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ORIGINAL ARTICLE

# Optimization of Rosella Extract-based Antioxidant Peel-off Mask Using Simple Lattice Design

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## ABSTRACT

**Objectives:** The skin is highly vulnerable to damage caused by free radicals, which disrupt biological components and accelerate aging. While endogenous antioxidants provide some protection, external sources are often needed. Rosella (*Hibiscus sabdariffa L.*) is a rich source of flavonoids and astaxanthin, proven antioxidants that inhibit matrix metalloproteinase-1, prevent collagen degradation, and reduce ultraviolet-induced damage. The aim of the study was to optimize the formulation of a peel-off mask, incorporating Rosella extract as an antioxidant.

**Materials and Methods:** Rosella extract was analyzed using liquid chromatography-mass - mass spectrometry (LC-MS) to identify its antioxidant components. Freeze-dried Rosella powder was granulated and incorporated into a gel mask using polyvinyl alcohol (PVA) or gelatin as the base polymer. Formula optimization was conducted using simplex lattice design and evaluated for physical properties, antioxidant activity, and stability.

**Results:** LC-MS analysis detected astaxanthin, quercetin, rutin, and kaempferol in Rosella extract. Granulated Rosella exhibited good flowability and a particle size distribution of 250-425 µm. The optimized formula was PVA-based, containing 12.5% PVA and 7.5% propylene glycol. The product demonstrated desirable physical properties, including a drying time of 5.29 minutes, a pH of 5.32, a spreadability of 5.34 cm, an adhesivity of 6.86 seconds, and a viscosity of 30,658 cP. Stability tests confirmed the formula remained stable for 3 months, under room temperature, freeze-thaw cycles, and centrifugation. The final product, containing 15% Rosella, exhibited 45.33% antioxidant activity.

**Conclusion:** The PVA-based Rosella peel-off mask demonstrated optimal physical properties, stability, and antioxidant properties, offering a promising approach to incorporating antioxidants into cosmetic formulations.

**Keywords:** Peel-off mask, Rosella (*Hibiscus sabdariffa L.*), antioxidant, scrub, multifunction cosmetics

## INTRODUCTION

Free radicals have garnered significant attention in the field of biology due to their crucial role in various physiological processes and their association with a wide range of disorders. They can be generated from both endogenous sources, such as mitochondria, peroxisomes, the endoplasmic reticulum, and phagocytic cells, as well as exogenous sources, including pollution, alcohol, and tobacco smoke. The skin is one of the body's most susceptible organs to damage caused by free radicals. Free radicals can negatively impact key biological components, including proteins, lipids, and nucleic acids, disrupting normal redox balance and leading to increased oxidative stress.[1]

The body produces some of the antioxidants required to neutralize free radicals, which are known as endogenous antioxidants. However, for the remaining antioxidant needs, the body relies on external (exogenous) sources. These exogenous antioxidants are commonly referred to as dietary antioxidants and can be found abundantly in vegetables, fruits, and grains.[2] Antioxidants also play a crucial role as active agents in cosmetics, helping to prevent the harmful effects of free radicals on the epidermis.

In today's fast-paced metropolitan lifestyle, individuals increasingly seek efficiency in various aspects, including maintaining their appearance. Hybrid cosmetics, which

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combine multiple functions in a single product, address this need effectively. According to a 2022 report, sales of hybrid beauty products increased by 24%. These products save time during application, reduce costs, and require less storage space. Data also show that 34% of French consumers reduced their cosmetic purchases following the COVID-19 pandemic, further emphasizing the demand for "multi-tasking" products. One such innovative hybrid product is a peel-off mask that incorporates micro-grains that function as a scrub, offering a practical and appealing solution.[3,4] Sustainability is another important factor driving the development of multifunctional products. Reducing the number of cosmetic products used directly contributes to minimizing cosmetic waste.[5]

Rosella (*Hibiscus sabdariffa* L.), a medicinal plant, is cultivated in tropical and subtropical regions, including Saudi Arabia, India, Thailand, Malaysia, and Indonesia. It contains various phytochemical components, including natural pigments, alkaloids, terpenoids, and phenolics.[6-8] Flavonoids, including flavonols and anthocyanin pigments, are present in Rosella petals. These compounds possess double-bond architectures that play a key role in protecting cells from ultraviolet (UV) radiation damage. A phytochemical analysis of Rosella petal extract conducted by the Faculty of Agricultural Technology at Udayana University revealed the following composition: flavonoids (42,938.72 mg/100 g), phenols [(1,758.68 mg/100 g gallic acid equivalent (GAE)], tannins (2,865.25 mg/100 g tannic acid equivalent), vitamin C (1,294.12 mg/100 g), antioxidant capacity (2,249.43 mg/L gallic acid equivalent antioxidant capacity), and positive saponins. These findings indicate that Rosella can be effectively utilized in cosmetics to enhance skin appearance by mitigating the harmful effects of oxidants. The chromophore groups (conjugated single and double bonds) present in flavonoids absorb UVA and UVB rays, thereby reducing UV-induced damage.[9]

This study aims to develop a multifunctional cosmetic formulation in the form of a peel-off mask with micro-grains that also function as a scrub, incorporating *Hibiscus sabdariffa* L. (Rosella) petal extract as the active ingredient, and to evaluate its antioxidant components.

