a colorimetric assay. This technique was modified to be compatible with a 96-well microplate format and was derived from the work of Naphatsorn and Chaiyavat (2017). The preparations were initially diluted to a concentration of 1 mg/mL and thereafter thoroughly mixed. The calibration standard was tannic acid, which was diluted with deionized water. In order to conduct the assay, 20 µL of each extract was added to a 96-well microplate, followed by 100 µL of 0.2 N Folin-Ciocalteu reagent. The mixture was then incubated for 4 minutes. Then, 80 µL of 7.5% (w/w) sodium carbonate solution was added, and the reaction was allowed to incubate for 2 hours in the dark at room temperature. The total tannin content was determined by recording the absorbance at 725 nm, and the results were expressed in tannic acid equivalents (TAE).

3.8 DPPH Radical Scavenging Activity

Ascorbic acid served as the reference standard for this assay, following the methodology established by Kanlayavattanakul et al. (2013), with a calibration curve generated through serial dilution in deionized water. The leaf extracts of *N. crenulata* were diluted to a concentration of 1 mg/mL for analysis. A 60 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared by dissolving the reagent in absolute ethanol and mixing thoroughly using a vortex mixer. In a 96-well microplate, 100 µL of the DPPH solution was combined with an equal volume of the diluted extract. The mixture was incubated in a dark chamber at room temperature for 30 minutes to complete reaction. The antioxidant capacity of the extracts, particularly their efficacy in scavenging DPPH radicals, was assessed by measuring absorbance with a microplate reader. The DPPH scavenging activity percentage was determined using the standard formula as follows.

$$DPPH(RSA\ \%) = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbnce\ of\ control}\right) \times 100$$

3.9 Ferric Reducing Antioxidant Power Assay (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) assay was performed using a modified method based on Naphatsorn and Chaiyavat (2017), tailored for a 96-well microplate format. The FRAP reagent was prepared by combining 20 mM ferric chloride ($FeCl_3$) in deionized water, 10 mM TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) in 40 mM HCl, and 0.1 M acetate buffer (pH 3.6) in a 1:1:10 volume ratio. In the assay, 100 µL of the diluted *N. crenulata* extract (1 mg/mL) was mixed with 100 µL of the FRAP reagent. The mixture was incubated at room temperature in a dark chamber for 4 minutes, facilitating the reduction of Fe^{3+} ions to Fe^{2+}, which subsequently formed a blue complex with TPTZ. Absorbance was quantified at 593 nm utilizing a microplate reader. Ascorbic acid served as the calibration standard, with the FRAP values for the extracts reported in milligrams of ascorbic acid equivalents (mg AAE).

3.10 Analysis of *in vitro* Astringent Activity

According to Ditthawutthikul (2021), a swine hemoglobin precipitation experiment was used to imitate tannin-protein interaction to evaluate *N. crenulata* leaf extracts' in vitro astringency. Deionized water was used to dilute a 1 mg/mL swine hemoglobin solution. Tannic acid was positive, and deionized water was negative controls. In a microcentrifuge tube, 500 µL of hemoglobin solution and 500 µL of *N. crenulata* extract (1 mg/mL) were combined using a vortex mixer. The mixture was incubated for 30 minutes to facilitate the reaction. After incubation, samples were centrifuged at 3000 rpm for 10 minutes. The absorbance of a 200 µL supernatant aliquot was measured at 407 nm using a UV-visible spectrophotometer. Astringent activity was measured by absorbance.

$$Astringent\ Activity(\%) = \left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbnce\ of\ control}\right) \times 100$$

3.11 Selection of Extract to Develop Into Cosmetic Formulation

The selection of *N. crenulata* leaf extract for cosmetic formulation was based on its extraction efficiency, phytochemical content, and antioxidant properties. Essence-type cosmetic formulations are widely available in the market, not only for anti-aging applications but also for their astringent properties. In this study, we have chosen to develop an anti-aging formulation with a primary focus on antioxidant properties. Among the tested extracts, 50% ethanol extract exhibited the highest extract yield, total tannin content (TTC), total flavonoid content (TFC), and antioxidant activity (DPPH and FRAP assays), despite demonstrating the lowest astringency. Given these favorable attributes, the 50% ethanol extract was selected for further formulation development.

To determine the optimal concentration of the extract in the cosmetic formulation, the dosage was calculated based on its IC_{50} value obtained from the DPPH radical scavenging assay. The final extract concentration was adjusted through testing sample formulations to achieve the most aesthetically favorable color and odor in the formulation.

3.12 Development of *N. crenulata* Extract Into Cosmetic Formulation

The analysis of bioactive compounds and antioxidant activity indicated that *N. crenulata* leaf extract in 50% ethanol is suitable for development into a cosmetic essence. The formula is presented in the table below. The ingredients were meticulously weighed and mixed uniformly. To evaluate stability, two additional formulations were created: one consisting solely of the base ingredients, and another that incorporated the extract along with preservatives. The stability of these formulations was subsequently assessed over time. The formulations were stored in tightly sealed centrifuge tubes and covered with aluminum foil to prevent light exposure.

Table 3.3 Essence Formula incorporating *N.crenulata* leaf extract

Ingredients	**Function**	**Extract + Base (% w/w)**	**Base (% w/w)**	**Extract (% w/w)**
Water	Solvent	qs 100	qs 100	qs 100
Glycerin	Humectant	3	3	–
Butylene Glycol	Humectant	2	2	–
Emogel oil™ (Glycerin (and) Glyceryl Acrylate/AcrylicAcid Copolymer(and) Propylene glycol (and) PVM/MA Copolymer)	Humectant	2	2	–
Liquid germall™ plus (Diazolidinyl Urea (and) Iodopropynyl Butylcarbamate (and) Propylene Glycol)	Preservative	0.5	0.5	0.5
Hydroxyethyl cellulose	Thickening agent	0.3	0.3	–

Table 3.3 (continued)

Ingredients	Function	Extract + Base (% w/w)	Base (% w/w)	Extract (% w/w)
Colem TT™ (Sodium Diethylenetriamine Pentamethylene Phosphonate/ Sodium Gluceptate)	Stabilizer against oxidation	0.1	0.1	–
Extract		0.062	–	0.062

3.13 Stability Assessment

3.13.1 Heating–cooling Cycle

The method adapted from Phonggasor (2020) was followed with minor adjustments. In each 50 mL centrifuge tube, 45 grams of formulations were poured into the 50 mL centrifuge tubes and tightly sealed. Then the tubes were protected from light by covering them with aluminum foil. Programmable Temperature and Humidity Tester (GT-7005-A) and set at 4°C and 45°C conditions, with each condition for 24 hours to complete one cycle. Stability was evaluated by comparing color, pH, viscosity, total flavonoid content, total tannin content, and antioxidant activity (measured by DPPH radical scavenging activity) at both the beginning and after five cycles.

3.13.2 Short Term Stability Test

A slightly modified version of the protocol by Phonggasor (2020) was used to evaluate the short-term stability of the formulations. The samples were stored at 4°C, room temperature and 45°C for one month. Initially and at the end of the test period, the formulations were analyzed by comparing color, pH, viscosity, total flavonoid content, total tannin content, and antioxidant activity (measured by DPPH radical scavenging activity) from the beginning to the end of the study.

1. Color measurement

The color of the formulations was assessed with colorimeter by measuring lightness (L*), redness–greenness (a*) and yellowness–blueness (b*).

2. pH measurement

The pH of the formulations was measured directly using pH meter.

3. Viscosity measurement

The viscosity measurement of the formulations was done by using a Rheometer with a cone head. 1 mL of the sample was put in place and the measurement was done at the fixed temperature of 25°C and expressed in mPa.s.

4. Measurement of bioactive content and antioxidant activity

Measurement of TFC, TTC and antioxidant activity by DPPH radical scavenging activity were done by using the same methods as described above with the adjustment of avoiding the sample dilution.

3.14 Statistical Analysis

In this study, all experimental data were collected in triplicate ($n = 3$) to ensure precision and reproducibility. The results are presented as mean ± standard deviation (SD). An ANOVA test was conducted to compare bioactive substances and antioxidant activity among the three extracts. Stability assessments involved comparing initial and final values of physical parameters (color, pH, and viscosity) and bioactive properties (total flavonoid content, total tannin content, and antioxidant activity) using paired *t*-tests to determine significant changes. The significance level was set at $p < 0.05$ for all analysis.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Microwave–assisted Extraction of *N. crenulata*

The leaves of *N. crenulata* were extracted utilizing a microwave-assisted extraction (MAE) technique. This study utilized ethanol as the solvent at three different concentrations: 50%, 70%, and 95%, to examine the impact of solvent polarity on yield. Extraction yield is a crucial factor in assessing the efficiency of the extraction process, especially for the isolation of bioactive compounds intended for cosmetic formulations. MAE technique employs microwave energy to directly heat the solvent and sample, resulting in a more efficient extraction of bioactive compounds (Gallo et al., 2010).

The extracts displayed a green hue and a fresh, earthy scent. The extraction yields of *N. crenulata* were determined as a percentage of the weight of dried leaf material in relation to the total weight of the recovered extract. The extraction yield was maximized at 50% ethanol, followed by 70% ethanol, while the lowest yield was recorded with 95% ethanol, (Table 4.1) indicating a clear relationship with solvent polarity. The data indicates a distinct correlation between solvent concentration and extraction yield, with 50% ethanol yielding the highest results and 95% ethanol yielding the lowest. The increased yield noted with 50% ethanol may be associated to its intermediate polarity, which enhances the solubility of both polar and non-polar bioactive compounds in the extract. This is significant for *N. crenulata* leaves due to their composition of phenolic, flavonoid, and tannin compounds, which exhibit higher solubility in water-ethanol mixtures. The 95% ethanol, due to its lower polarity, selectively extract a limited number of hydrophilic compounds, leading to a reduced overall yield. The 70% ethanol solution exhibited an intermediate yield of 11.45%, indicating that solvent polarity significantly influences the efficiency of bioactive compound extraction. The bioactive components are essential for the cosmetic efficacy of the extract, indicating that the 50% ethanol extract is likely to be the most effective when included in cosmetic formulations.

Prior research on microwave-assisted extraction of plant materials indicates that ethanol-water mixtures yield optimal results compared to pure ethanol. A study by Kubra and Kumar indicated that 50% aqueous ethanol resulted in higher extraction rates of polyphenols from ginger than 80% ethanol. The authors ascribed this phenomenon to the augmented swelling of plant material in the presence of water, which improved the contact surface area between the solvent and the plant matrix (Kubra & Kumar, 2013). It is also found that the extraction yields of oxymatrine from *Sophora flavescens* diminished as ethanol concentration increased from 60% to 90%, suggesting that lower ethanol concentrations are more effective for certain compounds (Xia et al., 2011). In the study on the optimization of microwave-assisted extraction of natural antioxidants from *Gordonia axillaris* fruit, researchers noted that the optimal ethanol concentration for extraction as 36.89% (Li et al., 2017).

The study shows that using 50% Ethanol as a solvent with MAE is effective in extracting bioactive chemicals from *N. crenulata*, however there is room for improvement in the method. Future studies may examine how extraction duration, temperature, and power affect yield and bioactive chemical concentration. Evaluation of various solvent systems may reveal more efficient or sustainable extraction methods. This study clarifies solvent polarity plays role in *N. crenulata* leaf extraction.

Table 4.1 Extraction Yield of *N. crenulata* Leaf Extract

Solvents used	**Extract yield (%w/w)**
95% EtOH	8.64 ± 0.09^{b}
70% EtOH	11.45 ± 3.18^{b}
50% EtOH	16.81 ± 0.89^{a}

Note Values within the same column marked with different superscript letters show statistically significant differences. ($p < 0.05$)

4.2 Determination of Total Phenolic Content (TPC)

TPC in cosmetics is an important metric, given the various advantages provided by phenolic compounds, especially their antioxidant properties. Phenolic compounds, including flavonoids and phenolic acids, possess the capacity to neutralize free radicals,

thus safeguarding the skin against oxidative stress and premature aging (Mapoung et al., 2021). The presence of these compounds in cosmetic formulations improves their effectiveness, making them significant components in anti-aging and skin protection products.

The Folin-Ciocalteu assay is a commonly employed technique for the quantification of TPC. The assay relies on the reduction of the Folin-Ciocalteu reagent by phenolic compounds in an alkaline environment, leading to a measurable color change via spectrophotometry (Hassan et al., 2019). The intensity of the blue color produced is directly proportional to the concentration of phenolic compounds in the sample, enabling quantification.

