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Formulation and evaluation of antioxidant activity of lip moisturizing serum containing dragon fruit pulp extract (*Selenicereus monacanthus* (Lem.) D.R.Hunt) and avocado pulp oil (*Persea americana* Mill.)

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ABSTRACT: This study aimed to formulate and evaluate a lip moisturizing serum containing dragon fruit extract (*Selenicereus monacanthus* (Lem.) D.R.Hunt) and avocado oil (*Persea americana* Mill.) with a focus on antioxidant activity, moisturizing efficacy, irritation potential, and physical stability. The combination of both ingredients was optimized at a 2:1 ratio of extract to oil, which exhibited the highest antioxidant activity. Based on this ratio, three serum formulations were developed: F0 (placebo), F1 (100×), and F2 (200×). The formulations were evaluated for their physicochemical properties, antioxidant capacity, moisturizing performance, irritation response, and stability using a cycling test. The results demonstrated that the antioxidant activity decreased after formulation but remained strong for F1 and F2 after formulation. Among all formulations, F2 exhibited optimal characteristics - strong antioxidant effect, high moisturizing ability, stable physicochemical properties, and no signs of irritation. These findings indicate that the 2:1 combination of dragon fruit extract and avocado oil can be successfully developed into a stable, effective, and antioxidant-rich natural lip-moisturizing serum.

KEYWORDS: Cosmetic formulation; DPPH assay; natural antioxidants; *Persea americana*; *Selenicereus monacanthus*; skin hydration.

INTRODUCTION

The skin of the lips is thin and lacks sebaceous glands, making it highly prone to dryness, cracking, and oxidative stress caused by exposure to ultraviolet (UV) rays, wind, and extreme temperatures [1]. Moisturizing lip preparations are necessary to maintain lip hydration and protect against skin damage. In recent years, there has been a growing interest in natural-based cosmetic products, including lip care products, owing to their safety and environmental friendliness [2].

Dragon fruit extract (*Selenicereus monacanthus* (Lem.) D.R.Hunt) is rich in active compounds such as polyphenols, flavonoids, and betalains, which are known for their high antioxidant activities. These compounds are known to scavenge free radicals that contribute to skin aging [3]. Avocado oil (*Persea americana* Mill.) contains essential fatty acids, tocopherols, and phytosterols, which act as emollients and support skin barrier repair [4], [5]. The combination of these two ingredients is expected to provide synergistic effects in moisturizing and protecting the lips from oxidative damage.

Recent trends in cosmetic formulations emphasize the development of natural lip care products utilizing plant-derived ingredients, such as oils, waxes, and botanical extracts, to provide antioxidant, anti-inflammatory, and moisturizing benefits. Several studies on herbal lip balm formulations have demonstrated that natural ingredients such as beeswax, coconut oil, almond oil, honey, and vitamin E can offer effective moisturization, protection against oxidative stress, and enhanced product stability compared to synthetic formulations [6].

Although the antioxidant properties and topical benefits of each ingredient have been widely studied individually, research on their combined application in lip-moisturizing serum formulations remains limited. Moreover, antioxidant activity may vary after formulation, making the evaluation of the final product crucial.

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Therefore, this study aimed to formulate and evaluate a lip moisturizing serum combining dragon fruit extract and avocado oil, focusing on its physicochemical properties, antioxidant activity using the DPPH method, moisturizing effect, irritation potential, and physical stability using the cycling test method.

▪ MATERIALS AND METHODS

Materials

Red dragon fruit extract (*Selenicereus monacanthus* (Lem.) D.R.Hunt) was obtained through a maceration process, and avocado oil (*Persea americana* Mill.) was extracted using a cold-press method. Additional excipients used in the serum formulation included xanthan gum, propylene glycol, butylated hydroxytoluene (BHT), phenoxyethanol, titanium dioxide, 96% ethanol, coloring agents, and distilled water.

Ethical approval for this study was obtained from the Health Research Ethics Committee, Faculty of Pharmacy, Universitas Pancasila, Indonesia. The clinical test on human panelists for skin moisture evaluation was approved under No. 168/KEPK-FFUP/VII/2025, and the animal irritation test was approved under the approval number. 152/KEPK-FFUP/VII/2025. All procedures were conducted in accordance with the CIOMS 2016 guidelines and complied with the seven WHO 2011 ethical standards.