## MATERIALS AND METHODS

*Materials*

Rosella powder was supplied by Herbilogy® (Indonesia), while PVP (Polyvinylpyrrolidone) was donated by PT. Pharos Indonesia. Polyvinyl alcohol (PVA), gelatin, and other materials used were of pharmaceutical grade. DPPH (2,2-Diphenyl-1-picrylhydrazyl) was purchased from PT. Pasifik Kimia Indonesia (Indonesia). All other chemicals were of analytical grade and used as received without further purification.

*Detection of antioxidant components in Rosella powder using LC-MS*

Rosella powder was prepared in dichloromethane to solubilize astaxanthin and in methanol to solubilize flavonoids. The prepared samples were collected into tubes and immediately centrifuged at 3500 × g for 10 minutes. The supernatant was transferred into amber vials and filtered through a 0.45 µm nylon syringe filter. The analysis was performed using a liquid chromatography - mass spectrometry (LC-MS) /MS Sciex 4500 QTrap instrument. Separation was conducted using a C-18 column (2.1 mm × 150 mm × 2.5 µm). The column temperature was maintained at 25 °C, while the autosampler was set to 4 °C. The mobile phases consisted of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B), with a flow rate of 0.5 mL/min. Separation and detection were optimized for astaxanthin and flavonoids, with retention times and mass-to-charge ratios (m/z) used to identify the compounds.

*Granulation of Rosella powder*

Rosella powder (11%) was mixed with a 5% PVP solution. The wet granules were sieved using a 425 µm mesh and dried via freeze-drying (Welch®). The dried granules were sieved again with a 425 µm mesh and characterized for the following properties: organoleptic properties: color, shape, and odor, measuring of particle size distribution (PSD) using Retsch AS 200, evaluating loss on drying (LOD) using a Mettler Toledo HE73, and assessment of flowability using an Erweka GTL instrument. These evaluations ensured the granules met the desired specifications for use in the peel-off mask formulation.

*Optimization formula of peel-off mask that contains Rosella*

The formulations were designed as multifunctional peel-off masks, with the additional capability of functioning as physical scrubs. This dual-purpose design combined the antioxidant benefits of Rosella powder with gentle exfoliation. The optimisation was done using a simple lattice design (SLD) with Design Expert version 13 from StatEase®. The studied parameters were the type of polymer, quantity of polymer, and plasticiser. The design formula was shown in Table 1.

First, PVA or gelatin, used as the base polymer, was dissolved in hot water at 70 °C and then allowed to cool to room temperature. Methylparaben, serving as a preservative, was dissolved in propylene glycol and subsequently added to the polymer premix. Rosella powder was pre-wetted with propylene glycol and, along with Rosella granules, incorporated into the premix. The mixture was homogenized using a DLAB® propeller mixer at a speed of 1,000 rpm until uniform consistency was achieved.

*Evaluation of a peel-off mask that contains Rosella*

*Organoleptic evaluation*

The finished product was assessed for appearance, odor, texture, and visual homogeneity. For homogeneity, 1 gram of the preparation was placed between two glass slides and visually inspected.

*Viscosity*

Viscosity was measured using a HAAKE® viscometer with an R7 spindle.

*Drying time*

One gram of the preparation was applied to the skin of the upper arm and spread over an area of 7 square cm. The drying

Table 1. Design formula of peel-off mask containing rosella

| Materials | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Rosella powder | 4% | 4% | 4% | 4% | 4% | 4% | 4% | 4% | 4% | 4% |
| Rosella granules | 11% | 11% | 11% | 11% | 11% | 11% | 11% | 11% | 11% | 11% |
| Propylene glycol | 0% | 2.5% | 5% | 7.5% | 10% | 1% | 2% | 3% | 4% | 5% |
| Methylparaben | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% | 0.1% |
| PVA | 20% | 17.5% | 15% | 12.5% | 10% | - | - | - | - | - |
| Gelatin | - | - | - | - | - | 10% | 9% | 8% | 7% | 6% |
| Distilled water | Ad to 100% | Ad to 100% | Ad to 100% | Ad to 100% | Ad to 100% | Ad to 100% | Ad to 100% | Ad to 100% | Ad to 100% | Ad to 100% |

Notes: Ad to 100% indicates the formula was adjusted to a total of 100% using distilled water, PVA: Polyvinyl alcohol

time was defined as the time required for the preparation to be continuously peeled off from the skin.

*Spreadability*

A sample of 0.25 grams of the preparation was placed between two glass slides. A 50-gram weight was applied to the top slide and left for 1 minute. The diameter of the spread preparation was then measured.