TPC of *N. crenulata* leaf extract was assessed to evaluate its potential for cosmetic applications, particularly as an antioxidant ingredient. The Folin-Ciocalteu colorimetric method was utilized to quantify the phenolic compounds in extracts derived from different ethanol concentrations (50%, 70%, and 95%). Table 4.2 presents a comparison of the TPC values, measured in milligrams of gallic acid equivalents (mg GAE) per gram of dried leaves. The 50% ethanol extract exhibited the highest phenolic content, measuring 11.76 ± 0.38 mg GAE/g. In contrast, the 95% ethanol extract recorded the lowest total phenolic content at 5.05 ± 0.07 mg GAE/g, while the 70% ethanol extract had a total phenolic content of 8.41 ± 0.05 mg GAE/g. The findings indicate a distinct trend in which TPC rises as ethanol concentration decreases, with the highest TPC observed at 50% ethanol. The intermediate polarity of the 50% ethanol solvent facilitates the effective extraction of both hydrophilic and lipophilic phenolic compounds. In contrast, 95% ethanol, as a more non-polar solvent, likely extracted fewer hydrophilic phenolic compounds, resulting in the lower total phenolic content observed.

The total phenolic content of *N. crenulata* aligns with findings from other studies, which report high phenolic content in various plant species when extracted using mid-polarity solvents. Huma et al. (2018) indicated that the optimal ethanol concentration for extracting total phenolic content from carob kibbles using microwave-assisted extraction is 44%. Wong et al. (2021) reported that 50% and 70% ethanolic extracts exhibited the highest TPC from *Ardisia elliptica* when utilizing the ultrasound extraction method.

Table 4.2 TPC values of *N. crenulata* Leaf Extract at Various Ethanol Concentrations

Solvents used	TPC (GAE mg/g dry plant)
95% EtOH	5.05 ± 0.07^{c}
70% EtOH	8.41 ± 0.05^{b}
50% EtOH	11.76 ± 0.38^{a}

Note Values within the same column marked with different superscript letters show statistically significant differences. ($p < 0.05$)

4.3 Determination of Total Flavonoid Content (TFC)

Flavonoids are acknowledged for their significant health benefits and are integral to numerous nutraceutical, pharmaceutical, medicinal, and cosmetic applications. Their significance is attributed to their potent antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, alongside their capacity to regulate essential cellular enzyme functions. Flavonoids serve as effective inhibitors of various essential enzymes (Panche et al., 2016). The measurement of TFC in *N. crenulata* leaf extract is essential for assessing its potential as a bioactive component in cosmetic formulations. This study determined TFC using the aluminum chloride colorimetric method, a recognized technique that quantifies the formation of a stable complex between flavonoids and aluminum chloride.

TFC of *N. crenulata* extracts is expressed in milligrams of quercetin equivalents (mg QE) per gram of dried leaves, as illustrated in Table 4.3. The extraction of flavonoids yielded varying results across different ethanol concentrations. The 50% ethanol extract demonstrated the highest flavonoid content at 19.35 ± 0.32 mg QE/g, followed by the 70% ethanol extract at 10.19 ± 0.02 mg QE/g, and the 95% ethanol extract, which recorded the lowest content at 8.57 ± 0.08 mg QE/g. The results indicate that solvent polarity has a substantial impact on the extraction efficiency of flavonoids.

The study conducted by Sari et al. (2020) employed MAE to extract flavonoids from *Padina australis*, a brown algae species, using varying ethanol concentrations of 30%, 50%, and 70%. The optimal ethanol concentration identified was 50.33% (Sari et al., 2020). The research conducted by Le et al. (2019) focused on optimizing the

extraction of total phenolic and flavonoid content from *Docynia indica* fruits. The findings indicated that an ethanol concentration of 65% was optimal for achieving the highest yield. Jiang et al. (2014) identified 40% ethanol as the optimal concentration for the MAE of total flavonoids from castor leaves. These findings align with our study, which identified mid-polarity solvents as the most effective for flavonoid extraction, owing to their capacity to dissolve both hydrophilic and lipophilic compounds.

Table 4.3 TFC values of *N. crenulata* Leaf Extract at Various Ethanol Concentrations

Solvents used	**TFC (QE mg/g dry plant)**
95% EtOH	8.57 ± 0.08^{c}
70% EtOH	10.19 ± 0.02^{b}
50% EtOH	19.35 ± 0.32^{a}

Note Values within the same column marked with different superscript letters show statistically significant differences. ($p < 0.05$)

4.4 Determination of Total Tannin Content (TTC)

Tannin content of *N. crenulata* leaf extracts was evaluated through a colorimetric assay, reported as tannic acid equivalents in mg per gram of dried leaves (TAE mg/g dried leaves), to assess the performance of various ethanol concentrations in microwave extraction. Table 4.4 presents a comparison of the results, indicating that the TTC is influenced by the concentrations of ethanol in the solvent. Of the three ethanol concentrations assessed, 50% ethanol demonstrated the greatest tannin yield at 11.51 mg TAE/g dry plant, significantly surpassing the yields of 95% ethanol (6.60 mg TAE/g) and 70% ethanol (8.04 mg TAE/g). This indicates that 50% ethanol achieves an optimal balance between the polarities of water and ethanol, thereby enhancing the extraction of tannins, which exhibit partial solubility in water.

The findings are consistent with Huma et al. (2018), which indicates that the optimal ethanol concentration for extracting tannin from carob kibbles via MAE is 57%. Additionally, Silva et al. (2021) reported that increasing ethanol concentration beyond 50% leads to a linear reduction in metabolite extraction, with a solid/solvent ratio of 50/50 yields the best recovery results in the MAE of *Syzygium cumini* (L.) Skeels

leaves. Intermediate ethanol concentrations (50-60%) are optimal for maximizing the extraction of phenolic compounds, such as tannins. Increased ethanol concentrations can diminish the extraction efficiency of total polyphenols and total tannin (Silva et al., 2021) because tannins exhibit limited solubility in less polar environments. The elevated tannin concentration in the 50% ethanol extract is significant in cosmetic formulation. Tannins exhibit astringent and antioxidant characteristics, which contribute to skin tightening and offer protection against oxidative stress.

Table 4.4 TTC values of *N. crenulata* Leaf Extract at Various Ethanol Concentrations

Solvents used	**TTC (TAE mg/g dry plant)**
95% EtOH	6.60 ± 0.11^{c}
70% EtOH	8.04 ± 0.12^{b}
50% EtOH	11.51 ± 0.07^{a}

Note Values within the same column marked with different superscript letters show statistically significant differences. ($p < 0.05$)

4.5 DPPH Radical Scavenging Assay

The antioxidant capacity of *N. crenulata* leaf extracts was assessed using the DPPH radical scavenging assay, a recognized method for evaluating the efficacy of plant-derived compounds in neutralizing free radicals. Extracts rich in antioxidants are essential in cosmetic formulations designed to mitigate oxidative stress, a factor that accelerates skin aging. The DPPH scavenging activity of obtained extracts were expressed as ascorbic acid equivalents (AAE) per gram of dry plant material. The findings indicated a distinct trend: a reduction in ethanol concentration corresponded with an enhancement in the extract's capacity to scavenge DPPH radicals. The 50% ethanol extract exhibited the greatest antioxidant activity (3.35 ± 0.04 mg AAE/g), succeeded by the 70% ethanol extract (2.45 ± 0.14 mg AAE/g) and the 95% ethanol extract (1.15 ± 0.01 mg AAE/g). The results indicate that 50% ethanol is the most efficient solvent for the extraction of antioxidant bioactive compounds from *N. crenulata* via microwave-assisted extraction (MAE). The increased antioxidant activity

observed in the 50% ethanol extract may be due to the enhanced solubility of phenolic and flavonoid compounds at lower ethanol concentrations.

Zhou et al. (2013) indicated that a 50.6% ethanol concentration is optimal for achieving maximal DPPH radical-scavenging capacity in the ultrasonic-assisted extraction (UAE) of *Clerodendrum cyrtophyllum* leaves. In 2020, Weremfo et al. conducted a meta-analysis on avocado (*Persea americana* Mill.) seeds and determined that the optimal ethanol solvent concentration for maximizing antioxidant activity, as measured by DPPH and ABTS assays, is 58.3%.

A parameter introduced for interpreting results from the DPPH method is the IC_{50} value. This refers to the substrate concentration that results in a 50% reduction of DPPH activity, measured by color change. The IC_{50} parameter indicates that increased antioxidant activity corresponds to a lower IC_{50} value (Molyneux & Philip 2004). A lower IC_{50} value signifies greater antioxidant potency, as a reduced concentration is required to attain the equivalent radical scavenging effect.

The IC_{50} value of standard ascorbic acid in our study is 13.87 µg/ml. The IC_{50} value for the 50% ethanolic extract is 624.6 ± 28.95 µg/mL. The IC_{50} value of standard ascorbic acid, a recognized and effective antioxidant, is significantly lower at 13.87 µg/mL than that of the 50% ethanol extract of *N. crenulata*, which is 624.60 µg/mL. This indicates that although the *N. crenulata* extract shows antioxidant activity, it is considerably less effective than pure ascorbic acid in scavenging DPPH free radicals. Qusti et al. (2010) classify antioxidants according to their IC_{50} values as follows: very strong (IC_{50}<0.01 mg/mL), strong (0.01 mg/mL< IC_{50}<1 mg/mL), moderate (1 mg/mL< IC_{50}<7 mg/mL), and weak (IC_{50}>7 mg/mL). The IC_{50} value of 624.60 µg/mL suggests that the extract necessitates a significantly higher concentration to attain 50% radical scavenging activity in comparison to ascorbic acid.

Plant extracts typically comprise a variety of compounds, many of which may work synergistically to enhance antioxidant activity. Thus, although the extract exhibits lower potency compared to the pure standard, it retains antioxidant potential, primarily attributed to the presence of phenolic and flavonoid compounds that may interact synergistically with other bioactive substances. Although the IC_{50} value of the *N. crenulata* extract is higher than that of pure ascorbic acid, its antioxidant potential remains beneficial for cosmetic formulations. Future studies should investigate various

extraction techniques, alternative solvents, and the optimization of ethanol concentration to improve the antioxidant efficacy of the extract and maximize the yield of bioactive compounds.

Table 4.5 DPPH radical scavenging activity of *N. crenulata* Leaf Extract at Various Ethanol Concentrations

Solvents used	DPPH (AAE mg/g dry plant)
95% EtOH	1.15 ± 0.01^{c}
70% EtOH	2.45 ± 0.14^{b}
50% EtOH	3.35 ± 0.04^{a}

Note Values within the same column marked with different superscript letters show statistically significant differences. ($p < 0.05$)

4.6 Ferric Reducing Antioxidant Power Assay

The Ferric Reducing Antioxidant Power (FRAP) assay assesses the antioxidant capacity of *N. crenulata* leaf extracts by quantifying their ability to convert ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions, resulting in the formation of a blue complex with TPTZ. The results indicate a distinct trend where antioxidant activity rises with decreasing ethanol concentration. The 50% ethanol extract exhibited the highest FRAP value (3.57 ± 0.05 mg AAE/g), followed by the 70% ethanol extract (2.63 ± 0.03 mg AAE/g), and the 95% ethanol extract (1.5 ± 0.02 mg AAE/g). This pattern indicates that mid-range ethanol concentrations are more effective in extracting the antioxidant compounds that reduce ferric capacity. The differences in FRAP values among ethanol concentrations can be attributed to the solubility of various antioxidant compounds, including phenolics and flavonoids, which are more effectively extracted at lower ethanol concentrations. This statement is also supported by the study of Luo et al. (2021) that identifies 49.61% as the optimal ethanol concentration for maximizing the antioxidant activity of *Akebia trifoliata* peels in MAE, also measured by the FRAP assay. Shang et al. (2020) also indicated that an ethanol concentration of 58.43% optimally maximizes the antioxidant capacity of Sweet Tea *(Lithocarpus polystachyus* Rehd.).