Preparation of dragon fruit extract

Dried dragon fruit powder was macerated in 96% ethanol for 3×24 h at room temperature with occasional stirring. The resulting filtrate was filtered and concentrated using a rotary evaporator until a thick extract was obtained.

Preparation of avocado oil

Fresh avocado pulp was crushed and pressed using the cold-press method without applying heat. The resulting oil was filtered, placed in dark containers, and stored at room temperature.

Formulation and preparation of lip moisturizing serum

The lip moisturizing serum was formulated using a combination of red dragon fruit extract (*Selenicereus monacanthus* (Lem.) D.R.Hunt) and avocado oil (*Persea americana* Mill.) in a 2:1 ratio. Three formulations were prepared: F0 (placebo, without active ingredients), F1 (active ingredients at ×100 concentration), and F2 (active ingredients at ×200 concentration). All formulations included common excipients such as xanthan gum, propylene glycol, butylated hydroxytoluene (BHT), phenoxyethanol, titanium dioxide, ethanol, colorant, and distilled water.

Table 1. Composition of lip moisturizing serum formulations.

Ingredients	F0 (%)	F1 (%)	F2 (%)	Function
Dragon fruit extract	–	0.8044	1.6088	Active
Avocado oil	–	0.5859	1.1718	Active
Xanthan gum	0.80	0.80	0.80	Gelling agent and stabilizer
Propylene glycol	15	15	15	Humectant and solvent
BHT	0.10	0.10	0.10	Antioxidant preservative
Phenoxyethanol	0.80	0.80	0.80	Antimicrobial preservative
Titanium dioxide	0.20	0.20	0.20	Opacifying agent and UV protector
Ethanol 96%	2.50	2.50	2.50	Co-solvent and penetration enhancer
Colorant (qs)	qs	qs	qs	Aesthetic enhancer (adjusts product color)
Distilled water	ad 100 mL	ad 100 mL	ad 100 mL	Vehicle (solvent base)

Antioxidant activity test (DPPH Method)

The antioxidant activity of the extract combination and serum formulations was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. Samples at various concentrations were mixed with 0.4 mM DPPH solution and incubated in the dark for 30 min. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of inhibition was calculated, and IC_{50} values were determined using linear regression analysis [7].

Physicochemical evaluation of serum

Serum formulations were evaluated for organoleptic properties (color, odor, consistency), pH (measured using a digital pH meter), homogeneity (glass slide test), viscosity (using a Brookfield viscometer), and spreadability (two-glass slide method with diameter measurement in centimeters).

Moisturizing effect test

The moisturizing performance was evaluated in 11 human volunteers. Lip hydration levels were measured before and after serum application using a skin analyzer. The percentage increase in moisture content was used as an indicator of the moisturizing effect [8].

Skin ırritation test

The irritation potential was tested on healthy white rabbits. The serum was topically applied to the shaved dorsal area of the rabbits, and signs of erythema or edema were observed for up to 72 h in accordance with standard topical irritation test protocols [9].

Cycling test

Stability testing was performed using the cycling test method, in which serum formulations were stored alternately at ±4°C and ±40°C for six cycles (12 days). Changes in color, pH, viscosity, and homogeneity were observed during the test period [10].

▪ RESULTS

Physicochemical evaluation of lip moisturizing serum

The lip moisturizing serum formulations (F0, F1, and F2) were evaluated for organoleptic properties, homogeneity, pH, viscosity, and spreadability. The results are presented in Table 2.

Table 2. Physicochemical evaluation of the lip moisturizing serum.

Parameter	F0	F1	F2	Standard criteria
Organoleptic	Stable (light pink, odorless, soft)	Stable (darker pink, odorless, soft)	Stable (deep pink, odorless, soft)	Stable color, odor, texture
Homogeneity	Homogeneous	Homogeneous	Homogeneous	No coarse particles
pH	6.72±0.0188	6.41±0.0069	5.87±0.0107	4.5–6.5
Viscosity	963.7±1.594 cP	969.2±0.460 cP	988.2±0.464 cP	45–5000 cP
Spreadability	6.06±0.053 cm	6.61±0.038 cm	6.13±0.049 cm	5–7 cm

