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Physicochemical Evaluation and Anti-Aging Activity of The Cream Formulation Containing *Aloe Vera* and Rosella Extracts

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ABSTRACT

Skin aging is a complex biological process influenced by intrinsic and extrinsic factors. Anti-aging cream is a cosmetic product designed to prevent premature aging. *Aloe vera* and rosella have been reported to exhibit various pharmacological activities, including anti-aging. Meanwhile, the use of a combination of these two natural products has not been widely reported. This research aims to determine the potential antioxidant and anti-collagenase activities of the combination of *Aloe vera* and rosella in cream products. This study analyzed the quality of cream in terms of physical parameters, chemical content, antioxidant activity, anti-collagenase properties, and sunscreen protection factor (SPF) test results of cream products. According to all test results, the recommended cream product is the C formula, which contains aloe vera and roselle extract in a 1:1 ratio. The C cream formula was pink, semi-solid, homogeneous, and had an O/W emulsion type. Total polysaccharide, o-acetyl polysaccharide, flavonoid, phenolic, and anthocyanin content in the C cream formula were 0.73%, 3.53%, 3.10×10^{-6} g QE/g, 6.11×10^{-6} g GAE/g, and 11.97 mg/kg, respectively. While the radical scavenging, anti-collagenase activities, and SPF value of the C cream formula were 37.85%, 54.66%, and 8.73, respectively. A cream containing Aloe vera gel and rosella flower extracts can be developed as an anti-aging cream product.

Keywords: Cream formula; Aloe gel; Rosella; Anti-collagenase; SPF

INTRODUCTION

Skin is the part of the human body that experiences premature aging. Fine lines, wrinkles, changes in skin pigmentation, and inflammation are characteristics of aging, often resulting from decreased collagen production and increased collagen degradation. The aging process is accelerated when the skin is overexposed to UV radiation. Several plant extracts are rich in bioactive compounds that exhibit antioxidant and anti-aging properties by inhibiting dermal enzyme activities and scavenging free radicals (Madan & Nanda, 2018).

Aloe (*Aloe vera* (L.) Burm.f.) is a native plant from South Africa that already cultivated in Asia, America, and Europe (Adlakha et al., 2022). *Aloe vera* showed anti-aging activity. Acemannan polysaccharide (o-acetyl polysaccharide) is a marker compound in aloe gel that acts as an antioxidant and is proven to overcome the adverse effects and suppress the production of free radicals (Liu et al., 2019). Acemannan (β-(1,4)-acetylated

soluble polymanose) functions as an inducer of collagen synthesis for wound healing in fibroblast cells, and pectin (a polysaccharide) compounds serve as drug delivery systems and coatings for biomaterials (Handayani et al., 2021). However, the antioxidant activity in *Aloe vera* was reduced during the preparation process. Rosella (*Hibiscus sabdariffa* Linn.) contains anthocyanin compounds. Anthocyanins can be used as natural colorants and antioxidants (Ayubi et al., 2024). Adding rosella extract to the *Aloe vera* anti-aging product can improve its antioxidant activity and maintain its UV protection and anti-collagenase activity. Cream was chosen to create the anti-aging formula in this study. Cream is a topical product that is easy to use, moisturizes the skin, and spreads the active ingredients contained (Okafo et al., 2023). Since antioxidant, anti-collagenase, and UV protection mechanisms have a role in anti-aging activity, this study aims to determine the potential anti-aging bioactivity of the combination of *Aloe vera* and rosella flower extracts, including antioxidant and collagenase enzyme inhibitory activities, as well as sun protection in anti-aging cream products.

MATERIALS AND METHODS

Plant Determination

This research was conducted at the Laboratory Facility of BRIN, Yogyakarta, Indonesia. *Aloe vera* raw materials were obtained from Nglipar aloe cultivation area, Gunungkidul, Yogyakarta, Indonesia. Raw materials of rosella simplisia were obtained from commercial herbal stores. Plant determination was conducted at the Center for Research and Development of Medicinal Plants and Traditional Medicines, Tawangmangu, Central Java, Indonesia.

