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Research Article

Effect of Randu Honey Concentration on Physicochemical Characteristics and Antioxidant Activity of Vitamin C Derived Cream

Pengaruh Konsentrasi Madu Randu Terhadap Karakteristik Fisikokimia dan Aktivitas Antioksidan Krim Turunan Vitamin C

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ABSTRACT

Vitamin C is a powerful yet unstable antioxidant. Ethyl ascorbic acid (EAA) and magnesium ascorbyl phosphate (MAP) are stable derivatives of Vitamin C. Randu honey is a natural ingredient with antioxidant properties that help counteract free radicals. This study aims to develop EAA cream and MAP cream formulations with added randu honey at various concentrations and to analyze how varying honey concentrations affect the creams' physicochemical characteristics and antioxidant activity. Physicochemical characterization included organoleptic assessment, pH measurement, viscosity determination, spreadability evaluation, and antioxidant activity testing using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The results yielded a white-to-yellowish, homogeneous, oil-in-water emulsion-type, odorless cream. The pH values of each formula were consistent with the skin's physiological pH. Viscosity increased, and spreadability decreased with increasing honey concentration. Antioxidant activity increased with increasing honey concentration, as indicated by a decrease in IC_{50}. In conclusion, honey concentration significantly affects the physicochemical characteristics and antioxidant activity of EAA creams and MAF creams. All cream formulations exhibited very strong antioxidant activity (IC_{50} < 50 µg/mL).

Keywords: Ethyl Ascorbic Acid, Cream, Honey, IC_{50}, Magnesium Ascorbyl Phosphate

ABSTRAK

Vitamin C merupakan antioksidan yang kuat namun tidak stabil. Etil asam askorbat (EAA) dan magnesium askorbil fosfat (MAF) adalah turunan vitamin C yang stabil. Madu randu merupakan bahan alami yang juga mempunyai sifat antioksidan untuk menangkal radikal bebas. Penelitian ini bertujuan untuk mengembangkan formula krim EAA dan krim MAF dengan penambahan madu randu pada berbagai konsentrasi, serta menganalisis pengaruh variasi konsentrasi madu terhadap karakteristik fisikokimia dan aktivitas antioksidan krim tersebut. Karakterisasi fisikokimia yang dilakukan meliputi penentuan organoleptis, nilai pH, viskositas, daya sebar dan aktivitas antioksidan krim menggunakan metode DPPH (2,2-diphenyl-1-picrylhydrazyl). Hasil yang didapatkan pada penelitian ini adalah sediaan berwarna putih kekuningan, berbentuk krim minyak/air, tidak berbau, dan homogen. Nilai pH masing-masing formula sesuai dengan pH fisiologis kulit. Viskositas formula meningkat dan daya sebar menurun seiring dengan meningkatnya konsentrasi madu. Aktivitas antioksidan meningkat seiring meningkatnya konsentrasi madu, yang ditunjukkan oleh penurunan nilai IC_{50}. Kesimpulannya, konsentrasi madu randu berpengaruh terhadap karakteristik fisikokimia dan aktivitas antioksidan pada krim EAA dan krim MAF. Semua formula krim mempunyai aktivitas antioksidan yang sangat kuat (IC_{50} < 50 µg/mL).

Kata Kunci: Etil Asam Askorbat, IC_{50}, Krim, Madu, Magnesium Askorbil Fosfat

I. INTRODUCTION

Skin aging is a biological process influenced by intrinsic and extrinsic factors and can also result from their interaction. Intrinsic factors, also known as chronological aging, are a natural process that progresses with age and is characterized by functional changes in skin tissue. This process is complex because it involves genetic mechanisms common not only to the skin but also to various other body tissues. In addition to intrinsic factors, extrinsic factors strongly influence skin aging and stem from exposure to the external environment (Hussein et al., 2025; Lee et al., 2021). Among these factors, ultraviolet (UV) radiation from sunlight is the main culprit that triggers photoaging (Shin et al., 2023). Both intrinsic and extrinsic factors in the skin aging process, especially exposure to sunlight with UVA wavelengths, can trigger the formation of reactive oxygen species (ROS) at the cellular level. Naturally, the body has a defense system of antioxidant compounds that neutralize and limit the harmful effects of ROS (Bouyahya et al., 2021). However, when ROS production is excessive, the skin's antioxidant defense becomes compromised, creating an imbalance between pro-oxidants and antioxidants. This condition, known as oxidative stress, plays a key role in accelerating skin aging. (Krutmann et al., 2017).