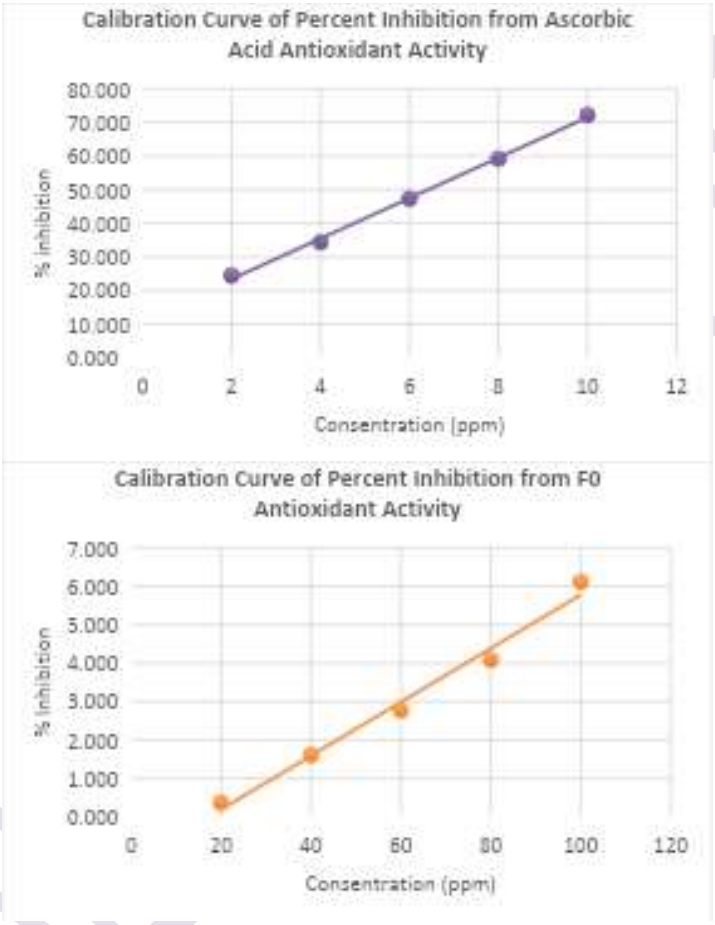
The pH values remained within the acceptable physiological range (4.5–6.5) [11], with minor differences attributable to the presence of the active ingredients. All formulations met the acceptable criteria for physical stability, showing no signs of phase separation, sedimentation, or instability after six cycles of temperature stress (cycling test).

Antioxidant activity evaluation

The antioxidant activity of the serum formulations was assessed using the DPPH radical-scavenging method. The results, presented as IC_{50} values, are shown in Table 3 and Figure 1.

Table 3. Antioxidant activity (IC_{50}) of the lip moisturizing serum.

Formula	% Inhibition range	Mean % inhibition	IC_{50} (ppm) ± SD	Antioxidant category
F0	0.29–6.26%	3.45%	732.873±4.74	Very weak
F1	7.71–51.06%	29.92%	98.764±0.25	Strong
F2	29.87–74.42%	50.01%	59.142±0.45	Strong