Extract Preparation

The *Aloe vera* gel was blended, and the supernatant was separated by centrifugation. Dried rosella flowers were extracted by aquadest at 90 °C for 15 min. *Aloe vera* gel and rosella extracts were processed into powders by the spray dry method (inlet 100 °C, outlet 80 °C) using maltodextrin as its additional compound ((Junaidi et al., 2021) with slight modification).

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The functional groups of compounds in the aloe gel and rosella aqueous extract were identified by an attenuated total reflectance (ATR)-FTIR spectrophotometer (Bruker, Vertex 10, Germany). The sample was placed in ATR crystal and the spectra was obtained at a wavenumber range of 4000–500 cm^{-1}.

Preparation of O/W Cream

A completely randomized design using different levels of *Aloe vera* and rosella extracts in the cream formula was used in this study. The six cream formulas were NC (negative control), A, B, C, D, and E, which contained *Aloe vera* and rosella extracts in a 0:0, 0:1, 1:0, 1:1, 1:3, and 3:1 ratio, respectively. The cream ingredient was made in three phases: phase 1 (oil ingredients), phase 2 (water ingredients), and phase 3 (powder extracts) (Table I). Each phase was prepared at 70 °C using a hotplate magnetic stirrer. Phase 2 was put into phase 1 and homogenized for 10 minutes. Then, phase 3 was mixed into the cream base (Phase 1 and 2) and homogenized for 10 minutes, then removed, cooled, and stored in a cream container ((Gajic et al., 2021) with slight modification).

Physical Cream Test

The physical test of the cream included organoleptic evaluation, homogeneity (10 times magnification), pH, spreadability (at 0, 50, 100, and 150 g), emulsion type, viscosity using a 25 mm plate rheometer, and color intensity using a chromameter (Konica Minolta CM-5).

Screening Phytochemical Cream

The phenol-sulfuric acid method was used for total polysaccharide measurement ((Sangthong et al., 2022) with slight modification). Various concentrations of d-glucose were used to make standard curve. The cream solution was mixed with 5% phenol solution. Then, the solution was stirred and incubated at room temperature for 10 minutes. Furthermore, H_2SO_4 was added, and hydrolyzed at 100° C for 20 min. The absorbance of the test solution was then measured using a spectrophotometer (UV-Vis Agilent Carry 60) at 490 nm.

The o-acetyl polysaccharide was done according to Metcalfe (2019) with slight modification. Acetylcholine chloride was used as a standard. The sample or standard was mixed with a Hydroxylamine Hydrochloride (HH) buffer solution for 5 minutes, then mixed with a 4N HCl and $FeCl_3$ solution, respectively. The solution was placed into a 96-well microplate, and the absorbance of the test solution was then measured at 540 nm (Multiskan GO, Thermo Scientific).

Table I. The concentration of *Aloe vera* and rosella in the cream formulas.

Materials (% w/w)	Concentration of Ingredients in Anti-Aging Cream Formulation					
	NC (0:0)	A (0:1)	B (1:0)	C (1:1)	D (1:3)	E (3:1)
Aloe vera powder extract	-	-	5	2.5	1.25	3.75
Rosella powder extract	-	5	-	2.5	3.75	1.25
Maltodextrin	5	-	-	-	-	-
Emulgade SE-PF, BHT, Isopropyl myristate, Xanthan gum, Glycerin, Na_2EDTA, Fragrance oil, and Aquadest	Up to 100					

Total flavonoids were done according to Artanti et al., (2023) with slight modification (Artanti et al., 2023). Quercetin was used as a standard. The sample or standard solution (20 µL) was mixed with 6 µL $NaNO_2$ solution (5% w/v), then incubated for 5 min, followed by adding $AlCl_3$ solution (10% w/v) (6 µL), and incubated again for 6 min in the dark room. After that, 80 µL 1N NaOH and distilled water were added and vortexed. The solution was placed into a 96-well microplate, and then the absorbance of the test solution was measured at 510 nm (Multiskan GO, Thermo Scientific).

Total anthocyanin content was determined according to Kemenkes RI, (2017) with slight modification). Cream was dissolved in ethanol P-HCl (1 N) (85:15). The solution was vortexed, and the pH was adjusted to 1. The solution was centrifuged for 15 min. The supernatant was collected and ethanol P-HCl (1 N) was added to the solution. The solution was placed into a 96-well microplate, and the absorbance of sample was measured at 535 nm (Spectrophotometer UV-Vis Agilent Carry 60).

