PHYTOCHEMICAL ANALYSIS AND LIPSTICK FORMULATION OPTIMIZATION OF WATERMELON RIND EXTRACT (*Citrullus lanatus*): PHYSICOCHEMICAL CHARACTERIZATION AND SAFETY EVALUATION

UNDERGRADUATE THESIS

Submitted to fulfill one of the requirements for obtaining
the Bachelor of Science (S.Si) degree in the Chemistry
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Proposed by:

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Bachelor's Program in Chemistry, Faculty of Mathematics and Natural Sciences

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(Muzakkir Fahmi)

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PREFACE

Assalamu'alaikum Warahmatullahi Wabarakatuh

All praise be to Allah SWT, the Most Gracious and Most Merciful, for His countless blessings and mercy, which have enabled the completion of this undergraduate thesis entitled **"PHYTOCHEMICAL ANALYSIS AND LIPSTICK FORMULATION OPTIMIZATION OF WATERMELON RIND EXTRACT (*Citrullus lanatus*): PHYSICOCHEMICAL CHARACTERIZATION AND SAFETY EVALUATION"**.

This thesis is prepared as one of the requirements to fulfill the final project for completing the undergraduate program in Chemistry at the Faculty of Mathematics and Natural Sciences, Universitas Islam Indonesia. It is hoped that this thesis can contribute significantly to the field of natural materials and the cosmetics industry, particularly in optimizing the utilization of fruit waste.

Throughout the writing process of this thesis, I have received guidance, support, and assistance from various parties. Therefore, I would like to express my deepest gratitude to:

1. Muzakkir Fahmi – I would like to appreciate myself for the perseverance and dedication I maintained to successfully complete this research. Congratulations to myself, and keep up the spirit for the next steps!
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I acknowledge that this thesis is far from perfect and still has limitations. Therefore, I highly welcome constructive criticism and suggestions for improvement and further refinement. As a closing note, I quote a profound saying from Imam Shafi'i:

"Whoever has not tasted the bitterness of learning will surely taste the humiliation of ignorance for a lifetime."

May the spirit to continue learning and growing always accompany us. Thank you.

Wassalamu'alaikum Warahmatullahi Wabarakatuh

Yogyakarta, January 17th 2025

Muzakkir Fahmi

PHYTOCHEMICAL ANALYSIS AND LIPSTICK FORMULATION OPTIMIZATION OF WATERMELON RIND EXTRACT (*Citrullus lanatus*): PHYSICOCHEMICAL CHARACTERIZATION AND SAFETY EVALUATION

ABSTRACT

Muzakkir Fahmi

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This study explored the potential of *Citrullus lanatus* rind extract as a natural antioxidant for sustainable lipstick formulation, involving phytochemical extraction, formulation optimization, physicochemical characterization, and safety evaluation. Sequential extraction with n-hexane, ethyl acetate, and ethanol was performed, with ethanol yielding the highest concentration of bioactive compounds. The lipstick formulation was optimized using the D-optimal mixture design to achieve a suitable melting point. Physicochemical characterization involved pH measurement, thermal stability analysis (TGA), and physical stability evaluation. The optimized lipstick had a melting point of 52.5 °C, thermal stability up to 160.92 °C, and a near-neutral pH of 7.20. Safety assessments confirmed microbiological contamination within ISO safety limits, with aerobic mesophilic bacteria at 50 cfu/g, yeast & mold at <100 cfu/g, and the absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Additionally, heavy metal analysis indicated that arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) levels complied with Malaysian and ASEAN cosmetic safety standards, with concentrations of 0.1722 ppm (As), <0.04 ppm (Pb), <0.02 ppm (Cd), and <0.02 ppm (Hg). These findings support the use of *C. lanatus* rind extract in cosmetics, advancing circular economy principles and sustainable product innovation.

Keywords: *Citrullus lanatus*, antioxidants, sequential extraction, phytochemicals, lipstick, D-optimal mixture design, safety analysis.

ANALISIS FITOKIMIA DAN OPTIMASI FORMULASI LIPSTIK EKSTRAK KULIT BUAH SEMANGKA (*Citrullus lanatus*): KARAKTERISASI FISIKOKIMIA DAN EVALUASI KEAMANAN

INTISARI

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Penelitian ini mengeksplorasi potensi ekstrak kulit buah semangka (*Citrullus lanatus*) sebagai antioksidan alami untuk formulasi lipstik berkelanjutan, mencakup ekstraksi fitokimia, optimasi formulasi, karakterisasi fisikokimia, dan evaluasi keamanan. Ekstraksi berurutan menggunakan n-heksana, etil asetat, dan etanol dilakukan, dengan etanol menghasilkan konsentrasi senyawa bioaktif tertinggi. Formulasi lipstik dioptimalkan menggunakan *D-optimal mixture design* untuk memperoleh titik leleh yang sesuai. Karakterisasi fisikokimia mencakup pengukuran pH, analisis stabilitas termal menggunakan termogravimetri (TGA), dan evaluasi stabilitas fisik. Formulasi lipstik yang dioptimalkan memiliki titik leleh sebesar 52,5 °C, stabilitas termal hingga 160,92 °C, dan pH mendekati netral (7,20). Evaluasi keamanan menunjukkan bahwa kontaminasi mikrobiologi berada dalam batas aman sesuai standar ISO, dengan bakteri mesofilik aerob sebesar 50 cfu/g, khamir & kapang <100 cfu/g, serta tidak terdeteksinya Pseudomonas aeruginosa, Staphylococcus aureus, dan Candida albicans. Selain itu, analisis logam berat mengonfirmasi bahwa kadar arsenik (As), timbal (Pb), kadmium (Cd), dan merkuri (Hg) memenuhi standar keamanan kosmetik di Malaysia dan ASEAN, dengan konsentrasi 0,1722 ppm (As), <0,04 ppm (Pb), <0,02 ppm (Cd), dan <0,02 ppm (Hg). Hasil ini mendukung pemanfaatan ekstrak kulit C. lanatus dalam formulasi kosmetik, sejalan dengan prinsip ekonomi sirkular serta inovasi produk yang ramah lingkungan.

Kata Kunci: *Citrullus lanatus*, antioksidan, ekstraksi berurutan, fitokimia, lipstik, *D-optimal mixture design*, analisis keamanan.

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CHAPTER I
INTRODUCTION

1.1 Background

Natural products have emerged as critical components in the cosmetics industry due to their multifunctionality, safety profile, and eco-friendliness. The increasing global demand for sustainable and health-conscious beauty products has shifted industry focus toward bioactive compounds derived from plants, marine life, and microorganisms. These natural ingredients offer numerous benefits, including skin rejuvenation, protection from environmental damage, and anti-aging effects, while minimizing adverse effects associated with synthetic chemicals. Their use aligns with consumer preferences for environmentally friendly products and contributes to advancing the industry's commitment to sustainability and innovation (Liu, 2022).

The increasing demand for natural antioxidants in cosmetic formulations reflects a shift in consumer preferences towards products offering health benefits and environmental sustainability. Antioxidants derived from natural sources, such as phenolic compounds from plant by-products, play a vital role in protecting the skin from oxidative stress, delaying aging, and enhancing skin health. Recent advancements in green extraction technologies have enabled the efficient retrieval of these bioactive compounds with minimal environmental impact, aligning with global sustainability goals. These natural antioxidants enhance the functional and therapeutic properties of cosmetic formulations while contributing to the valorization of agricultural by-products, thereby minimizing waste and fostering a circular economy (Leyva-Jiménez et al., 2022).

Lipstick is one of the most widely used cosmetic products in society. Its application not only serves to enhance aesthetics by highlighting the attractiveness and beauty of the lips but also provides social and psychological benefits for individuals. Although aesthetic function remains the primary factor in lipstick selection, consumer trends are shifting with the emergence of therapeutic lipsticks in the market. This type of lipstick is formulated with specific active ingredients to

provide pharmacological effects, such as preventing bacterial infections and addressing chapped lips. In line with the growing demand for natural-based cosmetic products, herbal cosmetic formulations are becoming increasingly popular. These products offer dual benefits: minimizing the risk of side effects from synthetic chemicals and contributing to health maintenance by providing additional nutrients to the body (Mawazi et al., 2022). Thus, cosmetic innovations that combine aesthetic and therapeutic aspects—in this case, lipstick—serve as an adaptive response to modern consumer preferences, which are increasingly aware of the importance of health and product safety.

The rising demand for natural antioxidants in cosmetics is supported by the growing awareness of their efficacy and eco-sustainability. Regulatory bodies, including the USDA (United States Department of Agriculture) and the EU (European Union), emphasize the importance of standardized definitions and certifications for terms like “organic” and “natural”. The USDA’s National Organic Program (NOP) outlines clear classifications, such as “100% organic” or “made with organic ingredients”, ensuring product authenticity. Similarly, the EU’s ISO 16128 standards provide guidelines for natural and organic cosmetics, emphasizing the inclusion of ingredients sourced from environmentally responsible practices. These initiatives ensure consumer trust while promoting the use of bioactive compounds like vitamins, polyphenols, and carotenoids derived from plant sources. This regulatory framework aligns with the global trend towards green cosmetics, combining sustainability with functional benefits such as anti-aging, hydration, and UV protection (Dini and Laneri, 2021).

A previous study by Kamairuddin et al. (2014) investigated the incorporation of natural additives in lipstick formulations by utilizing pitaya seed oil (*Hylocereus polyrhizus*). This oil is rich in unsaturated fatty acids (UFAs), which contribute to skin metabolism regulation by modulating sebum secretion and enhancing collagen nourishment as a structural support beneath the skin. Furthermore, research conducted by Nasution et al. (2022) explored the application of *Rhodomyrtus tomentosa* extract as a natural pigment in lipstick formulations. These studies

highlight the continuous advancement in cosmetic science, particularly in the development of natural-based lipstick formulations, aligning with the ongoing trend of innovation in the cosmetics industry.

Watermelon (*Citrullus lanatus*), a significant member of the *Cucurbitaceae* family, is an economically vital crop cultivated globally (Nadeem et al., 2022). It is rich in bioactive compounds such as carotenoids (e.g., lycopene and β-carotene), phenolic compounds, vitamins, amino acids (notably citrulline), and unsaturated fatty acids (Mashilo et al., 2022). These compounds confer a range of health benefits, including antioxidant, anti-inflammatory, antidiabetic, and cardioprotective effects. Watermelon is particularly notable for its high lycopene content, surpassing that of tomatoes, and its potent antioxidant properties. Despite its primary use as a fruit, watermelon by-products like rind and seeds are often discarded, representing a potential resource for bioactive compound extraction and valorization. Utilizing these by-products aligns with sustainable practices in food-waste management and the circular economy, offering opportunities for applications in nutraceuticals, functional foods, and cosmetics. This study emphasizes the underexplored potential of watermelon as a rich source of valuable bioactives and its role in advancing health and sustainability efforts (Zamuz et al., 2021).

Lipstick was chosen for this study due to its widespread use and high consumer demand worldwide. Given its direct application to the lips, ensuring its safety, stability, and incorporation of beneficial active ingredients is essential. This research introduces the antioxidant properties of *Citrullus lanatus* (hereafter will be written as *C. lanatus*) fruit rind extract as a novel functional ingredient in lipstick formulation, enhancing its therapeutic potential while maintaining desired physicochemical properties. To achieve this, the study explores the potential of *C. lanatus* fruit rind as an underutilized source of bioactive compounds with antioxidant activity, distinguishing it from conventional natural ingredients used in cosmetics. It focuses on extracting and identifying the phytochemical groups present in the rind to assess their functional benefits. Additionally, the lipstick

formulation is optimized using the D-optimal mixture design method, ensuring an ideal balance of ingredients to achieve optimal stability, texture, and performance, thereby contributing to the development of sustainable and natural cosmetic products.

Characterizing the physicochemical properties of the optimized lipstick ensures its quality, functionality, and consumer appeal. Furthermore, safety evaluations, including compatibility and toxicity assessments, provide critical insights into its suitability for human use. This comprehensive approach not only addresses the growing demand for eco-friendly, bioactive cosmetics but also supports the valorization of agricultural by-products, aligning with global sustainability goals.

1.2 Problem Statement

Based on the background above, there are several problems that can be formulated, namely:

1. How to extract and identify the phytochemical groups in *C. lanatus* fruit rind?
2. How to optimize the materials to formulate lipstick containing *C. lanatus* fruit rind extract?
3. How to characterize the physicochemical properties of the optimized lipstick?
4. How to evaluate the safety of the optimized lipstick?

1.3 Objective of Research

The objectives of the study are to:

1. Extract and identify the group of phytochemicals in the *C. lanatus* rind extract in three types of solvent of different polarity (n-hexane, ethyl acetete, and ethanol).
2. Optimize the materials for formulating the lipstick containing *C. lanatus* rind extract with respect to melting point using D-optimal mixture design.
3. Characterize the physicochemical properties of the optimized lipstick with respect to pH, physical stability, and thermogravimetric analysis.

4. Evaluate the safety of optimized lipstick by microbiological and heavy metal analysis.

1.4 Significance of Research

This research makes a significant contribution to sustainability and innovation in cosmetic science by exploring the potential of *C. lanatus* fruit rind as a natural antioxidant source for lipstick formulation. By utilizing agricultural by-products, this study promotes waste valorization and aligns with circular economy principles. The research advances knowledge in phytochemical extraction techniques, particularly using solvents of varying polarity, and demonstrates the application of D-optimal mixture design to optimize lipstick formulations.

Moreover, this study ensures product quality and consumer safety by characterizing the physicochemical properties of the formulated lipstick and evaluating microbiological and heavy metal safety. This research not only supports the development of eco-friendly cosmetics but also provides a foundation for future innovations in natural product chemistry and sustainable cosmetic formulations, addressing both scientific and industrial challenges.

CHAPTER II
LITERATURE REVIEW

2.1 Phytochemical Compounds in Watermelon (*C. lanatus*)

Phytochemicals, often referred to as "plant chemicals", are non-nutrient compounds found in plants that offer various health benefits and contribute to disease prevention. Phytochemicals are broadly categorized into primary and secondary constituents based on their roles in plant metabolism. Primary constituents include basic biomolecules such as sugars, amino acids, chlorophyll, and nucleic acid components like purines and pyrimidines. Conversely, secondary constituents comprise diverse bioactive compounds, including alkaloids, flavonoids, terpenes, phenolics, lignans, steroids, curcuminoids, saponins, and glycosides. These phytochemicals hold significant nutraceutical value, acting as bioactive compounds bridging the food and pharmaceutical industries. They exhibit diverse pharmacological properties such as anti-inflammatory, antioxidant, antibacterial, antifungal, anti-allergic, chemopreventive, neuroprotective, and anti-aging effects. Phytochemicals also support immune function, inhibit carcinogen formation, reduce oxidative stress, slow cancer cell growth, and alleviate inflammation (Nwozo et al., 2023).

Extensive research has been conducted on the extraction of phytochemical compounds from *C. lanatus*. A study by Kamal et al. (2022) optimized the extraction conditions of bioactive compounds from *C. lanatus* rind, conducted phytochemical screening, and evaluated its antioxidant activity. Using methanol as a solvent, the extraction was performed via maceration at a fixed solid-to-solvent ratio of 1:30 b/v. Optimization was carried out using response surface methodology (RSM) with a central composite design (CCD), assessing the effects of temperature (30–60 °C) and time (4–7 hours). The predicted maximum yield was 37.24%, achieved at 41.7 °C and 6 hours, which closely aligned with experimental results yielding 37.01% ± 0.66%.

Phytochemical screening revealed the presence of alkaloids, saponins, glycosides, phenols, and flavonoids, while steroids and terpenoids were absent. The

antioxidant activity of the methanol rind extract was significant, with total phenolic content (TPC) ranging from 56.98 to 88.25 mg GAE/g and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity marked by an IC50 value of 443.10 μg/mL. These findings highlight the significant antioxidant properties of watermelon rind extract due to its bioactive compounds, emphasizing its potential as a natural antioxidant source. Additionally, the study demonstrated the efficiency of RSM in optimizing the extraction process, showcasing the value of agricultural by-products for nutraceutical applications.

Masoko et al. (2022) investigated the nutritional content, phytochemical composition, and biological activity of various parts of *C. lanatus* var. *citroides* (citron watermelon), focusing on the flesh, seeds, and rind. The study explored the effects of temperature on rind bioactivity and employed various methods, including spectrometric analysis for nutrient quantification, chemical tests for phytochemical screening, and micro-broth dilution assays for antibacterial activity. The seeds exhibited the highest levels of protein, polyphenols, and antibacterial properties, particularly against *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The flesh showed higher antioxidant activity than the rind and seeds. However, higher temperatures negatively affected rind bioactivity, emphasizing the need for low-temperature processing.

Similarly, Arawande et al. (2024) highlighted the phytochemical composition and antioxidant properties of *C. lanatus* var. *citroides*, focusing on seeds and rind as sources of bioactive compounds. Using six solvents (methanol, ethanol, acetone, ethyl acetate, water, and chloroform), the study evaluated extraction yield, phytochemical content, and antioxidant activity. Methanol and water were identified as the most effective solvents for extracting bioactive compounds from the rind, yielding 13.56 ± 0.20% and 11.21 ± 0.22%, respectively. For seeds, methanol and ethyl acetate resulted in the highest extractable phytochemical content, with values of 66.67% and 58.33%, respectively.

The antioxidant properties were remarkable. For the rind, methanol extracts showed the highest DPPH radical scavenging activity (94.95 ± 0.61%), followed

by water (85.73 ± 0.23%) and raw samples (79.21 ± 0.39%). Total phenolic content was highest in water extracts (0.18 ± 0.007 mg/100 g), with methanol recording 0.11 ± 0.003 mg/100 g. Similarly, for seeds, methanol extracts exhibited superior DPPH radical scavenging activity (96.31 ± 0.21%) compared to chloroform (92.41 ± 0.11%). The total phenol content of the methanol extract was 0.25 ± 0.012 mg/100 g, much higher than that of chloroform (0.05 ± 0.00 mg/100 g). The methanol extract also showed the highest ferric reducing antioxidant power (FRAP) of 1.11 ± 0.01 GAE, followed by chloroform at 0.83 ± 0.08 GAE.

Neglo et al. (2021) evaluated the antioxidant and antimicrobial properties of various *C. lanatus* parts, including rind (epicarp), peel (mesocarp), flesh, and seeds, along with their phytochemical compositions. Antioxidant activity was measured using DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, while antimicrobial activity was assessed through well diffusion and broth dilution methods. The results showed that the rind showed the highest antioxidant and antimicrobial activities, while the pulp showed the lowest activity for both. The rind demonstrated the highest antioxidant and antimicrobial activity, while the flesh showed the lowest for both. The rind had the highest total phenolic content (0.087 ± 0.002 mg GAE/g), followed by seeds (0.042 ± 0.003 mg GAE/g), peel (0.026 ± 0.003 mg GAE/g), and flesh (0.010 ± 0.001 mg GAE/g). A strong correlation was observed between total phenolic content and biological activity, emphasizing the rind and seeds as valuable sources for nutraceutical and therapeutic applications. In contrast, the pulp showed the lowest phenolic activity and content among all the parts studied.

2.2 Lipstick Formulation

Lipstick is a cosmetic product in the form of a solid stick (roll-up stick). It is a decorative cosmetic component that enhances the lips, provides an attractive color, and keeps the lips moisturized. One solution to minimize harmful effects is to eliminate synthetic dyes (Yuniarsih et al., 2023).

Lipsticks typically consist of various components, including vegetable oils (such as castor oil and almond oil), mineral derivatives (such as Vaseline and white petrolatum), pigments, and waxes. While primarily designed for aesthetic purposes, these ingredients can also serve functional roles, such as providing bioactive benefits like UV protection in extreme weather conditions. Therefore, an ideal lipstick formulation must meet several key criteria: (1) thermal stability, with a melting point generally ranging between 55–75 °C, and resistance to extreme humidity fluctuations; (2) dermatological safety; (3) pleasant aroma and taste; (4) the ability to soften at lip temperature (32 °C); (5) strong mechanical and physical properties to maintain structural integrity; and (6) a flawless appearance free from defects such as air bubbles, cracks, or sweating during production (Esposito and Kirilov, 2021).

Various lipstick formulations have been developed through numerous studies. Esposito and Kirilov (2021) study explored the formulation and evaluation of organogel-based lipsticks using low-molecular-weight organogelators (LMOG), specifically 1,3:2,4-dibenzylidene-D-sorbitol (DBS) and 12-hydroxystearic acid (12-HSA), as alternatives to traditional waxes. Four lipstick formulations were prepared: two with LMOG (L1 with DBS and L2 with 12-HSA), one with a higher DBS concentration (L3), and a control formulation without LMOG (L4).

Lipsticks were formulated using a mixture of oils, waxes, and LMOGs through a molding method. Texture properties were assessed instrumentally, while sensory characteristics such as spreadability, hardness, and gloss were evaluated by a consumer panel. Thermal stability and photoprotection (SPF and UVA-PF) were tested using rheological studies and UV spectrophotometry. LMOG-based lipsticks showed improved photoprotection compared to the control, with 12-HSA (L2) exhibiting the highest SPF and UVA-PF. DBS-based formulations demonstrated superior thermal stability and mechanical strength. Sensory evaluations revealed significant differences in attributes like gloss and greasiness, aligning with instrumental findings. Overall, LMOG-based lipsticks, particularly those

containing 12-HSA, were harder and more stable under varying temperatures, making them a promising alternative for sustainable and photoprotective cosmetics.

Kamairudin et al. (2014) research addresses the development and optimization of pitaya (Hylocereus polyrhizus) seed oil-based natural lipstick formulations using a D-optimal mixture experimental design. The main objective of the study was to determine the optimal combination of ingredients to achieve the desired physical properties of the lipstick, especially the appropriate melting point. This study used five main ingredients, namely pitaya seed oil, virgin coconut oil, beeswax, candelilla wax, and carnauba wax. D-optimal mix design was applied to evaluate the effect of each ingredient on the melting point of the formulation, while statistical analysis using ANOVA was conducted to validate the prediction model.

The results showed that the optimal composition of the lipstick was 25% pitaya seed oil, 37% virgin coconut oil, 17% beeswax, 2% candelilla wax, and 2% carnauba wax. This formulation yielded a melting point of 46.0 °C, which was consistent with the theoretical prediction of 45.5 °C. The carnauba wax proved to have the greatest influence on the melting point increase due to its function in improving heat resistance. This study concludes that D-optimal mix design is an effective tool for optimizing cosmetic formulations by reducing time, cost, and number of experiments. This study also provides valuable guidance for the development of natural ingredient-based cosmetics.

Lwin et al. (2020) study formulated and evaluated lipstick using betacyanin pigments extracted from *Hylocereus polyrhizus* (red dragon fruit) as a natural dye. The goal was to create a safer and more natural alternative to synthetic dyes, often linked to heavy metal contamination and side effects. Lipstick formulation involved extracting betacyanin pigments using 50% ethanol through cold maceration. Pigments were quantified via UV-VIS spectrophotometry, and lipsticks were prepared with natural oils, waxes, and pigments using heating and mixing methods. Quality evaluations included melting point, pH, surface defects, aging stability, and antioxidant activity (DPPH assay). Safety evaluations comprised skin irritation tests (rat ear model), microbial analysis, and lead content determination. The results

showed acceptable lipstick quality with a melting point of 55.3°C, near-neutral pH (6.5), no surface defects, and good stability. Antioxidant activity was significant, with an IC50 value of 22.23 µg/ml. Safety tests showed negligible skin irritation, no microbial contamination, and low lead content (2.9 ppm), within regulatory limits.