*Adhesive strength*

A sample of 0.25 grams of the preparation was placed between two glass slides. A 1 kg weight was applied to the top slide and left for 5 minutes. An 80-gram weight was then attached to a string connected to the upper glass slide, and the time required for the upper slide to separate was recorded.

*Stability study*

A stability analysis of the finished product, packaged in amber glass Type II containers, was conducted under ambient conditions (30 ˚C ±2 ˚C/65% RH ±5% RH). This temperature and humidity range was selected to represent the typical environmental conditions of tropical climates, where the product is expected to be marketed. The selection aligns with the International Council for Harmonisation guidelines for stability testing in Zone IV (hot and humid regions). The evaluation included organoleptic properties, viscosity, drying time, spreadability, and adhesive strength. Furthermore, a freeze-thaw stability test was performed using three cycles at -5 ˚C and 40 ˚C. In addition, the product underwent centrifugation at 3700 rpm for 5 hours, followed by visual inspection for changes.

*The antioxidant property of the finished product using DPPH*

The antioxidant activity was evaluated using the DPPH assay. A 1 mL sample of the peel-off mask containing 15% Rosella (11% granules and 4% powder) was dissolved in water. DPPH was prepared in methanol and mixed with the product in test tubes, which were kept in the dark for 30 minutes. Absorbance was measured at 517 nm, and antioxidant activity was calculated using the formula:

| | |
|---|---|
| % of antioxidant activity=[(Ac-As)/Ac] x 100 | [1] |

Where absorbance of control: Absorbance of control, As: Absorbance of sample is the control reaction absorbance, and absorbance of sample is the testing specimen absorbance.

## RESULTS

*Antioxidant component detection in Rosella powder using LC-MS*

The LC-MS analysis confirmed the presence of key antioxidant compounds in Rosella powder, including astaxanthin and flavonoids such as quercetin, rutin, and kaempferol. Astaxanthin was identified in dichloromethane extracts as shown in the extracted ion chromatogram (Figure 1), with a retention time of 5.3-5.4 minutes and a mass-to-charge ratio (m/z) of 597/147.1. Flavonoids were detected in methanol extracts. Quercetin (Figure 2) was observed at a retention time of 9.9 minutes and m/z 301.0/150.9, while rutin (Figure 3) appeared at 29 minutes, with m/z 609.2/300.0. Kaempferol (Figure 4) was identified at 15 minutes with m/z 284.9/93.0. Identification was based on retention times and mass spectra that were matched against an LC-MS library.

*Granulation of Rosella powder*

Granulation was performed to optimize the particle size and flowability of Rosella powder for use in the peel-off mask. The PSD (Figure 5) revealed that over 70% of granules ranged between 250 and 425 µm, aligning with the ideal size for physical scrubs. Table 2 details the physical properties of the granules, which were spherical, red, and odorless, with a LOD of 4.92±0.11%, a flowability of 5.60±0.93 g/s, and an angle of repose of 31.5±1.2˚C.

*Optimization and evaluation of Rosella peel-off mask formula*

The peel-off mask formulations were optimized for physical and functional properties using PVA and gelatin as base polymers. On day 0, the physical properties of the masks, including homogeneity and organoleptic characteristics, were evaluated (Figure 6). Viscosity analysis (Figure 7) showed that PVA-based formulations had higher viscosity than gelatin-based formulations. The higher viscosity in PVA-based formulations can be attributed to its superior gel-forming properties and

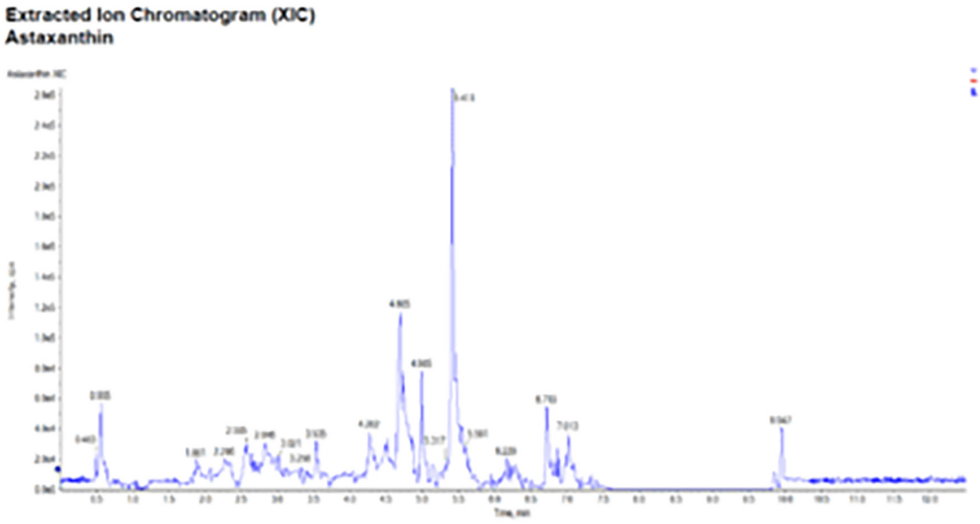


**Figure 1.** Extracted ion chromatogram from Rosella powder in dichloromethane for astaxanthin detection (Astaxanthin peak was 5.3-5.4 minutes and *m/z* 597/147.1)