Total phenolic content. Gallic acid was used as standard. The sample or standard solution (100 µL) was added to a 96-well microplate containing Folin-Ciocalteu reagent and 20% Na2CO3, followed by incubation for 30 min in the dark. The absorbance of the test solution was measured at 750 nm (Sturza et al., 2020).

Antioxidant Activity Test

The antioxidant activity of sample was analyzed using the 2,2-Azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) method following to Plácido et al., (2020) with slight modifications. Trolox was used as standard (0-80 µM). The sample, control (PBS), or standard solution (5 µL) was placed in a 96-well microplate. The ABTS solution (95 µL) was added under dark conditions. The microplate was incubated for 5 min, and the absorbance of the test solution was measured at 734 nm. The formula for calculating the percentage of ABTS antioxidant activity using formula 1.

Antioxidant Activity =

$$\left(\frac{(Abs(control) - Abs(sample))}{Abs(control)}\right) \text{ x } 100 \text{ ..... (1)}$$

Anti-Collagenase Test

The collagenase test was conducted using Alipour et al. (2019) method with slight modification. The collagenase enzyme was dissolved in 50 mM tricine buffer solution (0.2 - 1 U/mL). Vitamin C (100 µg) and 1,10-phenanthroline (10 mM) were used as controls. The sample or control solution (50 µL) was placed in a 96-well microplate, the enzyme was added and incubated for 15 min. Furthermore, the substrate was added to the mixture and immediately measured at 345 nm (37°C) at 0 and 90 min. The collagenase inhibitory activity was calculated using formula 2.

Inhibitory Activity (%) =

$$\frac{(activity\ of\ control - sample)}{activity\ of\ control} \text{ x } 100 \qquad \text{..... (2)}$$

Sun Protection Factor (SPF) Test

A Sun Protection Factor (SPF) test was conducted following the method of Bikiaris et al. (2023) with slight modifications. Cream products or ascorbic acid (1 mg/mL in 50% ethanol) were added to a 96-well microplate. The absorbance of the test solution was measured at 290-320 nm with an interval of 5 nm (Multiskan GO, Thermo Scientific). The SPF value of the sample was calculated using formula 3.

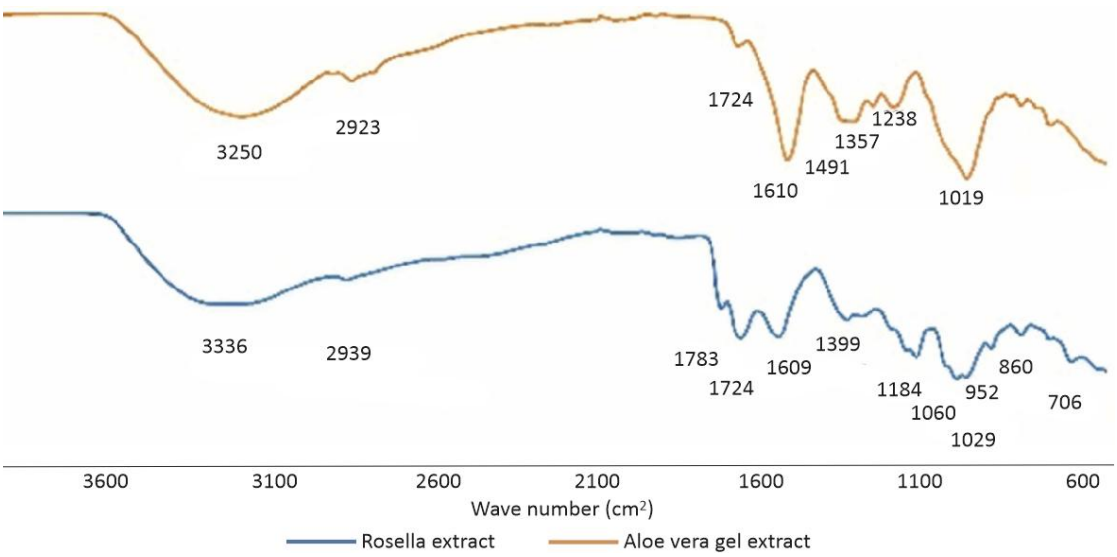

Figure 1. The FTIR spectra of aloe gel and rosella aqueous extract.