Table 4.6 *N. crenulata* Leaf Extract FRAP activity at Various Ethanol Concentrations

Solvents used	FRAP (AAE mg/g dry plant)
95% EtOH	1.5 ± 0.02^{c}
70% EtOH	2.63 ± 0.03^{b}
50% EtOH	3.57 ± 0.05^{a}

Note Values within the same column marked with different superscript letters show statistically significant differences. ($p < 0.05$)

4.7 Analysis of In-vitro Astringent Activity

The astringent activity of *N. crenulata* leaf extracts was determined through a hemoglobin precipitation assay. The findings indicated a notable variation in astringent activity based on the solvent used (Table 4.7). The 95% ethanol extract exhibited the greatest astringent activity at 64.94 ± 0.13%, followed by 70% ethanol at 39.48 ± 0.27% and 50% ethanol at 36.57 ± 1.68%. The findings suggest that increased ethanol concentrations amplify astringency, which may be significant for formulations targeting skin tightening and oil regulation. The extract exhibiting the highest astringency (95% ethanol) had the lowest total tannin content, as previously noted. The inverse relationship between tannin content and astringent activity may result from the diverse types of tannins and their varying solubility. Astringency is primarily caused by condensed tannins (proanthocyanidins) with varied chemical structures (Ma et al. 2016). The concentration of ethanol can markedly affect the interaction between tannins and salivary proteins. Obreque-Slier et al. (2010) demonstrated that ethanol modifies the binding affinity of tannins to salivary proteins, thereby influencing the perception of astringency. The high astringency observed in the 95% ethanol extract may be attributed to the increased ethanol concentration, which enhances tannin-protein interaction and facilitates the extraction of greater quantities of condensed tannins.

Astringent compounds frequently demonstrate metal-chelating abilities, which can enhance the free radical scavenging effects of antioxidants, thus offering dual protection against oxidative damage. The interaction of astringent and antioxidant

compounds can lead to the creation of new antioxidant species, including phenolic dimers, which contribute to increased oxidative inhibition (Bayram & Decker, 2023).

Astringents are compounds that cause contraction and tightening of tissues, particularly the skin and mucous membranes, by precipitating proteins. The reported phytochemical composition of *N. crenulata* suggests significant astringent activity, primarily attributed to its high tannin and phenolic content, along with the presence of flavonoids and alkaloids. Studies on *N. crenulata* have confirmed the presence of tannins in its leaves and bark (Sampathkumar et al., 2012; Pratheeba et al., 2019). These effects contribute to skin tightening, reduce pore size, and enhance skin firmness. Phenolic compounds engage with proteins via non-covalent interactions, including hydrophobic forces and electrostatic attractions, which affect the strength of their binding. Interactions of this nature can lead to conformational changes in proteins, which in turn influence their solubility and functionality (Shahidi & Dissanayaka, 2023). Ethanol increases the binding affinity of polyphenols to salivary proteins, modifying both the binding ratio and the thermodynamic properties of the interaction (Jahmidi-Azizi et al., 2023). This also indicates that higher ethanol-based extracts could enhance the perception of astringency by affecting the interactions between polyphenols and proteins.

Flavonoids, particularly quercetin, have been identified in *N. crenulata* leaves (Singh et al., 2017). Quercetin has been shown to interact with calmodulin, a calcium-binding protein, suggesting that it possesses astringent properties from its ability to modulate protein functions in biological systems (Nishino et al., 1984).

Table 4.7. *In vitro* astringent activity of *N. crenulata* Leaf Extract at Various Ethanol Concentrations

Solvents used	**Astringent activity %**
95% EtOH	64.94 ± 0.13^{a}
70% EtOH	39.48 ± 0.27^{b}
50% EtOH	36.57 ± 1.68^{c}

Note Values within the same column marked with different superscript letters show statistically significant differences. ($p < 0.05$)

4.8 Development of *N. crenulata* Extract Into Cosmetic Formulation

Essence was chosen as the type of formulation because of its versatility and common dosage form for antiaging cosmetics in the market. The 50% ethanol extract of *N. crenulata* leaves was selected for incorporating into formulation due to its significant antioxidant properties and the highest bioactive content compared to the other solvent extracts.

The base formula was developed utilizing standard cosmetic components, including glycerin and butylene glycol for hydration, while excluding colorants and fragrances to preserve the extract's natural properties. Three versions were developed for comparison: (1) base with extract, (2) base alone, and (3) extract alone. All formulations were maintained in sealed centrifuge tubes and wrapped in aluminum foil to mitigate light-induced degradation.

The concentration of the *N. crenulata* leaf extract used in the formulations was determined based on the IC_{50} value for DPPH radical scavenging activity, which was measured to be 624.6 ± 28.95 µg/mL. Four formulations were prepared as preliminary using extract concentrations of 1 time, 2 times, 3, and 4 times of the IC_{50} value. Among these, one time concentration was selected as the final formula based on its favorable color and scent attributes.

4.9 Stability Assessment

The stability of the formulations was evaluated through comparisons of physical appearance, color measurements using a colorimeter, pH values, and viscosity assessments via rheometer. Additionally, total tannin content, total phenolic content, and antioxidant activity measured by DPPH were assessed for extract containing samples both before and after five cycles of heating–cooling treatment, as well as before and after one month of storage.

4.9.1 Heating–Cooling Cycle

4.9.1.1 Organoleptic assessment

The formulations containing only the base exhibit a clear color, while those containing extracts display a green hue. No visible color changes were observed before and after five cycles of heating and cooling. The formulations containing only the base exhibit no odor, while those containing extracts produce a distinctive scent reminiscent of fresh leaves. No difference in odor was observed before and after five cycles.

4.9.1.2 Color measurement with colorimeter

The formulations were placed in a plastic cuvette for consistent value assessment, followed by measurement using a colorimeter. Assessments were done at both the beginning and conclusion of the five heating–cooling cycles. The ΔE value is calculated using the following formula:

$$\Delta E = \sqrt{(L2 - L1)^2 + (a2 - a1)^2 + (b2 - b1)^2}$$

Where: L1 and L2 are the lightness values before and after the test.

a1 and a2 represent the green-red coordinate values before and after the test.

b1 and b2 represent the blue-yellow coordinate values before and after the test.

The interpretation of ΔE values is crucial for evaluating color differences across various applications, including cosmetic formulations. The CIE L*a*b* color space offers a standardized approach for quantifying color differences, facilitating a systematic assessment of color stability and acceptability over time. ΔL, Δa, and Δb denote the variations in lightness and color coordinates between two measurements. Suliman et al. (2019) conducted a study that examines the relevant variables in the context of their research. The interpretation of ΔE values is essential for assessing the presence of a noticeable color difference between the initial and final measurements of a cosmetic formulation. Research classifies ΔE values into perceptibility thresholds. A ΔE value below 1.0 is deemed imperceptible to the human eye, whereas values ranging from 1.0 to 2.0 suggest a slight color difference that may become apparent under specific lighting conditions (Yoshida et al., 2010; Paravina et al., 2019). A ΔE value ranging from 2.0 to 3.0 is commonly recognized as the threshold for perceivable color differences, whereas values exceeding 3.0 are generally viewed as significant, suggesting a color change that may influence consumer perception and product quality

(Nemli et al., 2018; Sethi et al., 2015). Studies indicate that ΔE values exceeding 2.0 are noticeable in dental materials, implying that comparable thresholds may be relevant to cosmetic formulations (Paravina et al., 2015; Paravina et al., 2019).

All formulations possess the ΔE values of less than 2, showing that the formulations are stable in terms of color.

Table 4.8 Values of L*, a*, b* and ΔE calculation after color assessment with colorimeter

Formulation	**L1**	**L2**	**a1**	**a2**	**b1**	**b2**	***ΔE***
Base only	40.50 ± 0.43	40.56 ± 0.14	-0.13 ± 0.03	-0.11 ± 0.14	-1.23 ± 0.06	-1.11 ± 0.01	0.42 ± 0.08
Extract only	38.86 ± 0.23	38.89 ± 0.92	-0.73 ± 0.1	-0.89 ± 0.05	1.27 ± 0.02	1.6 ± 0.18	0.92 ± 0.34
Extract + Base	38.42 ± 0.09	39.06 ± 0.70	-0.77 ± 0.10	-0.82 ± 0.2	1.77 ± 0.24	1.88 ± 0.40	0.72 ± 0.52

Note Paired t-tests were conducted to compare L*, a*, and b* values before and after the stability test. No statistically significant differences were observed in any parameter ($p > 0.05$).

L1=lightness values before the test, L2=lightness values after the test

a1= green-red coordinate values before the test, a2= green-red coordinate values after the test

b1=blue-yellow coordinate values before the test, b2=blue-yellow coordinate values after the test

4.9.1.3 pH measurement

Following five heating-cooling cycles, the pH of all three formulations exhibited differing levels of alteration. The paired t-test results demonstrated a statistically significant reduction in pH for the formulations containing extracts ($p < 0.05$), while the base-only formulations showed non-significant pH changes ($p > 0.05$). The pH of the extract-only formulation decreased from an initial value of 5.07 ± 0.03 to 4.92 ± 0.01, resulting from thermal stress or oxidative processes during the cycles. The base-only formulations exhibited stability, with an initial pH of 5.82 ± 0.03, which

decreased slightly to 5.8 ± 0.04. The inclusion of preservatives and stabilizers in the base likely enhanced its resistance to pH fluctuations. The extract combined with base formulations exhibited a smaller pH change compared to formulations containing only the extract, shifting from an initial pH of 5.32 ± 0.02 to 4.92 ± 0.01. This reduction is likely attributable to the buffering capacity of the preservatives and stabilizers in the base ingredients, which lessened the impact of temperature cycling. The findings indicate that a well-formulated base can improve the pH stability of cosmetic products with botanical extracts. The ideal pH range for cosmetic products is typically between 4.5 and 5.5, aligning with the skin's natural pH (Bartels et al., 2014). Consequently, despite significant changes present in pH values of extract containing formulations, the measured pH levels remained within the desired range, ensuring compatibility with the skin.

Table 4.9 pH values comparison of before and after 5 heating–cooling cycles

Formulation	**Baseline**	**After HC cycle**
Base only	5.82 ± 0.03	5.8 ± 0.04
Extract only	5.07 ± 0.03	4.92 ± 0.01*
Extract + Base	5.32 ± 0.02	5.26 ± 0.01*

Note * indicates presence of significant changes between the values when compared with baseline ($p < 0.05$)

4.9.1.4 Viscosity measurement with Rheometer

After five heating-cooling cycles, the viscosity of all three formulations demonstrated varying degrees of change. The paired t-test results indicated a statistically significant decrease in pH for both the base and extract + base formulations ($p < 0.05$), whereas the extract-only formulations exhibited non-significant changes ($p > 0.05$). The extract-only formulations demonstrated minimal change, while the base-only formulations exhibited an average change exceeding 10%. The extract, when combined with base formulations, demonstrates a change exceeding 40% of the initial value. The significant reduction may be attributed to the synergistic interactions between the extract and the base components when subjected to thermal stress, leading

to destabilization. Bioactive compounds in the extract may interact with the base matrix, influencing overall viscosity and stability.

Dąbrowska et al. (2021) demonstrate that the chemical stability of active ingredients in cosmetic products depends on their compatibility with other formulation components and the storage conditions. The compatibility of interactions is essential, as incompatible interactions may result in phase separation or precipitation, thereby impacting the viscosity and overall stability of the formulation. A bioactive compound that is not well integrated into the base matrix can result in excessive viscosity or excessive fluidity, thereby causing instability in the formulation.

Table 4.10 Viscosity values comparison of before and after 5 heating–cooling cycles

Formulation	Baseline (mPa.s)	After HC cycle (mPa.s)
Base only	60.27 ± 1.76	54.09 ± 1.62*
Extract only	1.14 ± 0.26	1.44 ± 0.19
Extract + Base	50.92 ± 0.6	28 ± 0.75*

Note * indicates presence of significant changes between the values when compared with baseline ($p < 0.05$)

Measurements were performed at 25°C using a rheometer with a cone-and-plate geometry. A sample volume of 1 mL was used for the measurement.

4.9.1.5 Measurement of bioactive content and DPPH

The bioactive compounds (TTC, TFC) and DPPH activity of *N. crenulata* leaf extract was evaluated for extract containing formulations through five heating-cooling cycles, simulating the thermal stress formulations might experience during storage.

The initial TTC for the extract only containing formulations was 83.55 ± 1.28 mg TAE/g extract, which reduced slightly to 77.83 ± 3.62 mg TAE/g extract after the heating-cooling cycles, representing a small loss in tannin stability. Interestingly, the extract incorporated base formulations exhibited an increase in TTC from 106.95 ± 8.62 mg TAE/g extract to 134.5 ± 27.17 mg TAE/g extract. This unexpected rise might indicate interactions between the extract and the base components. But it doesn't have significant difference after statistical analysis (p>0.05). Butkevičiūtė et al. demonstrate

that the viscosity of the base matrix can affect the release of phenolic compounds, such as tannins, from semi-solid formulations. Lower viscosity formulations facilitate the diffusion of these compounds, resulting in higher measured concentrations after thermal cycling (Butkevičiūtė et al., 2022). This indicates that heating may improve the solubility of tannins, facilitating their greater release into the formulation, which may account for the observed increase in total tannin content.