Mahanthesh et al. (2020) study focused on the formulation and evaluation of herbal lipsticks using natural pigments extracted from *Rosa rubiginosa* (rose), *Bougainvillea spectabilis* (bougainvillea), *Beta vulgaris* (beetroot), and *Crocus sativus* (saffron). Pigments were extracted through maceration, filtration, and concentration methods. Lipstick formulations (F1 to F4) utilized castor oil, paraffin wax, beeswax, shikakai powder, lemon oil, vanilla essence, and natural dyes. The products were evaluated for parameters like melting point, breaking point, application strength, surface anomalies, pH, aging stability, fragrance stability, and skin irritation.

Among the formulations, F2 (bougainvillea) and F4 (saffron) exhibited superior properties, including better color stability, smooth application, and attractive appearance. All formulations had an ideal pH range (6.5–6.8), caused no skin irritation, and showed acceptable mechanical and thermal stability. F3 (beetroot) exhibited slightly lower pigmentation due to solvent effects during extraction. This study highlighted the good quality of herbal lipstick formulations with minimal side effects, making them a safe alternative to synthetic lipsticks.

Nasution et al. (2022) study investigated lipstick formulations using juice and extracts from Harimonting fruit (*Rhodomyrtus tomentosa*) as natural dyes. The deep purple color of the fruit is attributed to its flavonoid, tannin, and steroid content. Lipstick formulations were prepared using various concentrations of Harimonting juice (14%, 22%, and 30%) and extracts (10%, 13%, and 16%), with fruit extracts obtained through maceration with 75% ethanol.

The formulations were evaluated for pH, melting point, irritation, homogeneity, and spreadability. The pH values ranged from 4.4 to 5.4, aligning with the physiological lip pH (4.0–6.5). The melting point results (55.3–55.6 °C)

fell within the standard range for lipsticks (50–70 °C). However, juice-based lipsticks showed uneven color distribution with visible red granules, making them unsuitable as natural dyes. In contrast, extract-based lipsticks demonstrated uniform texture, smooth application, and no irritation, indicating their potential as natural dyes in lipstick production.

2.3 Literature Review Summary

Citation	Topic	Quantitative Results
Kamal et al., 2022	Optimization of phytochemical extraction and antioxidant activity of *C. lanatus* rind	- Extraction: Methanol, 1:30 b/v ratio, RSM method at 41.7°C for 6 hours yielded 37.01% ± 0.66%. - TPC: 56.98–88.25 mg GAE/g. - DPPH IC50: 443.10 μg/mL. - Alkaloids, saponins, phenols, flavonoids detected; steroids, terpenoids absent.
Masoko et al., 2022	Nutritional composition, phytochemicals, and bioactivity of various parts of *C. lanatus* var. citroides	- Seeds: Highest protein, polyphenols, and antibacterial properties against *Pseudomonas aeruginosa* and *Enterococcus faecalis.* - High temperatures reduce bioactivity in the rind.
Arawande et al., 2024	Effect of solvents on phytochemical extraction and antioxidant activity of *C. lanatus* rind	- Best solvents for rind: Methanol (13.56 ± 0.20%), water (11.21 ± 0.22%) - DPPH scavenging: Methanol (94.95 ± 0.61%), water (85.73 ± 0.23%) - TPC (rind): Water (0.18 mg/100g), methanol (0.11 mg/100g).
Neglo et al., 2021	Antioxidant and antimicrobial activity of various parts of *C. lanatus*	- Highest antioxidant and antimicrobial activity found in rind - TPC (rind): 0.087 ± 0.002 mg GAE/g. - Flavonoids and alkaloids present in rind and seeds.
Esposito and Kirilov, 2021	Lipstick formulation using LMOG (1,3:2,4-DBS and 12-HSA) as a wax substitute	- Stability: LMOG-based lipsticks showed superior thermal stability - SPF/UVA-PF: 12-HSA had the highest protection - DBS lipsticks exhibited superior mechanical strength.
Kamairudin et al. (2014)	Optimization of pitaya seed oil-based natural	The optimal formulation consisted of 25% pitaya seed oil, 37% virgin coconut

Citation	Topic	Quantitative Results
	lipstick formulations using a D-optimal mixture experimental design	oil, 17% beeswax, 2% candelilla wax, and 2% carnauba wax, yielding a melting point of 46.0 °C, which was close to the predicted 45.5 °C. Carnauba wax significantly impacted the melting point increase.
Lwin et al., 2020	Lipstick with natural betacyanin pigments from *Hylocereus polyrhizus* (red dragon fruit)	- Melting point: 55.3°C.- pH: 6.5. - Antioxidant activity: IC50 = 22.23 µg/mL (DPPH) - Lead content: 2.9 ppm - No microbial contamination or skin irritation observed.
Mahanthesh et al., 2020	Herbal lipstick using pigments from natural flowers (rose, bougainvillea, beetroot, saffron)	- Formulations: F2 (bougainvillea) and F4 (saffron) showed superior color stability and smooth application - pH: 6.5–6.8. - No irritation; good mechanical and thermal stability.
Nasution et al., 2022	Lipstick using juice and extract of *Rhodomyrtus tomentosa* as a natural dye	- pH: 4.4–5.4. - Melting point: 55.3–55.6°C. - Juice: uneven texture; unsuitable as a dye - Extract: smooth texture, uniform application, no irritation observed.

CHAPTER III
FUNDAMENTAL THEORIES

3.1 Phytochemical Compounds

Phytochemicals are plant-based bioactive compounds produced by plants as self-protection. Phytochemicals can come from various sources, such as whole grains, fruits, vegetables, nuts, and spices, and more than a thousand phytochemicals have been discovered to date. Some important phytochemical compounds include carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides. Phytochemicals have strong antioxidant activity and exhibit antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities (Kumar et al., 2023). Phytochemical compounds also help regulate gene transcription, enhance gap junction communication, strengthen the immune system, and provide protection against lung and prostate cancer (Kumar et al., 2023; Rowles and Erdman, 2020).

Phytochemicals are divided into primary and secondary metabolites based on their role in plant metabolism. Primary metabolites, which are crucial for plant survival, consist of carbohydrates, amino acids, proteins, lipids, and nucleic acid components such as purines and pyrimidines. Secondary metabolites are additional compounds produced by cells through pathways derived from primary metabolic processes. Compounds from secondary metabolites function as antivirals, antifungals, antibiotics, help defend plants from pathogens, as well as important UV-absorbing agents that protect leaves from potential light damage. With their strong biological properties, secondary metabolites have long been used in traditional medicine, as the medicinal benefits of plants are largely attributed to compounds from secondary metabolites. In addition, different parts of medicinal plants can exhibit unique therapeutic properties during certain stages of development (Rabizadeh et al., 2022). Nowadays, secondary metabolites play an important role in high-value industries such as pharmaceuticals, cosmetics, and chemicals production (Liu et al., 2021).

The phytochemical content in plants can be obtained through the extraction process. Various factors can affect the extraction efficiency, including the plant part used, material size, temperature, method, duration, solvent concentration, and solvent type. The polarity of the solvent used must match the active compound to ensure efficient extraction, as the principle of “like dissolves like” means that not all compounds will dissolve in every type of solvent (Prayoga et al., 2019).

3.2 Antioxidant

Antioxidants are chemical substances that provide electrons to free radicals with unpaired electrons, thereby reducing the oxidative effects caused by these radicals. Many plant-derived compounds function as natural exogenous antioxidants and have been shown to be clinically effective (Sukweenadhi et al., 2020).

Human cells regularly produce free radicals and reactive oxygen species (ROS) as a natural part of metabolism. When the production of free radicals in the body surpasses its defense mechanisms, oxidative stress may occur. Oxidative stress results from an imbalance between free radicals and endogenous antioxidants. Free radicals are produced through normal cellular metabolism, but can also form due to exposure to air pollution, vehicle exhaust, cigarette smoke and similar factors. These free radicals are highly reactive and generally unstable, leading to a chain reaction that can damage cell structures. If left unchecked, this process can lead to various diseases, including cancer, coronary heart disease, cataracts, premature aging, and other degenerative conditions (Kamoda et al., 2021).

Free radicals and reactive oxygen species (ROS) are produced in the body mainly through cellular respiration and various metabolic reactions. During these processes, oxygen molecules can undergo partial reduction, resulting in the formation of unstable and highly reactive molecules, such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$). These ROS can cause oxidative stress when accumulated beyond the body's ability to neutralize

them. In addition, environmental factors such as pollution, UV radiation, and smoking can increase ROS production (Gulcin, 2020).

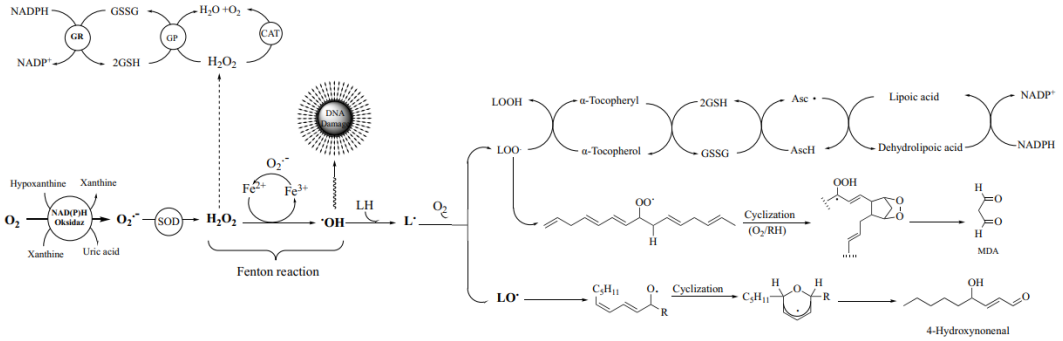


Figure 3.2.1 Overview of the reactions leading to the formation of ROS and their effects (SOD: Superoxide dismutase enzyme, CAT: Catalase enzyme, GR: Glutathione reductase, GP: glutathione peroxidase (Gulcin, 2020).

Antioxidants work by neutralizing free radicals and ROS, mainly through two mechanisms:

1. Hydrogen Atom Transfer (HAT): Antioxidants donate hydrogen atoms to free radicals, stabilizing them and stopping chain reactions that can damage cell structures.
2. Single Electron Transfer (SET): Antioxidants give an electron to free radicals, turning them into a more stable and less reactive form.

Through this mechanism, antioxidants help maintain cell integrity, protect DNA, and prevent oxidative stress-related diseases (Gulcin, 2020).

Antioxidants play a crucial role in reducing oxidative processes and mitigating ROS damage in both food systems and the human body. They prevent lipid peroxidation, preserving food quality by maintaining flavor, color, and texture. Additionally, antioxidants protect amino acids and proteins from oxidation and prevent harmful lipid-protein interactions, preserving protein function (Gulcin, 2020). Antioxidants are often used as dietary supplements and have been investigated for their role in preventing various diseases, including heart disease and cancer. Exogenous antioxidants, such as vitamins, flavonoids, anthocyanins, and certain minerals, are derived from natural sources, but can also be produced synthetically, such as butyl hydroxyanisole (BHA), butyl hydroxyoluene (BHT), and gallate (Rahaman et al., 2023).

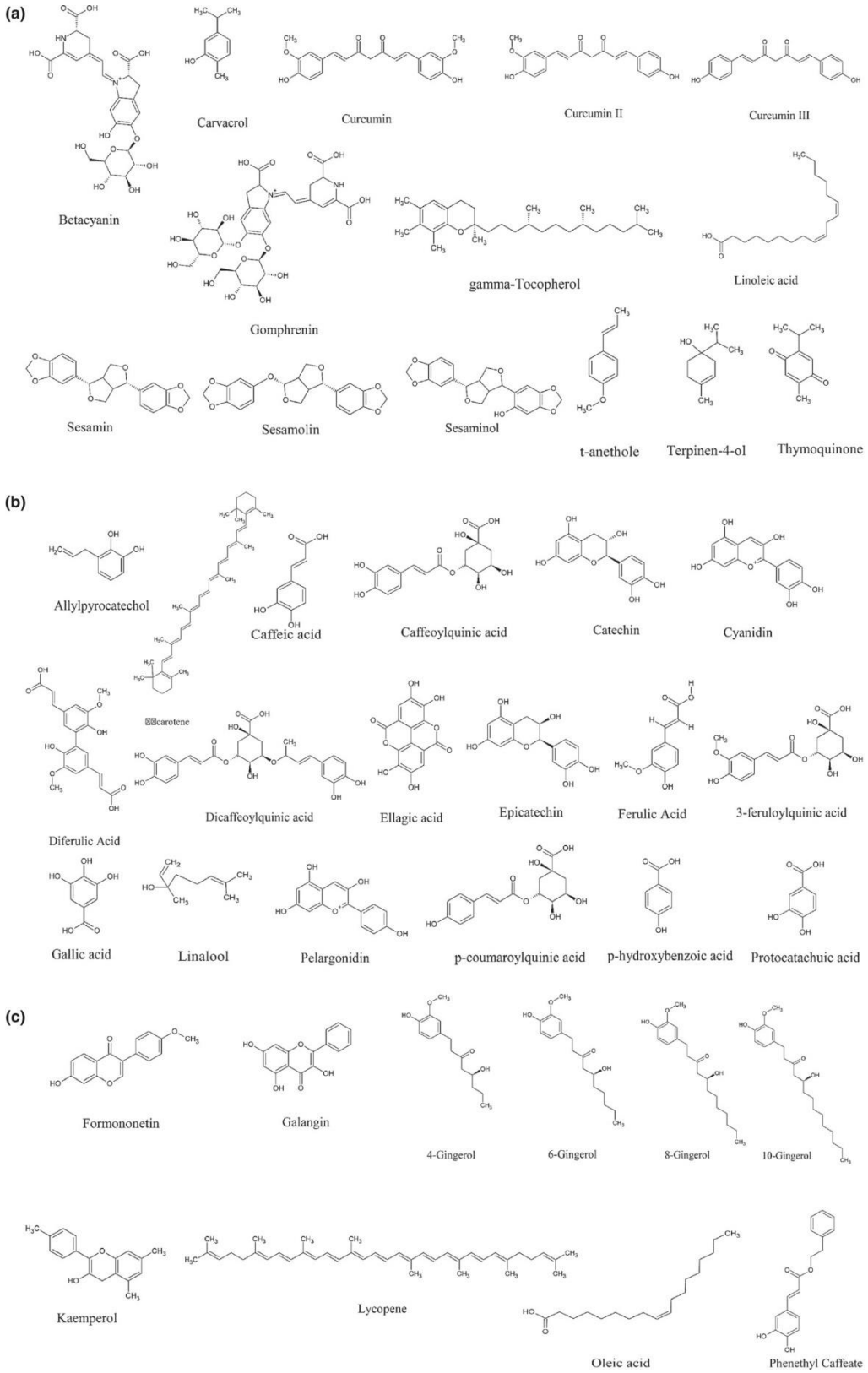


Figure 3.2.2 Chemical structures of the most representative bioactive compounds with antioxidant effects (Rahaman et al., 2023).

3.3 Extraction

Extraction is the process of separating materials, either solid or liquid, using a solvent. The chosen solvent must be able to dissolve the desired substance without dissolving other materials. The extraction process with solvents is based on their solubility properties. The type of solvent used in extraction can affect the results of obtaining active substance levels from plants. Therefore, choosing the right solvent will increase efficiency and optimization in the sample extraction process (Yuliani and Rasyid, 2019).

There are various methods in extraction such as maceration, microwave assisted extraction (MAE), and ultrasonic assisted extraction (UAE). Maceration involves immersing the material in a solvent until concentration equilibrium is reached. The advantages of the maceration method lie in its simple procedure and easy-to-use equipment. In addition, this method can be applied to both heat-resistant and non-heat-resistant materials (Nababan et al., 2018). MAE uses microwave radiation to heat the solvent quickly and efficiently. UAE, on the other hand, utilizes ultrasonic waves that create cavitation effects to heat the solvent and form extract compounds (Fauziyah et al., 2022).

Each extraction method has a different influence on the amount, content and quality of the extract. To improve efficiency, the development of extraction methods continues to be carried out in order to produce shorter extraction times, more efficient solvent use, higher yields, and more optimal activity (Fauziyah et al., 2022).

3.4 Watermelon (*Citrullus lanatus*)

Watermelon (*Citrullus lanatus*) (Figure 3.4.1) is a vine and produces delicious fruit that is widely cultivated for human consumption. It is an annual plant with a well-defined growth pattern suitable for hot climates. Its fruits usually vary in weight from 1–2 kg, but can reach 20 kg for juicy plants. In desert areas, watermelon is particularly valuable as an alternative water source. China is currently the world's largest producer of watermelon, followed by Turkey, India,

Iran, Algeria, Brazil, the United States, and South Korea. The scientific name of watermelon, Citrullus lanatus, is a combination of the Greek, '*Citrullus*' derived from the word '*citrus*' meaning orange and referring to the fruit, and the Latin word '*Lanatus*' meaning downy or woolly and referring to the microscopic hairs on its leaves and stems. Watermelon contains about 68% flesh or pulp, 30% rind, and 2% seeds (Nadeem et al., 2022).

Figure 3.4.1 Sliced watermelon (Personal Doc.)

The taxonomy and scientific classification of watermelon are as follows (Guo et al., 2020):

Kingdom : Plantae
Phylum : Embryophyta
Class : Dicotyledoneae
Order : Cucurbitales
Family : Cucurbitaceae
Genus : Citrullus
Species : *Citrullus lanatus*

Watermelon plants are included in the type of vines or creepers, using tendrils as support, and are annual. The root system spreads sideways and is shallow. The stem of the watermelon plant is angular and hairy, measuring 1.5 to 5.0 meters in length and growing along the soil surface. The stem is round, soft, slightly woody, and covered with fine hairs, with tendrils that can reach a length of 3.5 to 5.6 meters. The lateral branches resemble the main branches. Between the stem and leaf segments are tendrils, characteristic of the *Cucurbitaceae* family, which serve as

gripping devices when the plants are cultivated using trellises or support systems. The leaves are feather-like and small, with a hairy surface and heart-like shape at the base, pointed tip, wavy edges, and dark green in color. The leaves grow facing each other on relatively long stalks. Watermelon plants produce three types of flowers: male flowers, female flowers, and perfect flowers. Male flowers have no ovary, are trumpet-shaped, and have three stamens arranged on filaments 2.5 cm long, with an S-shaped anther structure, growing between the segments of the stem. Watermelons are available in various shapes, colors, and sizes. Based on their shape, watermelons can be oval, oblong, or cylindrical. The color of the rind can be light green, dark green, or yellow, with some rinds striped and others solid. Skin thickness also varies; thicker skins provide better durability for storage and transportation compared to thinner skins (Helmayanti et al., 2020).

Watermelons are commonly consumed by humans and are widely available around the world. Watermelon has an impressive nutrient content, a rich phytochemical profile, and a wide range of claimed medicinal and health benefits. The juice or pulp of watermelon is consumed by humans, while the rind and seeds constitute significant solid waste. Different carotenoid profiles are found in red- and yellow-fleshed watermelons; red-fleshed varieties are rich in lycopene and contain varying levels of β-carotene. The rind also contains high levels of citrulline, an amino acid that the body converts into arginine, which aids the urea cycle to remove ammonia. Watermelon seeds are nutrient dense, providing high amounts of protein, B vitamins, minerals, fats, and other phytochemicals (Arawande et al., 2024).

3.5 Lipstick

Lipstick (Figure 3.5.1) is a cosmetic product in the form of a solid stick (roll up stick). Lipstick is also a decorative cosmetic component that enhances the lips, provides attractive color and protects the lips to keep them moisturized. One solution is to remove synthetic colorants, which can be harmful (Yuniarsih et al., 2023). Lipstick uses are common in the cosmetic world, providing not only aesthetic benefits but also social, psychological and therapeutic benefits. Lips colored by lipstick not only enhance beauty and attractiveness, but also protect lips

from environmental damage. However, with the development of the times, lip care products not only focus on the aesthetic aspect, but also emphasize the therapeutic value. This is reflected in the popularity of medicinal lipsticks that contain active medicinal ingredients, provide protection against bacterial infections and keep lips moisturized (Afandi et al., 2017).

Figure 3.5.1 Lipstick (Personal Doc.).

Humans began using color as adornment around 3000 BC to attract game. The concept and term “cosmeceuticals” were first outlined by Raymond Reed in 1961, founder of the US Society of Cosmetic Chemists. The origin of the term comes from the Greek word “*kosm tikos*”, meaning “skill in ornamentation”. In 1984, Albert Kligman introduced the term “cosmeceuticals” to describe compounds that have both cosmetic and medicinal properties (Bijauliya et al., 2017). Lipstick, which has been used for over 500 years, was first discovered as a crude brick fragment in ancient Mesopotamia. The tradition of coloring lips has existed since prehistoric times. Lipstick was first introduced in France in 1869, made from animal fat and beeswax (Munawiroh et al., 2017). Cylindrical metal tubes for lipstick were introduced in 1915. Today, lipstick has become an essential product with a wide

array of color and texture options. The lipstick market records hundreds of colors available to meet the growing demand (Mawazi et al., 2022).

Lipsticks contain various ingredients of natural, chemical, or a combination of both. Nonetheless, both synthetic and natural-based lipsticks are available in the market. The use of synthetic-based lipsticks may cause adverse reactions. For example, the presence of lead and dyes in lipsticks is a serious concern. Metals such as nickel and copper, which are commonly found in cosmetics, can cause allergic reactions in certain individuals (Łodyga-Chruścińska et al., 2018).

Table 3.5.1 The synthetic components and their respective amounts used in lipstick formulations (Mawazi et al., 2022).

No.	Ingredients	Functions	Quantity (% *w*/*w*)
1	Paraffin wax	Glossy, hardness, stiffening agent	28
2	Butyl stearate	Lipstick base and solvent for dyestuff and dispersing agent	1–25
3	Microcrystalline wax	Lipstick base	2
4	Ozokerite wax	Lipstick base	3–10
5	Ceresin wax	Lipstick base or to increase the melting point of other waxes	3–10
6	Oleyl alcohol	Blending agent, emollient, oleaginous vehicle, solvent.	40–50
7	Methyl paraben	Preservative	0.1–1
8	Propyl paraben	Preservative	0.1–1
9	Propyl-p-hydroxybenzoate	Preservatives	0.1–0.2
10	Vitamin E	Antioxidant	00.05
11	Lanolin alcohol	Blending agents and plasticizing effect	2–5
12	Anhydrous lanolin	Blending agent	2–20
13	Titanium dioxide	Pigment shading agent, brightener	1–40
14	Zinc oxide	Pigment, brightener	1–40
15	Calcium, barium, and aluminium lakes	Colouring agents	10–15
16	Isopropyl myristate or isopropyl palmitate	Glossing agent	2–3
17	Acetoglycerides	Blending agents and plasticisers	2.5–7
18	Bromo mixture	Colouring agents	2–25

Some of the ingredients often used in making herbal lipsticks include (Table 3.4.2) castor oil, paraffin wax, beeswax, beet root juice, ripe shikakai fruit powder,

lemon oil, orange essence and vanilla essence (Mawazi et al., 2022). However, previous studies have shown little difference in the ingredients used in lipstick manufacturing.