Table II. Organoleptic and emulsion type of cream products

Comparison of *Aloe vera* and Rosella Extract	Color	Scent	Type of product	Homogeneity	Emulsion type
0:0 (NC)	White	rose	Semi-solid	homogenous	O/W
0:1 (A)	Pink (++++)	rose	Semi-solid	homogenous	O/W
1:0 (B)	White	rose	Semi-solid	homogenous	O/W
1:1 (C)	Pink (++)	rose	Semi-solid	homogenous	O/W
1:3 (D)	Pink (+++)	rose	Semi-solid	homogenous	O/W
3:1 (E)	Light Pink (+)	rose	Semi-solid	homogenous	O/W

$$SPF = CF \times \sum_{290}^{320} EE \times I \times Abs \quad \text{..... (3)}$$

Where CF is the correction factor (10), EE is the erythemogenic effect of radiation at wavelength (λ), I is the intensity of sunlight at wavelength (λ), and Abs is the absorbance of the test solution (λ).

Statistical Analysis

The Graphpad Prism software (10..3.1) with The Analysis of Variance (ANOVA) statistical test ($p<0.05$) and followed with Tukey's Post hoc test was used to analyze significant difference level between treatments.

RESULTS AND DISCUSSION

The FTIR spectroscopy analysis

The functional groups from compounds contained in aloe gel and rosella aqueous extracts were analyzed using FTIR spectroscopy (Figure 1). The rosella and aloe gel extracts exhibited vibrations at 3336 cm^{-1} and 3250 cm^{-1}, which correlated with the hydroxyl group (-OH). The vibration around 2920 cm^{-1} was correlated to the -CH_3, -CH_2, and -CH groups. The vibration around 1711 cm^{-1} correlated with the presence of the C=O group, while the vibration around 1600 cm^{-1} correlated with the C=C group. The vibration around 1000 cm^{-1} was correlated with the C-O vibration.

Physical Cream Test

The organoleptic test showed that all cream formulas were semi-solid and scented with rose oil (fragrance oil used in the creams). The color of base cream formula (negative control/NC) and the B formula was white. In contrast, the A, C, D, and E formulas were pink due to the concentration of rosella extract. The resulting cream is homogeneous and an O/W emulsion type (Table II). According to the tests conducted, the viscosity of the cream ranged from 301.22 to 489.22 mPa.s. The viscosity of all formulas did not

significantly differ, except the E formula (Figure 2A). Color tests were checked with the Hunter L (white), a* (red), and b* (yellow) color systems (Chandra et al., 2024). According to the color intensity test, the L* result showed that all the formulas were white instead of transparent. The value follows the concentration pattern of *Aloe vera* and rosella extracts. The a* result showed that the cream base (NC) was whitish, B formula tended to be greenish, and the others appeared more reddish. The b* result showed that A, C, D, and E formulas tend to be more yellow than the NC cream and B formula (Figure 2B). Adding rosella extract to the cream products lowered the L* value but increased the a* and b* values. The spreadability of the cream after being given a load of 50-150 g had an average spreadability of about 5.03-7.47 cm (Figure 2C). In general, the spreadability of all formulas were not influenced by the addition of variation concentration of extracts.

Phytochemical Analysis and Bioactivities

This study analyzed the phytochemical compounds, antioxidant, anticollagenase activities, and the SPF value of the cream product containing *Aloe vera* and rosella combination. The acidic level of the cream formulas ranges from 3.02 to 4.81 (Figure 3A). Acidic levels of the NC, B, and E formulas were in the range of cream requirements (pH 4-6), while the others were below the standard. Increasing the concentration of rosella causes the pH of the cream formulas to decrease. The result of antioxidant activity, as determined by the ABTS method, had a similar pattern to that of its phytochemical analysis. According to Figures 3-4, the results of the ABTS test (Figure 4A) were in line with the results of the total polysaccharide compounds, flavonoids, anthocyanins, and phenolics content of the formulas (Figure 3B-F). The A formula showed the highest free radical inhibitory activity. On the other hand, the results of the collagenase inhibition test showed that the C formula containing *Aloe vera* and rosella in an equal ratio presented the highest value (Figure 4B).