Adverse impacts of reactive oxygen species (ROS) in the body can be

minimized by the antioxidant defense system. The skin naturally relies on various endogenous antioxidants to neutralize free radicals (He et al., 2023). However, this natural capacity is often inadequate when excessive exposure to ultraviolet light, cigarette smoke, or other environmental pollutants triggers oxidative stress. Therefore, topical application of high-concentration antioxidants is an effective strategy for repairing and preventing skin damage. Topical antioxidant application has been shown to significantly increase antioxidant levels in skin layers by directly penetrating target tissue (Hussen et al., 2025; Martins et al., 2020).

One antioxidant compound that could help prevent skin aging is vitamin C, or ascorbic acid, which is known to fight free radicals and support skin cell function. Vitamin C, or ascorbic acid, is a powerful antioxidant. This compound is highly susceptible to oxidation when exposed to heat and air, unstable in alkaline solutions, but remains relatively stable in acidic media. Because of its low stability, vitamin C tends to degrade into L-dehydroascorbic acid (DHA), which can then convert into inactive forms such as L-gluconic acid and oxalic acid. This limitation can be overcome by converting vitamin C into more stable derivatives, such as 3-O-ethyl-L-ascorbic acid (EAA) and magnesium ascorbyl phosphate (MAP) (Caritá et al., 2020; Ravetti et al., 2019).

3-O-ethyl-L-ascorbic acid (EAA) is a modified form of L-ascorbate in which an ethyl group is attached to the third carbon, as shown in Figure 1. This change protects the hydroxyl group at the third position from ionization, thereby increasing the molecule's resistance to oxidation (Iliopoulos et al., 2020) .

Magnesium ascorbyl phosphate (MAP) is a stable, water-soluble, non-irritating derivative of ascorbic acid. MAP has been shown to improve skin viscoelasticity and hydration, and stimulate collagen synthesis. This has led to MAP being widely used in skincare products (Kandil et al., 2022; Khaled et al., 2024; Lakra et al., 2021). The molecular structure of MAP is shown in Figure 1.

Several studies have shown that the effectiveness of vitamin C can be enhanced when combined with other ingredients, including natural ones, which can provide synergistic effects. Previous studies demonstrated that formulations containing ascorbic acid (AA) in combination with other bioactive compounds, such as soy isoflavones, lycopene, α-tocopherol, fish oil, proteoglycans, Rosa moschata oil, ergothioneine, peptides, hyaluronic acid, and plant-derived extracts, may promote collagen deposition and improve various parameters associated with skin aging. In

another study, a serum containing encapsulated vitamin C, vitamin E, and raspberry leaf cell culture extract also improved several skin aging parameters, including pigmentation, elasticity, brightness, softness, dryness, and wrinkles (Boo, 2022; Rattanawiwatpong et al., 2020).

One natural ingredient widely recognized by the public is honey. Honey contains significant antioxidant compounds, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids, and proteins. Honey composition varies depending on several factors. The main factor is the source of the flowers honeybees consume. One of the flowers honeybees consume comes from the randu plant, which produces randu honey. Several studies have shown the antioxidant effectiveness of randu honey (Nasharuddin et al., 2022; Fadhilla et al., 2020; Wahdini et al., 2022; Yunus, 2024).

In this study, EAA creams and MAP creams were developed, each combined with randu honey at different concentrations. Cream was chosen as the dosage form because previous studies showed that cream can optimize skin penetration while limiting systemic effects. Moreover, cream provides moisturizing and emollient effects, as well as comfort due to its pleasant sensory characteristics (Barnes et al., 2021; Gavinet et al., 2024; Kurebayashi et al., 2024). The creams were evaluated for physicochemical characteristics, including organoleptic properties, pH value, emulsion type, flow properties, viscosity, and in vitro antioxidant activity using the DPPH method. This study aimed to develop MAP cream and EAA cream formulations with varying concentrations of randu honey as a topical antioxidant.