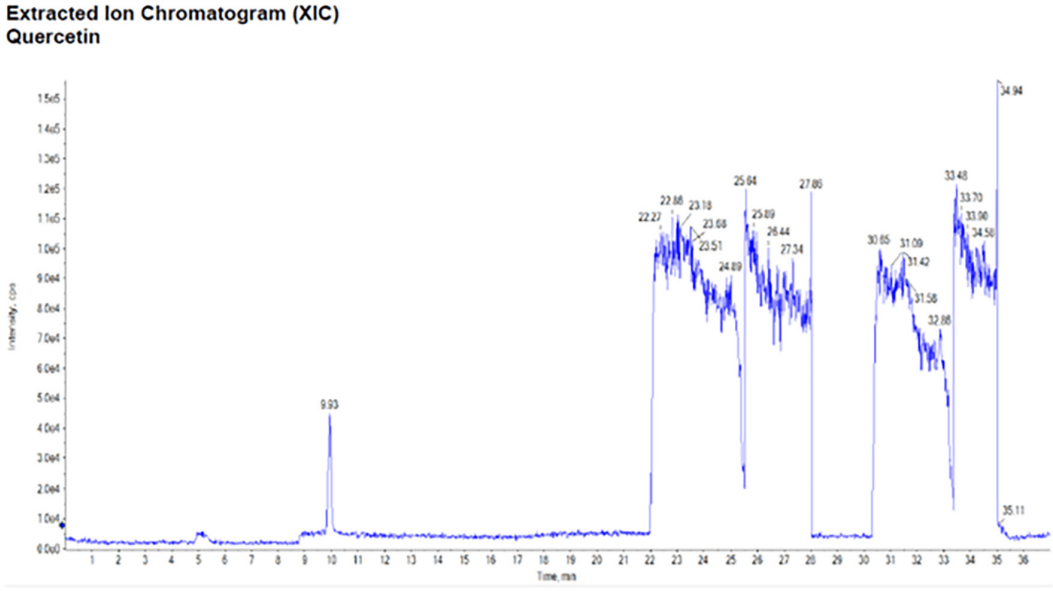


**Figure 2.** Total ion chromatogram from rosella powder in methanol for flavonoid detection (Quercetin peak was 9.9 minutes and *m/z* 301.0/150.9)

cohesive network structure. Gelatin-based formulations exhibited lower viscosity due to their higher hydrophilic content, which increased water absorption.

*Stability study*

The stability of the peel-off mask formulations was assessed under both ambient and stress conditions. Figure 8 presents the results of stability testing for the PVA-based formulation (F4) over three months, showing no significant changes in viscosity, drying time, pH, spreadability, or adhesivity. The freeze-thaw stability study (Figure 9) further demonstrated the robustness of the PVA-based formula, with no phase separation or degradation observed across three cycles. Comparative centrifugation tests (Figure 10) highlighted the superior stability of the PVA-based formulation (F4), which remained homogeneous, whereas the gelatin-based formulation (F6) showed phase separation.

## DISCUSSION

Figure 1 shows that astaxanthin was detected in the Rosella powder and three types of flavonoids were identified in the samples was rutin, quercetin, and kaempferol (Figures 2-4). The intensity of astaxanthin in the sample was approximately 200,000, while the flavonoid intensity was relatively low. Retention times were matched with a database; validated reference standards were not used. These results provide preliminary qualitative data, and we acknowledge that semi-

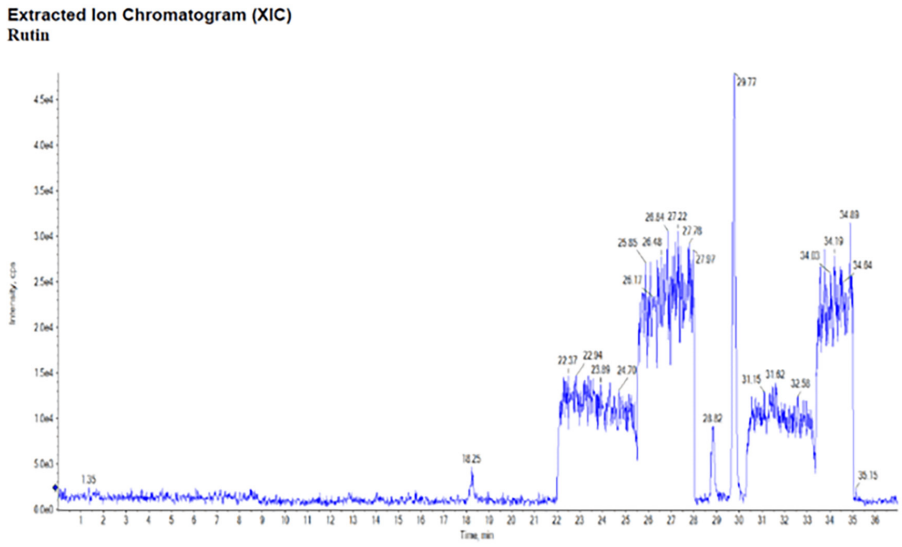


**Figure 3.** total ion chromatogram from Rosella powder in methanol for flavonoid detection (Rutin peak was 29 minutes and *m/z* 609.2/300.0)