The result of the SPF test showed that the cream base formula/NC had sun protection with an SPF value of 8.26 ± 0.12. The SPF value did not significantly different in all formulas. Nevertheless, according to the SPF test, the C formula (1:1) showed the highest SPF value. The C formula (combination of *Aloe vera* and rosella extract by 1:1) increased SPF value (8.73±0.02) compared to ascorbic acid (8.25±0.25) and cream base formula/NC (Figure 4C).

The development of formulas in cosmetic products needs to be evaluated. The plant samples in this study were identified using a determination test and FTIR spectroscopy. The result of plant determination confirmed that this study used Aloe (*Aloe vera* (L.) Burm.f.) and rosella (*Hibiscus sabdariffa* Linn.) as the active ingredient in the cream product. The FTIR can be used to identify plant samples through its functional group spectrum. Functional groups from the FTIR spectra (Figure 1) were interpreted according to several references (Abbasi et al., 2020; Indrianingsih et al., 2023). The vibration at 3336 cm^{-1} and 3250 cm^{-1} from rosella and aloe gel extract was associated with the hydroxyl (-OH) that generally comes from phenolic, polysaccharides, and lipids. The vibration around 1600 cm^{-1} was associated with C=C belonging to aromatic medicinal compounds or unsaturated fatty acids. The vibration around 1000 cm^{-1} was associated with the C-O group from proteins, fats, and carbohydrates. Acemannan is an aloe gel polysaccharide marker with several hydroxyl and acetyl groups (Shi et al., 2017). The vibration of C-O at 1740 cm^{-1}, the C-H vibration of the methyl group at 1375 cm^{-1}, and the C-O vibration of O-acetyl group at 1247 cm^{-1} proved the existence of acetyl groups of O-acetyl polysaccharide (Shi et al., 2017). Anthocyanin compounds in rosella extract can be identified by the presence of C=O, C-O, and C=C groups, which are characteristic of the anthocyanin structure (Sedyadi et al., 2016). The FTIR spectra of the samples used in this study were similar to the references. According to the FTIR analysis, the aloe gel and rosella extract used in this study contained O-acetyl polysaccharide and anthocyanin compound, respectively.

Physical measurements, including organoleptic properties, spreadability, viscosity, and color, were conducted to evaluate the cream products. The organoleptic test revealed that all creams were of the O/W emulsion type. The O/W emulsion type is easy to apply, spreads evenly on the skin, is easy to wash, and is non-sticky (Ijaz et al., 2022; Miastkowska et al., 2020). According to the results of spreadability tests (Figure 2C), all the cream formulas can spread widely when applied to the skin, thereby optimizing contact between the active substance and the skin and potentially affecting it (Okafo et al., 2023). The O/W emulsion type of the cream predominantly contains water, which also causes the wide spreadability of the cream formula.

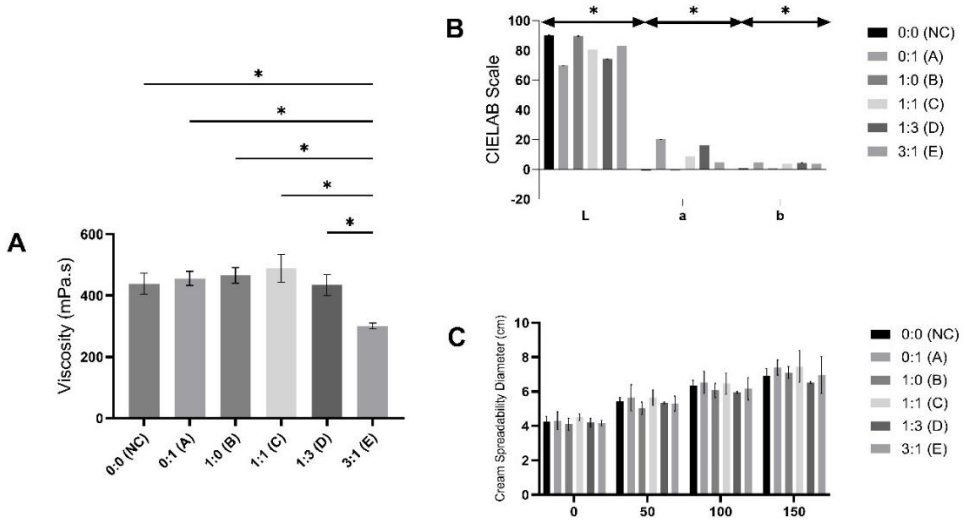

Figure 2. Physical analysis of aloe-rosela creams. A. Viscosity. B. Color measurement. C. Cream Spreadability diameter. Data are presented as means ± standard deviations. The asterisk indicates a statistically significant difference between entities ($p<0.05$).