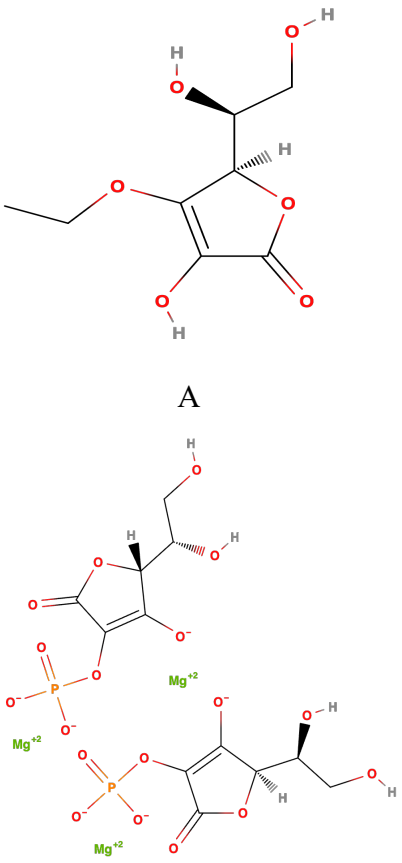


Figure 1. The molecular structure of 3-O-ethyl-L-ascorbic acid (A) and magnesium ascorbyl phosphate (B)

II. METHOD

A. Materials

The materials used in this study included magnesium ascorbyl phosphate (Merck KgaA), ethyl ascorbic acid (Greaf), cetyl alcohol, isopropyl myristate, sorbitan stearate, liquid paraffin, polysorbate 60, propylene glycol, and sodium metabisulfite, all of which were cosmetic grade and purchased from CV. Mutiara Group. The other materials used included Phenoxyethanol (and) Ethylhexylglycerin (euxyl® PE 9010, DKSH), Na_2HPO_4 p.a. (Merck KgaA), NaH_2PO_4, p.a. (Merck KgaA). 2,2-Diphenyl-1-picrylhydrazyl (Merck KgaA), Ethanol p.a. (Merck KgaA), and randu honey (Kembang Joyo).

B. Creams Formulation

Formulas F1-F4 were MAP-randu honey creams. Formulas F5-F8 were EAA-randu honey creams, as shown in Table I. The oil phase was heated and melted at 70-75°C. The aqueous phase was also heated at 70-75°C. After that, the melted oil phase was poured into the aqueous phase and stirred with an ultraturax at 3400 rpm to form a cream base until the temperature reached 40°C; then honey, active ingredient (MAP or EAA), and preservative were added to the cream base and stirred until room temperature.

Tabel I. Formulas of vitamin C derived creams

Function	Materials	Concentration (%)							
		F1	F2	F3	F4	F5	F6	F7	F8
Active ingredient	Randu honey	0	4	6	10	0	4	6	10
	MAP	2	2	2	2	-	-	-	-
	EAA	-	-	-	-	2	2	2	2
Oil phase	Cetyl alcohol	2	2	2	2	2	2	2	2
	Sorbitan stearate	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
	Isopropyl myristate	10	10	10	10	10	10	10	10
	Liquid paraffin	10	10	10	10	10	10	10	10
	BHT	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Beeswax	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Preservative	Phenoxyethanol-Ethylhexylglycerin	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Aqueous phase	Polysorbate 60	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
	Propylene glycol	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
	Sodium metabisulfite	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Phosphate buffer solution pH 6.5 up to	100	100	100	100	100	100	100	100

C. Evaluation of Physicochemical Characteristics

1. Organoleptics and homogeneity

Organoleptic assessment of the creams was performed by visually examining the color and physical form and identifying the smell by odor. A homogeneity test was performed by applying 0.1 g of the cream preparation to a glass slide, then observing for coarse particles or lumps. (Sitompul et al., 2024).

2. Emulsion type

Emulsion type was determined by the methylene blue coloring method. A number of samples and methylene blue were mixed until homogeneous, applied to a glass slide, covered with a cover glass, and then observed under a microscope (Olympus CX-23) at 40×10 magnification using an ocular micrometer lens and a 40×10 objective lens (Salehi et al., 2022).

3. The pH value

The cream's pH was measured using a pH meter (Horiba Laqua pH 1100) calibrated with standard pH 4.00 and 7.00 buffer solutions. The pH meter electrode was dipped into the cream, and the pH value was observed (Sitompul et al., 2024).