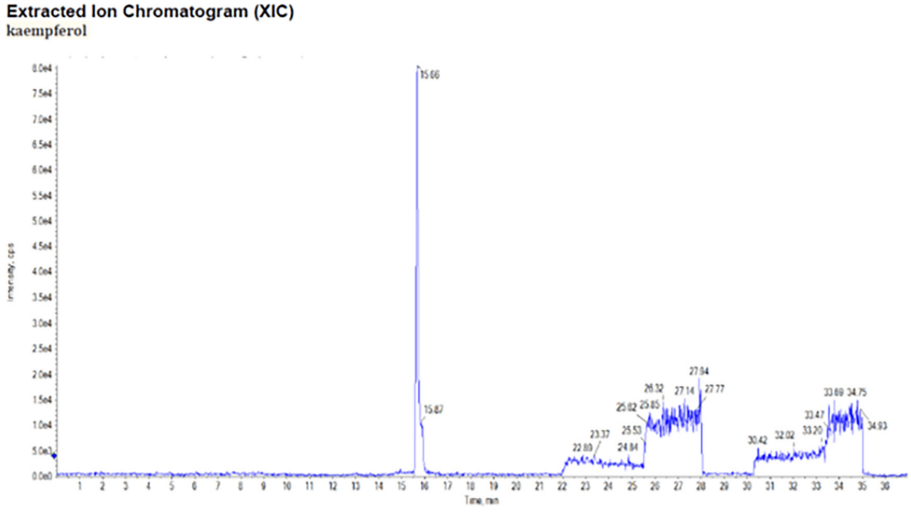


**Figure 4.** Total ion chromatogram from Rosella powder in methanol for flavonoid detection (Kaempferol peak was 15 minutes and *m/z* 284.9/93.0)

quantitative or quantitative LC-MS/MS analysis would offer stronger scientific support for the antioxidant capacity findings. Future studies will focus on purification and quantification steps using validated analytical standards to enable accurate comparison and potency determination.

Rosella powder, Herbilogy®, was processed through a 100-mesh sieve (150 µm). However, this particle size is too fine to be suitable for use as a skin scrub. Ideally, the particle size for a scrub should range from 150 to 600 µm, with an average size of approximately 250 µm.[10,11] Therefore, the granulation process should be performed to increase the particle size. Freeze-drying was selected as the granulation method because studies have shown that increasing temperature and pH leads to an increase in k values. (Note: The rationale for selecting freeze-drying based on the effects of temperature and pH on

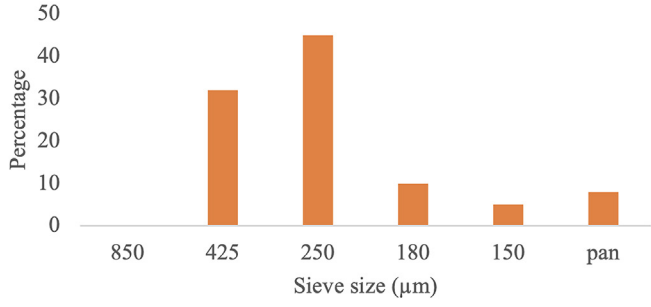


**Figure 5.** Particle size distribution of Rosella granules

k values should be clearly explained in the context.) Higher k values correspond to a faster reaction rate, which accelerates the degradation of antioxidants.[12]

**Table 2. Physical properties of Rosella granules**

| Parameters | Description |
|---|---|
| Organoleptic | Red light spherical granules, un-odorized |
| Loss on drying | 4.92±0.11% |
| Flowability | 5.60±0.93 g/s |
| Angle of repose | 31.5±1.2 ˚C |