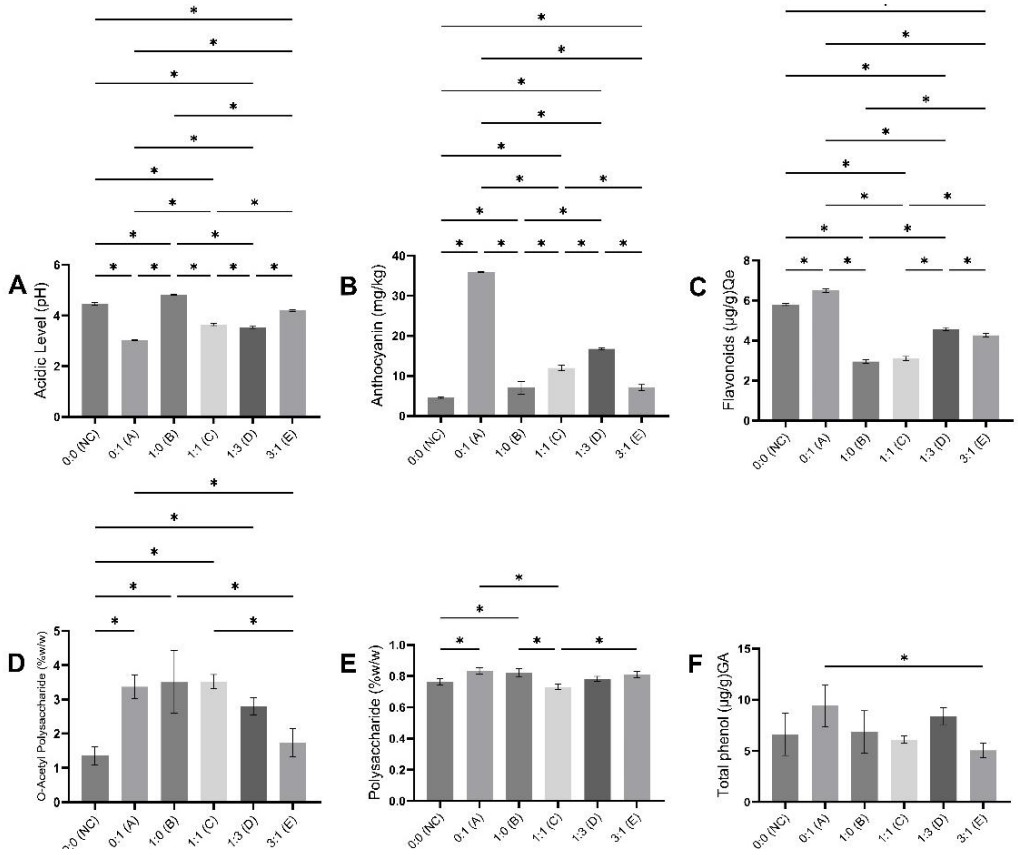

Figure 3. Phytochemical analysis of aloe-rosela creams. A. Acidic level, B. Polysaccharides, C. O-acetyl-polysaccharides, D. Flavonoids, E. Total Phenol, and F. Anthocyanin concentration of aloe-rosella creams. Data are presented as means ± standard deviations. The asterisk indicates a statistically significant difference between entities ($p<0.05$).