Table 3.5.2 The natural components and their respective quantities used in lipstick formulations (Mawazi et al., 2022).

No.	Ingredients	Functions	Quantity (% *w*/*w*)
1	Ripe fruit powder of *Shikakai*	Surfactant	12
2	Lemon oil	Antioxidant, preservative, flavouring agent	0.1–1
3	Orange essence	Flavouring agent	01.05
4	Mango butter from *Mangifera indica*	Lipstick base	10
5	Beetroot juice	Colouring agent	6
6	*Theobroma cocoa*	Colouring agent	40
7	Lycopene from *Solanum lycopersicum L* (Tomato)	Colouring agent	02.05
8	*Punica granatum* from pomegranate	Colouring agent	5–9
9	*Amaranthus Cruentus* L.	Colouring agent	0.5–1
10	Jati leaves (*Tectona grandis L.f.*)	Colouring agent	18–22
11	Ginger powder	Antimicrobial agent	2
12	Turmeric Powder	Antimicrobial agent	5–6
13	*Hylocereus polyrhizus*	Antimicrobial agent and colouring agent	4
14	Vanilla essence	Preservative	10
15	Olive oil	Blending agent and lipstick base	10–30

3.6 D-Optimal Mixture Design

The D-Optimal Mixture Design is a statistical approach used in experimental formulations where the proportions of components are interdependent and must sum to a constant value. This method is particularly effective in optimizing mixtures, as even small variations in component proportions can significantly impact the final product. In cosmetic formulations, for example, the response is influenced by the relative proportions of raw materials, active ingredients, and

additives rather than their absolute quantities. Doubling the amount of each ingredient would result in the same formulation profile, highlighting the importance of proportion over volume in mixture design (Hidayat et al., 2021).

To implement this approach, Design-Expert software, developed by Stat-Ease and released in 1996, was utilized in this study. The software provides a range of Design of Experiments (DOE) methods, including screening, characterization, and optimization. DOE optimization, which was applied in this research, requires the highest number of experiments but offers the most detailed insights. It is typically employed after reducing the number of influencing factors to fewer than six, allowing researchers to systematically determine the optimal formulation settings within the tested design space (Hidayat et al., 2021).

In mixture design, factor values range between 0 and 1, reflecting the proportional contributions of each component. One key methodology within this design is the Simplex Lattice Design (SLD), which is used to optimize formulations where the total of all components equals one (100%). This method requires at least two different components and determines the design space or test region by setting minimum and maximum limits for each factor. Based on these constraints, the software identifies test points using vertices, edge centers, overall centroids, and check runs. Some points may be repeated to calculate pure error. The resulting data are visualized through contour plots, enabling precise identification of the optimal formulation (Hidayat et al., 2021).

Data modeling in mixture design is conducted using mathematical models such as linear, quadratic, cubic, or special cubic. Model selection is based on several statistical criteria, including model significance, lack-of-fit significance, adjusted R^2, and predicted R^2, analyzed using ANOVA. A model is deemed appropriate if the model probability and lack-of-fit probability are below the alpha threshold (typically 5%), indicating a significant impact on the response (Hidayat et al., 2021).

Mixture design enables the identification of the optimal formulation by systematically analyzing response data across multiple parameters. This approach considers the interaction between different formulation components, ensuring a

balanced composition that meets predefined quality standards. The selection of the best formulation is based on achieving acceptable limits for all evaluated parameters, such as stability, texture, and overall performance. To quantify optimization, a desirability function is employed, which assigns a numerical value ranging from 0 to 1, where a desirability score approaching 1 indicates the most favorable and optimal formulation. This method provides a statistical and objective means of selecting the best composition, enhancing the efficiency and reliability of formulation development (Hidayat et al., 2021).

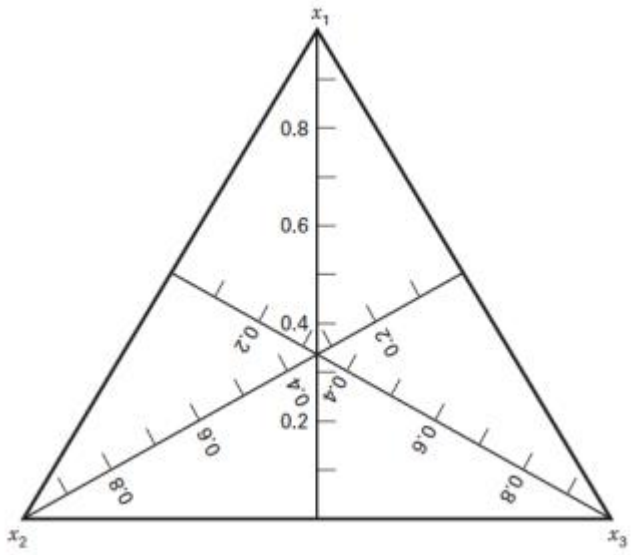


Figure 3.6.1 Test region of the mixture design with three factors (Hidayat et al., 2021).

3.7 Antibacterial Activity

Antibacterial activity refers to the ability of a substance to inhibit bacterial growth or eliminate bacteria. Antibacterial agents are generally classified into bactericidal and bacteriostatic substances. Bactericidal agents directly kill bacteria by disrupting vital cellular components, while bacteriostatic agents prevent bacterial growth and reproduction, allowing the immune system to eliminate the pathogens (Al-dolaimy et al., 2024).

The mechanism of antibacterial action varies depending on the target site within the bacterial cell. Some agents attack the bacterial cell wall, causing

structural damage that leads to cell lysis, while others disrupt the cell membrane, altering permeability and leading to leakage of essential intracellular contents. Additionally, antibacterial substances may interfere with DNA replication and protein synthesis, preventing bacteria from reproducing and carrying out essential metabolic functions (Ahmadi et al., 2022).

The efficacy of antibacterial agents is commonly evaluated using quantitative microbiological methods, such as the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests. MIC represents the lowest concentration of an antibacterial compound that effectively inhibits bacterial growth, while MBC is the minimum concentration required to kill the bacteria. These methods provide crucial insights into the potency and effectiveness of antibacterial substances, guiding their application in medicine, cosmetics, and other industries (Skrzypczak and Przybylski, 2022).

3.8 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is a thermal analysis technique used to evaluate the decomposition behavior and thermal stability of a material under controlled conditions. This method monitors mass changes, both loss and gain, as a function of temperature or time in a specific atmosphere, providing valuable insights into the material's stability, reactivity, reaction mechanisms, and decomposition kinetics (Pindelska et al., 2017). A simple diagram of the TGA instrument is shown in Figure 3.8.1.

TGA operates based on the principle of measuring the sample’s weight reduction as it is heated from room temperature to high temperatures, typically up to 900 °C. The TGA instrument is equipped with a high-precision microbalance that continuously records the sample’s mass, and the results are presented graphically to facilitate analysis (Lisdawati, 2017). The most common output is the thermogravimetric (TG) curve, which depicts the percentage of sample weight loss (wt%) relative to temperature (T). As the temperature rises, the sample undergoes thermal decomposition, leading to mass loss. In biomass analysis, decomposition

generally occurs at lower temperatures, where organic matter breaks down earlier, revealing important information about pyrolysis characteristics and the thermal behavior of raw materials. This data is crucial in optimizing processes related to material efficiency and reaction kinetics in various applications (Claudia B. P. et al., 2024).

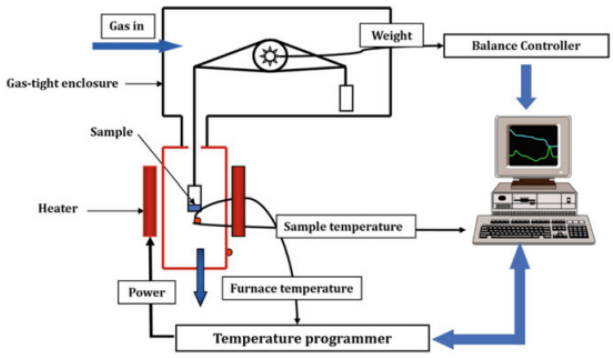


Figure 3.8.1 Schematic diagram showing the cross-sectional view of a TGA (Mehanny et al., 2020).

3.9 Inductively Couple Plasma Mass Spectroscopy (ICP-MS)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is an analytical technique used to measure trace elements in biological samples. Compared to older techniques such as atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES), ICP-MS has become the preferred choice in the past decade due to its ability to measure multiple elements simultaneously in a single analysis (multi-element capability), short analysis time, and simple sample preparation (Wilschefski and Baxter, 2019). A simple diagram of the ICP-MS instrument is shown in Figure 3.9.1.

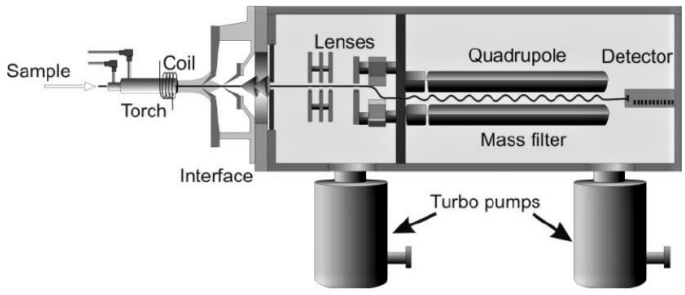


Figure 3.9.1 Schematic diagram showing the cross-sectional view of an ICP-MS (Wilschefski and Baxter, 2019).

ICP-MS is widely used in clinical applications for trace element analysis in biological samples, monitoring essential elements for biological functions (e.g. iodine, manganese, copper, selenium, and zinc) and toxic elements (e.g. arsenic, cadmium, mercury, lead) for exposure assessment. Imbalances in these elements may be associated with various pathological conditions. ICP-MS offers several advantages, including its ability to analyze multiple elements simultaneously, a wide analytical range, and exceptionally low detection limits. It enables high sample throughput with minimal sample volume and simple preparation. Additionally, high-resolution and tandem mass spectrometry (triple-quadrupole) enhance interference control, ensuring precise and reliable results (Wilschefski and Baxter, 2019).

There are six fundamental compartments in a single-quadrupole ICP-MS: the sample introduction system, the inductively coupled plasma (ICP), the interface, the ion optics, the mass analyzer, and the detector. ICP operates in two modes: capacitive (E mode) at low plasma density and inductive (H mode) at high density, depending on applied power. In E mode, an electric field sustains the plasma, while in H mode, a magnetic field enhances ionization. When power decreases, the plasma transitions back, but due to hysteresis, density remains high, causing different transition points. This effect is influenced by factors like power, pressure, and gas composition (Lee, 2018). ICP-MS utilizes the H mode, as its higher ionization efficiency ensures better sensitivity and accuracy in elemental analysis.

The sample introduction system is designed primarily for liquid samples, though solid samples can be analyzed using specialized techniques. The interface contains two metal cones (sample and skimmer cones) that extract ions from the plasma and introduce them into the mass spectrometer under vacuum conditions. The ion optics system, composed of electrostatic lenses, guides the ion beam to the mass analyzer while filtering out photons and neutral species that could interfere with detection. The mass analyzer, commonly a quadrupole, separates ions based on their mass-to-charge ratio for precise element identification. Finally, the detector, typically an electron multiplier, amplifies ion signals for highly sensitive

measurements, achieving detection limits in the µmol/L range. The detection limits in ICP-MS are significantly superior to flame atomic absorption and are comparable to (or even better than) graphite furnace atomic absorption, as shown in Figure 3.8.2 (Wilschefski and Baxter, 2019).

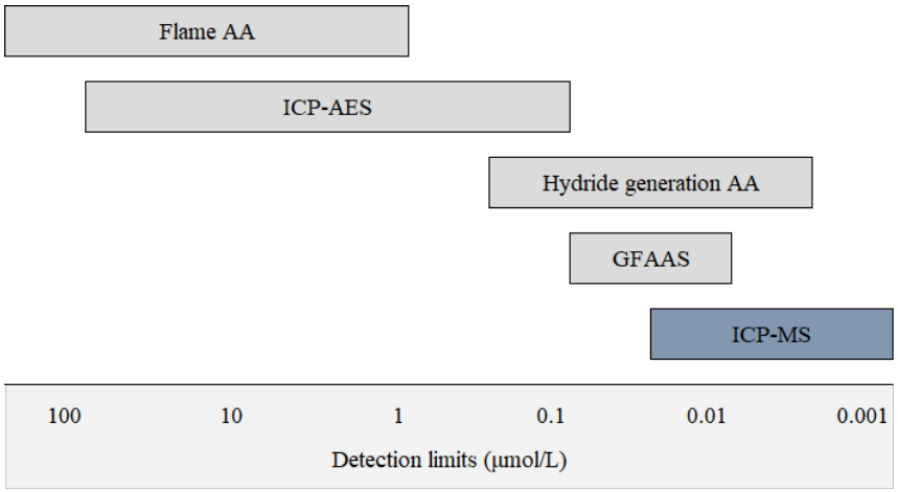


Figure 3.9.2 Typical detection limits of various elemental analysis techniques, including atomic absorption (AA), inductively coupled plasma atomic emission spectroscopy (ICP-AES), graphite furnace atomic absorption (GFAAS), and inductively coupled plasma mass spectrometry (ICP-MS) (Wilschefski and Baxter, 2019).

CHAPTER IV
RESEARCH METHODOLOGY

4.1 Tools of Research

The tools used in this research included aluminum tray (Mr. DIY), drying oven (Shinsaeng Finetech), beaker flasks in various volumes of 50 mL, 100 mL, 250 mL, 500 mL, and 1000 mL (Isolab), erlenmeyer flask (Iwaki), reaction tube (Iwaki) grinder, Büchner filter funnel (Favorit), filter paper (Smith), diaphragm vacuum pump (Vacuubrand ME 2 NT), 500 mL suction flask (HmBG), analytical balances (Sartorius BT 244 S and Adam PGW 4502e), bench scale (Adam LBK6), 1000 mL round bottom flask (Schott Duran), rotary evaporator (Buchi R-210), vaseline, heater circulating pump (Radnoti), glass funnel, thermometer, hot plate stirrer (Pro HPS-7), magnetic stirrer bar, stirring rod, spatula, lipstick mold kit, freezer, refrigerator, melting point apparatus (Dynalon DMP100), capillary tube, thermogravimetric analyzer (PerkinElmer TGA 7), inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer ELAN DRC II ICP-MS), vortex mixer, pH meter (Mettler-Toledo Delta 320), spreader, petri dish (Oxoid), and incubator (Memmert).

4.2 Materials of Research

The materials used in this research included watermelon (*C. lanatus*) sourced from Bahau, Negeri Sembilan, Malaysia, n-hexane ($CH_3(CH_2)_4CH_3$, R&M Chemicals), ≥99.5% ethyl acetate ($CH_3COOCH_2CH_3$, EMSURE Merck), 95% ethanol (C_2H_5OH), olive oil, virgin coconut oil, castor oil, candelilla wax, carnauba wax, beeswax, titanium dioxide (TiO_2), colorant, preservative, lipstick casing tube, watermelon fragrance, Wagner's reagent (R&M Chemicals), 99% chloroform ($CHCl_3$, R&M Chemicals), 99% glacial acetic acid (CH_3COOH, Chemiz), concentrated hydrochloric acid (HCl, EMSURE Merck), sodium hydroxide pellets (NaOH, R&M Chemicals), 96% iron(III) chloride ($FeCl_3$, PC Laboratory Reagent), 95% sulfuric acid (H_2SO_4, Chemiz), concentrated nitric acid (HNO_3, Merck), high-purity hydrogen peroxide (H_2O_2, Merck), a gold-lutetium (Au-Lu) solution (50

mg/L in 5% HNO_3, Merck), ampicillin (Pfizer), sodium chloride (NaOH, R&M Chemicals), tryptic soy agar (TSA, Himedia), and distilled water (H_2O). All materials were purchased in Malaysia, with some imported from Germany.

4.3 Work Procedure

This study was conducted in multiple stages, including the extraction of phytochemical compounds from *C. lanatus* fruit rind, phytochemical analysis of the resulting extract, formulation and optimization of lipstick incorporating *C. lanatus* fruit rind extract, characterization and safety evaluation of the optimized lipstick. Additionally, the safety evaluation of the optimized lipstick was conducted by SIRIM Berhad (Malaysia) to ensure compliance with quality and safety standards.

4.3.1 Extraction of *C. lanatus* Fruit Rind Phytochemical Compounds

The extraction method was adapted from Kamal et al. (2022) with minor modifications. Fresh *C. lanatus* fruits were first cut, and the rinds were carefully separated from the flesh. The rinds were then chopped into small pieces to accelerate the drying process. Drying was conducted by spreading the chopped rinds on aluminium trays and placing them in a drying oven for 48 hours (2 × 24 hours). Once dried, the rinds were finely ground using a grinder to increase surface area, facilitating efficient extraction.

The powdered *C. lanatus* rind was extracted sequentially using solvents of increasing polarity to achieve a comprehensive isolation of bioactive compounds. The process was conducted using a sample-to-solvent ratio of 1:5 (w/v), beginning with the nonpolar solvent n-hexane, followed by the semipolar solvent ethyl acetate, and concluding with the polar solvent ethanol. In the final step, ethanol-only extraction was performed to ensure the maximum recovery of polar bioactive compounds from the rind. Each extraction step was carried out separately to maximize the selective dissolution of different classes of bioactive compounds present in the rind.

The first extraction was performed using the nonpolar solvent n-hexane in a closed glass container while stirring with a magnetic stirrer for 24 hours. After the

extraction process, the *C. lanatus* rind extract was separated from the residue by vacuum filtration using a Büchner funnel lined with filter paper, connected to a vacuum pump. The remaining extraction residue was then dried in an oven at a temperature above the boiling point of n-hexane (>68 °C) to remove any residual solvent.

Following filtration, the solvent was removed from the *C. lanatus* rind extract by evaporation using a rotary evaporator (Buchi R-210). The evaporation process was conducted at a temperature slightly below the solvent's boiling point and was halted when the extract volume was reduced by approximately 80–90%, leaving a concentrated extract. The resulting thickened *C. lanatus* rind extract was then stored in a refrigerator for preservation. Subsequent extractions were carried out sequentially using the semipolar solvent (ethyl acetate) and the polar solvent (ethanol), following the same procedure as described for the first extraction.

4.3.2 Phytochemical Analysis of *C. lanatus* Rind Extract

Phytochemical screening of *C. lanatus* rind extract was conducted to identify the presence of alkaloids, flavonoids, terpenoids and steroids, tannins, and cardiac glycosides based on methods described by Arawande et al. (2023), Hendrisno et al. (2023), and Jani et al. (2020).

4.3.2.1 Alkaloid Test

The presence of alkaloids in the extract was determined using Wagner's reagent, a commonly used reagent for alkaloid detection. In this test, six drops of Wagner's reagent were added to 2 mL of the extract, and the mixture was observed for any color changes. The formation of reddish or brown precipitates indicated a positive result for alkaloids, confirming their presence in the sample (Jani et al., 2020).

4.3.2.2 Flavonoid Test

The flavonoid content of the extract was assessed using iron(III) chloride ($FeCl_3$) solution, which reacts with flavonoids to produce characteristic color changes. A few drops of 96% (w/v) $FeCl_3$ solution were added to 2 mL of the sample, and the reaction was monitored. The development of an intense green

coloration confirmed the presence of flavonoids in the extract (Arawande et al., 2024).

4.3.2.3 Terpenoid and Steroid Test

The presence of terpenoids and steroids in the extract was assessed using the Salkowski test. A few drops of the sample were mixed with 2 mL of $CHCl_3$, followed by the addition of 1 mL of concentrated H_2SO_4. The formation of a reddish-brown coloration at the interface between the $CHCl_3$ and H_2SO_4 layers indicated a positive result for terpenoids and steroids (Hendrisno et al., 2023).

4.3.2.4 Tannin Test

Approximately 1 mL of the sample was combined with 2 mL of a 10% $FeCl_3$ solution, which was prepared by diluting 96% (w/v) $FeCl_3$ solution. The formation of a blue-black coloration indicated the presence of tannins (Arawande et al., 2024).

4.3.2.5 Cardiac Glycoside Test

The detection of cardiac glycosides was performed using the Keller-Killiani test. To 1 mL of the sample**,** 1 mL of CH_3COOH and 1 mL of 10% $FeCl_3$ solution were added. Subsequently, H_2SO_4 was carefully layered along the side of the test tube to create a distinct interface. The appearance of green or blue precipitates confirmed the presence of cardiac glycosides (Arawande et al., 2024).

4.3.3 Preparation and Optimization of Lipstick Containing *C. lanatus* Rind Extract

4.3.3.1 Preliminary Study

The initial lipstick formulation was prepared to determine the appropriate composition range for the mixture design. Based on previous research, the optimal formulation consists of 39.40% castor oil, 20.00% beeswax, 5.00% carnauba wax, 5.00% candelilla wax, and 17.6% solvent (Kamairudin et al., 2014). Therefore, six preliminary formulations were developed based on this formulation to establish the minimum and maximum limits for each component to be used in the D-optimal design.

4.3.3.2 Experimental Design

This study follows the approach of Kamairudin et al. (2014) in designing a lipstick formulation incorporating *C. lanatus* rind extract. The primary components of the formulation include olive oil, virgin coconut oil (VCO), castor oil, candelilla wax, carnauba wax, beeswax, titanium dioxide (TiO_2), preservatives, and previously extracted *C. lanatus* rind extract.

To optimize the formulation and analyze the influence of components on the lipstick's melting point, the D-optimal mixture design was applied with five factors: (A) olive oil, (B) VCO, (C) beeswax, (D) candelilla wax, and (E) carnauba wax. The D-optimal criterion was used to determine optimal candidate points within the formulation design space. In this approach, the proportion of each component (*Xj*) was constrained within lower (*Lj*) and upper (*Uj*) bounds to prevent exploration across the entire simplex region, with the general constraint expressed as follows: Σ Xj = 1 and $Lj \leq Xj \leq Uj$ (Kamairuddin et al., 2014).

The minimum and maximum limits for each component are listed in Table 4.3.3.2.1, which were established based on a formulation range approximating the best experimental results from preliminary studies. From 25 candidate experiments, a subset of 20 experiments was selected based on the criterion of maximizing the determinant of the information matrix.

Table 4.3.3.2.1 Limitations on the proportion of the independent variable for the lipstick formulation.

Independent Variable (*Xj*)	**Lower Limit (*Lj*)**	**Upper Limit (*Uj*)**
(A) Olive oil	10	35
(B) VCO	25	45
(C) Beeswax	5	25
(D) Candelilla wax	1	5
(E) Carnauba wax	1	5

Each lipstick formulation was prepared following the D-optimal mixture design in a randomized order to minimize the influence of external factors on the observed response. The independent variables in this study consist of the five main components mentioned earlier, collectively comprising 84% of the total formulation weight. Additionally, the formulation includes dependent variables,

namely castor oil, colorant, titanium dioxide, preservative, and *C. lanatus* rind extract.

Table 4.3.3.2.2 D-optimal mixture design of lipstick formulations containing *C. lanatus* fruit rind extract.