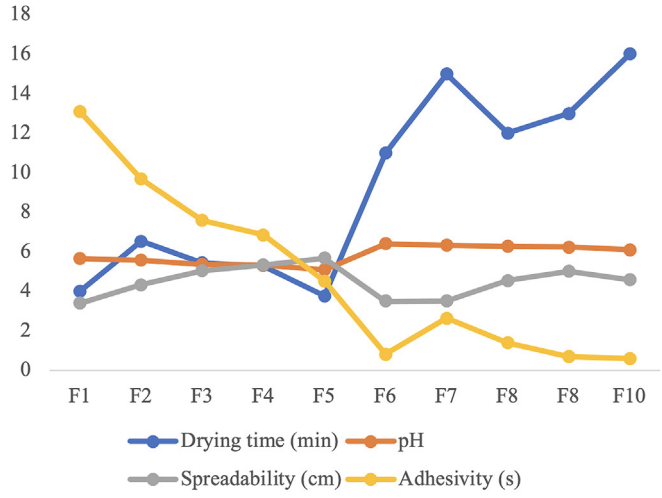


**Figure 6.** Physical Properties of Rosella peel-off mask at day 0

The granulation process was carried out using a PVP solution. The wet granules were solidified at -20 ˚C and dried for 24 hours. Wet granulation was selected as it ensures a more effective binding process through uniform wetting and produces spherical granules with improved flowability.[13] The evaluation results are presented in Figure 5 and Table 2. The granules exhibited good flowability, as indicated by the flow rate and angle of repose, placing them in the "good" category. This suggests that scaling up the batches using commercial equipment is highly feasible. The particle size was primarily distributed within the range of 250-425 µm, meeting the requirements for effective scrub particles. From the granulation process, more than 70% of the particles were found to have a size of ≥250 micrometers, which aligns with the objective of the granulation as a scrub process. Fines of <20% are commonly observed in granulation processes and do not affect the intended function.[14] Particle size plays a crucial role in skin friction; excessively large particles are only suitable for areas of the body with a thick stratum corneum or significant dead skin cells.[15]

The Rosella peel-off mask was formulated using two types of polymers: PVA and gelatin. Gelatin, with the appropriate bloom strength, provides good gel strength while remaining flexible enough to conform to the contours of the user's face. PVA is non-irritating to both skin and eyes. Both polymers are included on the generally recognized as safe list of materials.[16] The formula was designed using SLD, as shown in Table 1. The final dosage form was evaluated for physical parameters, presented in Figures 6 and 7.

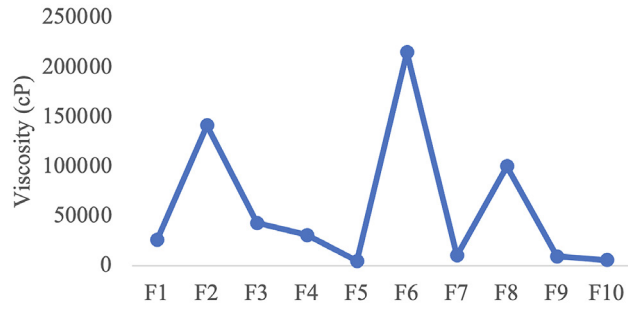


**Figure 7.** Viscosity parameters of Rosella peel-off mask at day 0

Based on the one-way ANOVA quadratic model, the concentrations of propylene glycol and PVA had no significant effect on the drying time parameter (*p* value=0.2247). Similarly, for the gelatin-based formula, the one-way ANOVA linear model indicated no significant effect on the drying time parameter (*p* value =0.4656). The PVA-based formula exhibited a shorter drying time compared to the gelatin-based formula. This difference is attributed to the lower water content in the PVA-based formula. In contrast, gelatin swells in an aqueous solution due to its abundance of hydrophilic groups. These hydrophilic groups, such as hydroxyl and amino groups, increase the equilibrium swelling ratio, enhancing the material's capacity to absorb water.[17] The increase in absorption capability is observed within the concentration range of 5 to 30, whereas this formula exhibits a narrow.

Er range of absorption capability. A higher absorption capability prevents the water present in the formula from being easily removed.

As shown in the day-0 evaluation (Figure 6), there is no significant difference in pH values across formulas with varying concentrations of PVA or gelatin. However, the pH of the gelatin-based formula is slightly higher than that of the PVA-based formula. This difference is attributed to the intrinsic pH values of the materials, with PVA having a pH range of 4.5-6.5 and gelatin (type B) a range of 5-7.5.[16]

The ability of a mask's material to spread makes it easier to cover the entire surface of the face, forming a thin and perfect layer.[18] The test is carried out by measuring the diameter of a number of preparations after being given a load of a certain weight (Deuschle et al.[19]). Spreadability is inversely correlated with viscosity.[20] In the PVA-based formula, spreadability increased as the concentration of PVA decreased. This trend aligns with the viscosity profile, which decreased from F2 to F3, as shown in Figure 7. According to the one-way ANOVA quadratic model, the concentrations of propylene glycol and PVA significantly affected viscosity (*p* value=0.0114). The coefficient values were 10.241 for propylene glycol and $2.5\times10^5$ for PVA, indicating that PVA had a more substantial impact on the viscosity profile.