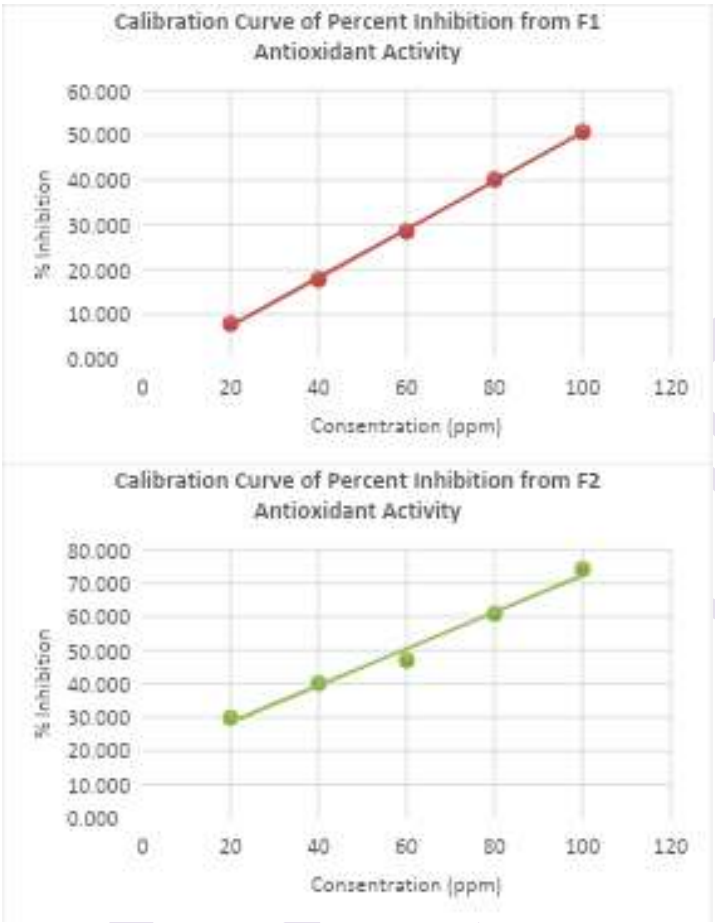


Figure 1. Calibration curve of DPPH radical inhibition showing a linear relationship between concentration and absorbance.

One-way ANOVA indicated a statistically significant difference in the IC_{50} values of the three formulations ($p < 0.05$). Post-hoc Tukey HSD analysis showed that all pairwise comparisons (F0 vs. F1, F0 vs. F2, and F1 vs. F2) were statistically significant ($p < 0.05$), confirming that the active ingredients significantly enhanced antioxidant activity.

Moisturizing effect

The moisturizing effect was evaluated in vivo using a skin analyzer on 11 volunteers over five consecutive days of application. The results are summarized in Table 4 and illustrated in Figures 2 and 3.

Table 4. Moisture increase (%) after serum application.

Day	F0 (%) ± SD	F1 (%) ± SD	F2 (%) ± SD	Commercial product (%) ± SD
1	44.52±1.99	55.45±1.91	60.82±1.84	-26.51±1.92
2	24.23±1.93	39.23±1.55	44.09±1.99	-23.50±1.74
3	34.86±1.89	42.56±1.53	46.87±1.46	-24.97±1.78
4	31.47±1.35	37.79±1.33	41.18±1.64	-25.45±0.68
5	28.66±0.56	35.90±1.20	39.13±1.05	-25.44±0.29

Negative values indicate a decrease in measured skin moisture after application of the commercial product, This may be due to alcohol or volatile components causing temporary surface dehydration.

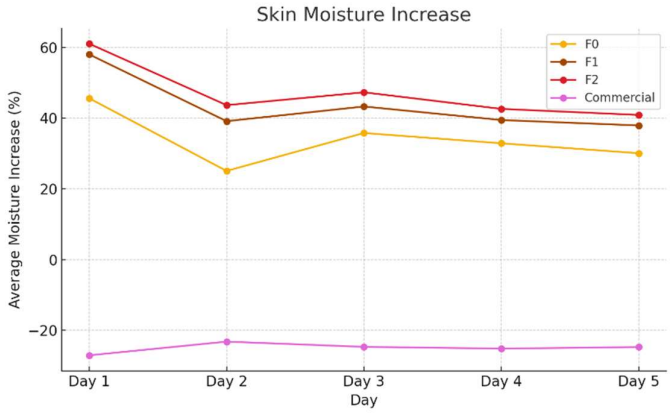


Figure 2. Moisture increased over 5 days.

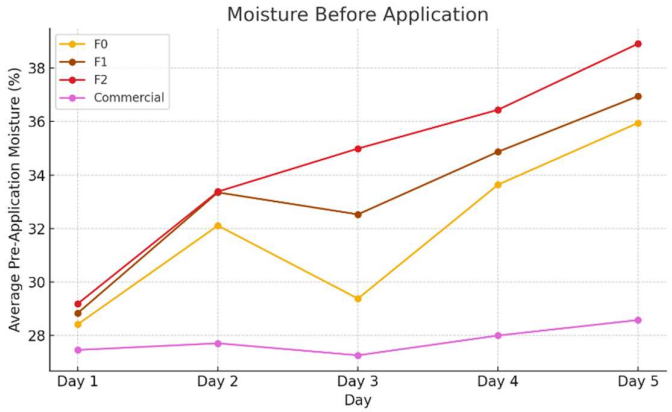


Figure 3. Pre-application moisture levels were measured.