Flavonoids, known for their susceptibility to oxidation and thermal degradation, showed a substantial decrease in both formulations. The extract only formulations' TFC dropped from 135.49 ± 8.03 mg QE/g extract to 87.02 ± 6.93 mg QE/g extract, while the extract and base combined formulation decreased from 80.24 ± 3.41 mg QE/g extract to 58.31 ± 2.46 mg QE/g extract. This suggests that the flavonoid compounds in the extract are sensitive to the thermal fluctuations encountered during the cycles. Additionally, the presence of the base did not seem to offer protective effects, potentially exacerbating the degradation.

The DPPH radical scavenging activity of the formulations provided insights into the antioxidant capacity of the extract before and after thermal cycling. For the extract only formulation, the antioxidant activity increased significantly from 21.0 ± 0.63 mg AAE/g extract to 24.31 ± 0.84 mg AAE/g extract. Conversely, the extract + base formulation exhibited no significant change in antioxidant activity (15.96 ± 0.6 mg AAE/g extract to 16.06 ± 0.86 mg AAE/g extract) even with the significant decrease in value of total flavonoid content, suggesting that the base helped maintain the antioxidant properties of the extract.

The stability of *N. crenulata* leaf extract in cosmetic formulations was highly dependent on the formulation matrix. While the extract alone showed stability in TTC (no significant change), decrease in TFC values significantly and a significant change in antioxidant activity, the presence of the base altered these dynamics. The base appeared to reduce the flavonoid content while potentially enhancing the tannin content but maintaining stability in antioxidant capacity. These findings suggest that incorporating stabilizers or optimizing the formulation matrix may be necessary to ensure the long-term stability of the bioactive compounds in cosmetic products.

Table 4.11 Bioactive compound and DPPH activity comparison of before and after 5 heating–cooling cycles

Formulation	**Baseline TTC (mg TAE/g extract)**	**After HC, TTC (mg TAE/g extract)**
Extract only	83.55 ± 1.28	77.83 ± 3.62
Extract + Base	106.95 ± 8.62	134.5 ± 27.17
Formulation	**Baseline TFC (mg QE/g extract)**	**After HC, TFC (mg QE/g extract)**
Extract only	135.49 ± 8.03	87.01 ± 6.93*
Extract + Base	80.24 ± 3.41	58.31 ± 2.46*
Formulation	**Baseline DPPH (mg AAE/g extract)**	**After HC, DPPH (mg AAE/g extract)**
Extract only	21.08 ± 0.63	24.31 ± 0.85*
Extract + Base	15.96 ± 0.6	16.06 ± 0.86

Note * indicates presence of significant changes between the values when compared with baseline ($p < 0.05$)

4.9.2 Short Term Stability Test

The evaluation included both heating–cooling cycles and the stability of the formulations, which were subjected to three temperature settings: 4°C, room temperature and 45°C, over a duration of one month. Organoleptic measurements, color measurements using a colorimeter, pH values, and viscosity assessments via rheometer were compared across all formulations. Additionally, total tannin content, total phenolic content, and antioxidant activity measured by DPPH were evaluated for extract-containing formulations both before and after one month of storage.

4.9.2.1 Organoleptic assessment

The formulations comprising solely the base demonstrate a clear color, whereas those incorporating extracts exhibit a green hue. No apparent color changes were noted. The formulations comprising solely the base demonstrate no odor, whereas those incorporating extracts yield a distinctive aroma like fresh leaves before the stability test. No difference in odor was observed between formulations stored at 4°C

and room temperature, as well as in base-only formulations at 45°C. However, the extract containing formulations stored at 45°C emits an aroma reminiscent of brewed tea.

4.9.2.2 Color measurement with colorimeter

The measurements were conducted using a colorimeter, following the same methodology as outlined for the heating–cooling cycles stability test. Assessments were conducted at the start and end of a one-month period. The ΔE value is determined using the identical formula.

Table 4.12 Values of L*, a*, b* and ΔE calculation after one month at different temperature settings

Formulation	**Temp (ºC)**	**L1**	**L2**	**a1**	**a2**	**b1**	**b2**	**ΔE**
Base only	Room	40.50 ± 0.43	39.95 ± 0.03	-0.13 ± 0.03	-0.04 ± 0.04	-1.23 ± 0.06	-1.09 ± 0.11	0.62 ± 0.36
Extract only	Room	38.86 ± 0.23	38.72 ± 0.29	-0.73 ± 0.1	-0.82 ± 0.07	1.27 ± 0.02	1.31 ± 0.12	0.42 ± 0.06
Extract + Base	Room	38.38 ± 0.14	38.45 ± 0.26	-0.77 ± 0.10	-0.77 ± 0.02	1.75 ± 0.27	1.75 ± 0.06	0.3 ± 0.08
Base only	4°C	40.50 ± 0.43	40.37 ± 0.52	-0.13 ± 0.03	-0.04 ± 0.04	-1.23 ± 0.06	-1.09 ± 0.11	0.26 ± 0.11
Extract only	4°C	38.86 ± 0.23	37.92 ± 0.3	-0.73 ± 0.1	-0.83 ± 0.07	1.27 ± 0.02	1.23 ± 0.03	0.97 ± 0.38
Extract + Base	4°C	38.38 ± 0.14	38.24 ± 0.08	-0.77 ± 0.10	-0.92 ± 0.05	1.75 ± 0.27	1.85 ± 0.1	0.30 ± 0.13

Table 4.12 (continued)

Formulation	Temp (ºC)	L1	L2	a1	a2	b1	b2	ΔE
Base only	45°C	40.50 ± 0.43	40.37 ± 0.52	-0.13 ± 0.03	-0.04 ± 0.04	-1.23 ± 0.06	-1.09 ± 0.11	0.26 ± 0.11
Extract only	45°C	38.86 ± 0.23	37.92 ± 0.30	-0.73 ± 0.1	-0.83 ± 0.07	1.27 ± 0.02	1.23 ± 0.03	0.97 ± 0.38
Extract + Base	45°C	38.38 ± 0.14	38.24 ± 0.08	-0.77 ± 0.10	-0.92 ± 0.05	1.75 ± 0.27	1.85 ± 0.1	0.3 ± 0.13

Note Paired t-tests were conducted to compare L*, a*, and b* values before and after the stability test. No statistically significant differences were observed in any parameter ($p > 0.05$).

L1=lightness values before the test, L2=lightness values after the test

a1= green-red coordinate values before the test, a2= green-red coordinate values after the test

b1=blue-yellow coordinate values before the test, b2=blue-yellow coordinate values after the test

4.9.2.3 pH measurement

The pH stability of formulations with *N. crenulata* leaf extract was assessed over one month under three storage conditions: room temperature (RT), 4°C (refrigeration), and 45°C (hot air oven). Variations in pH can considerably affect the efficacy and safety of cosmetic formulations, particularly those incorporating natural extracts, which tend to be more susceptible to environmental influences.

At baseline, the pH of all formulations was within the acceptable range for cosmetic products intended for skin application. The formulation containing only the base exhibited a pH of 5.82, whereas the formulation containing solely the extract began at a marginally lower pH of 5.07, suggesting that the bioactive compounds in *N.*

crenulata extract may have an acidic nature. The combination of the extract and base formulation resulted in a pH of 5.32, suggesting that the base exerted a buffering effect that mitigated the natural acidity of the extract.

After one month at room temperature, all formulations showed a notable reduction in pH ($p < 0.05$). The formulation containing only the extract experienced the most significant reduction, decreasing from 5.07 to 4.94. This indicates that the bioactive components of the extract may experience gradual degradation at room temperature, resulting in the release of acidic by-products or modifications to the balance of pH-sensitive compounds. Dąbrowska et al. (2021) emphasized that the chemical stability of active ingredients in cosmetic formulations is notably influenced by their compatibility with other components and the storage conditions, particularly temperature variations. Fredes et al. (2018) observed that anthocyanins, a class of flavonoids, exhibit stability in acidic conditions (pH 1–3) but may degrade at elevated pH levels, leading to the formation of phenolic acids. The degradation of anthocyanins results in the release of acidic by-products, which may affect the overall pH of the formulation. The combination of the base and extract and base formulations also resulted in significant pH decreases but lesser extent than the extract only formulation. The buffering capacity of the base likely reduced pH fluctuations to a certain degree.

At 45°C, all formulations exhibited notable pH changes, thereby confirming the instability of the formulations at elevated temperatures. The formulation containing only the extract exhibited the most significant decrease in pH, from 5.07 to 4.74, indicating that the compounds within the extract may degrade more quickly when subjected to heat. The thermal degradation of bioactive molecules frequently results in the production of acidic by-products, which contributes to a decrease in pH. The formulations containing only the base and those combining extract and base also demonstrated notable reductions; however, the buffering capacity of the base seemed to restrict the degree of pH change relative to the formulation containing solely extract.

Chu et al. (2016) also found that elevated storage temperatures significantly degrade plant extracts, particularly impacting their color and antioxidant properties. The research indicated that extracts stored at higher temperatures (e.g., 60°C) demonstrated a significant decline in color stability, whereas those maintained at lower temperatures preserved their properties more effectively. This indicates that

refrigeration may enhance the chemical stability of plant extracts by decelerating degradation processes that transpire at elevated temperatures.

All formulations exhibited statistically significant pH variations ($p < 0.05$) under the various storage conditions, as verified by paired t-tests. The results highlight the formulations' sensitivity to environmental factors, especially temperature showing most changes at 45°C and least changes at 4°C. The observed pH changes significantly impact the stability and shelf life of cosmetic products that include *N. crenulata* leaf extract. The formulations may necessitate the incorporation of stabilizers or pH buffers to ensure long-term pH stability.

The pH stability of *N. crenulata* leaf extract formulations is greatly impacted by temperature. Cold storage at 4°C results in minimal pH variation, whereas formulations subjected to room temperature and increased heat experience major pH shifts. The findings indicate a necessity for additional formulation optimization to improve product stability, especially in geographic regions with elevated temperatures. Future research may investigate the application of pH-adjusting agents or natural stabilizers to enhance the shelf life of products containing *N. crenulata* extract.

Table 4.13 pH values after one month at different temperature settings

Formulation	**Condition kept**	**Baseline**	**After one month**
Base only	13	5.82 ± 0.03	5.72 ± 0.02*
Base only	4°C	5.82 ± 0.03	5.72 ± 0.01*
Base only	45°C	5.82 ± 0.03	5.57 ± 0.01*
Extract only	Room Temp	5.07 ± 0.03	4.94 ± 0.02*
Extract only	4°C	5.07 ± 0.03	4.93 ± 0.05*
Extract only	45°C	5.07 ± 0.03	4.74 ± 0.05*
Extract + Base	Room Temp	5.32 ± 0.02	5.19 ± 0.01*
Extract + Base	4°C	5.32 ± 0.02	5.22 ± 0.00*
Extract + Base	45°C	5.32 ± 0.02	5.1 ± 0.01*

Note * indicates the presence of significant changes between the values when compared with baseline ($p < 0.05$)

4.9.2.4 Viscosity measurement with Rheometer

The viscosity of cosmetic formulations is a crucial parameter that affects product application, texture, and consumer satisfaction. Stability testing was performed to assess alterations in the viscosity of *N. crenulata* leaf extract formulations following one month of storage at various temperature conditions (room temperature, 4°C, and 45°C). The results provide insights into the long-term physical stability of these formulations and their appropriateness for commercial cosmetic applications and optimal storage temperature.

At baseline measurements, the viscosity of the formulation comprising solely base ingredients was 60.27 ± 1.76 mPa.s, attributed to the inclusion of viscosity-modifying agents such as hydroxyethyl cellulose, commonly utilized in cosmetic formulations. The formulation containing extract exhibited a significantly lower viscosity of 1.14 ± 0.26 mPa.s, relating to the lack of gelling or thickening agents and the high-water content. The extract-base formulation, incorporating *N. crenulata* leaf extract and the base, demonstrated a viscosity of 50.92 ± 0.6 mPa.s, suggesting an interaction between the extract and base components resulting in lower value than the base only formulation.