Std	A (%)	B (%)	C (%)	D (%)	E (%)
1	10.000	43.275	24.751	1.012	4.961
2	32.683	33.269	8.060	5.000	4.987
3	11.068	39.878	24.988	5.000	3.066
4	22.240	45.000	14.673	1.050	1.037
5	35.000	26.984	16.012	1.008	4.996
6	22.716	38.512	16.719	5.000	1.054
7	32.818	25.000	19.346	3.734	3.103
8	10.053	45.000	18.954	4.996	4.997
9	10.118	45.000	24.985	2.896	1.000
10	26.564	26.391	24.995	5.000	1.050
11	26.976	45.000	5.589	4.990	1.444
12	35.000	34.586	10.184	3.209	1.022
13	26.599	29.857	25.000	1.173	1.371
14	19.755	30.710	24.995	3.541	5.000
15	24.692	45.000	7.363	1.960	4.985
16	26.143	30.727	17.131	4.998	5.000
17	28.248	38.453	12.023	1.000	4.275
18	19.073	38.650	24.272	1.000	1.005
19	16.569	40.724	18.533	3.181	4.994
20	19.526	34.980	20.094	5.000	4.400
21	10.000	43.275	24.751	1.012	4.961
22	35.000	34.586	10.184	3.209	1.022
23	26.976	45.000	5.589	4.990	1.444
24	32.818	25.000	19.346	3.734	3.103
25	35.000	26.984	16.012	1.008	4.996

A: olive oil; B: VCO; C: beeswax; D: candelilla wax; E: carnauba wax

The experimental plan, consisting of 20 trials, is presented in Table 4.3.3.2.2, with the proportion of each component expressed in % w/w. Additionally, the list of dependent variables and their proportional constraints are detailed in Table 4.3.3.2.3. Based on this design, test formulations were prepared and analyzed to determine the melting point of the lipstick, ensuring that the composition met the desired physicochemical properties.

The lipstick formulation process involved the gradual mixing of ingredients using a hot plate stirrer. Initially, oil and wax were heated separately according to

their type in a 50 mL beaker until the temperature reached 80 °C, with continuous stirring. Once fully melted, the oil and wax phases were combined into the same beaker, maintaining constant stirring to achieve homogeneity. Following this, additional ingredients, including the coloring agent, TiO_2, preservative, fragrance, and *C. lanatus* rind extract, were incorporated. Stirring was continued for 30 minutes to ensure uniform dispersion of all components.

After the mixing process, the hot lipstick mixture was carefully poured into a lipstick mold kit and left to cool at room temperature for an initial setting period. To enhance solidification and structural integrity, the mold was then transferred to a freezer until the lipstick fully solidified. The resulting formulations were subsequently analyzed for their melting point, ensuring compliance with the experimental design parameters. Based on this design, the test formulations were prepared and analyzed to determine the melting point of the lipstick.

Table 4.3.3.2.3 Dependent variable in lipstick formulations.

Components	Amount (%)
Castor oil	10
Colorant	3
TiO_2	1
Preservative	1
C. lanatus rind extract	1

4.3.3.3 Evaluation of Lipstick Melting Point

Ensuring a consistent standard for lipstick is crucial, particularly in terms of its melting point, which serves as a key indicator of storage stability and usability. A melting point test was conducted to assess whether the formulated lipstick meets the required thermal stability for safe human use. This test is essential for determining appropriate storage conditions, as it identifies the temperature limit at which the product remains stable.

The melting point was determined using the slip melting point method, a conventional technique for evaluating waxy solids. In this method, a 10-mm column of the solid was cast in a capillary tube with an internal diameter of approximately 1 mm and a length of 80 mm. The tube was then immersed in a temperature-controlled water bath, and the melting point was recorded as the temperature at

which the product gradually melted and exited the tube. The procedure was repeated three times to ensure accuracy and consistency. The test was performed using a melting point apparatus (Dynalon DMP100), with the target melting point set within the range of 40–56 °C, as referenced in Kamairudin et al. (2014).

4.3.3.4 Statistical Analysis

In mixture design, the optimal conditions of independent variables are determined to predict variations in material composition and preparation parameters. The selection of the optimal formulation and natural lipstick preparation is based on achieving a moderate melting point (R_1). The D-optimal model for the five-component system is expressed through the following quadratic regression equation (Equation 1) (Kamairuddin et al., 2014):

$$Y = b + b_1X_1 + b_2X_2 + \cdots + b_nX_n + \varepsilon \quad \text{Eq. (1)}$$

where Y represents the dependent variable, b is the constant, and b_i, b_{ii}, and b_{ij} denote the linear, quadratic, and interaction coefficients, respectively. The statistical significance of the independent variables is analyzed using Analysis of Variance (ANOVA). Only statistically significant independent variables ($p < 0.05$) are included in the reduced model, while non-significant variables ($p > 0.05$) are eliminated. To ensure a good model fit, the R^2 value is recommended to be at least 0.80 (Kamairuddin et al., 2014).

4.3.3.5 Model Verification

To validate the model, a quantitative comparison was conducted between the experimental and theoretical predicted values. Additionally, the percentage of the calculated values was determined. The predicted error was defined as the difference between the experimental and predicted values, expressed as a proportion of the predicted value (Kamairuddin et al., 2014)

4.3.4 Characterization of Optimized Lipstick

4.3.4.1 Melting Point

A melting point test was performed on the lipstick samples to ensure that the formulation is suitable for human use. This test is essential for determining appropriate storage conditions, as it indicates the temperature limit at which the

product remains stable. Using a melting point apparatus (Dynalon DMP100), the target melting point was set within the range of 40–56 °C (Kamairudin et al., 2014).

4.3.4.2 pH Measurement

Sample of the prepared lipstick was dissolved in n-hexane:ethanol (50% v/v) at 100 ppm. The pH value of the lipstick was tested at 25 ℃ using pH meter (Mettler-Toledo Delta 320). Three pH standard buffer solutions (pH 4.01, pH 7.00 and pH 10.01) were used for calibration. Three measurements were conducted for the sample and the average were determined (Ben-Chioma et al., 2015). The same procedures also were carried out on the conventional lipstick. The pH measurement of the prepared lipstick was compared to the conventional lipstick in the market.

4.3.4.3 Stability Study

The approach used in the stability study was adapted from the research of Yasir et al. (2023) and Navarro-Pérez et al. (2021) with minor adjustments. The stability of the prepared lipstick sample in casing was observed at 5, 27, 40 °C as well as for freeze-thaw cycle. For the freeze-thaw cycle, the sample was put in the refrigerator at ± 5 ℃ for 24 hours. Then, it was taken out and left at 27 ℃ for 24 hours. On the third day, the same sample was again put in the refrigerator for 24 hours. The step was repeated for six consecutive days. For other stability studies, the observation was carried out at 5, 27 and 40 ℃ for the duration of three months.

4.3.4.4 Thermal Stability

Thermal stability analysis was conducted using thermogravimetric analyzer (TGA) (PerkinElmer TGA-7). The TGA method is based on the work of Nciri et al. (2022) with custom settings. Sample of lipstick was weighed in 20 to 30 mg and placed in a platinum sample pan, then heated from 50 ℃ to 600 ℃ with a heating rate of 10 ℃/min with nitrogen flow rate of 20 mL/min. The thermogram was plotted as a change in weight versus temperature.

4.3.5 Safety Evaluation of the Optimized Lipstick

4.3.5.1 Microbiological Analysis

Microbiological analysis was conducted to evaluate the safety of the formulated lipstick by assessing microbial contamination levels. The tests were

performed in accordance with ISO standards for cosmetic microbiology, ensuring compliance with international safety guidelines. The microbiological evaluation included the enumeration and detection of aerobic mesophilic bacteria (ISO 21149:2017), the enumeration of yeast and mold (ISO 16212:2017), as well as the detection of *Pseudomonas aeruginosa* (ISO 22717:2015), *Staphylococcus aureus* (ISO 22718:2015), and *Candida albicans* (ISO 18416:2015).

To prepare the test discs, 20 µL of the lipstick sample was applied to a blank sterile disc (Oxoid, 6 mm) and air-dried for 1 hour before use. Bacterial strains were first cultured on Tryptic Soy Agar (TSA) at 35 °C for 18–24 hours, then suspended in Tryptone-NaCl water and adjusted to 1.00E+08 cfu/mL to standardize the bacterial density for testing.

For the disc diffusion test, 50 mL of molten TSA was inoculated with the microbial suspension (1.00E+05 cfu/mL) and 5 mL was layered over a solidified TSA base in a sterile petri dish. Once the top agar solidified, test discs impregnated with the lipstick sample were placed in duplicate, alongside a positive control (Ampicillin 10 µg) and a negative control (sterile water). The plates were incubated at 35 °C for 48 hours, after which the inhibition zones were measured and recorded.

The enumeration of aerobic mesophilic bacteria, yeast, and mold was performed according to ISO 21149:2017 and ISO 16212:2017, respectively. Serial dilutions of the prepared sample were plated onto selective agar media and incubated under controlled conditions to quantify microbial growth. Meanwhile, the detection of specific pathogenic microorganisms, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, was carried out in accordance with ISO 22717:2015, ISO 22718:2015, and ISO 18416:2015. For these tests, separate plating and incubation procedures were conducted using selective culture media and confirmatory identification techniques.

After the incubation period, microbial colony counts were recorded for the enumeration tests, while the presence or absence of pathogenic microorganisms was determined for the detection assays. These results provided critical insights into the microbiological safety of the lipstick formulation.

4.3.5.2 Heavy Metal Analysis

The presence of Arsenic (As), Lead (Pb), Cadmium (Cd), and Mercury (Hg) in the formulated lipstick was determined in accordance with the Guidelines for Control of Cosmetic Products in Malaysia, 2nd Edition – August 2022 and CPCT/TP/MM/In-House 018 based on AOAC Official Method 2015.01.

The determination of arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg) in lipstick samples was carried out using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, PerkinElmer ELAN DRC II ICP-MS) following AOAC Method 2015.01. A total of 0.25 g of the lipstick sample was weighed into a clean microwave digestion vessel, followed by the addition of 4 mL of concentrated (HNO_3) and 1 mL of high-purity H_2O_2. Additionally, 0.1 mL of Au-Lu solution (50 mg/L in 5% HNO_3) was added to stabilize mercury and evaluate analyte loss during digestion.

The sample was digested using a microwave digestion system with the following heating program: the temperature was increased to 190 °C within 20 minutes, held at 190 °C for 10 minutes, and then allowed to cool before opening to prevent the release of pressurized gases. After digestion, the sample solution was cooled to room temperature and transferred to a 50 mL HDPE centrifuge tube that had been acid-cleaned. The solution volume was then adjusted to 20 mL with deionized water.

The digested sample solution was diluted at least fourfold with 1% HNO_3 before analysis using ICP-MS. The instrument was calibrated using multi-element standards with concentrations ranging from 0.01 to 20 µg/L for As, Cd, Pb, and Hg. Calibration was performed using the standard curve method and verified with an initial calibration verification (ICV) solution from a second independent standard source.

Quality control was ensured by analyzing three method blanks to verify the absence of contamination and performing sample duplication for every 10 samples to assess precision. Additionally, a spike test was conducted to verify accuracy within the lipstick matrix, and certified reference materials (CRM) were analyzed

to confirm result accuracy. The concentrations of As, Pb, Cd, and Hg in the sample were determined based on the calibration curve, which was corrected using internal standards (Rh, In, Tm).

CHAPTER V
RESULT AND DISCUSSION

5.1 Extraction of *C. lanatus* Fruit Rind Phytochemical Compounds

The extraction process was carried out in several stages to efficiently isolate bioactive compounds from *C. lanatus* rind. The method was adapted from Kamal et al. (2023) with minor modifications. Fresh *C. lanatus* fruits were first cut, and the rinds were carefully separated from the flesh. The rinds were then chopped into small pieces to accelerate the drying process (Figure 5.1.1 (a)). Drying was conducted by spreading the chopped rinds on aluminium trays and placing them in a drying oven at a controlled temperature for 48 hours. Once dried (Figure 5.1.1 (b)), the rinds were finely ground using a grinder (Figure 5.1.1. (c)) to increase their surface area, facilitating a more efficient extraction process.

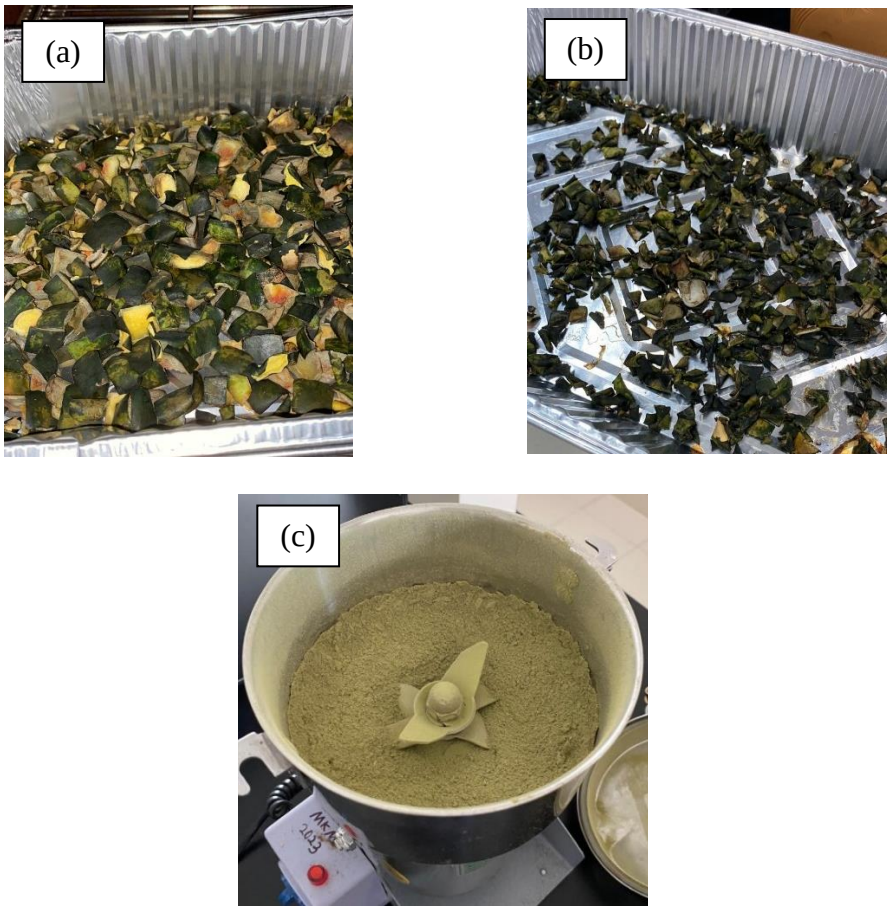


Figure 5.1.1 (a) *C. lanatus* rind cut into small pieces, (b) dried *C. lanatus* rind, and (c) finely ground *C. lanatus* rind.

To maximize the extraction of bioactive compounds, sequential solvent extraction was employed using solvents of increasing polarity. The process was conducted with a sample-to-solvent ratio of 1:5 (w/v), beginning with the nonpolar solvent n-hexane, followed by the semipolar solvent ethyl acetate, and concluding with the polar solvent ethanol. In the final step, ethanol-only extraction was performed to ensure the maximum recovery of polar bioactive compounds, as ethanol is known for its high extraction efficiency, safety, environmental friendliness, and suitability for food and medicinal applications (Chen et al., 2020).

The first extraction was carried out using n-hexane, a nonpolar solvent, in a closed glass container under continuous agitation with a magnetic stirrer for 24 hours. This step allows for the selective extraction of lipophilic compounds while minimizing oxidation and contamination. After the extraction process, the *C. lanatus* rind extract was separated from the solid residue using vacuum filtration through a Büchner funnel lined with filter paper, connected to a vacuum pump. This method ensures the efficient removal of solid impurities, leaving behind a clear filtrate. The extraction residue was then dried in an oven at a temperature above the boiling point of n-hexane (>68 °C) to eliminate any residual solvent, preventing unwanted interference in subsequent extractions.

Following filtration, the solvent was removed from the *C. lanatus* rind extract through evaporation using a rotary evaporator (Buchi R-210). The process was conducted at a temperature slightly below the solvent's boiling point to prevent the thermal degradation of bioactive compounds, ensuring the integrity of thermolabile constituents. Evaporation was halted once approximately 80–90% of the solvent had been removed, resulting in a thickened, dark green concentrated extract (Figure 5.1.2). The intense green coloration suggests a high concentration of bioactive constituents, particularly chlorophylls and other nonpolar phytochemicals. These bioactives were subsequently analyzed through phytochemical screening tests to determine the presence of specific compound groups. To maintain stability and prevent oxidative degradation, the extract was stored in a refrigerator at a controlled temperature, ensuring the preservation of its phytochemical properties for further analysis and formulation.

Figure 5.1.2 *C. lanatus* rind extract with a dark green color.

Subsequent extractions were performed sequentially using ethyl acetate and ethanol, following the same procedure. Ethyl acetate, a semipolar solvent, was expected to extract compounds with moderate polarity, while ethanol, a highly polar solvent, was used in the final stage to maximize the recovery of highly polar bioactive compounds. The stepwise solvent extraction method was employed to enhance extraction yield and selectivity by utilizing differences in solvent polarity (Migo-Sumagang et al., 2023). This approach is widely applied in the extraction of high-value bioactive compounds, particularly for pharmaceutical and cosmetic formulations, due to its efficiency in isolating diverse phytochemicals.

The extraction yield varied across different solvents, reflecting the influence of solvent polarity on the efficiency of bioactive compound isolation. Determination of the extraction yield was calculated by dividing the weight of the dried extract by the initial weight of *C. lanatus* rind powder, then multiplying the result by 100 to express it as a percentage. This calculation method ensures a standardized comparison of extraction efficiency across solvents, further validating the effectiveness of sequential extraction in maximizing bioactive compound recovery. Among the solvents used, ethanol exhibited the highest yield (6.03%), attributed to its strong ability to dissolve a broad range of polar bioactive compounds. The extraction yield increased sequentially, starting from n-hexane (2.93%), followed by ethyl acetate (5.23%), and culminating in ethanol, demonstrating that stepwise

solvent extraction effectively isolates different classes of phytochemicals. The final yield values were recorded in Table 5.1.1.

Table 5.1.1 Yield of *C. lanatus* rind extract from sequential extraction.

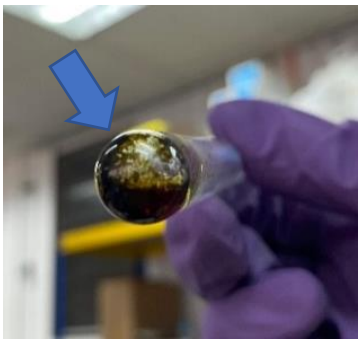
Solvent	Mass Before Extraction (g)	Mass After Extraction (g)	Extract Yield (%)
n-hexane	33,4806	31,9748	2,93
Ethyl acetate	31,9748	30,2265	5,23
Ethanol	30,2265	29,3312	6,03

5.2 Phytochemical Analysis of *C. lanatus* Rind Extract

The phytochemical analysis of *C. lanatus* rind extract was performed using sequential extraction yield. After extraction, phytochemical analysis was performed using various tests, including alkaloid, flavonoid, terpenoid and steroid, tannin, and cardiac glycoside tests.

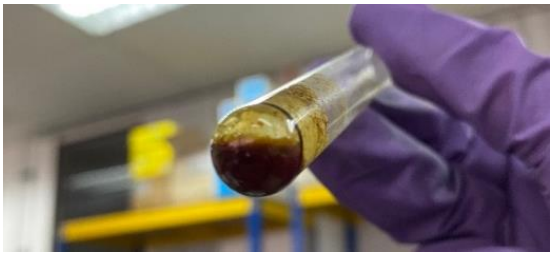
5.2.1 Alkaloid Test

The detection of alkaloids was conducted using Wagner's method. In this test, six drops of Wagner's reagent were added to 2 mL of extract, and the presence of alkaloids was confirmed by the formation of a reddish or brown precipitate (Jani et al., 2020). Positive alkaloid results were observed in the ethyl acetate and ethanol extracts, as indicated by the appearance of reddish precipitates, which are highlighted with arrows in Figures 5.2.1.1 (a) and (b). In contrast, the n-hexane extract did not show any reddish precipitate, confirming a negative result for alkaloids, as illustrated in Figure 5.2.1.1 (c).



(a)

(b)



(c)

Figure 5.2.1.1 Alkaloid test results for *C. lanatus* rind extract with (a) ethyl acetate, (b) ethanol, and (c) n-hexane.

Wagner's reagent is prepared by reacting potassium iodide (KI) with iodine (I_2) in an aqueous solution, forming a triiodide complex (I_3^-), as described in the reaction below:

$$K^+ - I_2 + I^+ - I^- \rightarrow K^+ + I^+ + 2I^-$$

This reagent is commonly used for alkaloid detection due to its ability to interact with the nitrogen atoms in alkaloid structures. During the test, when Wagner's reagent is added to an extract containing alkaloids, the triiodide ions (I_3^-) react with the lone pair of electrons on the nitrogen atoms present in alkaloid molecules. This interaction results in the formation of a coordinate covalent bond, leading to the precipitation of alkaloid-iodine complexes in the form of a reddish-brown precipitate. The intensity of the precipitate depends on the concentration and structure of the alkaloids present in the extract (Erlidawati et al., 2023).

This reaction mechanism is based on the electrophilic nature of iodine, which readily forms charge-transfer complexes with the electron-donating nitrogen atoms in alkaloids. Since alkaloids typically contain basic nitrogen groups, they easily form stable complexes with iodine, making Wagner's reagent a reliable qualitative test for their detection. The reaction of the alkaloid test with Wagner's reagent is illustrated in Figure 5.2.1.2.

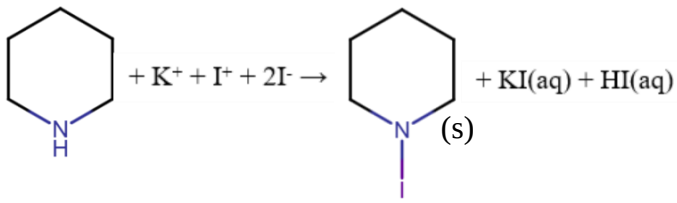


Figure 5.2.1.2 Mechanism of the alkaloid test reaction using Wagner's reagent (Erlidawati et al., 2023).

5.2.2 Flavonoid Test

Flavonoid detection was performed by reacting 0.1 mL of extract with dilute NaOH, which resulted in the formation of a bright yellow coloration. The subsequent addition of concentrated HCl caused the yellow color to fade, confirming the presence of flavonoids (Arawande et al., 2024). Among the tested extracts, the ethanol (Figure 5.2.2.1 (a)) extract yielded a positive result, as evidenced by the observed color change, while the ethyl acetate (Figure 5.2.2.1 (b)) and n-hexane (Figure 5.2.2.1 (c)) extracts showed negative results.