F2 consistently exhibited the highest moisturizing effect, followed by F1. One-way ANOVA and Tukey's HSD analyses confirmed that F2 had significantly better moisturizing performance than F0 ($p < 0.05$), supporting the synergistic moisturizing role of dragon fruit extract and avocado oil.

Irritation test

The irritation potential of each formulation was assessed in three healthy rabbits over 72 h. The results are presented in Table 5.

Table 5. Irritation test results for rabbit skin.

Formula	Time	Erythema	Edema	Irritation index	Category
Healthy Control	24h / 48h / 72h	0	0	0	Non-irritant
F0	24h / 48h / 72h	0	0	0	Non-irritant
F1	24h / 48h / 72h	0	0	0	Non-irritant
F2	24h / 48h / 72h	0	0	0	Non-irritant

All formulations were classified as non-irritants in rabbit models; however, the sample size was limited (n = 3). Further safety assessments using larger animal groups and human patch testing are recommended for future product development.

Cycling test

Stability was assessed using cycling tests over six cycles of temperature variation. Key parameters (organoleptic properties, pH, viscosity, spreadability, and antioxidant activity) remained stable, as shown in Table 6.

Table 6. Stability test results after cycling.

Parameter	F0	F1	F2	Standard criteria
Organoleptic	Stable	Stable	Stable	No change
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Uniform
pH	6.72 ± 0.0188	6.41 ± 0.0069	5.87 ± 0.0107	4.5–6.5
Viscosity (cP)	963.7 ± 1.594	969.2 ± 0.460	988.2 ± 0.464	45–5000
Spreadability (cm)	6.06 ± 0.053	6.61 ± 0.038	6.13 ± 0.049	5–7
IC_{50} before cycling test (ppm)	732.873 ± 4.74	98.764 ± 0.25	59.142 ± 0.45	<100 (Strong)
IC_{50} after cycling test (ppm)	821.93 ± 6.605	100.59 ± 0.597	66.12 ± 0.344	<100 (Strong)

Figure 4. The visual appearance of the lip moisturizing serum formulations (F0, F1, and F2) before and after the cycling test showed consistent color, texture, and homogeneity, indicating good organoleptic stability.

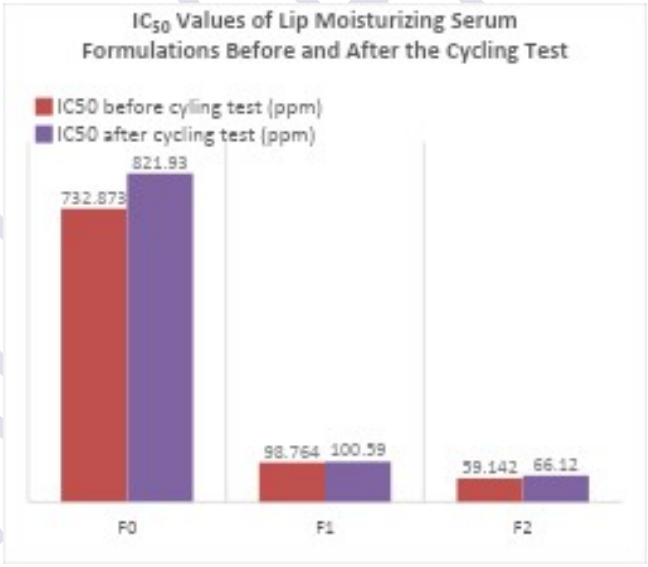


Figure 5. Comparison of IC_{50} values before and after cycling test.

The stability test confirmed that all serum formulations maintained their physical and antioxidant properties throughout the cycling process. Minor increases in the IC_{50} values were within acceptable limits and did not significantly reduce the antioxidant strength.

▪ DISCUSSION

The results demonstrated that the lip moisturizing serum containing dragon fruit extract and avocado oil exhibited favorable physicochemical characteristics, strong antioxidant activity, and good moisturizing effects without causing irritation to the skin. These findings suggest that the combination of hydrophilic antioxidants

from dragon fruit and lipophilic compounds from avocado oil contributes to the overall stability and bioefficacy of the formulation. The observed synergistic performance highlights the potential of integrating natural botanical extracts and oils for the development of effective lip care products.