Following a one-month period at room temperature, both the base-only formulation and the extract and base combination demonstrated statistically significant decreases in viscosity ($p < 0.05$). The base-only formulation decreased from 60.27 ± 1.76 to 53.3 ± 1.85 mPa.s, whereas the extract plus base formulation exhibited a more significant reduction from 50.92 ± 0.6 to 23.26 ± 0.56 mPa.s. The presence of *N. crenulata* extract may facilitate phase destabilization or the degradation of thickeners over time. The extract only formulation exhibited a minor increase in viscosity (from 1.14 ± 0.26 to 1.66 ± 0.21 mPa.s), likely attributable to solvent evaporation or alterations in the extract's physical properties over time. The minimal alterations may be due to the extremely low initial viscosity of the formulation.

At 4°C, the viscosity of all formulations exhibited greater stability compared to room temperature; however, significant changes ($p < 0.05$) were observed in the formulations containing the base. The formulation with only the base exhibited a minor decrease from 60.27 ± 1.76 to 57.9 ± 1.54 mPa.s, whereas the formulation combining the base and extract demonstrated a larger reduction from 50.92 ± 0.6 to

41.97 ± 1.45 mPa.s. The lessened rate of viscosity alteration at lower temperatures indicates that refrigeration minimizes the degradation of viscosity-modifying agents, thereby maintaining the structural integrity of the formulations. Similar to the room temperature findings, the extract-only formulation exhibited a minor increase in viscosity (from 1.14 ± 0.26 to 1.47 ± 0.44 mPa.s), with no significant changes ($p > 0.05$), likely attributable to the decreased rate of solvent evaporation in refrigerated conditions.

At elevated temperatures (45°C), all formulations exhibited increased viscosity changes than other storage conditions; however, the extract-only formulation did not demonstrate significant changes ($p > 0.05$). The formulation with only the base decreased from 60.27 ± 1.76 to 47.17 ± 1.31 mPa.s, whereas the formulation combining the extract and base showed a significant reduction from 50.92 ± 0.6 to 12.5 ± 0.33 mPa.s. This indicates that the viscosity-modifying agents in the base are susceptible to thermal degradation, and the inclusion *of N. crenulata* extract may additionally destabilize the formulation. The extract containing only formulation exhibited an insignificant rise in viscosity (from 1.14 ± 0.26 to 1.96 ± 0.25 mPa.s), likely attributable to solvent loss or the concentration of specific extract components. The observed changes indicate the potential instability of formulations at elevated temperatures.

Viscosity stability is greatly influenced by storage conditions and the inclusion of *N. crenulata* extract. Statistically significant reductions are observed in all base-containing formulas, with higher temperatures correlating to greater reductions in viscosity, indicating temperature dependence. Formulations that included both the extract and base exhibited notable reductions in viscosity over time, especially at elevated temperatures. This indicates that the presence of *N. crenulata* extract accelerates to its degradation, suggesting the potential need for additional stabilizers or pH buffers to improve long-term stability. Future research may concentrate on optimizing the formulation to enhance resistance to thermal degradation.

Table 4.14 Viscosity values in mPa.s after one month at different temperature settings

Formulation	Condition kept	Baseline (mPa.s)	After one month (mPa.s)
Base only	Room Temp	60.27 ± 1.76	53.3 ± 1.85*
Base only	4°C	60.27 ± 1.76	57.9 ± 1.54*
Base only	45°C	60.27 ± 1.76	47.17 ± 1.31*
Extract only	Room Temp	1.14 ± 0.26	1.66 ± 0.21
Extract only	4°C	1.14 ± 0.26	1.47 ± 0.44
Extract only	45°C	1.14 ± 0.26	1.96 ± 0.25
Extract + Base	Room Temp	50.92 ± 0.6	23.26 ± 0.56*
Extract + Base	4°C	50.92 ± 0.6	41.97 ± 1.45*
Extract + Base	45°C	50.92 ± 0.6	12.5 ± 0.33*

Note * indicates presence of significant changes between the values when compared with baseline ($p < 0.05$)

The Measurements were performed at 25°C using a rheometer with a cone-and-plate geometry. A sample volume of 1 mL was used for the measurement.

4.9.2.5 Measurement of bioactive content and DPPH

The stability of bioactive compounds, including total tannin content (TTC), total flavonoid content (TFC), and DPPH radical scavenging activity, was assessed in formulations with *N. crenulata* leaf extract over a one-month duration under various storage conditions. The compounds are essential for the antioxidant and astringent properties of the extract, which are vital for cosmetic applications. The storage conditions comprised room temperature, refrigeration at 4°C, and elevated temperature in a hot air oven at 45°C. The findings illustrate the behavior of these compounds over time and under different environmental conditions, which is crucial for maintaining product efficacy.

At room temperature, TTC remained stable in both the extract-only formulation (83.55 ± 1.28 to 83.58 ± 0.96 mg TAE/g extract) and the extract-base combination (106.95 ± 8.62 to 107.42 ± 3.01 mg TAE/g extract), with no statistically significant changes observed ($p > 0.05$). This suggests that tannins exhibit resistance to

degradation under ambient conditions. In contrast, TFC in the extract-only formulation experienced a significant decrease (from 135.49 ± 8.03 to 71.57 ± 4.33 mg QE/g extract, $p < 0.05$), indicating notable flavonoid degradation. However, the extract-base formulation exhibited a smaller, non-significant decrease (from 80.24 ± 3.41 to 69.66 ± 7.52 mg QE/g extract, $p > 0.05$), suggesting that the base provides some protection against flavonoid degradation. DPPH radical scavenging activity remained stable for both formulations, with the extract-only formulation showing a minor decline (from 21.08 ± 0.63 to 19.58 ± 2.16 mg AAE/g extract, $p > 0.05$) and the extract-base combination exhibiting a slight increase (from 15.96 ± 0.60 to 16.68 ± 0.38 mg AAE/g extract, $p > 0.05$). These findings indicate that the antioxidant properties of the formulations are largely retained at room temperature.

At 4°C, a significant reduction in TTC was observed in the extract-only formulation (from 83.55 ± 1.28 to 77.46 ± 1.05 mg TAE/g extract, $p < 0.05$), indicating tannin degradation, possibly due to moisture condensation or microbial activity. However, the extract-base combination exhibited a non-statistically significant decrease (from 106.95 ± 8.62 to 97.35 ± 2.96 mg TAE/g extract). The TFC showed significant reductions in both formulations, with the extract-only formulation decreasing from 135.49 ± 8.03 to 69.79 ± 4.65 mg QE/g extract ($p < 0.05$), and the extract-base formulation decreasing from 80.24 ± 3.41 to 49.80 ± 0.21 mg QE/g extract ($p < 0.05$). This indicates that refrigeration slows degradation but does not entirely prevent it. Interestingly, the DPPH radical scavenging activity of the extract-only formulation increased significantly (from 21.08 ± 0.63 to 24.55 ± 0.54 mg AAE/g extract, $p < 0.05$), which could be attributed to the formation of smaller, more active antioxidant compounds. In contrast, the extract-base formulation exhibited a decrease in antioxidant activity (from 15.96 ± 0.60 to 13.32 ± 2.74 mg AAE/g extract), though the change was not statistically significant ($p > 0.05$).

At elevated temperatures (45°C), TTC in the extract-only formulation decreased significantly (from 83.55 ± 1.28 to 73.81 ± 1.80 mg TAE/g extract, $p < 0.05$), indicating thermal degradation of tannins. Conversely, the extract-base combination exhibited an increase (from 106.95 ± 8.62 to 115.69 ± 5.16 mg TAE/g extract), which may be due to the stabilizing effect of the base ingredients such as Colemn TT. TFC in the extract-only formulation exhibited a significant decrease (from 135.49 ± 8.03 to

83.51 ± 1.11 mg QE/g extract, $p < 0.05$), confirming that flavonoids are highly sensitive to heat. However, the extract-base formulation showed an unexpected increase (from 80.24 ± 3.41 to 93.23 ± 5.48 mg QE/g extract, $p > 0.05$), which may result from thermal breakdown of the base components, releasing bound flavonoids into the formulation. This process can result in an apparent increase in measurable TFC. Studies have shown that heat treatment can lead to the release of phenolic compounds from their bound forms, enhancing their detectable levels. For instance, when mulberry juice was subjected to thermal treatment at 25–45°C, a decrease in total flavonoid content (TFC) was observed. However, exposure to higher temperatures ranging from 45 to 100°C resulted in an increase in TFC (Lin & Xiao, 2024).

Antioxidant activity measured through DPPH assay increased slightly in both formulations at high temperatures. The extract-only formulation rose from 21.08 ± 0.63 to 23.27 ± 2.37 mg AAE/g, while the extract-base formulation increased from 15.96 ± 0.60 to 17.82 ± 0.58 mg AAE/g, although these changes were not statistically significant ($p > 0.05$). This phenomenon aligns with findings by Molaveisi et al. (2019), suggesting that heat may facilitate the release of smaller, more potent antioxidant compounds from larger precursors.

The stability of bioactive compounds in *N. crenulata* leaf extract formulations is highly influenced by storage temperature. Room temperature conditions provide stability for tannins and antioxidant activity but lead to flavonoid degradation. Cold storage (4°C) offers partial preservation but still results in significant losses of flavonoids and tannins, whereas antioxidant activity can increase under such conditions. High temperatures (45°C) cause flavonoid degradation but can enhance antioxidant activity, suggesting complex interactions between bioactive compounds and formulation components. Understanding these stability patterns is crucial for optimizing the formulation and storage of *N. crenulata* extract in cosmetic applications.

Table 4.15 Total tannin content comparison before and after one month stability test

Formulation	Condition kept	Baseline (TAE mg/g extract)	After one month (TAE mg/g extract)
Extract only	Room Temp	83.55 ± 1.28	83.58 ± 0.96
Extract only	4°C	83.55 ± 1.28	77.46 ± 1.05*
Extract only	45°C	83.55 ± 1.28	73.81 ± 1.8*
Extract + Base	Room Temp	106.95 ± 8.62	107.42 ± 3.01
Extract + Base	4°C	106.95 ± 8.62	97.35 ± 2.96
Extract + Base	45°C	106.95 ± 8.62	115.69 ± 5.16

Note * indicates the presence of significant changes between the values when compared with baseline ($p < 0.05$)

Table 4.16 Total flavonoid content comparison before and after one month stability test

Formulation	Condition kept	Baseline (QE mg/g extract)	After one month (QE mg/g extract)
Extract only	Room Temp	135.49 ± 8.03	71.57 ± 4.33*
Extract only	4°C	135.49 ± 8.03	69.79 ± 4.65*
Extract only	45°C	135.49 ± 8.03	83.51 ± 1.11*
Extract + Base	Room Temp	80.24 ± 3.41	69.66 ± 7.52
Extract + Base	4°C	80.24 ± 3.41	49.8 ± 0.21*
Extract + Base	45°C	80.24 ± 3.41	93.23 ± 5.48

Note * indicates the presence of significant changes between the values when compared with baseline ($p < 0.05$)

Table 4.17 DPPH comparison before and after one month stability test

Formulation	Condition kept	Baseline (AAE mg/g extract)	After one month (AAE mg/g extract)
Extract only	Room Temp	21.08 ± 0.63	19.58 ± 2.16
Extract only	4°C	21.08 ± 0.63	24.55 ± 0.54*
Extract only	45°C	21.08 ± 0.63	23.27 ± 2.37
Extract + Base	Room Temp	15.96 ± 0.6	16.68 ± 0.38
Extract + Base	4°C	15.96 ± 0.6	13.32 ± 2.74
Extract + Base	45°C	15.96 ± 0.6	17.82 ± 0.58

Note * indicates the presence of significant changes between the values when compared with baseline ($p < 0.05$)

CHAPTER 5

CONCLUSION

This study demonstrated the effectiveness of microwave-assisted extraction (MAE) in isolating bioactive compounds, specifically phenolics, flavonoids, and tannins, from *N. crenulata* leaves for potential cosmetic applications. The application of 50% ethanol as a solvent demonstrated the highest extraction efficiency (16.81 ± 0.89% w/w), supported by elevated TPC (11.76 ± 0.38 mg GAE/g dry leaves), TFC (19.35 ± 0.32 mg QE/g dry leaves), and TTC (11.51 ± 0.07 mg TAE/g dry leaves). Additionally, significant antioxidant properties were indicated by DPPH (3.35 ± 0.04 mg AAE/g dry leaves) and FRAP assays (3.57 ± 0.05 mg AAE/g dry leaves). The 95% ethanol extract exhibited the highest astringent activity (64.94 ± 0.13%) despite a lower tannin yield, highlighting the complex interactions that affect astringency beyond mere tannin concentration.