4. Antioxidant activity

Antioxidant activity was determined by the DPPH method. A certain amount of cream was mixed with a certain volume of ethanol, then sonicated to obtain the sample solution. The sample solutions in ethanol (5, 10, 15, 20, 25, and 35 µg/mL) were mixed with DPPH solution in ethanol (40 µg/mL) in microplate wells at a 1:1 volume ratio and incubated for 90 min in the dark. Absorbance was measured using a spectrophotometer (Shimadzu UV-800) at 517 nm, and the inhibition percentage was calculated (Equation 1).

$$\% \text{ Inhibition} = (A-B)/A \times 100\% \quad \textbf{(1)}$$

Notes: A is the absorbance of the DPPH blank solution, and B is the absorbance of the sample solution. The IC_{50} value was determined from the regression equation obtained by plotting the percentage inhibition against sample concentration (Khairi et al., 2018; Putri et al., 2021; Sitompul et al., 2024).

D. Data Analysis

The data on viscosity, spreadability, droplet size, pH, and antioxidant activity (IC_{50}) were analyzed in triplicate using one-way ANOVA with SPSS version 27, with $p < 0.05$ indicating statistical significance. Organoleptic characteristics, flow properties, emulsion type, and homogeneity were assessed descriptively.

III.RESULTS AND DISCUSSION

A. Creams Formulation

In this study, creams containing 2% MAP and various concentrations of randu honey (0, 4, 6, and 10%) were formulated as F1, F2, F3, and F4, respectively. Additionally, creams containing 2% EAA and randu honey at 0, 4, 6, and 10% were

prepared as formulas F5, F6, F7, and F8, respectively. The target formulas were oil-in-water creams. The required HLB value for the oil phase to form the cream is 12.04, so the concentration of the emulsifier mixture, i.e., polysorbate 60 (HLB = 14.9) and sorbitan oleate (HLB=4.7), was 3.6% and 1.4 %, respectively. The matching between the required HLB for the oil phase (rHLB) and the HLB of the emulsifier mixture determines the stability of the resulting cream (Smejkal et al., 2024). In addition, BHT is also used in this formula as an antioxidant to prevent oxidation of the oil phase in the cream base (Ghosh et al., 2020). Meanwhile, sodium metabisulfite protects the active ingredients MAP and EAA from oxidation-related degradation. Sodium metabisulfite is a water-soluble antioxidant; it prevents oxidation of water-soluble active ingredients (Shojaee et al., 2017).

B. Physicochemical Characteristics

1. Organoleptics and homogeneity

All cream formulas produced a yellowish-white, odorless, homogeneous semi-solid product.

2. Emulsion type

All formulas produced oil-in-water creams. The test results showed a blue external phase, while the droplets were clear. This is because methylene blue is water-soluble, as shown in Figure 2; these results indicate that all formulas produced oil-in-water creams. This conforms to the planned cream specifications, as the calculated oil-phase rHLB value was 12.04, the HLB value for oil-in-water creams. Oil-in-water creams rinse off easily and are non-greasy, making them more preferred by cosmetic consumers (Fransiska et al., 2021).

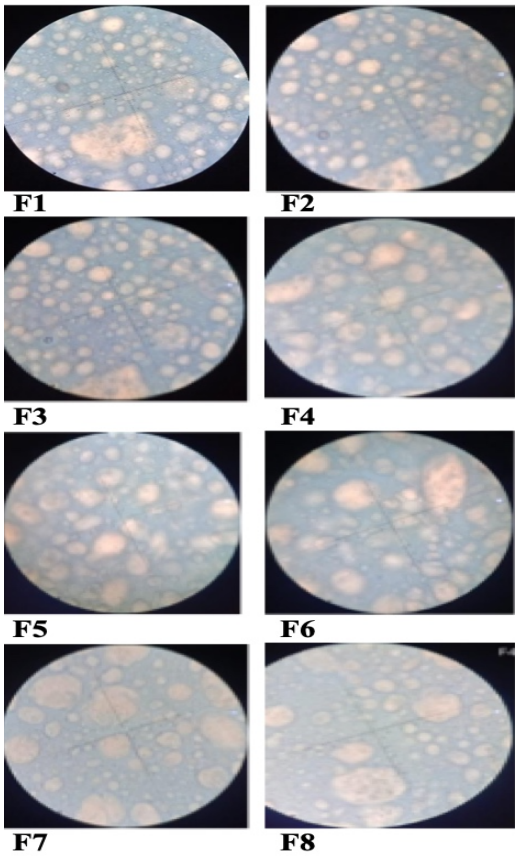


Figure 2. Emulsion type of F1-F8

3. Flow properties and viscosity

Test Results indicated that all cream formulations exhibited non-Newtonian flow characteristics. Viscosity decreases with increasing shear rate (rpm), as shown in Figure 3. This phenomenon indicated pseudoplastic, or shear-thinning, flow