The consistent superiority of F2 over F1 cannot be explained solely by concentration differences. This result indicates the possibility of synergistic interactions between the hydrophilic antioxidants (anthocyanins and phenolic acids) in dragon fruit extract and the lipophilic antioxidants (tocopherols and unsaturated fatty acids) in avocado oil. Anthocyanins primarily act in the aqueous phase by scavenging hydrophilic radicals, whereas tocopherols function in the lipid phase by terminating lipid peroxidation chains. The interaction between these two antioxidant systems regenerates tocopherol radicals and prolongs overall antioxidant protection through redox cycling. Furthermore, avocado oil improves anthocyanin stability by forming a lipid microenvironment that reduces oxidation and enhances penetration, providing complementary effects in both phases. These findings suggest that F2's enhanced efficacy results from nonlinear synergistic mechanisms between polar and nonpolar antioxidants rather than a mere increase in concentration.

Antioxidant activity

The antioxidant activity of the lip moisturizing serum, evaluated using the DPPH radical scavenging method, showed that the combination of dragon fruit extract and avocado oil effectively enhanced the free radical scavenging capacity. Formula F2 demonstrated the strongest antioxidant activity and was significantly different from F1 and the placebo F0 ($p<0.05$), confirming that the increased proportion of active components contributed to greater antioxidant potency.

The results of this study align with and further strengthen the evidence presented in previous studies on the individual antioxidant potentials of both components. Tahsin et al. (2022) reported that avocado oil exhibited antioxidant activity with an IC_{50} value of 62.99 ppm, attributed to the presence of tocopherols and unsaturated fatty acids that interrupt free-radical chain reactions [12]. Similarly, Widianingsih (2016) found that the methanolic extract of red dragon fruit possessed strong antioxidant activity, with an IC_{50} value of 67.45 ppm, owing to its high content of phenolic acids, such as gallic, vanillic, and protocatechuic acids [13]. In the present study, the combined formulation (F2) achieved an even stronger antioxidant effect (IC_{50} = 59.14 ppm), suggesting that the interaction between these two antioxidant systems generated a synergistic enhancement, rather than a simple additive effect.

This synergism can be mechanistically explained by the complementary functions of the active constituents of these herbs. Anthocyanins and other phenolic compounds from dragon fruit are hydrophilic antioxidants that scavenge radicals in the aqueous phase, whereas tocopherols and fatty acids in avocado oil are lipophilic antioxidants that protect the lipid phase by terminating peroxidation chain reactions. Phenolic compounds may regenerate oxidized tocopherols through redox cycling, maintaining the antioxidant network and preventing the degradation of both antioxidant pools. Additionally, the lipid matrix provided by avocado oil may improve anthocyanin stability by reducing oxidative degradation, resulting in enhanced and sustained antioxidant activities.

After the cycling stability test, all formulations maintained an acceptable antioxidant capacity, with only a minor increase in IC_{50} values. The slight rise observed in F2 (59.14 → 66.12 ppm) was statistically insignificant ($p>0.05$), indicating that the synergistic antioxidant network remained stable even during thermal stress. Importantly, the IC_{50} value of F2 after storage still fell within the "strong" antioxidant classification (<100 ppm), demonstrating that the formulation retained its antioxidant potency and structural integrity throughout the stability evaluation.

Moisturizing effect

The moisturizing efficacy of the serum formulations, evaluated *in vivo* using a skin analyzer over a five-day period, showed that both F1 and F2 significantly improved skin hydration compared to F0 and a commercial benchmark serum. The highest moisturizing effect was observed in F2, particularly on day one (60.82±1.84%), consistent with the "burst hydration" phenomenon. This initial surge in hydration can be attributed to the humectant action of propylene glycol and polysaccharides in the dragon fruit extract, combined with the occlusive properties of avocado oil [10], [14].

Importantly, the long-term increase in baseline skin moisture content observed throughout the study period suggests a cumulative hydration effect. This aligns with Lodén's theory (2003), which postulates that effective moisturizers not only hydrate but also enhance the barrier function and moisture retention of the stratum corneum [14].

The superior performance of F2 compared to F1 may not be solely attributed to its higher concentration of active ingredients but also to the potential nonlinear synergistic interactions between anthocyanins in the dragon fruit extract and bioactive lipids, such as tocopherols and oleic acid, in the avocado oil. Anthocyanins provide humectant and antioxidant effects, whereas avocado oil forms a lipid film that reduces transepidermal water loss and stabilizes radicals in the lipid phase. This synergistic mechanism may explain the enhanced and sustained moisturizing and antioxidant performance of F2 beyond a simple concentration-dependent effect.