Stability testing indicated that bioactive-rich extracts are optimally preserved most at room temperature followed by cold storage at 4°C, with notable degradation occurring at higher temperatures (45°C), underscoring the importance of appropriate storage conditions to sustain bioactivity. Color stability was consistent across storage conditions, pH value remains within the recommended range for skin despite the decline; however, viscosity exhibited a gradual decline, especially at elevated temperatures. This research highlights the potential of *N. crenulata* extracts as a source of antioxidants for anti-aging cosmetics. Given the susceptibility of flavonoids to thermal degradation, future studies should explore encapsulation and other innovative stabilization strategies to improve formulation longevity. Investigating synergistic combinations with other natural ingredients and performing clinical trials would facilitate in fully utilizing the cosmetic potential of *N. crenulata* extracts.

This study demonstrated the effectiveness of MAE and optimal ethanol solvent concentrations for *N. crenulata* leaves extraction, while also giving insights for future advancements in bioactive-based cosmetics utilizing often overlooked parts of the plant.

REFERENCES

Adegbusi, H. S., Ismail, A., Mohd Esa, N., & Azuan Mat Daud, Z. (2022). Application of Folin-Ciocalteau colorimetric method in the determination of total tannin in maize and soybean food products. *International Food Research Journal*, *29*(5).

Al Mamari, H. H. (2021). Phenolic compounds: Classification, chemistry, and updated techniques of analysis and synthesis. In *Phenolic compounds-chemistry, synthesis, diversity, non-conventional industrial, pharmaceutical and therapeutic applications*. IntechOpen.

Ali, A., Akhtar, N., Khan, M. S., Rasool, F., Iqbal, F. M., Khan, M. T., Elahi, E. (2013). Moisturizing effect of cream containing *Moringa oleifera* (Sohajana) leaf extract by biophysical techniques: in vivo evaluation. *Journal of Medicinal Plants Research*, *7*(8), 386-391.

Amarowicz, R., TROSZYŃSKA, A., Baryłko-Pikielna, N. I. N. A., & Shahidi, F. (2004). Polyphenolics extracts from legume seeds: correlations between total antioxidant activity, total phenolics content, tannins content and astringency. *Journal of Food Lipids*, *11*(4), 278-286.

Amarowicz, R., TROSZYŃSKA, A., KOSIŃSKA, A., Lamparski, G., & Shahidi, F. (2008). Relation between sensory astringency of extracts from selected tannin-rich foods and their antioxidant activity. *Journal of Food Lipids*, *15*(1), 28-41

Amr, A. S., Ahmad, M. N., & Abdullah, M. A. (2020). Oak (*Quercus aegilops* L.) Root Bark Tannins Astringency, Antioxidant Potential, and Use as Functional Tea. *Jordan Journal of Agricultural Sciences*, *16*(2), 27-38.

Aung, H. M., Kanpipit, N., & Thapphasaraphong, S. (2024). Effects of Thanaka (*Hesperethusa crenulata*) Stem Bark Extract on Collagen Activation and Anti-Melanogenesis for Cosmetic Applications. *Tropical Journal of Natural Product Research*, *8*(1).

Barbehenn, R. V., & Constabel, C. P. (2011). Tannins in plant–herbivore interactions. *Phytochemistry*, *72*(13), 1551-1565.

Bayram, I., & Decker, E. A. (2023). Underlying mechanisms of synergistic antioxidant interactions during lipid oxidation. *Trends in Food Science & technology*, *133*, 219-230.

Ben Salah, N., Casabianca, H., Ben Jannet, H., Chenavas, S., Sanglar, C., Fildier, A., & Bouzouita, N. (2015). Phytochemical and biological investigation of two Diplotaxis species growing in Tunisia: *D. virgata* & *D. erucoides*. *Molecules*, *20*(10), 18128-18143.

Bhuvaneswari, T., Thiyagarajan, M., Geetha, N., & Venkatachalam, P. (2014). Bioactive compound loaded stable silver nanoparticle synthesis from microwave irradiated aqueous extracellular leaf extracts of *Naringi crenulata* and its wound healing activity in experimental rat model. *Acta Tropica*, *135*, 55-61.

Bjørklund, G., Shanaida, M., Lysiuk, R., Butnariu, M., Peana, M., Sarac, I., Chirumbolo, S. (2022). Natural compounds and products from an anti-aging perspective. *Molecules*, *27*(20), 7084.

Bowen-Forbes, C. S., Zhang, Y., & Nair, M. G. (2010). Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *Journal of food composition and analysis*, *23*(6), 554-560.

Buranasudja, V., Vimolmangkang, S., Sanookpan, K., Binalee, A., Bao Nguyen, A., Dong Thach, U., Minh Nguyen, H. (2023). Antioxidant, Anti-Skin-Aging, Anti-Inflammatory, and Anti-Acetylcholinesterase Activities of *Rourea oligophlebia* Extracts. *Chemistry & Biodiversity*, *20*(4), e202201096.

Butkeviciute, A., Ramanauskiene, K., Kurapkiene, V., & Janulis, V. (2022). Dermal Penetration Studies of Potential Phenolic Compounds Ex Vivo and Their Antioxidant Activity In Vitro. *Plants*, *11*(15), 1901.

Butkevičiūtė, A., Ramanauskienė, K., & Janulis, V. (2022). Release of Apple Extract and Phenolic Compounds from Five Semi-Solid Formulations. In *Scientific Collection «InterConf»: Proceedings of the 3rd International Scientific and Practical Conference «Scientific Goals and Purposes in XXI Century: July 19-20, 2022, Seattle, USA/Editors Anna Svoboda, Mariia Granko. Seattle: ProQuest LLC; Scientific Publishing Center «InterConf», 2022, No 117, July*.

Cai, Y.-Z., Sun, M., Xing, J., Luo, Q., & Corke, H. (2006). Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sciences, 78*(25), 2872-2888.

Cefali, L. C., Ataide, J. A., Fernandes, A. R., Sanchez-Lopez, E., Sousa, I. M. d. O., Figueiredo, M. C., Souto, E. B. (2019). Evaluation of in vitro solar protection factor (SPF), antioxidant activity, and cell viability of mixed vegetable extracts from *Dirmophandra mollis* Benth, *Ginkgo biloba* L., *Ruta graveolens* L., and *Vitis vinífera* L. *Plants, 8*(11), 453.

Chaari, M., Akermi, S., Elhadef, K., Said-Al Ahl, H. A. H., Hikal, W. M., Mellouli, L., & Smaoui, S. (2024). Microwave-assisted extraction of bioactive and nutraceuticals. In *Bioactive Extraction and Application in Food and Nutraceutical Industries* (pp. 79-102). Springer.

Chen, S., Wang, X., Cheng, Y., Gao, H., & Chen, X. (2023). A review of classification, biosynthesis, biological activities and potential applications of flavonoids. *Molecules, 28*(13), 4982.

Chu, B.-S., Wilkin, J. D., House, M., Roleska, M., & Lemos, M. A. (2016). Effect of sucrose on thermal and pH stability of *Clitoria ternatea* extract. *International Journal of Food Processing Technology, 3*(1), 11-17.

de Hoyos-Martínez, P. L., Merle, J., Labidi, J., & Charrier–El Bouhtoury, F. (2019). Tannins extraction: A key point for their valorization and cleaner production. *Journal of Cleaner Production, 206*, 1138-1155.

Dentinho, M. T. P., Moreira, O. C., & Bessa, R. J. B. (2018). The use of polyethylene glycol to reduce the anti-nutritional effects of tannins in *Cistus ladanifer* L. *Forest systems, 27*(1), 11.

Dhanira, A., Elya, B., & Basah, K. (2020). Antioxidant activity test of fractions from star fruit leaves (*Averrhoa Carambola* L.) from three regions in West Java. *International Journal of Applied Pharmaceutics, 12*(Special Issue 1), 97-100.

dos Santos Nascimento, L. B., Gori, A., Raffaelli, A., Ferrini, F., & Brunetti, C. (2021). Phenolic compounds from leaves and flowers of *Hibiscus roseus*: Potential skin cosmetic applications of an under-investigated species. *Plants, 10*(3), 522.

Dąbrowska, M., & Nowak, I. (2021). Lipid nanoparticles loaded with selected iridoid glycosides as effective components of hydrogel formulations. *Materials*, *14*(15), 4090.

Erpel, F., Mateos, R., Pérez-Jiménez, J., & Pérez-Correa, J. R. (2020). Phlorotannins: From isolation and structural characterization, to the evaluation of their antidiabetic and anticancer potential. *Food Research International*, *137*, 109589.

Fernandes, A. J. D., Ferreira, M. R. A., Randau, K. P., de Souza, T. P., & Soares, L. A. L. (2012). Total flavonoids content in the raw material and aqueous extractives from *Bauhinia monandra* Kurz (Caesalpiniaceae). *The Scientific World Journal*, *2012*(1), 923462.

Fleming, E. E., Ziegler, G. R., & Hayes, J. E. (2016). Salivary protein levels as a predictor of perceived astringency in model systems and solid foods. *Physiology & Behavior*, *163*, 56-63.

Fraga-Corral, M., García-Oliveira, P., Pereira, A. G., Lourenço-Lopes, C., Jimenez-Lopez, C., Prieto, M. A., & Simal-Gandara, J. (2020). Technological application of tannin-based extracts. *Molecules*, *25*(3), 614.

Fraga-Corral, M., Otero, P., Echave, J., Garcia-Oliveira, P., Carpena, M., Jarboui, A., Prieto, M. A. (2021a). By-products of agri-food industry as tannin-rich sources: A review of tannins' biological activities and their potential for valorization. *Foods*, *10*(1), 137.

Fredes, C., Becerra, C., Parada, J., & Robert, P. (2018). The microencapsulation of maqui (*Aristotelia chilensis* (Mol.) Stuntz) juice by spray-drying and freeze-drying produces powders with similar anthocyanin stability and bioaccessibility. *Molecules*, *23*(5), 1227.

Gallo, M., Ferracane, R., Graziani, G., Ritieni, A., & Fogliano, V. (2010). Microwave assisted extraction of phenolic compounds from four different spices. *Molecules*, *15*(9), 6365-6374.

Garcia Bartels, N., Lünnemann, L., Stroux, A., Kottner, J., Serrano, J., & Blume-Peytavi, U. (2014). Effect of diaper cream and wet wipes on skin barrier properties in infants: a prospective randomized controlled trial. *Pediatric dermatology*, *31*(6), 683-691.

Gaweł-Bęben, K., Bujak, T., Nizioł-Łukaszewska, Z., Antosiewicz, B., Jakubczyk, A., Karaś, M., & Rybczyńska, K. (2015). *Stevia rebaudiana* Bert. leaf extracts as a multifunctional source of natural antioxidants. *Molecules*, *20*(4), 5468-5486.

Gordaliza, M. (2007). Natural products as leads to anticancer drugs. *Clinical and Translational Oncology*, *9*, 767-776.

Grabber, J. H., Zeller, W. E., & Mueller-Harvey, I. (2013). Acetone enhances the direct analysis of procyanidin-and prodelphinidin-based condensed tannins in Lotus species by the butanol–HCl–iron assay. *Journal of Agricultural and Food Chemistry*, *61*(11), 2669-2678.

Graça, M. A., & Bärlocher, F. (2020). Radial diffusion assay for tannins. *Methods to study litter decomposition: a practical guide*, 163-167.

Grenier, A., Legault, J., Pichette, A., Jean, L., Bélanger, A., & Pouliot, R. (2021). Antioxidant, anti-inflammatory, and anti-aging potential of a *Kalmia angustifolia* extract and identification of some major compounds. *Antioxidants*, *10*(9), 1373.

Gunalan, S., Thangaiah, A., Rathnasamy, V. K., Janaki, J. G., Thiyagarajan, A., Kuppusamy, S., & Arunachalam, L. (2023). Microwave-assisted extraction of biomolecules from moringa (*Moringa oleifera* Lam.) leaves var. PKM 1: A optimization study by response surface methodology (RSM). *Kuwait Journal of Science*, *50*(3), 339-344.

Guo, Y., Chen, X., Qi, J., & Yu, B. (2016). Simultaneous qualitative and quantitative analysis of flavonoids and alkaloids from the leaves of *Nelumbo nucifera* Gaertn. using high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry. *Journal of Separation Science*, *39*(13), 2499-2507.