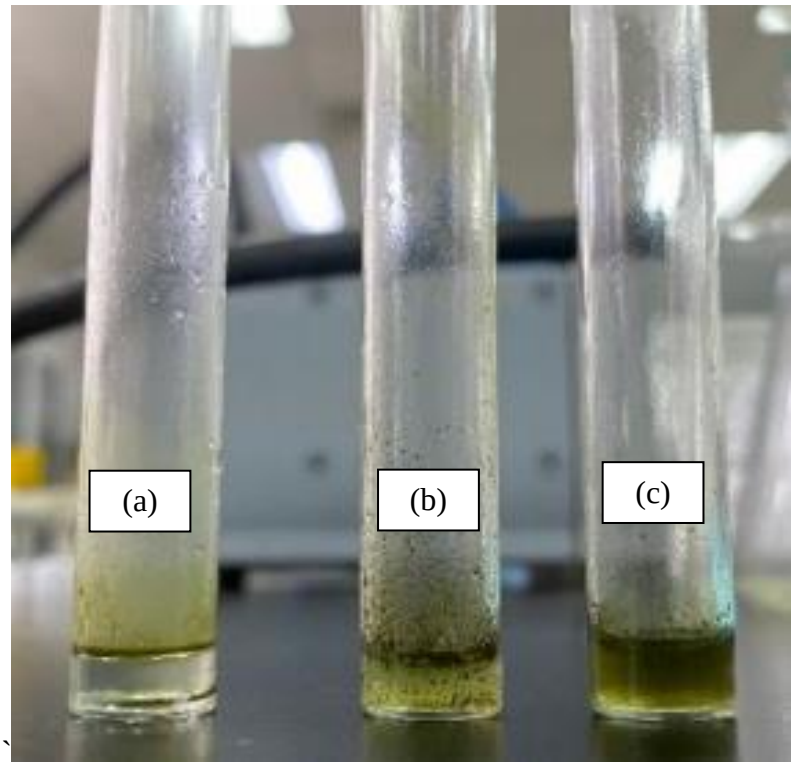


Figure 5.2.2.1 Flavonoid test results for *C. lanatus* rind extract from left to right: (a) ethanol, (b) ethyl acetate, and (c) n-hexane.

The color change in this test occurs due to the interaction between flavonoid compounds and NaOH. Flavonoids contain -OH groups attached to ring A or B of their structure, which form flavonoid-sodium complexes in an alkaline environment, producing a yellow coloration. When HCl is added, it protonates the hydroxyl groups, disrupting the complex and leading to the disappearance of the yellow color (Sari et al., 2019). This confirms the presence of flavonoid compounds, as they exhibit pH-dependent structural changes that influence their

optical properties. The proposed reaction mechanism for this test is illustrated in Figure 5.2.2.2.

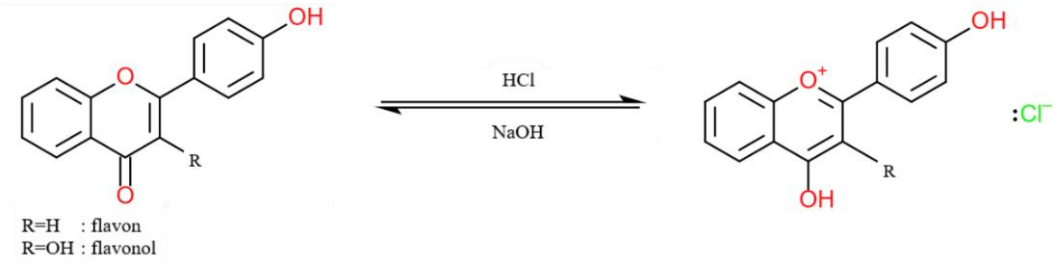


Figure 5.2.2.2 Mechanism of the flavonoid test reaction (Sari et al., 2019).

The positive result in the ethanol extract suggests that flavonoids in *C. lanatus* rind are predominantly polar, making them more soluble in ethanol, a highly polar solvent. Conversely, the absence of flavonoids in the ethyl acetate and n-hexane extracts indicates that these compounds have low affinity for less polar or nonpolar solvents, reinforcing the idea that flavonoids are best extracted using polar solvents. This finding aligns with previous studies that emphasize the high solubility of flavonoids in ethanol-based extractions, particularly for plant-derived bioactive compounds (Arawande et al., 2024).

These results further support the phytochemical profile of *C. lanatus* rind, indicating that it contains flavonoids as part of its bioactive constituents. Since flavonoids are known for their antioxidant, anti-inflammatory, and antimicrobial properties, their presence in the ethanol extract highlights the potential functional benefits of *C. lanatus* rind in cosmetic and pharmaceutical applications.

5.2.3 Terpenoid and Steroid Test

The presence of terpenoids and steroids in the extracts was assessed using the Salkowski method, which relies on the interaction between triterpenoid compounds and H_2SO_4 to produce characteristic color changes. In this test, a few drops of the sample were mixed with 2 mL of $CHCl_3$, followed by the addition of 1 mL of concentrated H_2SO_4. A reddish-brown coloration at the liquid interface confirmed the presence of terpenoids (Arawande et al., 2024).

Among the tested extracts, only the ethanol (Figure 5.2.3.1 (c)) extract showed a positive result, as indicated by the development of a reddish-brown color. In

contrast, the ethyl acetate (Figure 5.2.3.1 (b)) and n-hexane (Figure 5.2.3.1 (a)) extracts showed no color change, indicating the absence of terpenoid and steroid compounds in those fractions.

The formation of a reddish-brown coloration occurs due to the reaction between triterpenoid compounds and H_2SO_4 in the presence of $(CH_3CO)_2O$. This reaction is influenced by the structural differences in functional groups attached to the C4 atom of triterpenoid molecules, leading to variations in color intensity (Hendrisno et al., 2020). The proposed reaction mechanism is illustrated in Figure 5.2.3.2.

The positive result in the ethanol extract suggests that terpenoids and steroids in *C. lanatus* rind are primarily polar, making them more soluble in ethanol, a highly polar solvent. The absence of terpenoid and steroid compounds in the ethyl acetate and n-hexane extracts indicates that these compounds are either not sufficiently nonpolar to be extracted with n-hexane or that their moderate polarity prevents efficient extraction with ethyl acetate. This result aligns with findings from previous studies, which reported that ethanol-based extractions yield a higher concentration of triterpenoid compounds due to their strong affinity for polar solvents (Hendrisno et al., 2020).

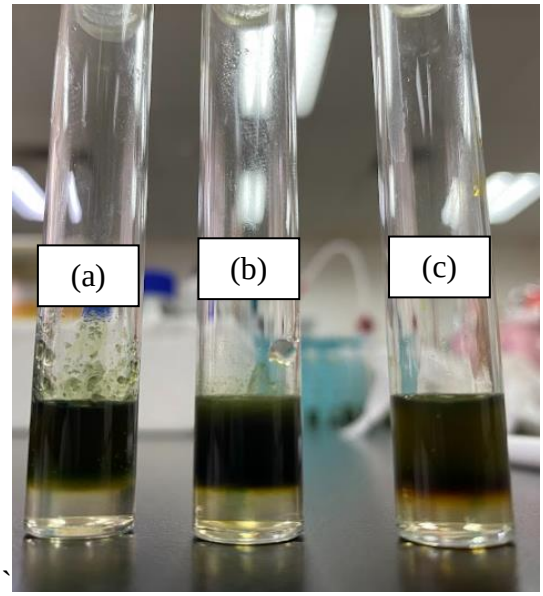


Figure 5.2.3.1 Terpenoid and steroid test results for *C. lanatus* rind extract from left to right: (a) n-hexane, (b) ethyl acetate, and (c) ethanol.

Since triterpenoids and steroids are known for their anti-inflammatory, antimicrobial, and antioxidant properties, their presence in the ethanol extract highlights the potential functional benefits of *C. lanatus* rind for cosmetic and pharmaceutical applications.

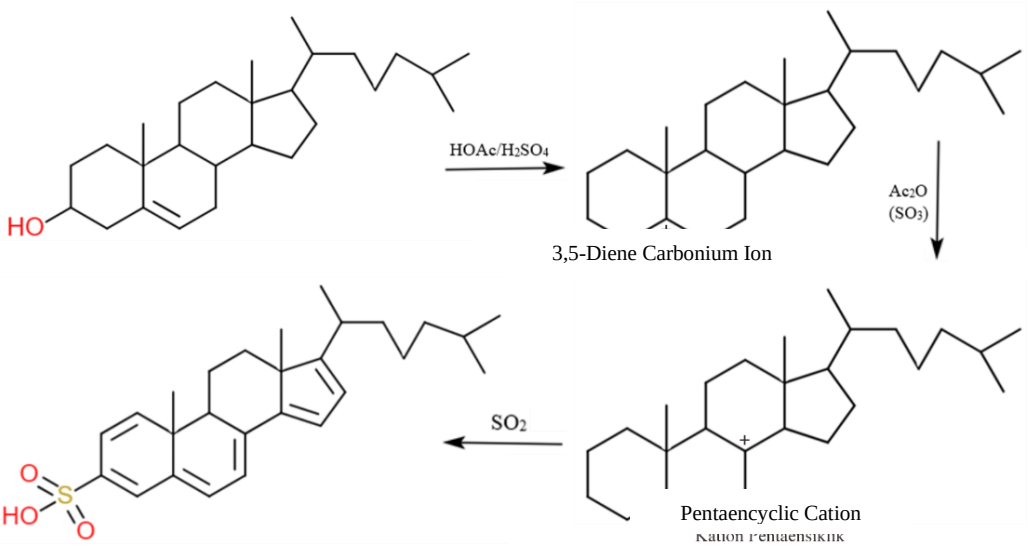


Figure 5.2.3.2 Mechanism of the terpenoid test reaction (Hendrisno et al., 2020).

5.2.4 Tannin Test

The presence of tannins in the extracts was evaluated using the ferric chloride ($FeCl_3$) test, a qualitative method based on the formation of iron-tannin complexes, which produce distinct color changes. Tannins, as polyphenolic compounds, have a strong affinity for iron (Fe^{3+}) ions, leading to the formation of a dark-colored coordination complex. This reaction occurs due to the ability of tannins to donate electrons from their hydroxyl (-OH) groups, allowing them to chelate Fe^{3+} ions and form a stable complex.

In this test, 1 mL of extract was combined with 2 mL of a 10% $FeCl_3$ solution, resulting in a blue-black coloration, confirming the presence of tannins (Arawande et al., 2024). Among the tested extracts, only the ethanol extract (Figure 5.2.4.1 (c)) exhibited a positive result, as indicated by the formation of a dark blue-black color. In contrast, the ethyl acetate (Figure 5.2.4.1 (b)) and n-hexane extracts (Figure 5.2.4.1 (a)) showed no observable color change, suggesting that tannins were either absent or present in undetectable concentrations in these extracts.

The selective presence of tannins in the ethanol extract is attributed to solvent polarity. Since ethanol is a polar solvent, it efficiently extracts highly polar phytochemicals such as tannins, whereas ethyl acetate and n-hexane, being less polar and nonpolar solvents respectively, are less effective in dissolving these compounds. This result aligns with previous studies, reinforcing that tannins are more soluble in polar solvents, which enhances their extraction efficiency and detectability.

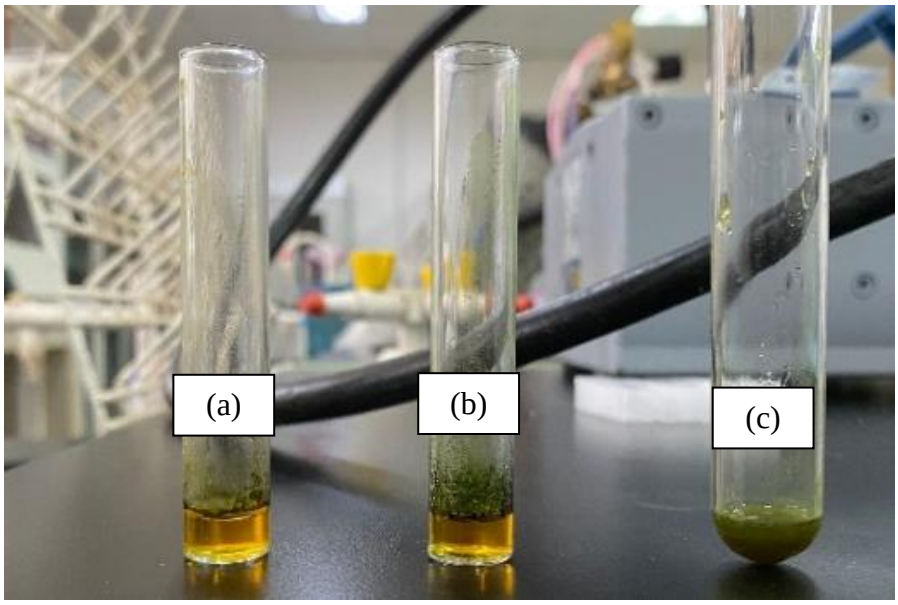


Figure 5.2.4.1 Tannin test results for *C. lanatus* rind extract from left to right: (a) n-hexane, (b) ethyl acetate, and (c) ethanol.

The color change mechanism in the tannin test occurs due to the ability of tannins to form coordination complexes with Fe^{3+} ions. In this reaction, Fe^{3+} acts as the central metal ion, while tannins, which contain oxygen atoms, function as ligands by coordinating with the Fe^{3+} ion through lone electron pairs present on the oxygen atoms. The degree of color intensity depends on the type and structure of tannins, as different tannin subclasses (hydrolyzable or condensed tannins) may exhibit variations in the complexation process (Lestari et al., 2020). The reaction mechanism is illustrated in Figure 5.2.4.2.

The positive result in the ethanol extract suggests that tannins in *C. lanatus* rind are predominantly polar, making them highly soluble in ethanol, a strongly polar solvent. Conversely, the absence of tannins in the ethyl acetate and n-hexane

extracts indicates that these compounds have low affinity for less polar or nonpolar solvents, reinforcing the idea that polar solvents are most effective for tannin extraction. These findings are consistent with previous research, which reports that ethanol-based extractions yield higher concentrations of tannins, particularly from plant-derived bioactive compounds (Lestari et al., 2020).

Since tannins are known for their antioxidant, antimicrobial, and astringent properties, their presence in the ethanol extract highlights the potential functional applications of *C. lanatus* rind in cosmetic, pharmaceutical, and nutraceutical formulations.

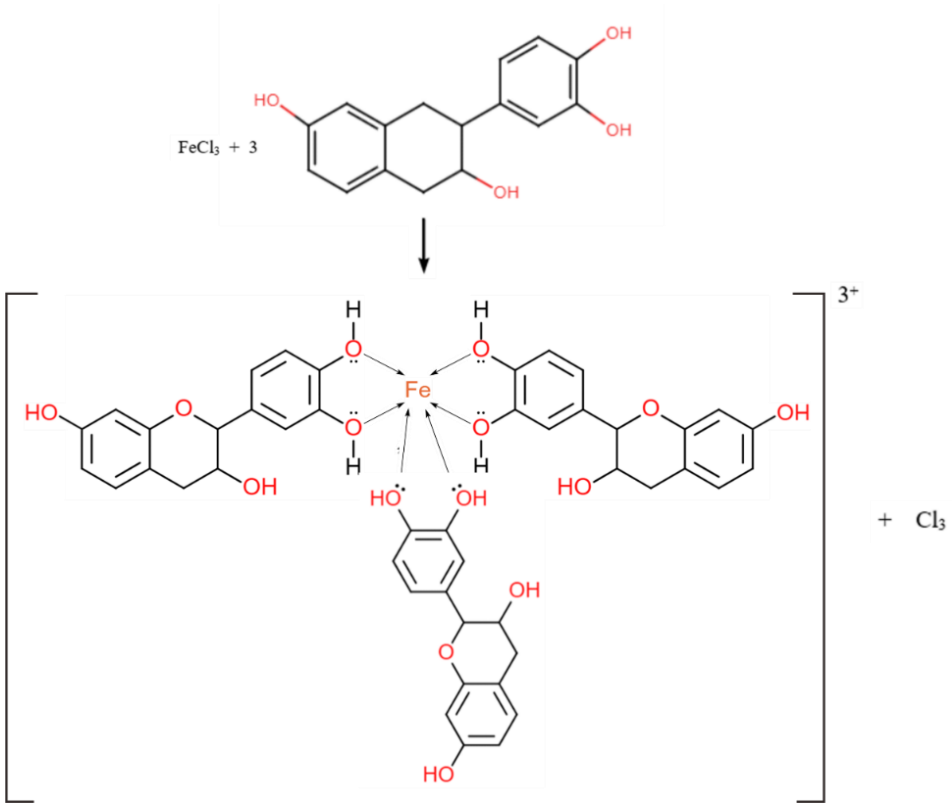


Figure 5.2.4.2 Mechanism of the tannin test reaction (Lestari et al., 2020).

5.2.5 Cardiac Glycoside Test

The presence of cardiac glycosides in the extracts was assessed using the Keller-Killiani method, a qualitative test based on colorimetric reactions involving Fe^{3+} ions. In this procedure, 1 mL of CH_3COOH and 1 mL of a 10% $FeCl_3$ solution were added to 1 mL of the sample. This mixture was then carefully layered with 1 mL of concentrated H_2SO_4 along the inner wall of the test tube. The formation of a

green or blue precipitate confirmed the presence of cardiac glycosides (Arawande et al., 2024). After testing, all solvent extracts tested positive, as indicated by the color changes observed (Figure 5.2.5.1 (a-c)).

The observed color change mechanism is attributed to the interaction between Fe^{3+} ions and the chemical structure of Digitalis glycosides. Cardiac glycosides typically contain steroid aglycones, which undergo oxidation and complex formation with Fe^{3+} ions, resulting in the distinctive blue or green coloration (Kiliani, 1896). The reaction mechanism is influenced by the presence of deoxy sugars in cardiac glycosides, which enhance the color intensity and precipitation process.

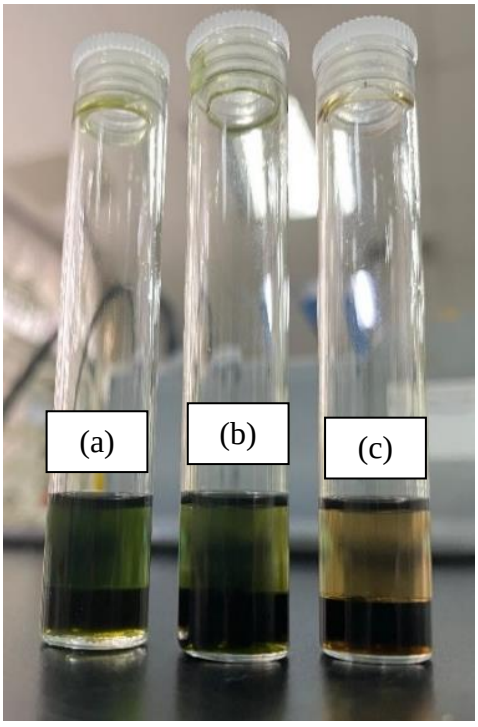


Figure 5.2.5.1 Cardiac glycoside test results for *C. lanatus* rind extract from left to right: (a) ethanol, (b) ethyl acetate, and (c) n-hexane.

The positive results in all tested solvents indicate that cardiac glycosides in *C. lanatus* rind exhibit a wide range of solubility, being effectively extracted by polar (ethanol), semipolar (ethyl acetate), and nonpolar (n-hexane) solvents. This suggests that cardiac glycosides have amphiphilic properties, allowing them to dissolve in both polar and nonpolar media. These findings are consistent with

previous studies reporting that cardiac glycosides can be extracted using various solvents depending on the structural composition of the glycoside moiety.

Since cardiac glycosides are known for their cardiotonic effects, their presence in *C. lanatus* rind highlights its potential pharmacological applications, particularly in cardiovascular treatments. However, further studies are needed to quantify their concentration and evaluate their bioavailability and safety for medicinal use.

5.2.6 Overall Test Results and Comparison of the Preliminary Study

The overall test results for *C. lanatus* rind extract obtained from sequential extraction are presented in Table 5.2.6.1. The presence or absence of specific phytochemical compounds in different solvent extracts is influenced by the polarity of both the compounds and the extraction solvents.

Table 5.2.6.1 Phytochemical analysis results of *C. lanatus* rind extract obtained through sequential extraction.

Phytochemical	**Result**		
	Ethanol	**Ethyl acecate**	***n*-Hexane**
Alkaloids	+	+	-
Flavonoids	+	-	-
Terpenoids & Steroids	+	-	-
Tannins	+	-	-
Cardiac glycosides	+	+	+

+: Present; -: Absent

Ethanol extract showed the broadest range of detected phytochemicals, including alkaloids, flavonoids, terpenoids & steroids, tannins, and cardiac glycosides, indicating that polar bioactive compounds are predominant in *C. lanatus* rind. Ethanol, being a highly polar solvent, efficiently extracts phenolic and nitrogen-containing compounds, which explains the detection of flavonoids and alkaloids in this fraction.

In contrast, the ethyl acetate extract contained alkaloids and cardiac glycosides but lacked flavonoids, terpenoids & steroids, and tannins. As a semipolar solvent, ethyl acetate has a moderate affinity for extracting certain alkaloids and glycosides, but it may not be as effective in dissolving highly polar flavonoids or tannins.

The n-hexane extract demonstrated the least variety of phytochemicals, with only cardiac glycosides detected. This is expected, as n-hexane is a nonpolar solvent, which primarily dissolves lipophilic compounds such as fats, oils, and

certain terpenoids, rather than polar secondary metabolites like flavonoids or tannins. The absence of most phytochemicals in n-hexane extract further supports that the dominant bioactive compounds in *C. lanatus* rind are polar or semipolar in nature.

However, a comparison with previous studies by Kamal et al. (2023) and Neglo et al. (2021) highlights variations in the detected phytochemicals, which may be attributed to differences in extraction methods, solvents, plant varieties, and plant parts used in analysis.

The results from Kamal et al. (2023) (Table 5.2.6.2) align with this study in detecting alkaloids, flavonoids, and glycosides in *C. lanatus* rind extract. However, saponins and phenols, which were reported as present in their study, were not explicitly detected in this research. This discrepancy may be due to differences in solvent selection and extraction techniques, as well as variations in plant origin, growing conditions, and sample processing methods. Ethanol was the primary solvent in this study, while Kamal et al. (2023) may have used alternative or more exhaustive extraction methods, potentially leading to a higher recovery of saponins and phenols.

Table 5.2.6.2 Qualitative phytochemical screening of *C. lanatus* rind (Kamal et al., 2023).

Phytochemical	**Result**
Alkaloids	+
Saponins	+
Terpenoids	-
Steroids	-
Glycosides	+
Phenols	+
Flavonoids	+

+: Present, −: Absent

A broader comparison with Neglo et al. (2021) (Table 5.2.6.3) reveals further variations, particularly when considering different parts of the *C. lanatus* fruit (rind, pulp, and seed). Their study confirmed the presence of alkaloids, flavonoids, tannins, glycosides, and free reducing sugars in the rind, which aligns with this study's findings for alkaloids, flavonoids, tannins, and glycosides. However, their results showed an absence of terpenoids and steroids, which matches this study's

findings where terpenoids & steroids were only detected in the ethanol extract. Interestingly, Neglo et al. (2021) reported the presence of triterpenoids in the pulp and seeds but not in the rind, whereas this study detected terpenoids & steroids in the ethanol extract of the rind. This suggests that terpenoid content may vary significantly between plant parts, with higher concentrations possibly present in the pulp and seeds due to their lipid-rich composition.