Irritation test

The safety profiles of all serum formulations were confirmed via primary irritation tests on rabbits. All observations showed zero irritation index scores, indicating no signs of erythema or edema throughout the 72 h observation period. This result meets the Draize criteria for a "non-irritant" classification, ensuring the suitability of the formulations for application on sensitive skin areas, such as the lips [15].

Although all formulations were classified as non-irritant, the irritation test involved only three rabbits, which limited the generalizability of the findings. Larger-scale safety evaluations, including human dermatological testing, are necessary to confirm the safety profile of this product for cosmetic application.

Cycling test

Cycling tests revealed that all formulations maintained acceptable stability in terms of organoleptic properties, homogeneity, pH, viscosity, spreadability, and their antioxidant activity. The pH of all formulations remained within the physiologically acceptable range (4.5–6.5) [11], with F1 and F2 showing optimal values for compatibility with the lip skin. The viscosity values remained within the standard range for topical preparations (45–5000 cP) [16], with F2 exhibiting a slightly higher viscosity due to its increased concentration of bioactive compounds and polysaccharides [17]. Spreadability was inversely proportional to viscosity, which was consistent with the Newtonian fluid behavior.

The notable increase in the IC_{50} value of F0 after the cycling test suggests a reduction in its residual antioxidant capacity. This phenomenon may be attributed to the degradation of excipients, such as butylated hydroxytoluene (BHT), or changes in the polymeric matrix (xanthan gum) under thermal stress. Although F0 contained no active antioxidant extract or oil, the presence of these excipients may have initially contributed to weak radical scavenging activity, which decreased upon exposure to repeated temperature fluctuations.

The slight increase in the IC_{50} values of F2 after the cycling test (59.14 → 66.12 ppm) indicates a minor reduction in antioxidant activity following repeated temperature stress. However, statistical analysis showed that this change was not significant (p>0.05), suggesting that the antioxidant stability of F2 remained within acceptable limits. Importantly, despite the slight increase, the IC_{50} value of F2 still fell within the "strong" antioxidant activity classification range (50–100 ppm), indicating that the formulation retained its antioxidant potency after the stability test. The minimal change in F2's antioxidant capacity after thermal cycling further supports the hypothesis that the combination of anthocyanins and tocopherols contributes to enhanced oxidative stability through mutual regeneration and radical interception across the aqueous and lipid domains.

Statistical analyses (ANOVA and Tukey's HSD)revealed significant differences in pH, viscosity, and spreadability among the formulations, attributed to compositional differences. However, all formulations remained physically and chemically stable throughout the storage period, with no signs of phase separation or degradation.

Formulation justification

The formulation strategy employed a scientifically grounded selection of ingredients: dragon fruit extract as a source of anthocyanins and polysaccharides with antioxidant and hydrating properties; avocado oil as an occlusive agent rich in essential fatty acids and tocopherols; xanthan gum for gel formation and texture

stabilization; propylene glycol as a humectant; butylated hydroxytoluene (BHT) as an antioxidant stabilizer; phenoxyethanol as a preservative; and titanium dioxide and food-grade dyes for aesthetic appeal. The formulation was carefully balanced to achieve both functionality and consumer acceptability of the final product.

Limitations and future perspectives

Although the findings demonstrated promising antioxidant and moisturizing effects, this study was limited by the small sample size used in the irritation test and the absence of direct mechanistic assays to confirm anthocyanin–tocopherol interactions. Future studies should employ advanced analytical methods, such as HPLC-based degradation profiling and antioxidant regeneration assays, to verify the hypothesized synergism and assess long-term safety using human patch testing. These investigations will provide a more comprehensive understanding of the formulation mechanism and its potential for commercial cosmetic applications.

▪ CONCLUSION

Formula F2, combining dragon fruit extract (*Selenicereus monacanthus* (Lem.) D. R. Hunt) and avocado oil (*Persea americana* Mill.) at a 2:1 ratio demonstrated optimal antioxidant and moisturizing activities with acceptable physicochemical stability and safety. These findings support the potential of this formulation as a natural, antioxidant-rich lip serum candidate suitable for further cosmetic development.

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