Gutiérrez-Grijalva, E. P., Ambriz-Pére, D. L., Leyva-López, N., Castillo-López, R. I., & Heredia, J. B. (2016). Dietary phenolic compounds, health benefits and bioaccessibility. *Archivos Latinoamericanos De Nutricion*, *66*(2), 87-100.

Hammami, M., Chaabani, E., Yeddes, W., Wannes, W. A., & Bourgou, S. (2023). Phenolic Compounds and Skin Permeability: An *in Silico* Investigation. *Avicenna Journal of Medical Biochemistry*, *11*(1), 11-18.

Hashemi-Moghaddam, H., Kalatejari, A., Afshari, H., & Ebadi, A. H. (2013). Microwave accelerated distillation of essential oils from the leaves of *Eucalyptus microtheca*: Optimization and comparison with conventional hydrodistillation. *Asian J Chem*, *25*, 5423-5427.

Hassan, H. A., Attia, E. Z., Desoukey, S. Y., Mohamed, K. M., & Kamel, M. S. (2019). Quantitative analysis of total phenolic and total flavonoid constituents of some Ficus species. *Journal of advanced Biomedical and Pharmaceutical Sciences*, *2*(1), 38-40.

Henderson, T., Nigam, P. S., & Owusu-Apenten, R. K. (2015). A universally calibrated microplate ferric reducing antioxidant power (FRAP) assay for foods and applications to Manuka honey. *Food Chemistry*, *174*, 119-123.

Huang, Q., Liu, X., Zhao, G., Hu, T., & Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Animal Nutrition*, *4*(2), 137-150.

Hudz, N., Sedláčková, V. H., Kukhtenko, H., & Svydenko, L. (2023). Facets of the Elaboration of the *Salvia sclarea L*. Extracts. *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, *7*(1).

Huma, Z. E., Jayasena, V., Nasar-Abbas, S. M., Imran, M., & Khan, M. K. (2018). Process optimization of polyphenol extraction from carob (*Ceratonia siliqua*) kibbles using microwave-assisted technique. *Journal of Food Processing and Preservation*, *42*(2), e13450.

Hung, Y.-T., Chen, P.-C., Chen, R. L. C., & Cheng, T.-J. (2010). Sequential determination of tannin and total amino acid contents in tea for taste assessment by a fluorescent flow-injection analytical system. *Food Chemistry*, *118*(3), 876-881.

Hussain, M. B., Hassan, S., Waheed, M., Javed, A., Farooq, M. A., & Tahir, A. (2019). Bioavailability and metabolic pathway of phenolic compounds. In M. M. Ramos (Ed.), *Plant physiological aspects of phenolic compounds* (pp. 67–83). IntechOpen.

Jahmidi-Azizi, N., Oliva, R., & Winter, R. (2023). Alcohol-Induced Conformation Changes and Thermodynamic Signatures in the Binding of Polyphenols to Proline-Rich Salivary Proteins. *Chemistry–A European Journal*, *29*(67), e202302384.

Jiang, Y. H., Jiang, X. L., & Cai, C. G. (2014). Study on microwave-assisted extraction technology of total flavonoid from castor leaves. *Advanced Materials Research*, *1004*, 868-872.

Johnson, J. B., Mani, J. S., & Naiker, M. (2022). Development and validation of a 96-well microplate assay for the measurement of total phenolic content in ginger extracts. *Food Analytical Methods*, *15*(2), 413-420.

Kalili, K. M., & de Villiers, A. (2011). Recent developments in the HPLC separation of phenolic compounds. *Journal of Separation Science*, *34*(8), 854-876.

Kaliyadan, F., Al Dhafiri, M., & Aatif, M. (2021). Attitudes toward organic cosmetics: A cross-sectional population-based survey from the Middle East. *Journal of Cosmetic Dermatology*, *20*(8), 2552-2555.

Kanlayavattanakul, M., Lourith, N., Ospondpant, D., Ruktanonchai, U., Pongpunyayuen, S., & Chansriniyom, C. (2013). Salak plum peel extract as a safe and efficient antioxidant appraisal for cosmetics. *Bioscience, Biotechnology, and Biochemistry*, *77*(5), 1068-1074.

Kanthraj, M., Namitha, K. C., Vaishali, & Trilokchandran, B. (2024). A review: Microwave assisted extraction. International Journal of Scientific Research in Engineering and Management.

Kelly, M., Vardhanabhuti, B., Luck, P., Drake, M. A., Osborne, J., & Foegeding, E. A. (2010). Role of protein concentration and protein–saliva interactions in the astringency of whey proteins at low pH. *Journal of Dairy Science*, *93*(5), 1900-1909.

Kumar, N., & Chaiyasut, C. (2017). Health promotion potential of vegetables cultivated in northern Thailand: a preliminary screening of tannin and flavonoid contents, 5α-reductase inhibition, astringent activity, and antioxidant activities. *Journal of Evidence-based Complementary & Alternative Medicine*, *22*(4), 573-579.

Latha, K. V., Arumugasamy, K., & Manonmani, K. (2005). Preliminary Phytochemical And Antimicrobial Activites On The Leaves Of *Naringi crenulata* Roxb. *Ancient Science of Life*, *25*(2), 53-56.

Le, X. D., Nguyen, M. C., Vu, D. H., Pham, M. Q., Pham, Q. L., Nguyen, Q. T.,Nguyen, T. V. (2019). Optimization of microwave-assisted extraction of total phenolic and total flavonoid contents from fruits of *Docynia indica* (Wall.) Decne. using response surface methodology. *Processes*, *7*(8), 485.

Lee, J., & Kwon, K. H. (2022). Future perspective safe cosmetics: Focused on associated with ISO natural organic index. *Journal of Cosmetic Dermatology*, *21*(12), 6619-6627.

Li, D.-C., & Jiang, J.-G. (2010). Optimization of the microwave-assisted extraction conditions of tea polyphenols from green tea. *International Journal of Food Sciences and Nutrition*, *61*(8), 837-845.

Li, Y., Li, S., Lin, S.-J., Zhang, J.-J., Zhao, C.-N., & Li, H.-B. (2017). Microwave-assisted extraction of natural antioxidants from the exotic *Gordonia axillaris* fruit: Optimization and identification of phenolic compounds. *Molecules*, *22*(9), 1481.

Lim, M. W., Aroua, M. K., & Gew, L. T. (2021). Thanaka (*H. crenulata*, *N. crenulata*, *L. acidissima* L.): a systematic review of its chemical, biological properties and cosmeceutical applications. *Cosmetics*, *8*(3), 68.

Lima, M. B., Andrade, S. I. E., Barreto, I. S., Almeida, L. F., Silva, E. C., & Araújo, M. C. U. (2012). Quantitative spot test analysis of soluble tannin in green tea using a portable diffuse reflectometer. *Analytical Methods*, *4*(8), 2329-2333.

Lin, S., & Xiao, J. (2024). Impact of thermal processing on dietary flavonoids. *Advances in Food and Nutrition Research*, *108*, 1-34.

Luo, M., Zhou, D.-D., Shang, A., Gan, R.-Y., & Li, H.-B. (2021). Influences of microwave-assisted extraction parameters on antioxidant activity of the extract from *Akebia trifoliata* peels. *Foods*, *10*(6), 1432.

Ma, W., Waffo-Teguo, P., Jourdes, M., Li, H., & Teissedre, P.-L. (2016). Chemical affinity between tannin size and salivary protein binding abilities: Implications for wine astringency. *PloS one*, *11*(8), e0161095.

Macáková, K., Kolečkář, V., Cahlíková, L., Chlebek, J., Hošt'álková, A., Kuča, K., & Opletal, L. (2014). Tannins and their influence on health. In A. M. Holban & A. M. Grumezescu (Eds.), *Recent advances in medicinal chemistry* (pp. 159–208). Elsevier.

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition, 81*(1), 230S-242S.

Mapoung, S., Semmarath, W., Arjsri, P., Umsumarng, S., Srisawad, K., Thippraphan, P., Limtrakul, P. (2021). Determination of phenolic content, antioxidant activity, and tyrosinase inhibitory effects of functional cosmetic creams available on the Thailand market. *Plants*, *10*(7), 1383.

Meliniasari, A. R., & Mas'od, A. (2024). Understanding factors shaping green cosmetic purchase intentions: Insights from attitudes, norms, and perceived behavioral control. *Int. J. Acad. Res. Bus. Soc. Sci*, *14*, 1487-1496.

Melone, F., Saladino, R., Lange, H., & Crestini, C. (2013). Tannin structural elucidation and quantitative 31P NMR analysis. Hydrolyzable tannins and proanthocyanidins. *Journal of Agricultural and Food Chemistry, 61*(39), 9316-9324.

Micek, I., Nawrot, J., Seraszek-Jaros, A., Jenerowicz, D., Schroeder, G., Spiżewski, T., Gornowicz-Porowska, J. (2021). Taxifolin as a promising ingredient of cosmetics for adult skin. *Antioxidants*, *10*(10), 1625.

Mizgier, P., Kucharska, A. Z., Sokół-Łętowska, A., Kolniak-Ostek, J., Kidoń, M., & Fecka, I. (2016). Characterization of phenolic compounds and antioxidant and anti-inflammatory properties of red cabbage and purple carrot extracts. *Journal of Functional Foods*, *21*, 133-146.

Mo, E. K., Han, B. H., Kim, S. M., Yang, S. A., Kang, S. K., Oh, C. J.,Sung, C. K. (2012). Identification of d-friedoolean-13-en-3-one (Taraxerone) as an antioxidant compound from sedom (*Sedum sarmentosum*). *Food Science and Biotechnology*, *21*, 485-489.

Molaveisi, M., Beigbabaei, A., Akbari, E., Noghabi, M. S., & Mohamadi, M. (2019). Kinetics of temperature effect on antioxidant activity, phenolic compounds and color of Iranian jujube honey. *Heliyon*, *5*(1).

Molino, S., Casanova, N. A., Rufián Henares, J. Á., & Fernandez Miyakawa, M. E. (2019). Natural tannin wood extracts as a potential food ingredient in the food industry. *Journal of Agricultural and Food Chemistry*, *68*(10), 2836-2848.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*, *26*(2), 211-219.

Nemli, S. K., Gungor, M. B., Bagkur, M., Bal, B. T., & Arici, Y. K. (2018). *In vitro* evaluation of color and translucency reproduction of maxillofacial prostheses using a computerized system. *The Journal of Advanced Prosthodontics*, *10*(6), 422-429.

Nishino, H., Naitoh, E., Iwashima, A., & Umezawa, K. (1984). Quercetin interacts with calmodulin, a calcium regulatory protein. *Experientia*, *40*, 184-185.

Nolvachai, Y., & Marriott, P. J. (2013). GC for flavonoids analysis: Past, current, and prospective trends. *Journal of Separation Science*, *36*(1), 20-36.

Obreque-Slier, E., Pena-Neira, A., & Lopez-Solis, R. (2010). Enhancement of both salivary protein− enological tannin interactions and astringency perception by ethanol. *Journal of Agricultural and Food Chemistry*, *58*(6), 3729-3735.

Okuda, T., & Ito, H. (2011). Tannins of constant structure in medicinal and food plants—hydrolyzable tannins and polyphenols related to tannins. *Molecules*, *16*(3), 2191-2217.

Ozcan, T., Akpinar-Bayizit, A., Yilmaz-Ersan, L., & Delikanli, B. (2014). Phenolics in human health. *International Journal of Chemical Engineering and Applications*, *5*(5), 393-396.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, *5*, e47.

Paravina, R. D., Ghinea, R., Herrera, L. J., Bona, A. D., Igiel, C., Linninger, M., Mar Perez, M. d. (2015). Color difference thresholds in dentistry. *Journal of Esthetic and Restorative Dentistry*, *27*, S1-S9.

Paravina, R. D., Pérez, M. M., & Ghinea, R. (2019). Acceptability and perceptibility thresholds in dentistry: A comprehensive review of clinical and research applications. *Journal of Esthetic and Restorative Dentistry*, *31*(2), 103-112.

Pongsua, P. (2016). *Extraction and biological activities of water-soluble polysaccharides from edible mushrooms for cosmetic applications* (Master's thesis, Mae Fah Luang University). School of Cosmetic Science, Mae Fah Luang University.

Pratheeba, T., Vivekanandhan, P., Faeza, A. K. N., & Natarajan, D. (2019). Chemical constituents and larvicidal efficacy of *Naringi crenulata* (Rutaceae) plant extracts and bioassay guided fractions against *Culex quinquefasciatus* mosquito (Diptera: Culicidae). *Biocatalysis and Agricultural Biotechnology*, *19*, 101137.