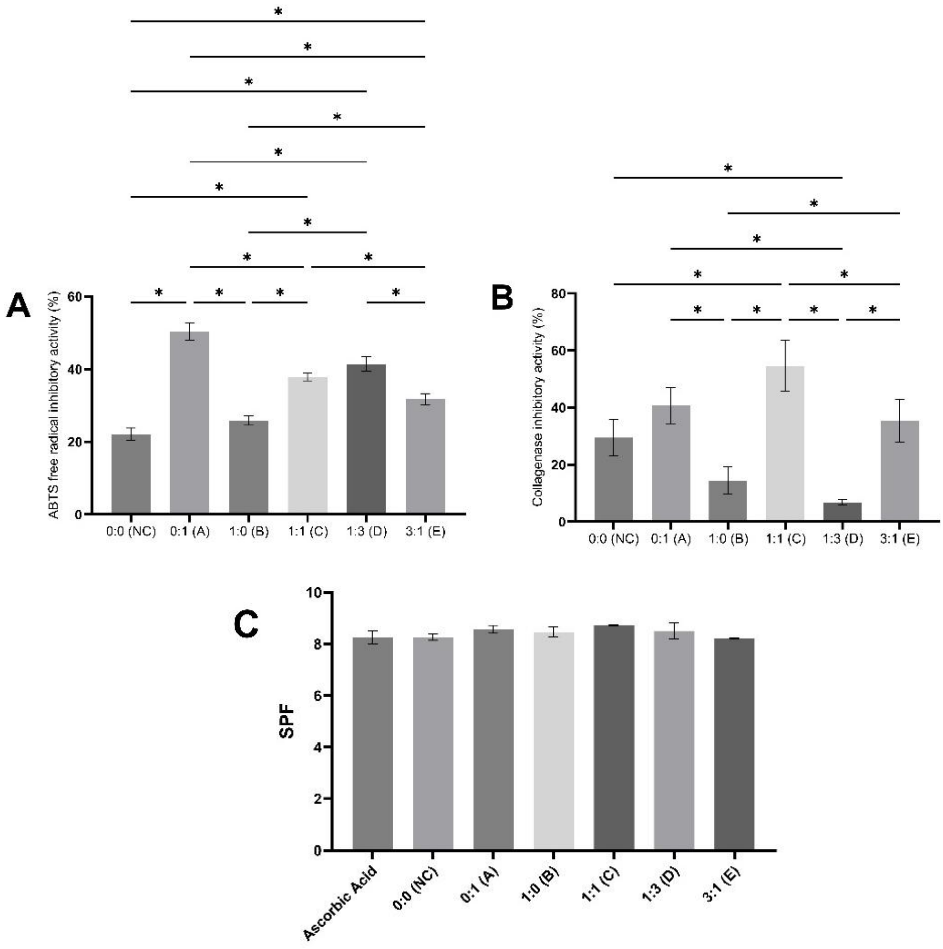

Figure 4. Bioactivities of cream formulas containing *Aloe vera* and rosella. A. The ABTS radical scavenging activity. B. The collagenase inhibitory activity. C. The SPF value. Data are presented as means ± standard deviations. The asterisk indicates a statistically significant difference between entities ($p<0.05$).

In general, the viscosity of all formulas was not influenced by the addition of various concentrations of extracts, except in the E formula (Figure 2A). Thus, the A-D formulas were more stable compared to the E formula. The O/W emulsion type partly influences the viscosity of the cream, which tends to contain more water (Sandi & Susiani, 2021). Additionally, using ingredients such as isopropyl myristate, glycerin, distilled water, and xanthan gum at appropriate concentrations makes the cream softer. The temperature and duration of stirring when making cream can also influence the viscosity. The high temperatures and a long stirring time made the water evaporate, causing the high viscosity of the cream. Temperature of 70 °C and 15-20 minutes were used to stabilize the active ingredients in the cream. Viscosity is one of the factors affecting cream stability. Particles will be hard to move in high-viscosity cream products, thus creating a more stable cream product (Dina et al., 2017).

According to the color intensity test (Figure 2C), the value follows the concentration pattern of *Aloe vera* and rosella extracts. The color of the cream depends on the amount of carrier substance in the preparation (Dayal et al., 2018). The a* result showed that the cream base (NC) was whitish, B formula tended to be greenish, and the others appeared more reddish, which was influenced by the rosella content (Tungadi et al., 2022). The b* result showed that A, C, D, and E formulas tend to be more yellow than the NC cream and B formula due to the concentration of rosella extract used. The increasing rosella concentration causes the cream to have a higher yellow level (Aryanti et al., 2019).