For the gelatin-based formula, the spreadability did not show significant variation due to the narrow range of concentrations, a lack that was also reflected in the viscosity parameter. Based on the one-way ANOVA linear model, neither propylene glycol

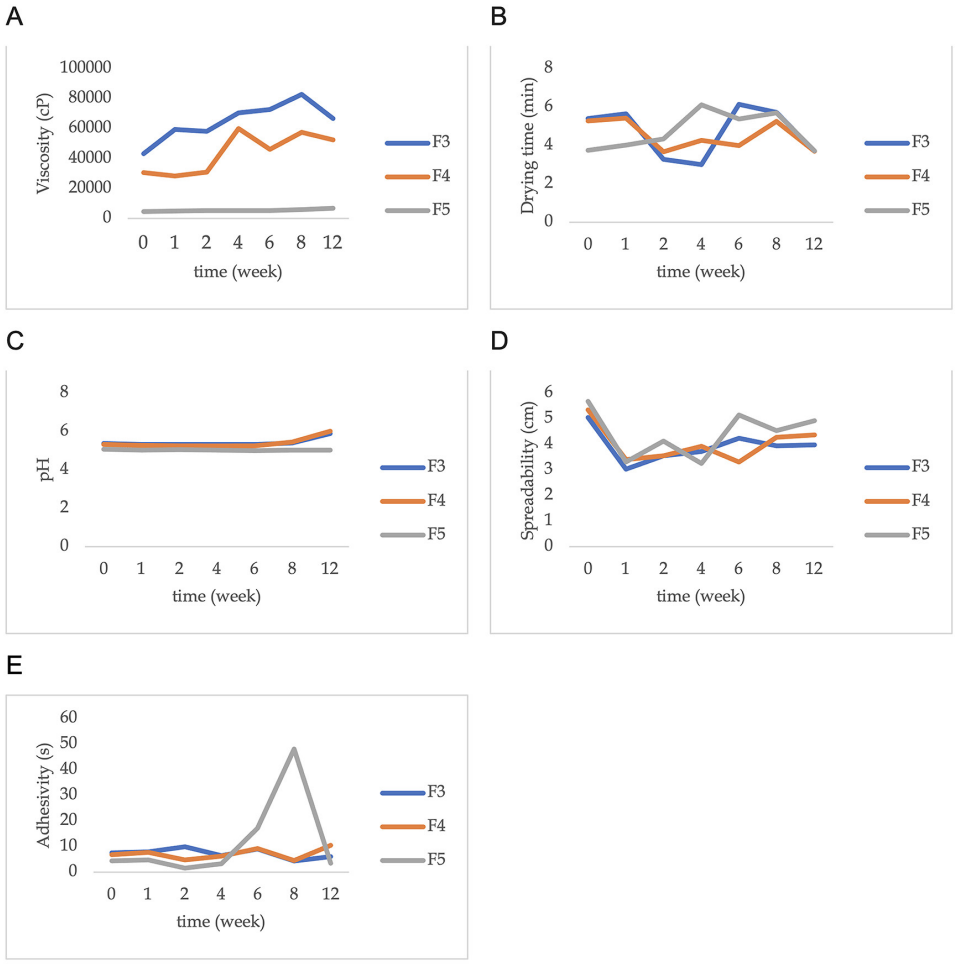


**Figure 8.** Stability study of Rosella peel-off mask PVA-based for (A) viscosity parameters, (B) drying time parameters, (C) pH parameters, (D) spreadability parameters, (E) adhesivity parameters

PVA: Polyvinyl alcohol

nor gelatin concentration had a significant effect on viscosity ($p$ value=0.1642). In contrast, according to the one-way ANOVA linear model, the concentrations of propylene glycol and PVA significantly influenced adhesivity ($p$ value=0.0041). The coefficient values were 4.36 for propylene glycol and 12.41 for PVA, demonstrating that PVA had a more pronounced impact on the adhesivity profile. Lower PVA concentrations resulted in reduced adhesiveness. The high adhesiveness of the hydrogel is attributed to the presence of carboxyl and hydroxyl groups in the PVA-COOH chain, which contribute significantly to the adhesion properties.[21]

The selected formula for the PVA-based formulation was F4, while F6 was chosen for the gelatin-based formulation based on the results of SLD numerical optimization. All formulations were stored at room temperature in amber glass containers and subsequently evaluated for viscosity, drying time, pH, spreadability, and adhesiveness. All gelatin-based formulations were found to be unstable in less than a month and therefore, were not considered optimal formulations. Similarly, the PVA-based formulations F1 and F2 were unstable after one month. In contrast, formulations F3, F4, and F5 remained stable for 7

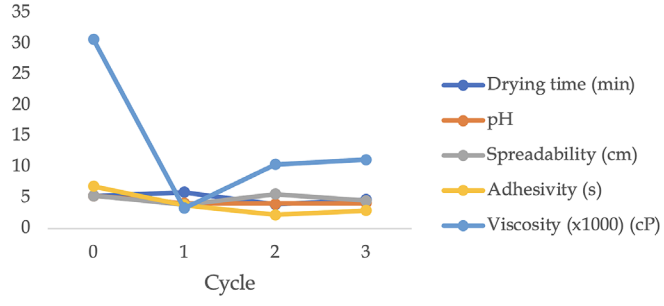


**Figure 9.** Freeze thaw stability study of Rosella peel-off mask PVA-based polymer (F4)

PVA: Polyvinyl alcohol

weeks at room temperature, and they maintained all parameters without phase separation for up to 3 months, as shown in Figure 8. Centrifugation results depicted in Figure 10 indicated phase separation in F6, whereas no phase separation was observed in F4.