Similarly, saponins were identified in both the rind and pulp by Neglo et al. (2021) but were not detected in this study, reinforcing the role of solvent polarity and plant part composition in phytochemical solubility. Another notable difference is the presence of free reducing sugars in Neglo et al. (2021), which were not analyzed in this study. Since free reducing sugars are highly polar compounds, they are likely extracted efficiently with aqueous or hydroalcoholic solvents, which may explain their absence in this study's ethanol, ethyl acetate, and n-hexane extracts. These comparative findings emphasize the influence of plant part selection, solvent polarity, and extraction technique on phytochemical profiles. While there are similarities in detecting alkaloids, flavonoids, glycosides, and tannins, differences in terpenoid, steroid, saponin, and phenolic content suggest that extraction conditions play a crucial role in determining the final composition. The presence of certain compounds in previous studies but their absence in this study could be attributed to variations in plant material, geographical factors, or differences in extraction efficiency.

Table 5.2.6.3 Phytochemical contents of various parts of *C. lanatus* fruit (Neglo et al., 2021).

Phytochemical	**Result**		
	Rind	**Pulp**	**Seed**
Alkaloids	+	+	+
Saponins	+	+	-
Triterpenoids	-	+	+
Steroids	-	-	-
Flavonoids	+	-	+
Tannins	+	-	-
Free reducing sugars	+	+	+

+: Present, −: Absent

5.3 Preparation and Optimization of Lipstick Containing *C. lanatus* Rind Extract

5.3.1 Variables Screening

A preliminary study was conducted to establish the appropriate levels for the five independent variables in the lipstick formulation. Based on the experimental results, the lower and upper limits for each component were determined to ensure optimal melting point stability. The formulation successfully maintained a melting point above 40 °C by adjusting the concentration ranges of olive oil (10%–35%), virgin coconut oil (25%–45%), beeswax (5%–25%), candelilla wax (1%–5%), and carnauba wax (1%–5%). These limits were set based on D-optimal mixture design constraints, ensuring that the composition remained within a balanced formulation space while achieving the desired physicochemical properties.

In addition to these independent variables, the formulation also included dependent variables, which were kept constant throughout the study. These consisted of castor oil (10%), colorant (3%), TiO_2 (1%), preservative (1%), and *C. lanatus* rind extract (1%). The inclusion of these components was essential to maintain the desired aesthetic, structural, and functional properties of the lipstick while ensuring stability and safety for consumer use.

5.3.2 Models Fitting and D-Optimal Analysis

The optimal conditions for independent variables were determined using the D-optimal mixture design, which allows for predicting the effects of variations in ingredient composition on the melting point of lipstick. Melting point analysis is a crucial aspect as it serves as an indicator of safe storage limits. The formulated lipstick must have an appropriate melting point to prevent it from becoming too soft, melting under its own weight, or slipping from its casing.

The lipstick formulation process involved the gradual mixing of ingredients using a hot plate stirrer. Initially, oil and wax were heated separately according to their type in a 50 mL beaker until the temperature reached 80 °C, with continuous stirring. Once fully melted, the oil and wax phases were combined into the same beaker, maintaining constant stirring to achieve homogeneity (Figure 5.3.2.1). Following this, additional ingredients, including the coloring agent, TiO_2,

preservative, fragrance, and *C. lanatus* rind extract, were incorporated. Stirring was continued for 30 minutes to ensure uniform dispersion of all components.

After the mixing process, the hot lipstick mixture was carefully poured into a lipstick mold kit and left to cool at room temperature for an initial setting period (Figure 5.3.2.2). To enhance solidification and structural integrity, the mold was then transferred to a freezer until the lipstick fully solidified. The resulting formulations were subsequently analyzed for their melting point, ensuring compliance with the experimental design parameters.

Figure 5.3.2.1 Lipstick ingredients being stirred using a magnetic stirrer.

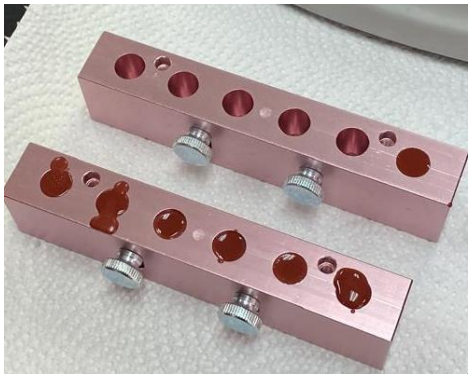


Figure 5.3.2.2 Lipstick poured into a mold kit for solidification.

The variation in melting point within the lipstick formulations containing *C. lanatus* rind extract was analyzed using the D-optimal mixture design, where melting point (Y) served as the response variable. This design allowed for the evaluation of five independent variables, namely (A) olive oil, (B) virgin coconut oil, (C) beeswax, (D) candelilla wax, and (E) carnauba wax, which influence the

melting characteristics of the final product. The experimentally obtained melting point values were compared with the predicted results, as presented in Table 5.3.2.1, providing insights into the formulation's thermal behavior and optimization potential.

Table 5.3.2.1 D-optimal mixture design with predicted and experimental values of melting point of the prepared lipstick containing *C. lanatus* rind extract.

Std	A (%)	B (%)	C (%)	D (%)	E (%)	Melting point (°C)	
						Predicted	Actual
1	10.000	43.275	24.751	1.012	4.961	62.04	63.00
2	32.683	33.269	8.060	5.000	4.987	63.02	64.00
3	11.068	39.878	24.988	5.000	3.066	60.23	60.00
4	22.240	45.000	14.673	1.050	1.037	52.18	52.00
5	35.000	26.984	16.012	1.008	4.996	57.70	57.00
6	22.716	38.512	16.719	5.000	1.054	54.50	56.00
7	32.818	25.000	19.346	3.734	3.103	55.10	55.00
8	10.053	45.000	18.954	4.996	4.997	66.35	66.00
9	10.118	45.000	24.985	2.896	1.000	54.18	53.00
10	26.564	26.391	24.995	5.000	1.050	51.58	52.00
11	26.976	45.000	5.589	4.990	1.444	56.87	55.00
12	35.000	34.586	10.184	3.209	1.022	51.47	50.00
13	26.599	29.857	25.000	1.173	1.371	49.53	48.00
14	19.755	30.710	24.995	3.541	5.000	61.40	62.00
15	24.692	45.000	7.363	1.960	4.985	63.02	63.00
16	26.143	30.727	17.131	4.998	5.000	62.63	62.00
17	28.248	38.453	12.023	1.000	4.275	58.69	57.00
18	19.073	38.650	24.272	1.000	1.005	50.66	53.00
19	16.569	40.724	18.533	3.181	4.994	63.44	63.00
20	19.526	34.980	20.094	5.000	4.400	62.27	61.00
21	10.000	43.275	24.751	1.012	4.961	62.04	62.00
22	35.000	34.586	10.184	3.209	1.022	51.47	51.00
23	26.976	45.000	5.589	4.990	1.444	56.87	60.00
24	32.818	25.000	19.346	3.734	3.103	55.10	55.00
25	35.000	26.984	16.012	1.008	4.996	57.70	60.00

A: olive oil; B: VCO; C: beeswax; D: candelilla wax; E: carnauba wax

The actual values obtained are in good agreement with the predicted values in almost all runs. The predicted of the melting point was made based on the experimental data. The final equation for the model describing the melting point can be written as Equation (2) as follow:

$$\text{Melting point } (°C) = +66.52A + 58.53B + 65.70C + 35.96D - 13.07E \quad \text{Eq. (2)}$$

where A is the olive oil; B is virgin coconut oil; C is beeswax; D is candelilla wax, and E is carnauba wax. The residual analysis was performed using the normal probability plot as given in Figure 5.3.2.3 The plot was found to be normally distributed which resembled a straight line with no outlier point encountered. The plot of predicted value versus actual value (Figure 5.3.2.4) gives indication of good agreement between actual and predicted response of melting point.

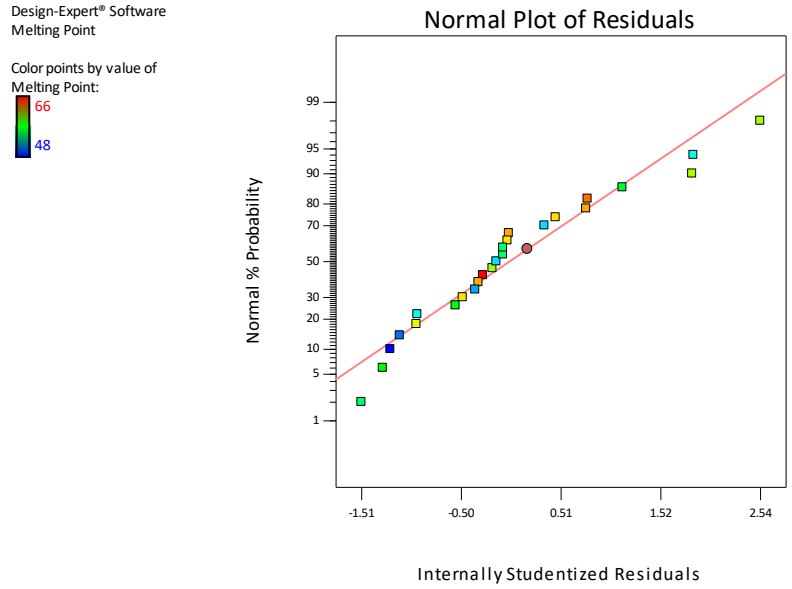


Figure 5.3.2.3 Normal plots of residual from D-optimal design of melting point of the prepared lipstick.

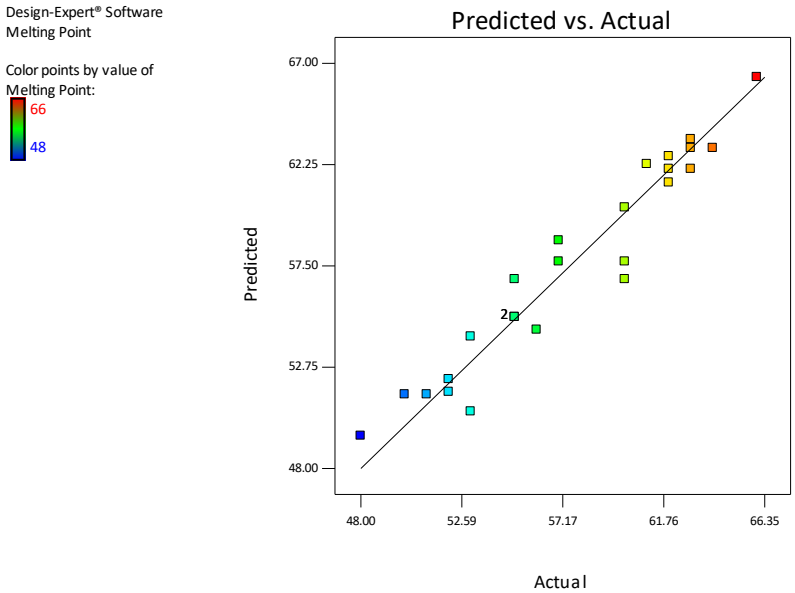


Figure 5.3.2.4 Scatter plots of predicted versus actual values of melting point of the prepared lipstick.

The experimental data was statistically analyzed using ANOVA (Analysis of Variance) to determine the best-fitted model for the five independent variables influencing the lipstick's melting point. The summary statistics of the model, presented in Table 5.3.2.2, indicate that the linear model provided the best representation of the relationship between the studied parameters, as evidenced by its high R^2 (0.9332), adjusted R^2 (0.9199), and predicted R^2 (0.8907) values. Additionally, the good agreement between predicted R^2 and adjusted R^2 values further confirms the suitability of this model. In contrast, while the quadratic and special cubic models showed higher R^2 values (0.9480 and 0.9704, respectively), their adjusted and predicted R^2 values were notably lower, indicating that they may not be as reliable for predicting outcomes. The cubic model was not defined due to insufficient degrees of freedom.

Table 5.3.2.2 Summary statistics of the models.

Source	Std. Dev.	*R*-square	Adjusted *R*-square	Predicted *R*-square
Linear	**1.42**	**0.9332**	**0.9199**	**0.8907**
Quadratic	1.47	0.9480	0.8752	0.6241
Special cubic	1.90	0.9704	0.8579	ND
Cubic	ND	ND	ND	ND

ND: not defined

The ANOVA results in Table 5.3.2.3 further validate the significance of the model. The high model F-value (69.89) and its "Prob > F" value of <0.0001 indicate that the model is statistically significant, with only a 0.01% probability that this large F-value occurred due to random noise. The lack of fit "Prob > F" value of 0.9124 suggests that the lack of fit is insignificant, meaning the developed model fits well within the experimental design space. Furthermore, the adequate precision value (signal-to-noise ratio) of 26.400 exceeds the recommended threshold of 4, confirming that the model has a strong signal for predicting responses accurately.

Overall, these statistical findings confirm that the linear model is the most suitable for predicting the melting point of the lipstick formulation within the studied range. The model effectively describes the influence of olive oil, virgin

coconut oil, beeswax, candelilla wax, and carnauba wax on the response variable, ensuring reliable predictions for optimizing the formulation.

Table 5.3.2.3 ANOVA for the D-optimal mixture design of the model.

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	Prob > *F*
Model	567.41	4	141.85	69.89	<0.0001[a]
Linear Mixture	567.41	4	141.85	69.89	<0.0001[a]
Residual	40.59	20	02.03		
Lack of fit	22.59	15	01.51	00.42	0.9124[b]
Pure error	18.00	5	0,166667		
Cor Total	608.00	24			

[a]Significant at "Prob > F" less than 0.05

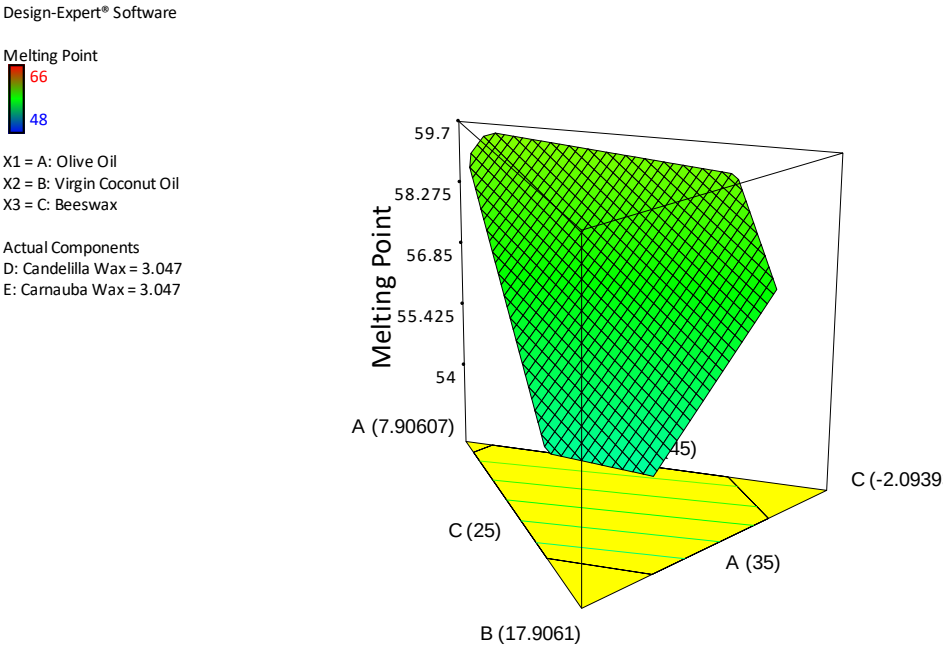
[b]Insignificant at "Prob > F" more than 0.05

Figure 5.3.2.5 (a) and (b) present the 3D and 2D surface plots, illustrating the influence of olive oil (A), virgin coconut oil (VCO) (B), and beeswax (C) on the melting point of the lipstick, while keeping candelilla wax (D) and carnauba wax (E) constant. The plots demonstrate that the melting point increases proportionally with the rising amounts of olive oil, VCO, and beeswax. This effect is attributed to the role of these waxes in enhancing the thermal stability of the formulation, as they contribute to a higher melting point in the final product (Kamairuddin et al., 2014).

Among the three components, olive oil and beeswax exert the most significant influence on the melting point due to their heat endurance properties, which allow the lipstick to maintain its structure under varying temperature conditions. This correlation is further supported by Equation (2), reinforcing their role in determining the formulation's thermal behavior.

Additionally, Figure 5.3.2.6 and Figure 5.3.2.7 provide alternative 3D and contour plots, where different combinations of three components are plotted at a time, while the other two parameters remain constant. These visual representations offer further insights into the interactions between different formulation components, aiding in the optimization of the lipstick's melting point and overall stability.

(a)



(b)

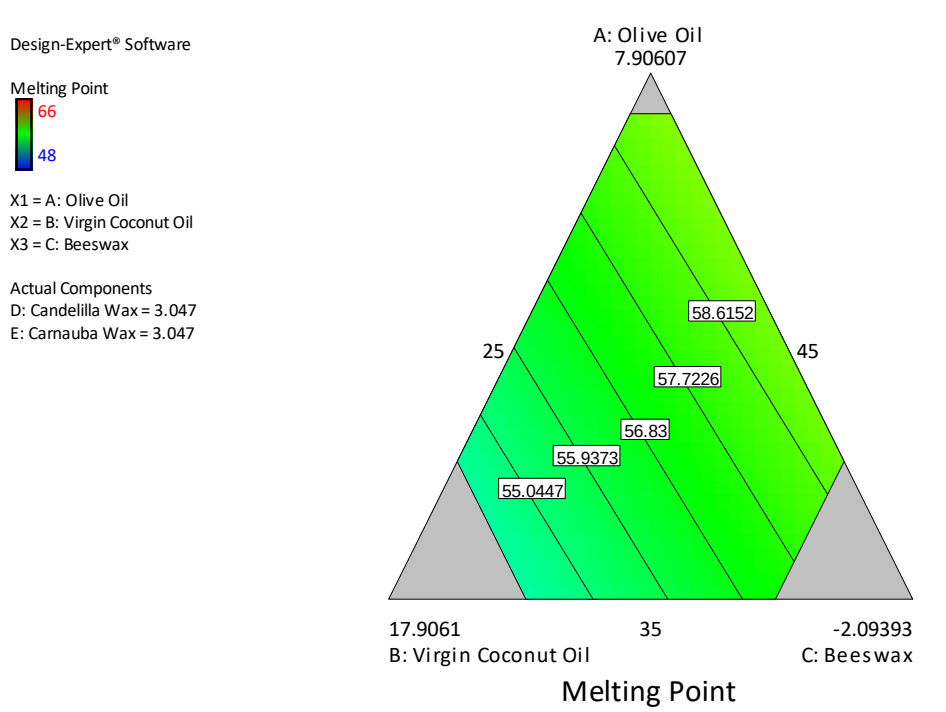
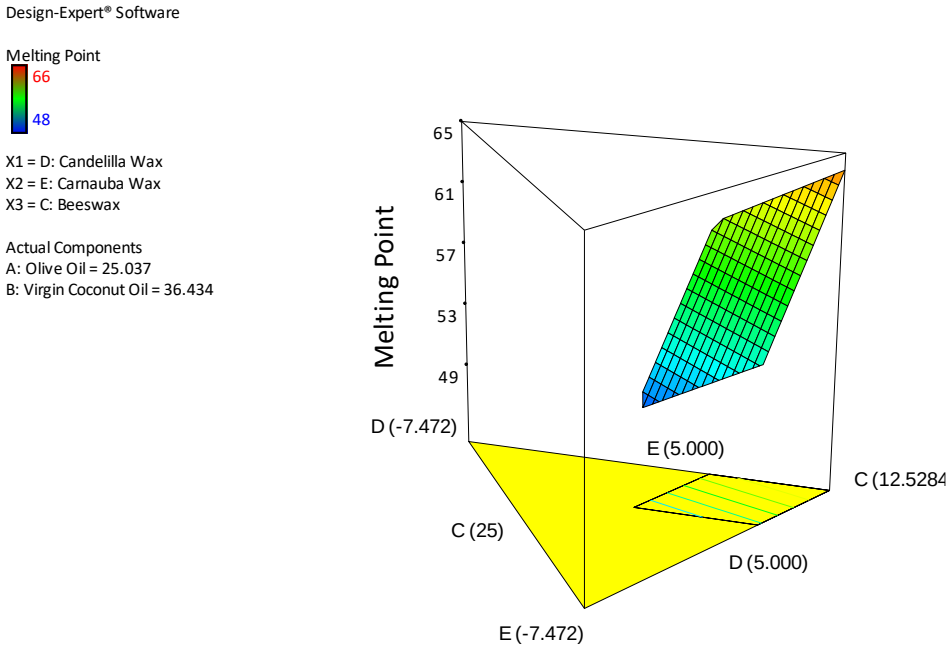


Figure 5.3.2.5 (a) 3D and (b) 2D surface plots showing the interaction effect between A (olive oil), B (VCO) and C (beeswax) with respect to melting point at constant D (candelilla wax) and E (carnauba wax).

(a)



(b)

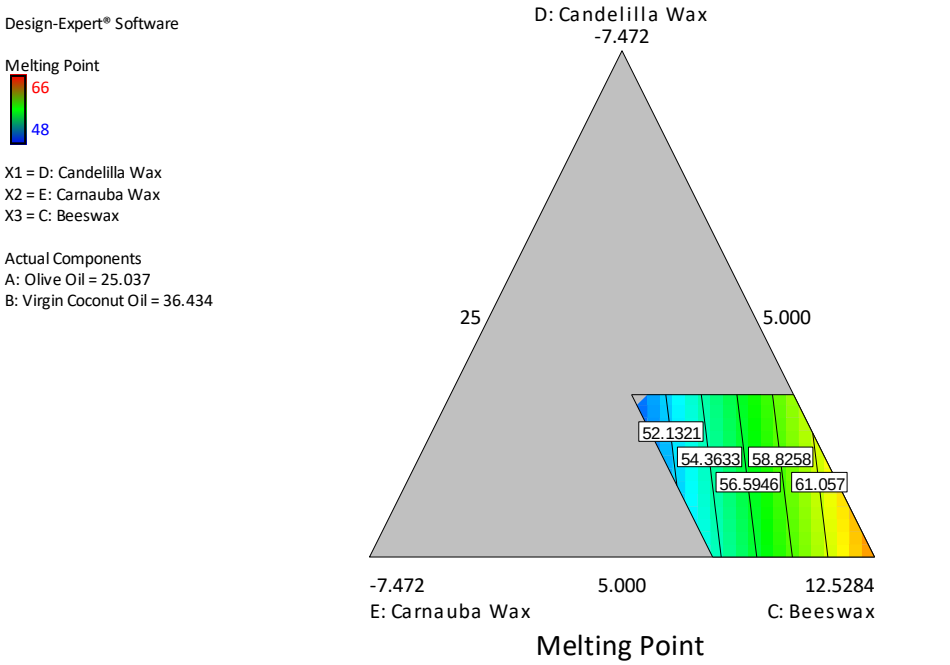
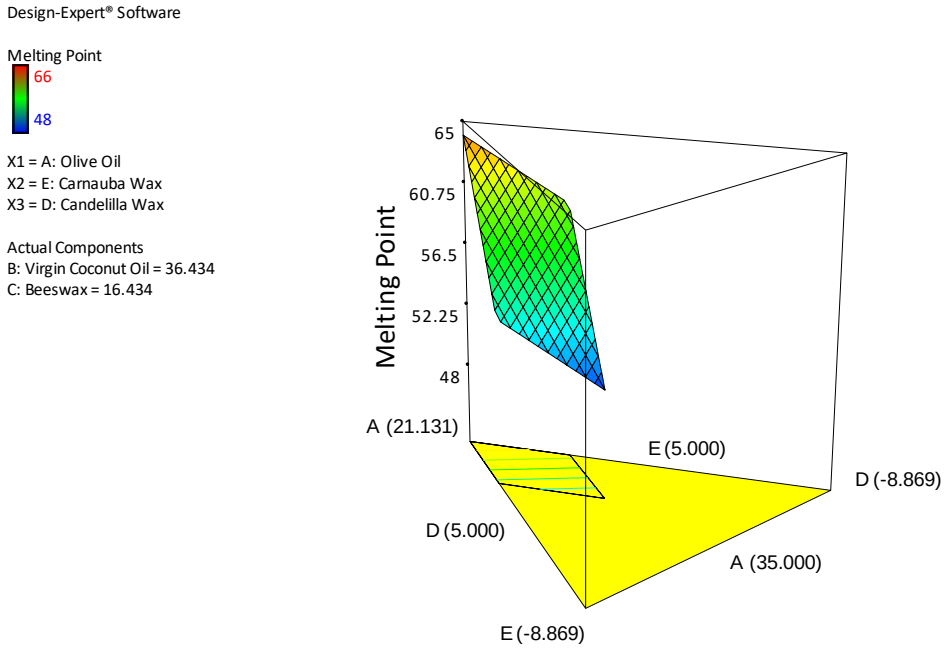


Figure 5.3.2.6 (a) 3D and (b) 2D surface plots showing the interaction effect between C (beeswax), D (candelilla wax) and E (carnauba wax) with respect to melting point at constant A (olive oil) and B (VCO).