The pH of the cream formulas were less than 5 (Figure 3A). Rosella contains acid compounds such as citric acid, malic acid, and ascorbic acid, with a pH value around 1.42 (Mahmudatussa'adah et al., 2023). Thus, the concentration of rosella extract influences the pH of the cream. Increasing the concentration of rosella causes the pH of the cream formulas to decrease. The acidic level of the cream formula was similar with the anti-aging cream product containing alpha hydroxy acid (AHA). Cream products with AHA have low pH level compared to general cream products. The mechanism of action of AHA is to stimulate collagen synthesis, improve skin barrier function and hydration, and remove dead skin cells (exfoliate), leading to a smoother and rejuvenated skin texture. The anti-aging cream containing AHA generally works in an acidic environment (pH 3-5). The anti-aging cream containing AHA is recommended for daily use at a concentration of around 5-10%, but this can be adjusted according to all supporting factors in the formulation (Karwal & Mukovozov, 2023). The cream product with a lower pH (ranging from 3-5) can be used to develop exfoliating product formulations.

The phytochemicals and bioactivities of different products containing the same plant extract can vary. Combination of *Aloe vera* and rosella in the granul beverage product revealed its antioxidant and anti-elastase activities (Chandra et al., 2024). In this study, we analyzed the antioxidant and anticollagenase activities of combination of *Aloe vera* and rosella in the cosmetic product. The A formula showed the highest free radical inhibitory activity, likely due to the high concentration of active phytochemicals in rosella extract (Figures 3-4), which counteracts and neutralizes free radicals (Ayubi et al., 2024).

In contrast, the results of the collagenase inhibition test showed that the C formula, containing Aloe vera and rosella in an equal ratio, presented the highest value (Figure 4B). This result did not align with their flavonoids, anthocyanins, phenolic, total polysaccharide contents, and ABTS scavenging activity. In addition, the O-acetyl polysaccharide compounds were found equally in *Aloe vera* and rosella extracts. Both rosella and *Aloe vera* showed anticollagenase activity. Combination *Aloe vera* and rosella in granule beverage product revealed its antioxidant and anti-elastase activities (Chandra et al., 2024). Similar to collagenase, elastase also plays a role in the aging process. Inhibiting the enzymes elastase and collagenase reduces the speed of the aging process (Eun et al., 2020). Aloe hydrogel repaired wound in rat (Meza-Valle et al., 2021). Collagenase is one of the enzymes that have a role in inhibiting the wound process through collagen degradation (Jeong et al., 2022). Rosella extract inhibited collagen degradation caused by the MMP-1 enzyme (Ramadhani et al., 2019). The C formula in this study was probably presented in a synergistic combination of rosella and Aloe vera concentrations, resulting in increased anticollagenase activity. While the combination is optimally synergistic, it can increase bioactivity (Ghimeray et al., 2015; Handayani et al., 2023).

Ultraviolet (UV) irradiation was exposed in the environment through sunlight, leading to various skin maladies, including inflammation, degenerative aging, and cancer. Therefore, anti-UV substances should be used when preparing a cosmetic formula. The activity of sunscreen products is primarily marketed by SPF, which determines the fraction of sunburn protection. The higher SPF increases the protection against UV light (Bikiaris et al., 2023). The SPF analysis of the cream product (Figure 4C) aligned with several studies. Kanthik et al. (2020) revealed that *Aloe vera* extract did not significantly affect SPF value (Kanthik et al., 2020). However, combining *Aloe vera* gel extract and titanium dioxide synergistically enhanced sun protection efficacy. On the other hand, Monica et al. (2020) stated that rosella extract had sun protection ability with an SPF value of 12.973 (Monica et al., 2020). We suggest that combining *Aloe vera* and rosella in the C formula synergistically enhances the physicochemical and bioactivities of the cream product.

CONCLUSION

The characteristics of the cream formula containing aloe (*Aloe vera* L. Burm.f.) and rosella flower (*Hibiscus sabdariffa* L.) extracts are semi-solid emulsions and O/W emulsion type. Increasing rosella extract concentration in the cream decreases the pH value and free radical activity. The C formula enhances SPF value and inhibits collagenase enzyme activity due to the synergistic effect of a combination of *Aloe vera* and rosella extracts. Combining *Aloe vera* and rosella extract in cream formulas can be developed to produce anti-aging cream products.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest in this work.

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