properties in all formulations. Pseudoplastic properties are crucial for evenly distributing the cream when applied to the skin (Nunes et al., 2021).

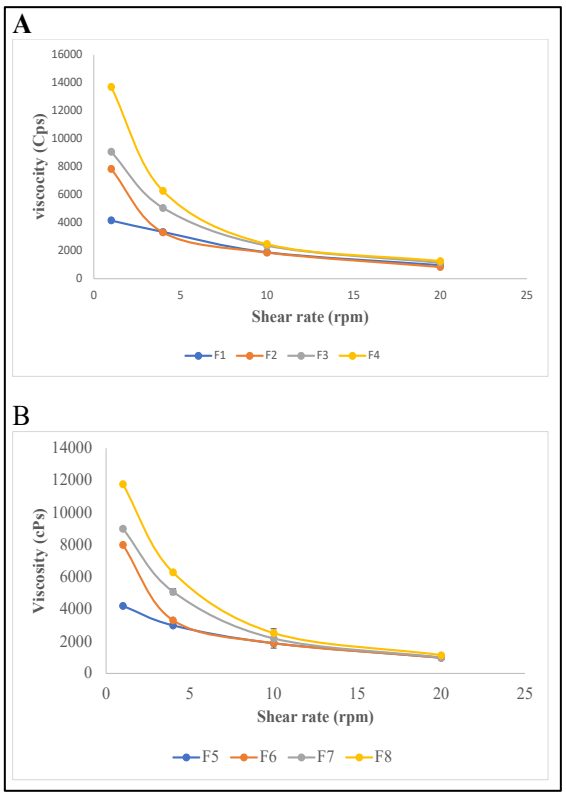


Figure 3. Flow properties of F1-F4 (A) and F5-F8 (B)

The viscosity of the creams was evaluated based on SNI 16-4399-1996, which specifies an acceptable viscosity range of 2000–50,000 cP for cream formulations (Maesaroh et al., 2020). All cream formulations had viscosities between 4000–11,000 cP, as shown in Table II. Therefore, all cream formulations met the SNI viscosity requirements.

The viscosity of the cream increased as the concentration of randu honey in the formula increased, as shown in Table II. Viscosity is an important parameter that represents a formulation's resistance to flow. Higher viscosity increases flow resistance, which in turn affects the cream's spreadability.

4. Spreadability

Spreadability is an essential quality attribute of cream formulations that affects product performance, consumer acceptance, and user compliance. It refers to the ability of semisolid formulations to distribute evenly on the skin after application. Adequate spreadability ensures proper surface coverage, supports effective delivery of active ingredients, and maintains consistent performance during product dispensing and use (Khan et al., 2024; Franco-gil et al., 2024). The test results show that the spreadability of creams in formulations F1–F8 ranges from 6–7 cm, as shown in Table II, which remains within the criteria for good spreadability (5–7 cm) (Nurman et al., 2019). However, as randu honey concentration increased, spreadability tended to decrease because cream viscosity increased. This effect was most noticeable in the formula with 10% honey, which showed the lowest spreadability compared with the other formulas. Therefore, honey concentration significantly affects the cream's viscosity and spreadability.

5. Droplet size

Droplet size influences cream

stability, uniformity, and quality. Smaller droplets enhance physical stability by decreasing phase coalescence and separation. Droplet size also influences drug release, skin penetration, and sensory properties such as softness and spreadability (Chiarentin et al., 2025; Chondrou et al., 2024).

The size of internal phase droplets in formulas F1–F8 ranged from 15–16 μm, as shown in Table II, and showed no significant differences between formulas ($p>0.05$). This uniformity resulted from consistently controlled cream-manufacturing conditions, including surfactant type and amount, temperature, mixing speed, and mixing duration. These factors play an important role in determining droplet size in cream preparations (Simões et al., 2019).