(a)



(b)

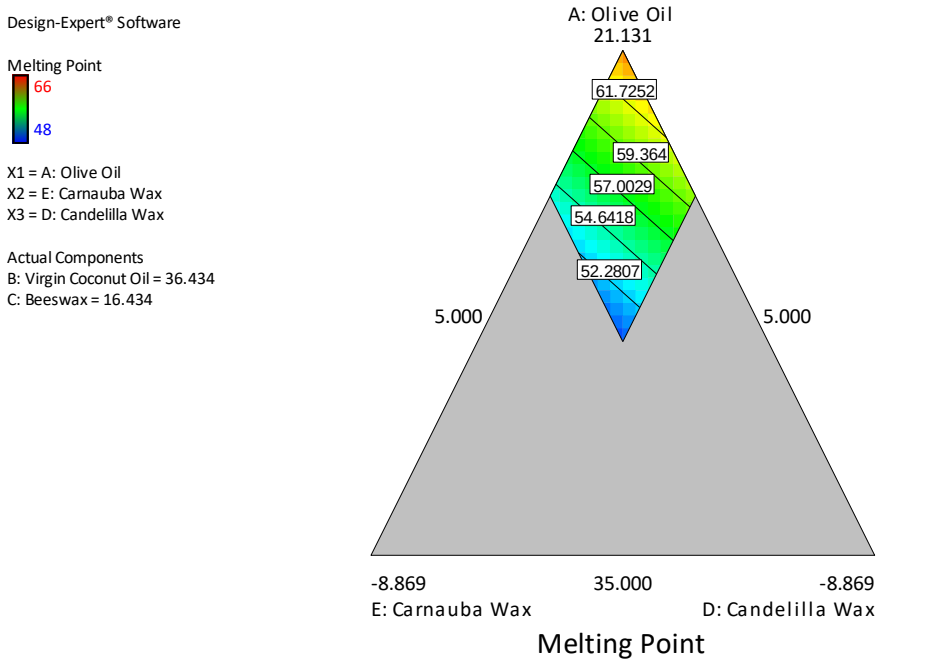


Figure 5.3.2.7 (a) 3D and (b) 2D surface plots showing the interaction effect between A (olive oil), D (candelilla wax) and E (carnauba wax) with respect to melting point at constant B (VCO) and C (beeswax).

5.3.3 Model Validation

Within the experimental range studied, the optimization function of Design Expert Software was used to predict the desirable composition of the optimum formulation. An optimum lipstick formulation with a melting point in the range of 40 °C to 56 °C was chosen in this study as refer to the previous studies (Kamairuddin et al., 2014) and the melting point most of the commercialized products. The lipstick was experimentally prepared under suggested optimum composition and the melting point were measured in triplicate. Experimentally, the melting point showing good agreement between the predicted and actual values. The predicted melting points recorded for the optimum composition are given in Table 5.3.3.1. The experiments were conducted under the specified conditions, and the observed responses were compared with the predicted values by calculating the residual standard error (RSE), as follows (Kamairuddin et al., 2014):

Residual Standard Error (RSE) % = (Actual Value – Predicted Value) / Predicted Value × 100

Table 5.3.3.1 Predicted and actual values for the optimized *C. lanatus* lipstick formulation.

A (%)	B (%)	C (%)	D (%)	E (%)	Melting point (°C)		RSE (%)
					Predicted	Actual	
24.19	36.5	18.84	3.4	1.06	52.50	52.00	0.95

RSE: Residual standard error

5.4 Characterization of the Optimized Lipstick

5.4.1 Melting Point

The melting point test was conducted on the lipstick samples to ensure that the product formulation is suitable for human use and to determine appropriate storage conditions. This test is important as it indicates the temperature limit at which the product remains stable and does not undergo deformation.

In this study, the optimum melting point range of 40 °C to 56 °C was selected based on Kamairudin et al. (2014) research and the melting point range of most marketed lipstick products. The optimal composition of the lipstick formulation was determined using the optimization function of the Design Expert Software. The

lipstick sample with the optimal composition was then experimentally prepared, and the melting point was measured three times (triplicate) to ensure the accuracy of the results. Experimentally, the melting point showed a good alignment between the predicted and actual values. A summary of the predicted and actual melting point values for the optimal formulation is presented in Table 5.4.1.1.

Table 5.4.1.1 Predicted and actual values for the optimized *C. lanatus* lipstick formulation.

A (%)	B (%)	C (%)	D (%)	E (%)	Melting point (°C)		RSE (%)
					Predicted	Actual	
24.19	36.5	18.84	3.4	1.06	52.50	52.00	0.95

RSE: Residual standard error

The results indicate that the tested lipstick formulation has good thermal stability within the specified temperature range, making it suitable for human use and storage under normal conditions.

5.4.2 pH Measurement

To evaluate the pH of the formulated lipstick, the sample was dissolved in a n-hexane:ethanol solution (50% v/v) with a concentration of 100 ppm. pH measurements were conducted at 25 °C using a pH meter, which was calibrated using three standard buffer solutions (pH 4.01, pH 7.00, and pH 10.01). Each sample was measured three times, and the average value was calculated to ensure accuracy (Ben-Chioma et al., 2015). For comparison, the same procedure was applied to conventional lipsticks available on the market.

The measurement results showed that the pH of the *C. lanatus* lipstick was 7.20 ± 0.02, while the conventional lipstick had a pH of 7.12 ± 0.01 (Table 5.4.2.1). The pH difference between the two lipsticks was very small, indicating no significant variation. According to the result, the optimized *C. lanatus* rind lipstick has a pH of 7.20, which falls within the natural pH range of lips, typically between 4.5 and 8. Maintaining an appropriate pH level is crucial for ensuring the product's safety and comfort during use. If the pH is too low, the formulation may become overly acidic, potentially causing irritation or discomfort to the delicate skin of the lips. Conversely, if the pH is too high, it may lead to excessive alkalinity, which can

disrupt the skin's natural moisture balance, resulting in dryness and chapping (Sitoastri and Hutabarat, 2024).

Therefore, the pH of 7.20 indicates that the lipstick is well-balanced, minimizing the risk of irritation while helping to maintain the lips' natural hydration and overall health. This result shows that the pH of the formulated lipstick falls within a similar range to commercial products, making it suitable for use as a cosmetic.

Table 5.4.2.1 pH measurement of optimized *C. lanatus* lipstick and conventional lipstick.

Product	pH
C. lanatus lipstick	7.20±0.02*
Conventional lipstick	7.12±0.01*

*Values are expressed in the mean ± standard deviations, triplicates of three independent samples

5.4.3 Stability Study

The stability of the optimized *C. lanatus* lipstick was tested under various conditions according to Yasir et al. (2023) and Navarro-Pérez et al. (2021) with minor adjustments, including freeze-thaw cycles and storage at different temperatures (5, 27, and 40 °C) for three months. For the freeze-thaw cycle test, the lipstick samples were stored in a refrigerator at approximately 5 °C for 24 hours, then left at room temperature (27 °C) for the following 24 hours. This process was repeated every day for six consecutive days. Stability at constant temperatures of 5, 27, and 40 °C was observed by storing the lipstick at these temperatures for three months.

The stability test results showed that the *C. lanatus* lipstick remained stable under all tested conditions. The samples did not show any signs of physical or functional damage during the freeze-thaw cycle or storage at 5, 27, and 40 °C for three months. These findings indicate that the lipstick formulation has excellent stability, making it suitable for various environments and storage conditions. The term "stable" in this study refers to the absence of physical or functional degradation in the optimized lipstick formulation during the stability evaluation. Stability testing was conducted to assess whether any phase separation occurred during storage. A stable formulation maintains its intended shape and consistency,

meaning it does not melt or soften under room temperature conditions. Additionally, the color remains unchanged, indicating no oxidation or degradation of the pigment components. Furthermore, the fragrance remains stable, ensuring that no undesirable odors develop over time (Rasyadi et al., 2021). These parameters confirm that the lipstick retains its original quality and performance throughout the storage period.

Table 5.4.3.1 The result of *C. lanatus* lipstick stability tests.

Stability test	Condition
Freeze-thaw cycle	STABLE
Storage at 5°C	STABLE
Storage at 27°C	STABLE
Storage at 40°C	STABLE

5.4.4 Thermal Stability

The thermal stability and decomposition behavior of the optimized *C. lanatus* lipstick were analyzed using a thermogravimetric analyzer (TGA), following the method of Nciri et al. (2022) with specific settings. A 20–30 mg lipstick sample was carefully weighed and placed in a platinum sample holder. The sample was then heated from 50 °C to 600 °C at a controlled heating rate of 10 °C/min under a nitrogen flow of 20 mL/min. A thermogram was generated, illustrating the weight change of the sample as a function of temperature.

The TGA results indicated that the *C. lanatus* lipstick remained thermally stable up to 160.92 °C, with no significant weight loss observed before this temperature. Beyond 160.92 °C, a progressive weight loss event was detected on the thermogram, spanning from 160.92 °C to 508.58 °C, indicating the thermal degradation of different components within the formulation. The decomposition process began between 160–293 °C, where the weight loss was attributed to the degradation of minor components, including additives such as TiO_2 and bioactive compounds from *C. lanatus* rind extract. These compounds exhibited lower thermal stability, leading to their volatilization and breakdown at relatively moderate temperatures. As the temperature increased beyond 293 °C, a more significant weight loss was observed, which corresponded to the thermal decomposition of the primary components of the lipstick, namely the wax and oil phases. These structural

components, essential for the lipstick's consistency and stability, required higher temperatures to degrade, further demonstrating their role in enhancing the formulation's thermal resistance.

The thermogram indicated a single-stage decomposition, where both minor and major components degraded progressively within the same phase. This suggests strong interactions between the wax-oil matrix and bioactive components, contributing to the formulation's thermal stability. The continuous degradation profile implies that the matrix remains stable at moderate temperatures before significant breakdown occurs at higher thermal thresholds. These findings confirm the structural integrity of the lipstick, making it suitable for use under varying temperature conditions. Additionally, the absence of multiple degradation phases suggests uniform dispersion of the extract within the matrix, further enhancing stability. This thermal behavior highlights the formulation's potential resilience in different environmental settings.

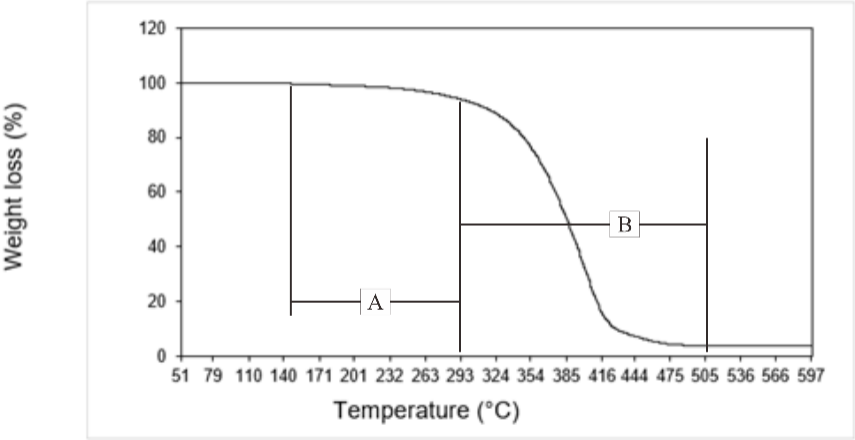


Figure 5.4.4.1 TGA thermogram of the optimized *C. lanatus* lipstick, showing the decomposition of minor components (e.g., *C. lanatus* rind extract) in (A) and the degradation of major components (e.g., oils and waxes) in (B).

5.5 Safety Evaluation of the Optimized Lipstick

5.5.1 Microbiological Analysis

Products from the pharmaceutical and cosmetic industries are highly susceptible to microbial contamination due to the presence of chemical components that support microbial growth. Contamination may occur during production,

storage, or use. Such contamination can cause a variety of adverse effects, including changes in consistency and appearance, phase separation, changes or reduction in activity, and even the development of toxicity in the affected product (Roy et al., 2023). Contamination can also be a potential source of infection. The ability of microorganisms to grow and multiply in cosmetic products has been known for many years. These microorganisms can cause damage or chemical changes to cosmetic products to the point of posing a real risk of injury to users if pathogenic microorganisms are involved (Huang et al., 2024; Siya et al., 2019).

To assess the microbiological safety of the *C. lanatus* lipstick, microbiological testing was conducted following ISO 21149:2017, ISO 16212:2017, ISO 22717:2015, ISO 22718:2015, and ISO 18416:2015. The acceptance criteria were based on ISO 17516:2014 and the ASEAN Guideline on Microbiological Limits in Cosmetic Products, which set the maximum permissible limit for total aerobic mesophilic microorganisms at ≤ 1000 cfu/g (in certain type of products at ≤ 100 cfu/g (ISO 17516:2014) and ≤ 500 cfu/g (ASEAN Guideline)).

To prepare the test discs, 20 µL of the lipstick sample was applied to a blank sterile disc (Oxoid, 6 mm) and air-dried for 1 hour. Bacterial strains were cultured on Tryptic Soy Agar (TSA) at 35 °C for 18–24 hours, then suspended in Tryptone-NaCl water and adjusted to 1.00E+08 cfu/mL. For the disc diffusion test, 50 mL of molten TSA was inoculated with the microbial suspension (1.00E+05 cfu/mL), and 5 mL was layered over a solidified TSA base in a sterile petri dish. Once solidified, test discs were placed in duplicate alongside a positive control (Ampicillin 10 µg) and a negative control (sterile water). After incubation at 35 °C for 48 hours, inhibition zones were measured and recorded.

Table 5.5.1.1 Microbiological analysis results of optimized *C. lanatus* lipstick.

Test	**Test item (in 1 g)**	**Microbiological limit in cosmetic[a] (cfu/g)**
Aerobic Mesophilic Bacteria	50 cfu	
Yeast & Mould	100 cfu	
Detection of *Pseudomonas aeruginosa*	Absence	1000
Detection of *Staphylococcus aureus*	Absence	
Detection of *Candida albicans*	Absence	

[a] Based on Cosmetics - Microbiology - Microbiological limits. ISO 17516:2014 and ASEAN Guideline on Microbiological Limit in Cosmetic Products

The microbiological test results confirmed that the *C. lanatus* lipstick complied with international microbiological safety limits. The total aerobic mesophilic bacteria count was recorded at 50 cfu/g, which is well below the maximum limit of ≤ 1000 cfu/g. The yeast and mold count was < 100 cfu/g, further indicating the product's microbiological stability (Table 5.5.1.1). Additionally, the presence of pathogenic microorganisms was assessed, and the results confirmed the absence of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* in all tested samples. These findings demonstrate that the *C. lanatus* lipstick formulation is microbiologically safe for consumer use, meeting the regulatory requirements of ISO 17516:2014 and the ASEAN Guideline on Microbiological Limits in Cosmetic Products.

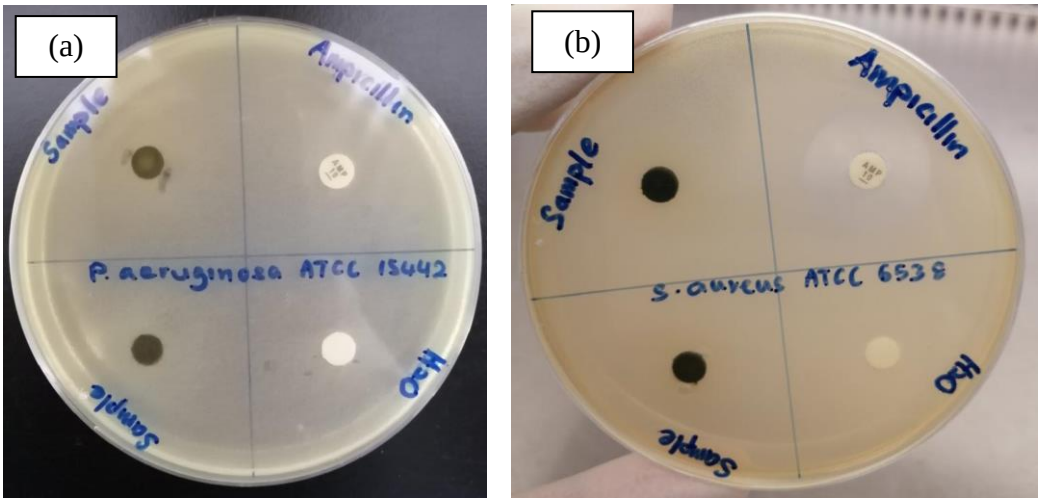


Figure 5.5.1.1 Disc diffusion test results of sample against (a) *Pseudomonas aeruginosa* ATCC 15442 and (b) *Staphylococcus aureus* ATCC 6538 on TSA medium after 48 hours of incubation.

Phytochemical compound such as flavonoids found in *C. lanatus* rind extract have been shown to exhibit strong antibacterial activity, leading to the absence of *S. aureus* and *P. aeruginosa* in treated samples. This effect is primarily attributed to flavonoids' ability to disrupt bacterial cell membranes through multiple mechanisms. They interact with the lipid bilayer, either by embedding themselves into the hydrophobic interior or by forming hydrogen bonds with polar lipid head groups, depending on their polarity. This interaction can alter membrane structure, affecting its thickness and fluidity, which in turn influences membrane protein

function (Figure 5.5.1.2). Additionally, flavonoids inhibit both intracellular and extracellular enzymes, disrupting bacterial metabolism and leading to cell death. Some flavonoids also act as pro-oxidants at high concentrations, inducing the production of reactive oxygen species (ROS) that increase membrane permeability and structural damage. Furthermore, they can cause leakage of intracellular components, such as potassium ions and proteins, signaling severe membrane disruption. By reducing membrane fluidity, flavonoids further impair essential cellular processes (Górniak et al., 2019). Collectively, these mechanisms contribute to their antibacterial properties, explaining why *C. lanatus* rind extract effectively inhibits *S. aureus* and *P. aeruginosa*.

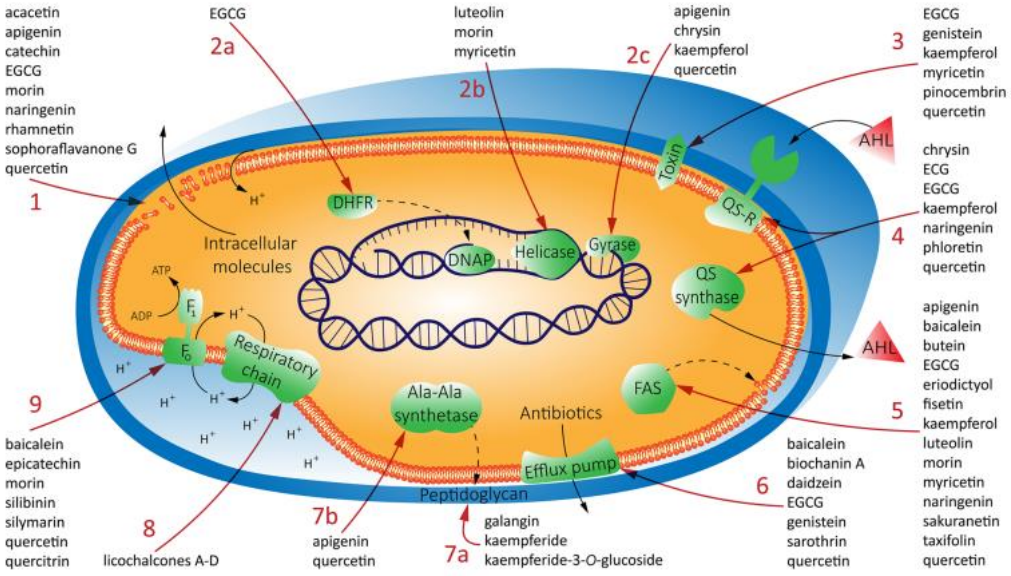


Figure 5.5.1.2 Diagram illustrating the antibacterial mechanisms of flavonoids. Flavonoids exert antibacterial effects through membrane disruption (1), nucleic acid synthesis inhibition (2), and virulence suppression (3, 4). They also interfere with cell envelope formation (5, 7), block efflux pumps (6) to counter antimicrobial resistance, and disrupt energy metabolism (8, 9), ultimately leading to bacterial cell death (Górniak et al., 2019).

5.5.2 Heavy Metal Analysis

The concentration of heavy metals, including arsenic (As), lead (Pb), cadmium (Cd), and mercury (Hg), in the *C. lanatus* lipstick was analyzed to ensure compliance with safety regulations. The analysis was conducted following the Guidelines for Control of Cosmetic Products in Malaysia, 2nd Edition – August

2022, National Pharmaceutical Regulatory Division, Ministry of Health, Malaysia (Annex I, Part 14 – August 2022) and CPCT/TP/MM/In-House 018, based on AOAC Official Method 2015.01.

For sample preparation, approximately 0.25 g of lipstick was digested using a microwave digestion system to ensure complete breakdown of the organic matrix and release of metal ions. The digestion process involved adding 4 mL of concentrated nitric acid (HNO_3) and 1 mL of high-purity hydrogen peroxide (H_2O_2) to the sample, followed by the addition of 0.1 mL of a gold-lutetium (Au-Lu) solution (50 mg/L in 5% HNO_3) to stabilize mercury and evaluate potential analyte loss. The digestion was carried out at 190°C for 10 minutes. After cooling, the digested sample was transferred to a 50 mL acid-cleaned HDPE centrifuge tube and diluted with deionized water to a final volume of 20 mL. Further dilution was performed with 1% HNO_3 to bring the metal concentrations within the optimal analytical range, minimizing potential spectral interferences and preventing signal saturation.

The analysis was conducted using ICP-MS, which offers high sensitivity and selectivity for trace metal detection. The instrument was calibrated using a series of multi-element standard solutions with concentrations ranging from 0.01 to 20 µg/L for As, Cd, Pb, and Hg. A calibration curve was established to ensure a linear correlation between concentration and signal intensity. The accuracy of the calibration was verified using an initial calibration verification (ICV) solution from an independent standard source, ensuring that the instrument's performance remained within acceptable limits.