The F4 formula was subsequently subjected to a freeze-thaw stability study and remained stable in terms of drying time,

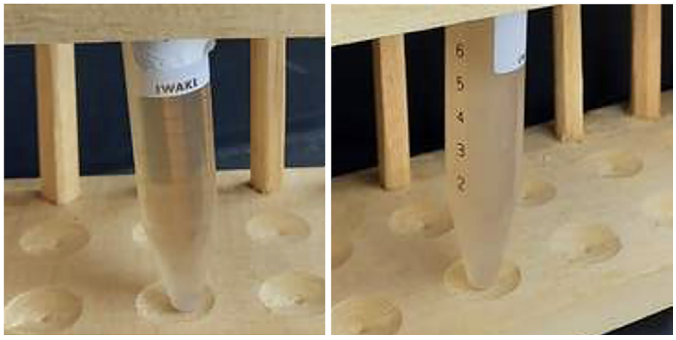

**Figure 10.** (a) Organoleptic of rosella peel-off mask PVA-based Polymer (F4) after centrifugation, (b) organoleptic of Rosella peel-off mask gelatin-based Polymers (F6) after centrifugation (Notes: Rosella granules were taken out from the formula to ensure that the separation phase of the base can be seen clearly)

PVA: Polyvinyl alcohol

pH, and spreadability parameters (Figure 9). However, the adhesiveness parameter showed a slight decrease, likely due to the freeze-thaw process triggering cross-linking, which reduces the availability of free functional groups responsible for the adhesion process.[22] Viscosity decreased significantly after the first freeze-thaw cycle, likely due to syneresis or solvent extraction caused by increased crystallinity levels during temperature fluctuations. With an increasing number of cycles, the percentage of crystalline regions within the hydrogel also increased, leading to a stiffer structure.[23,24]

According to Wu et al.,[12] the primary antioxidants in Rosella are polyphenols, with dehydrated Rosella calyces found to contain a total polyphenol concentration of approximately 683.13 mg GAE per 100 g. The DPPH radical scavenging activity ranged from 20% to 60% at sample concentrations of 1-5 mg/mL and exceeded 80% when the sample concentration reached 7.5 mg/mL. The findings of this study are consistent with these results, showing an antioxidant activity of 45.332% at a concentration of 2 mg/mL when compared to ascorbic acid as the standard.

Furthermore, this research can be extended to in vivo studies to evaluate its effectiveness in reducing the impact of free radicals. Additionally, user perspective testing can be conducted to assess its pharmaceutical aspects. The Rosella powder can also be purified to enhance its effectiveness in the formulation, and the active components responsible for its effects can be quantified. This study was conducted in a non-GMP laboratory environment, and as a result, microbiological testing was not performed; future studies will address this limitation by preparing the formulations in a GMP-compliant facility to ensure appropriate microbial control and testing.

## CONCLUSION

This study successfully developed a multifunctional peel-off mask with scrub properties by incorporating a natural source of flavonoids and astaxanthin with antioxidant potential, *Hibiscus sabdariffa* L. (Rosella) extract. The formulation was optimized through a PVA-based system using a simplex lattice design to ensure optimal physical properties and stability. LC-MS analysis verified the presence of key antioxidant compounds in the extract, such as astaxanthin, quercetin, rutin, and kaempferol. Granulated Rosella powder, sized between 250-425 µm, exhibited good flowability and functioned effectively as a scrub agent. The optimized formula, containing 15% Rosella (11% granules and 4% powder), displayed favorable peel-off characteristics, including quick drying time, appropriate pH, excellent spreadability, and strong adhesiveness, while maintaining stability under ambient conditions, freeze-thaw cycles, and centrifugation stress. The product achieved an antioxidant activity of 45.33% relative to ascorbic acid. These results indicate that Rosella-based peel-off masks represent a viable solution for incorporating natural antioxidants into multifunctional cosmetic products, supporting sustainable and practical skincare innovations.

### *Ethics*

**Ethics Committee Approval:** Not required.

**Informed Consent:** Not required.

### *Acknowledgments*

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### *Footnotes*

#### Authorship Contributions

Concept: P.R., Design: P.R., Data Collection or Processing: P.R., A.K., R.S., G.L., Analysis or Interpretation: P.R., A.K., R.S., G.L., Literature Search: P.R., I.Y.K., Writing: P.R., I.Y.K.

**Conflict of Interest:** The authors declare no conflicts of interest.

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