Périno-Issartier, S., Abert-Vian, M., & Chemat, F. (2011). Solvent free microwave-assisted extraction of antioxidants from sea buckthorn (*Hippophae rhamnoides*) food by-products. *Food and Bioprocess Technology*, *4*, 1020-1028.

Qusti, S. Y., Abo-khatwa, A. N., Lahwa, M. A. B., & Qusti, S. (2010). Screening of antioxidant activity and phenolic content of selected food items cited in the Holy Quran. *EJBS*, *2*(1), 40-51.

Rahath Kubra, I., Kumar, D., & Rao, L. J. M. (2013). Effect of microwave-assisted extraction on the release of polyphenols from ginger (*Zingiber officinale*). *International Journal of Food Science & Technology*, *48*(9), 1828-1833.

Rambabu, M., Basha, S. K. M., & Narasimha Murthy, C. V. (2017). Antimicrobial activity of leaf extracts of *Naringi crenulata*. *International Journal of Latest Trends in Engineering and Technology*, *8*(2), 072-076.

Ratajczak, P., Landowska, W., Kopciuch, D., Paczkowska, A., Zaprutko, T., & Kus, K. (2023). The growing market for natural cosmetics in Poland: Consumer preferences and industry trends. *Clinical, Cosmetic and Investigational Dermatology*, 1877-1892.

Rothe, J., Fischer, R., Cotterchio, C., Gastl, M., & Becker, T. (2023). Analytical determination of antioxidant capacity of hop-derived compounds in beer using specific rapid assays (ORAC, FRAP) and ESR-spectroscopy. *European Food Research and Technology*, *249*(1), 81-93.

Rubió, L., Motilva, M.-J., & Romero, M.-P. (2013). Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. *Critical Reviews in Food Science and Nutrition*, *53*(9), 943-953.

Ruesgas-Ramón, M., Figueroa-Espinoza, M. C., & Durand, E. (2017). Application of deep eutectic solvents (DES) for phenolic compounds extraction: Overview, challenges, and opportunities. *Journal of Agricultural and Food Chemistry*, *65*(18), 3591-3601.

Saewan, N., & Jimtaisong, A. (2013). Photoprotection of natural flavonoids. *Journal of Applied Pharmaceutical Science*, *3*(9), 129-141.

Salminen, J. P., & Karonen, M. (2011). Chemical ecology of tannins and other phenolics: we need a change in approach. *Functional Ecology*, *25*(2), 325-338.

Sampaopan, Y., & Suksaeree, J. (2022). Microwave-assisted extraction and content determination of astilbin in *Lysiphyllum strychnifolium* stems. *Brazilian Journal of Pharmaceutical Sciences*, *58*, e20577.

Sampathkumar, S., & Ramakrishnan, N. (2012). Pharmacognostical analysis of *Naringi crenulata* leaves. *Asian Pacific Journal of Tropical Biomedicine*, *2*(2), S627-S631.

Santos, E. L., Maia, B., Ferriani, A. P., & Teixeira, S. D. (2017). Flavonoids: Classification, biosynthesis and chemical ecology. *Flavonoids-From Biosynthesis to Human Health*, *13*, 78-94.

Sanz, M., Cadahia, E., Esteruelas, E., Muñoz, A. n. M., Fernandez de Simon, B., Hernandez, T., & Estrella, I. (2010). Phenolic compounds in chestnut (*Castanea sativa* Mill.) heartwood. Effect of toasting at cooperage. *Journal of Agricultural and Food Chemistry*, *58*(17), 9631-9640.

Sarada, K., Margret, R. J., & Mohan, V. R. (2012). Hepatoprotective and antioxidant activity of ethanol extracts of *Naringi crenulata* (Roxb) Nicolson against CCl_4-induced hepatotoxicity in rats. *International Journal of Pharmaceutical Sciences and Research, 3*(3), 874.

Sari, B. L., Triastinurmiatiningsih, T., & Haryani, T. S. (2020). Optimasi Metode Microwave-Assisted Extraction (MAE) untuk Menentukan Kadar Flavonoid Total Alga Coklat *Padina australis*. *ALCHEMY Jurnal Penelitian Kimia*, *16*(1), 38-49.

Seiverling, E. V., Trubiano, J. P., Williams, J. C., Ahrns, H. T., Craft, D. W., & England, M. R. (2017). Analysis of the antimicrobial properties of thanaka, a Burmese powder used to treat acne. *Journal of Biosciences and Medicines*, *5*(7), 1-6.

Sen, S., & Chakraborty, R. (2011). The role of antioxidants in human health. In D. Armstrong (Ed.), *Oxidative stress: Diagnostics, prevention, and therapy* (pp. 1–37). American Chemical Society.

Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A. M., & Saura-Calixto, F. (2009). Tannins: current knowledge of food sources, intake, bioavailability and biological effects. *Molecular Nutrition & Food Research, 53*(S2), S310-S329.

Sethi, T., Kheur, M., Coward, T., & Patel, N. (2015). Change in color of a maxillofacial prosthetic silicone elastomer, following investment in molds of different materials. *The Journal of Indian Prosthodontic Society*, *15*(2), 153-157.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods*, *18*, 820-897.

Shahidi, F., & Dissanayaka, C. S. (2023). Phenolic-protein interactions: insight from in-silico analyses–a review. *Food Production, Processing and Nutrition*, *5*(1), 2.

Shang, A., Luo, M., Gan, R.-Y., Xu, X.-Y., Xia, Y., Guo, H., Li, H.-B. (2020). Effects of microwave-assisted extraction conditions on antioxidant capacity of sweet tea (*Lithocarpus polystachyus* Rehd.). *Antioxidants, 9*(8), 678.

Sharma, K., Kumar, V., Kaur, J., Tanwar, B., Goyal, A., Sharma, R., Kumar, A. (2021). Health effects, sources, utilization and safety of tannins: A critical review. *Toxin Reviews*, *40*(4), 432-444.

Shay, P.-E., Trofymow, J. A., & Constabel, C. P. (2017). An improved butanol-HCl assay for quantification of water-soluble, acetone: methanol-soluble, and insoluble proanthocyanidins (condensed tannins). *Plant Methods*, *13*, 1-11.

Shirmohammadli, Y., Efhamisisi, D., & Pizzi, A. (2018). Tannins as a sustainable raw material for green chemistry: A review. *Industrial Crops and Products*, *126*, 316-332.

Silva, C. C. A. R., Gomes, C. L., Danda, L. J. A., Roberto, A. E. M., Carvalho, A. M. R. D. E., Ximenes, E. C. P. A., Rolim, P. J. (2021). Optimized microwave-assisted extraction of polyphenols and tannins from *Syzygium cumini* (L.) Skeels leaves through an experimental design coupled to a desirability approach. *Anais da Academia Brasileira de Ciências*, *93*(2), e20190632.

Singh, N., Meena, M. K., & Patni, V. (2017). HPTLC method for isolation, identification and quantification of quercetin from in vivo and in vitro samples of *Naringi crenulata*. *Indian Journal of Pharmaceutical and Biological Research*, *5*(03), 26-31.

Siqueira, C. F. D. Q., Cabral, D. L. V., Peixoto Sobrinho, T. J. D. S., de Amorim, E. L. C., de Melo, J. G., Araújo, T. A. D. S., & de Albuquerque, U. P. (2012). Levels of tannins and flavonoids in medicinal plants: Evaluating bioprospecting strategies. *Evidence-Based Complementary and Alternative Medicine*, *2012*, Article 434782.

Smeriglio, A., Barreca, D., Bellocco, E., & Trombetta, D. (2017). Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. *British journal of pharmacology*, *174*(11), 1244-1262.

Suliman, S., Sulaiman, T. A., Olafsson, V. G., Delgado, A. J., Donovan, T. E., & Heymann, H. O. (2019). Effect of time on tooth dehydration and rehydration. *Journal of Esthetic and Restorative Dentistry*, *31*(2), 118-123.

Sun, F. L., Chen, G. N., Wu, B., Zhu, W. N., & Yan, Y. (2014). Research on the extraction and antibacterial activity of tea polyphenols. *Applied Mechanics and Materials*, *644*, 5157-5160.

Tran, P. L. P., & Le, H. P. (2022). Plant flavonoids as potential natural antioxidants in phytocosmetics. *Journal of Technical Education Science*, *17*(Special Issue 01), 86-93.

Vallinayagam, S., Rajendran, K., & Sekar, V. (2021). Pro-apoptotic property of phytocompounds from *Naringi crenulata* in HER2+ breast cancer cells in vitro. *Biotechnology & Biotechnological Equipment*, *35*(1), 303-314.

Vermerris, W., Nicholson, R., Vermerris, W., & Nicholson, R. (2006). Families of phenolic compounds and means of classification. *Phenolic Compound Biochemistry*, 1-34.

Villa, C., Robustelli Della Cuna, F. S., Russo, E., Ibrahim, M. F., Grignani, E., & Preda, S. (2022). Microwave-assisted and conventional extractions of volatile compounds from *Rosa* x *damascena* Mill. fresh petals for cosmetic applications. *Molecules*, *27*(12), 3963.

Vuolo, M. M., Lima, V. S., & Junior, M. R. M. (2019). Phenolic compounds: Structure, classification, and antioxidant power. In *Bioactive compounds* (pp. 33-50). Elsevier.

Watrelot, A. A. (2021). Tannin content in Vitis species red wines quantified using three analytical methods. *Molecules*, *26*(16), 4923.

Wei, A., & Shibamoto, T. (2010). Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. *Journal of Agricultural and Food Chemistry*, *58*(12), 7218-7225.

Weremfo, A., Adulley, F., & Adarkwah-Yiadom, M. (2020). Simultaneous optimization of microwave-assisted extraction of phenolic compounds and antioxidant activity of avocado (*Persea americana* Mill.) seeds using response surface methodology. *Journal of Analytical Methods in Chemistry*, *2020*(1), 7541927.

Wigley, B. J., Charles-Dominique, T., Hempson, G. P., Stevens, N., TeBeest, M., Archibald, S., Donaldson, J. (2020). A handbook for the standardized sampling of plant functional traits in disturbance-prone ecosystems, with a focus on open ecosystems. *Australian Journal of Botany*, *68*(8), 473-531.

Wong, P. L., Ramli, N. S., Tan, C. P., Azlan, A., & Abas, F. (2021). Metabolomic analysis reveals the valuable bioactive compounds of *Ardisia elliptica*. *Phytochemical Analysis*, *32*(5), 685-697.

Xia, E.-Q., Cui, B., Xu, X.-R., Song, Y., Ai, X.-X., & Li, H.-B. (2011). Microwave-assisted extraction of oxymatrine from *Sophora flavescens*. *Molecules*, *16*(9), 7391-7400.

Yahfoufi, N., Alsadi, N., Jambi, M., & Matar, C. (2018). The immunomodulatory and anti-inflammatory role of polyphenols. *Nutrients*, *10*(11), 1618.

Yoshida, A. K. I., Miller, L., Da Silva, J. D., & Ishikawa-Nagai, S. (2010). Spectrophotometric analysis of tooth color reproduction on anterior all-ceramic crowns: part 2: color reproduction and its transfer from in vitro to in vivo. *Journal of Esthetic and Restorative Dentistry*, *22*(1), 53-63.

Zeng, N., Zhang, G., Hu, X., Pan, J., & Gong, D. (2019). Mechanism of fisetin suppressing superoxide anion and xanthine oxidase activity. *Journal of Functional Foods*, *58*, 1-10.

Zhang, M., Sun, J., & Chen, P. (2017). Development of a comprehensive flavonoid analysis computational tool for ultrahigh-performance liquid chromatography-diode array detection-high-resolution accurate mass-mass spectrometry data. *Analytical Chemistry*, *89*(14), 7388-7397.

Zhou, J., Zheng, X., Yang, Q., Liang, Z., Li, D., Yang, X., & Xu, J. (2013). Optimization of ultrasonic-assisted extraction and radical-scavenging capacity of phenols and flavonoids from *Clerodendrum cyrtophyllum* Turcz leaves. *PloS One*, *8*(7), e68392.

Álvarez, A., Poejo, J., Matias, A. A., Duarte, C. M. M., Cocero, M. J., & Mato, R. B. (2017). Microwave pretreatment to improve extraction efficiency and polyphenol extract richness from grape pomace. Effect on antioxidant bioactivity. *Food and Bioproducts Processing*, *106*, 162-170.