Quality control procedures included analyzing three method blanks to check for contamination, conducting duplicate sample analysis for every 10 samples to assess precision, and performing a spike recovery test to verify accuracy in the lipstick matrix. Additionally, Certified Reference Materials (CRM) were analyzed to confirm the reliability of the obtained results. The final concentrations of As, Pb, Cd, and Hg in the lipstick samples were determined using the established calibration curve, with corrections applied using internal standards (Rh, In, Tm) to compensate for potential matrix effects and instrumental drift.

The results showed that the concentrations of all tested heavy metals were below the permissible limits set by the Guidelines for Control of Cosmetic Products in Malaysia (Annex I, Part 14 – August 2022). The concentration of As was 3.68 ppm, which is below the maximum limit of 5 ppm. Meanwhile, Pb, Cd, and Hg were reported as <0.04 ppm, <0.02 ppm, and <0.02 ppm, respectively. These values indicate that their concentrations were below the instrument's limit of reporting (LOR), meaning they were detected at levels too low to be quantified accurately. The permissible limits for these metals are 20 ppm for Pb, 5 ppm for Cd, and 1 ppm for Hg, confirming that the lipstick formulation complies with safety standards. These findings demonstrate that the formulated *C. lanatus* lipstick is free from harmful levels of heavy metal contamination, ensuring its safety for consumer use.

Table 5.5.2.1 Levels of heavy metals in optimized *C. lanatus* lipstick.

Metal contaminants	**Concentration (ppm)**	**Concentration limit[a] (ppm)**
Arsenic (As)	3.68	5
Lead (Pb)	<0.04	20
Cadmium (Cd)	<0.02	5
Mercury (Hg)	<0.02	1

[a] based on Guidelines for Control of Cosmetic Products in Malaysia, 2nd Edition – August 2022, National Pharmaceutical Regulatory Division Ministry of Health, Malaysia (Annex I, Part 14 – August 2022).

CHAPTER VI
CLOSING

6.1 Conclusion

This study successfully investigated the phytochemical composition of *Citrullus lanatus* (*C. lanatus*) rind extract, its extraction efficiency, and its application in formulating a stable lipstick product. The findings demonstrated that the sequential solvent extraction method effectively isolated bioactive compounds from C. lanatus rind, with ethanol extraction yielding the highest extract percentage (6.03%), followed by ethyl acetate (5.23%) and n-hexane (2.93%). Phytochemical analysis confirmed the presence of alkaloids, flavonoids, terpenoids & steroids, tannins, and cardiac glycosides, with ethanol extract exhibiting the broadest range of detected phytochemicals, indicating its superior solvent capability for polar compounds.

A comparison with previous studies showed variations in phytochemical detection, emphasizing the impact of extraction techniques, solvents, and plant part selection. The findings reinforced that the polar bioactive compounds dominate *C. lanatus* rind, making ethanol the most effective solvent for their extraction. The formulation of a lipstick containing *C. lanatus* rind extract was optimized using a D-optimal mixture design. The optimal composition included olive oil (24.19%), virgin coconut oil (36.5%), beeswax (18.84%), candelilla wax (3.4%), and carnauba wax (1.06%), resulting in a melting point of 52.00 ℃, which is suitable for consumer use. Statistical analysis using ANOVA confirmed that the linear model provided the best prediction for melting point optimization.

The optimized lipstick formulation exhibited desirable physicochemical properties, including a stable melting point range (40–56 °C), a neutral pH (7.20), and high structural integrity across different temperature conditions. Thermal stability analysis confirmed that the product remained intact up to 160.92 ℃, indicating strong resistance to environmental temperature fluctuations. Microbiological tests confirmed that the lipstick met ISO 17516:2014 and ASEAN microbiological safety standards, with total bacterial and fungal counts within

acceptable limits. The absence of pathogenic microorganisms (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*) further verified its safety. Additionally, heavy metal analysis confirmed that arsenic, lead, cadmium, and mercury levels were within permissible limits, ensuring the product's safety for consumer application.

6.2 Recommendation

Further research on bioavailability is needed to quantify the long-term efficacy of the bioactive compounds in cosmetic applications. Given the antioxidant, antimicrobial, and potential therapeutic properties of *C. lanatus* rind extract, future research could explore its use in other cosmetic or pharmaceutical formulations. Extended stability testing under varying humidity and light exposure conditions would provide deeper insights into the long-term shelf life of the product. Conducting a consumer acceptance test to assess user perception, texture, and overall satisfaction would be beneficial for commercial development. This study demonstrated that *C. lanatus* rind extract is a viable natural ingredient for cosmetic applications, particularly in lipstick formulation. Its phytochemical richness and stability highlight its potential as an eco-friendly and functional cosmetic additive.

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APPENDICES

Appendix 1. Calculation of *C. lanatus* Rind Extract Yield Percentage from Sequential Extraction

$$\% = \frac{C.\,lanatus \text{ rind extract mass}}{C.\,lanatus \text{ powder mass before extraction}} \text{ x } 100\%$$

a. *n*-Hexane

$$\% = \frac{0.9815 \text{ g}}{33.4806 \text{ g}} \text{ x } 100\%$$

$$\% = 2.9315\%$$

b. Ethyl Acetate

$$\% = \frac{1.6715 \text{ g}}{31.9748 \text{ g}} \text{ x } 100\%$$

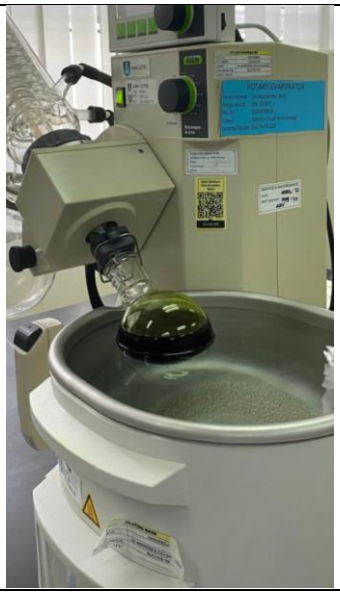
$$\% = 5.2276\%$$

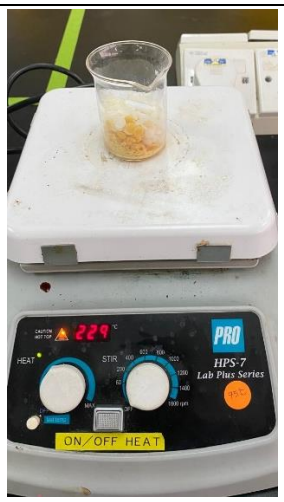
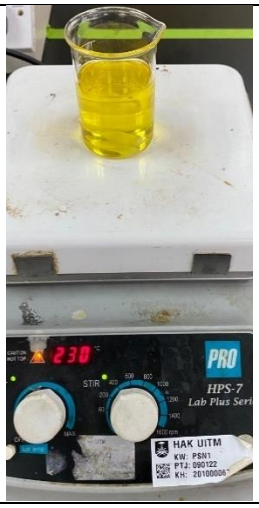
c. Ethanol

$$\% = \frac{1.8238 \text{ g}}{30.2265 \text{ g}} \text{ x } 100\%$$

$$\% = 6.0338\%$$

Appendix 2. Figures

		
(a)	(b)	(c)

		
(d)	(e)	(f)
		
(g)	(h)	(i)

Description:

(a) Filtration of *C. lanatus* rind extract from its residue using a Büchner funnel and vacuum pump.
(b) Solvent evaporation from *C. lanatus* rind extract using a rotary evaporator.
(c) Weighing the extract mass to determine the yield percentage.
(d) Heating wax as the main ingredient for lipstick production.
(e) Wax and oil mixture after the wax has melted at 80 °C.
(f) Lipstick mixing process after adding colorant, extract, TiO_2, and preservative.
(g) Pouring the liquid lipstick into the lipstick mold kit.
(h) Lipstick prototype after solidification.
(i) Packaged lipstick prototype in a lipstick casing.

Appendix 3. Safety Evaluation Data

A. Microbiological Analysis

SIRIM Berhad
Industrial Biotechnology Research Centre, Building 19
Tel: 03-55446953/6960
Fax: 03-55446988

TEST REPORT

REPORT NO: R401/24/B19/07	PAGE: 1 of 2

Applicant	:	MAKTAB RENDAH SAINS MARA (MRSM) SERTING 72120 Bandar Seri Jempol, Negeri Sembilan, MALAYSIA.
Manufacturer / Company	:	-
Reference standard / Method of Test	:	1. Cosmetics – Microbiology – Enumeration and detection of aerobic mesophilic bacteria. ISO 21149:2017 2. Cosmetics – Microbiology – Enumeration of yeast and mould. ISO 16212:2017 3. Cosmetics – Microbiology – Detection of *Pseudomonas aeruginosa*. ISO 22717:2015 4. Cosmetics – Microbiology – Detection of *Staphylococcus aureus*. ISO 22718:2015 5. Cosmetics – Microbiology – Detection of *Candida albicans*. ISO 18416:2015
Test item	:	**LIPSTIC**
Description of test item	:	Received one (1) test item for testing with the following identification: Marking : **S1** Appearance : **Red, solid**
Job No.	:	J401/24
Receipt of Test Item Date	:	27 March, 2024
Experimental Start Date	:	16 April, 2024
Experimental End Date	:	23 April, 2024
Issue Date	:	25 April, 2024

Approved signatories,

(MOHD KHAIRUL AZWAN AHMAD)
MJMM 0481
Analyst,
Industrial Biotechnology Research Centre,
SIRIM Berhad.

(MOHD MAHAYUDDIN HUSSIN)
MJMM 0482
Reviewer,
Industrial Biotechnology Research Centre,
SIRIM Berhad.

SIRIM Berhad
(No. Syarikat 367474 - V)
1, Persiaran Dato' Menteri
Seksyen 2, Peti Surat 7035
40700 Shah Alam
MALAYSIA
Tel : 60-3-55446000
Hotline : 60-3-55103535
Fax : 60-3-55108095
Website : www.sirim.my

REPORT NO: R401/24/B19/07	PAGE: 2 of 2

Results:

Total aerobic mesophilic bacteria and yeast & mould of the following test items:

Test item : **LIPSTIC**
Marking : **S1**
Appearance : **Red, solid**

Test	Test item (in 1 g)	Acceptance criteria	
		ISO 17516	ASEAN
Aerobic Mesophilic Bacteria	5.00E+01 CFU	**PASS**	**PASS**
Yeast & Mould	< 1.00E+02 CFU		
Detection of *Pseudomonas aeruginosa*	Absence	**PASS**	**PASS**
Detection of *Staphylococcus aureus*	Absence	**PASS**	**PASS**
Detection of *Candida albicans*	Absence	**PASS**	**PASS**

Microbiological quality control limits for specified microorganisms
(Based on Cosmetics - Microbiology – Microbiological limits. ISO 17516:2014 and ASEAN Guideline on Microbiological Limit in Cosmetic Products)

Type of microorganisms	Guideline	Limit in 1 g or 1 ml of product	
		Certain type of products [b]	Other products
Total aerobic mesophilic microorganisms [a]	ISO 17516	≤ 1.00E+02	≤ 1.00E+03
	ASEAN	≤ 5.00E+02	

Notes:
CFU - Colony Forming Unit
[a] *Aerobic Mesophilic Bacteria plus Yeast & Mould*
[b] *Products specifically intended for children under three years of age, the eye area or the mucous membranes*

INDUSTRIAL BIOTECHNOLOGY RESEARCH CENTRE * SIRIM BERHAD

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Industrial Biotechnology Research Centre, Building 19
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TEST REPORT

REPORT NO: R402/24/B19/85	PAGE: 1 of 3

Applicant : MAKTAB RENDAH SAINS MARA (MRSM) SERTING
72120 Bandar Seri Jempol, Negeri Sembilan, MALAYSIA.

Test item : **PLANT EXTRACT**

Description of test item : Received one (1) test item for testing with the following identification:

Marking : **PLANT EXTRACT**
Appearance : **Dark green, liquid**

Job No. : J402/24

Receipt of Test Item Date : 27 March, 2024

Experimental Start Date : 16 April, 2024

Experimental End Date : 23 April, 2024

Issue Date : 25 April, 2024

Approved signatories,

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MJMM 0481
Analyst,
Industrial Biotechnology Research Centre,
SIRIM Berhad.

(MOHD MAHAYUDDIN HUSSIN)
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Reviewer,
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REPORT NO: R402/24/B19/85	PAGE: 2 of 3

Reference standard / Method of Test : In-house method – Disc diffusion method

Preparation of impregnated test disc

20 µL of test item was used to impregnate a blank sterile test disc (Oxoid, 6 mm diameter). Impregnated test discs were dried at room temperature for 1 hour before being used in the test.

Preparation of microbial suspension

Prior to sensitivity testing, bacterial strains were cultured onto Tryptic Soy Agar (TSA) and incubated for 18 to 24 hours at 35 °C. A loopful of colony was then inoculated into 10 mL of Tryptone-NaCl water. The density of bacterial suspension required for the test was adjusted to 10^8 CFU/mL.

Disc diffusion test

50 mL of molten Tryptic Soy Agar (TSA) was inoculated with microbial suspension to a final concentration of 10^5 CFU/mL. 5 mL of the inoculated, molten TSA was poured over solidified 10 mL TSA in 90 mm disposable, sterile petri dish. After the top agar has solidified, the test discs which had been impregnated with test item were aseptically placed on the surface of the agar in duplicate. Each test plate comprises of four test discs equidistance to each other; one positive control, which is an antibiotic disc, two impregnated test discs and one control disc. The antibiotic disc was impregnated with Ampicilin 10 µg and the control disc was impregnated with sterile water. The bacterial-inoculated plate were incubated at 35 °C for 48 hours. After the incubation period, the plates were examined for inhibition zone. The inhibition zone was then measured (diameter; in mm) using calipers and recorded.

REPORT NO: R402/24/B19/85	PAGE: 3 of 3

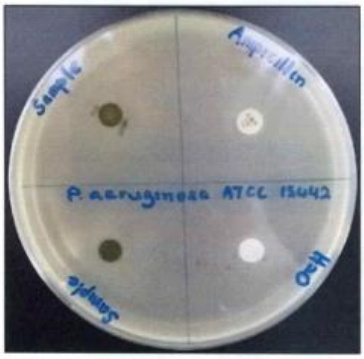
Result :

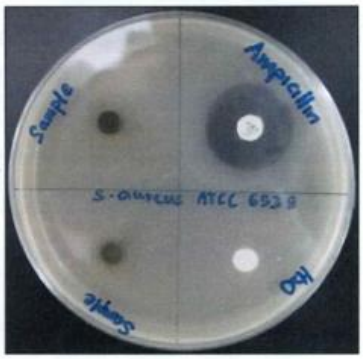
In vitro antibacterial of the following test item

Test item : **PLANT EXTRACT**
Marking : **PLANT EXTRACT**
Appearance : **Dark green, liquid**

Test microorganisms	Zone of inhibition (in mm)			
	Test item disc (Replicate 1)	Test item disc (Replicate 2)	Antibiotic disc	Control disc
Pseudomonas aeruginosa ATCC 15442	**ND**	**ND**	**ND**	**ND**
Staphylococcus aureus ATCC 6538	**ND**	**ND**	**21**	**ND**

** ND - not detected*





SIRIM BERHAD
INDUSTRIAL BIOTECHNOLOGY RESEARCH CENTRE

B. Heavy Metal Analysis

SIRIM QAS International Sdn. Bhd.
(Company No.: 199601037981 (410334-X))
No.1, Persiaran Dato' Menteri, P.O.BOX 7035, Section 2,
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Tel: 017-602 1247
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SAMM 087

TEST REPORT

REPORT NO : 2024CE1124	PAGE : 1 OF 3

THIS TEST REPORT IS ISSUED IN SECURED PDF SOFTCOPY

Applicant	: MAKTAB RENDAH SAINS MARA (MRSM) SERTING 72120 Bandar Seri jempol, Negeri Sembilan, Malaysia
Manufacturer	: MAKTAB RENDAH SAINS MARA (MRSM) SERTING 72120 Bandar Seri jempol, Negeri Sembilan, Malaysia
Product	: COSMETIC / SKINCARE PRODUCTS
Reference Standard / Method of Test	: 1) Guidelines for Control of Cosmetic Products in Malaysia, 2nd Edition – August 2022, National Pharmaceutical Regulatory Division Ministry of Health, Malaysia. 2) CPCT/TP/MM/In-House 018 based on AOAC Official Method 2015.01.
Description of sample	: Received one (1) sample for testing which was identified as follows: Product Name: COSMETIC / SKINCARE PRODUCTS Product Details: Refer to Page 2
Date Received of Complete Application	: 23 April 2024
Job No.	: J20243670772
Description of Test Results	: The test results for the submitted test samples as described in the following pages of this test report complied with the requirements of the above reference standard at the respective clauses tested.
Issued Date	: 13 May 2024

Approved Signatory;

L/3099/9189/21

(ChM. WAN MUHAMAD AFEEQ AFNANI BIN WAN ALI)
Testing Executive

SIRIM QAS International Sdn. Bhd. CHEMICAL, POLYMER AND COMPOSITE SECTION (CPCT)

(DALHA BIN KATNI @ RAHMAT)
Head
Chemical, Polymer and Composite Section
Testing Services Department

REPORT NO : 2024CE1124	PAGE : 2 OF 3

TEST RESULTS

Product Name : **Cosmetic / Skincare Products**
Model : **Lipstick**
Type : **Solid**
Size : **25 g**

No.	Type of Tests	Testing Method	Requirements of Guidelines for Control of Cosmetic Products [a]	Results	Remarks
1.	Heavy Metals	CPCT/TP/MM/In - House 018 – based on AOAC 2015.01			
	a) Arsenic (As), ppm		5 max	3.68	Pass
	b) Lead (Pb), ppm		20 max	< 0.04 [b]	Pass
	c) Cadmium (Cd), ppm		5 max	< 0.02 [b]	Pass
	d) Mercury (Hg), ppm		1 max	< 0.02 [b]	Pass

Note :

Testing period – 8th May. 2024 to 10th May. 2024.
Testing location – Chemical & Consumer Laboratory, Building 16, SIRIM Complex, Shah Alam, Selangor

1) [a] Guidelines for Control of Cosmetic Products in Malaysia, 2nd Edition – August 2022, National Pharmaceutical Regulatory Division Ministry of Health, Malaysia (Annex I, Part 14 – August 2022).
   [b] Limit of reporting.
2) ppm denotes part per million.
3) max denotes maximum.
4) < denotes less than.
5) A simple acceptance rule is used for the conformity statement. The level of risk regarding the probability of false acceptance is up to 50% according to ILAC G8:09.

SIRIM QAS International Sdn. Bhd.
CHEMICAL, POLYMER AND COMPOSITE SECTION (CPCT)

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CONDITIONS RELATING TO THE USE OF SIRIM QAS INTERNATIONAL TEST REPORT

1. A Test Report will be issued in respect of Testing Services conducted and shall relate only to the sample actually tested. SIRIM QAS International makes no warranty whatsoever and the Applicant shall not represent in any manner that any duplication or mass production of the Product is same as the sample actually tested or that SIRIM QAS International has tested any of the duplicated or mass-produced Product. Measurement uncertainty shall be included in the Test Report when there is no statement of conformity required. When a statement of conformity to a specification or standard is applied, the Simple Acceptance Rule is used. Unless otherwise stated, the Acceptance Rule with Guard Band is used.

2. For quantitative test results (with values), when a statement of conformity to a specification or standard is applied, the Simple Acceptance Rule shall be used. Unless otherwise stated, the Acceptance Rule with Guard Band is used, and additional charge will be incurred accordingly.

3. For qualitative test results (visual observation), when requested by applicant, a statement of conformity in the Test Report shall be reported. Should there is no request by applicant, a statement of conformity in the Test Report can be reported based on our discretion.

4. The Test Report shall not be misused, amended, changed, varied or modified in any manner whatsoever by the Applicant or otherwise.

5. If the Test Report is to be furnished to any third party or to the public, each such Test Report shall be furnished in full, legible and in its entirety.

6. The Test Report shall not be reproduced and shall not in any event be used for any advertising purposes or whatsoever without written approval from the Head of Quality, Occupational Safety and Health & Environment (QOSHE) of SIRIM QAS International of No 1, Persiaran Dato' Menteri, Building 8, Section 2, P.O. Box 7035, 40700 Shah Alam, Selangor Darul Ehsan.

7. Customer (Applicant/Manufacture/Factory,etc.) is not permitted to use any SIRIM QAS International, SIRIM or other SIRIM's subsidiaries logo or words on packaging, sample's manual, technical specification, items and products.

8. Subject to consent and written approval from the Head of Quality, Occupational Safety and Health & Environment (QOSHE) of SIRIM QAS International, the customer (Applicant/Manufacture/Factory,etc.) may use SIRIM QAS International logo or word on the promotional materials and the Applicant shall only include the phrase, "A sample of this product has been tested by SIRIM QAS International ...(Test Report No) ...(dated) ...(for what test) ...(to which standard)" or such similar words which stress that only the sample was actually tested. This phrase shall only be used for the purpose of product advertisement or product promotion (eg; brochures/flyers/official website). For avoidance of doubt, the statement shall not be used on the sample, packaging of the sample, items and products.

9. In the event there is an investigation from a Government Regulatory Agency concerning the Applicant's Test Report, SIRIM QAS International may disclose the information pertaining to the Test Report for purposes of such investigation.

10. In the event the Applicant is found in breach of this provision, SIRIM QAS International, SIRIM and/or other SIRIM's subsidiaries without prejudice to any other rights and remedies may take whatever action necessary including but not limited to:
    a) Informing and placing a notice in the media;
    b) Obtaining an injunction from Court (cost on a solicitor-client basis to be borne by the Applicant);
    c) Refusing to accept any further Product for Testing Services from the Applicant or whosoever related to the Applicant, whether subsidiary or otherwise;
    d) Instructing the Applicant to withdraw and recall the advertisement, statement or document in question and advertise a clarification and apology to SIRIM QAS International, SIRIM and/or other SIRIM's subsidiaries twice in a national publication of SIRIM QAS International's choice at the Applicant's sole cost; and
    e) Informing or lodging a report pertaining the Applicant's Test Report with the relevant authorities.

11. SIRIM QAS International is committed in supporting an environmentally-friendly business practices by reducing paper consumption, therefore we do not issue any hard copy of Test Report to the Applicant. However, additional certified true copy(ies) or softcopy of the Test Report may be issued upon request by the Applicant upon payment of the relevant fee. The certified true copy(ies) or softcopy of test report shall only be given for test report issued not more than three (3) years from the date of issuance.

12. Issuance of Amendment Report due to the following reasons are chargeable to the Applicant :
    a) Changes in details of the Applicant name and/or address;
    b) Changes in details of the Manufacturer's name and/or address;
    c) Changes in details of the Factory location name and/or address;
    d) Changes in details of the model and/or type designation

13. However, issuance of Supplementary Report due to the following reasons are FOC :
    a) Misprints and typo errors;
    b) Missing technical information as agreed in PP1 form;
    c) Test data not reported;
    d) Mistake in reporting of test data

14. Corrections to report shall only be allowed if the date of issuance of the original report has not exceeded 6 months and shall be limited to a maximum 3 times, after either case whichever occurs earlier, an Amendment or a Supplementary Report shall not be issued.