6. The pH value

The pH value of the creams was approximately 5-6, as shown in Table II. The cream's pH tended to become more acidic as the randu honey concentration increased. This is because randu honey is acidic, with a pH of 4.66 (Nasharuddin et al., 2022). However, the overall cream formula remained within the skin's physiological pH range (Sitompul et al., 2024). The pH of the cream formulation was adjusted to the skin's pH to minimize the risk of irritation (Simões et al., 2019).

Table II. Results of viscosity, spreadability, droplet size and pH value of the creams

Fomula	Viscosity (cPs)	Spreadability (cm)	Droplet size (μm)	pH value
F1	4158.00 ± 81.96	7.00 ± 0.10	16.00 ± 0.08	6.35±0.03
F2	7840.33 ± 148.02	6.57 ± 0.12	16.31 ± 0.22	6.30±0.01
F3	9066.67 ± 104.51	6.43 ± 0.12	16.32 ± 0.19	6.28±0.01
F4	13712.00 ± 261.07	6.07 ± 0.12	16.43 ± 0.33	6.25±0.04
F5	4200.33 ± 56.58	6.90 ± 0.10	15.07 ± 0.25	6.40±0.01
F6	7995.66 ± 45.70	6.80 ± 0.10	15.37 ± 0.28	6.15±0.01
F7	9002.33 ± 102.80	6.49 ± 0.09	15.66 ± 0.28	5.91±0.01
F8	11767.67 ± 173.96	6.00 ± 0.10	16.10 ± 0.27	5.83±0.01

7. Antioxidant activity

In this research, antioxidant activity was examined using the DPPH method. The DPPH assay is a simple, fast, and efficient colorimetric method for evaluating free radical scavenging activity. This method is widely used to assess the radical-scavenging ability of active ingredients and pharmaceutical products. DPPH is a stable organic compound containing nitrogen atoms and has a maximum absorption at 517 nm, with a deep purple color. When it reacts with antioxidant compounds, the radical is reduced, indicated by a color change to yellow. The change in color intensity can then be analyzed using a spectrophotometer and plotted against concentration variations (Khairi et al., 2018).

The results showed that MAP creams and EAA creams had very strong antioxidant activity, which increased with the addition of randu honey. The antioxidant activity of MAP creams and EAA creams increased significantly with increasing concentrations of randu honey. This was shown by lower IC_{50} values with increasing honey concentrations in the MAP and EAA cream formulations, as shown in Table III. All cream formulations had very strong antioxidant activity with IC_{50} values < 50 µg/mL.

Antioxidant activity is classified into four categories: very strong with an IC_{50} value of less than 50 µg/mL, strong in the range of 50–100 µg/mL, moderate between 101–150 µg/mL, and weak with an IC_{50} value in the range of 250–500 µg/mL (Jumina et al., 2019). All MAP-randu honey creams and EAA-randu honey creams were oil-in-water (O/W) creams, so the water-soluble honey, MAP, and EAA were present in the external phase. This resulted in very high antioxidant activity in all of the cream formulas. These results also align with previous studies showing that O/W creams containing hydrophilic berry extracts in the external phase provided very strong antioxidant activity (Makarova et al., 2017).

Table III. Results of IC_{50} of the creams

Formula	IC_{50} (µg/mL)
F1	32.22 ± 0.36
F2	22.78 ± 0.42
F3	16.69 ± 0.31
F4	7.68 ± 0.09
F5	30.43 ± 0.55
F6	20.82 ± 0.32
F7	12.61 ± 0.23
F8	6.39 ± 0.1

IV.CONCLUSION

The physicochemical properties of MAP creams and EAA creams were influenced by variations in randu honey concentration. Increasing randu honey concentration was directly proportional to increased viscosity in MAP creams and EAA creams, which decreased spreadability; however, the spreadability value was still considered good. The pH of

MAP creams and EAA creams tended to decrease as randu concentration increased, but it remained within the skin's physiological pH range. In addition, the antioxidant activity of the creams increased significantly with higher randu honey concentrations, and all cream formulations exhibited very strong antioxidant activity, with IC_{50} values < 50 μg/mL.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this research.

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