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Original Article

Formulation development of *Polygonum minus* creams for cosmeceutical application: Characterization and stability studies

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Abstract

Polygonum minus, rich in flavonoid compounds renowned for potent antioxidant and anti-aging properties, is prized for cosmeceutical applications. This study aimed to fabricate, characterize, and evaluate the stability of creams incorporating bioactive compounds from *P. minus* aqueous extract. Three formulations (Placebo, KC1 (5% w/w), and KC2 (10% w/w) were prepared with varying concentrations of *P. minus* standardized aqueous extract while maintaining a consistent cream base composition. Rheological analysis, texture analysis, and stability studies were conducted on the prepared herbal creams, compared with placebo and a commercial vitamin E cream. KC2 exhibited the lowest viscosity (-367.8±6.28 g.sec) and superior spreadabiliy (125.86±1.02 g.sec) compared to placebo (-472.1±5.21 g.sec; 157.22±2.42 g.sec), KC1 (-589.28±2.64 g.sec; 184.35±6.09 g.sec) and commercial vitamin E cream (-1143.79±25.08 g.sec; 381.35±2.26 g.sec). Furthermore, the cream maintained its organoleptic properties and demonstrated excellent physical and chemical stability throughout six months. These results are crucial in supporting the suitability for use in topical skincare products.

Keywords: *Polygonum minus*, cream formulation, rheological analysis, texture analysis, stability study

1. Introduction

Cosmeceuticals are topical products imbued with active compounds that elicit therapeutic effects, positively influencing the intricate biological functions of the skin. These effects encompass the provision of essential antioxidants, enhancing the overall visual attractiveness, luminosity, texture, potential for direct protection against ultraviolet (UV) radiation, and anti-aging properties (Ghazi, 2022; Rita, Taofiq, Ferreira, & Barros, 2021). In recent years, there has been a notable resurgence in the acceptance and utilization of topical herbs as essential components in skincare and dermatological formulations. In 2018, the worldwide natural cosmetics industry reached an estimated valuation of approximately 34.12 billion USD and is projected to grow at a rate of approximately 5.01% from 2019 to 2025 (Natural Cosmetics Market Size, 2019). This trend is driven by a growing awareness and preference for natural and plant-based bioactive products among consumers seeking alternatives to synthetic ingredients. Notably, plant-derived phyto constituents, also referred to as phytocosmetics, stand out for their heightened anti-aging antioxidant capabilities, safety, low toxicity, and cost-effectiveness (Magalhães, Santos, Ribeiro, Alvarenga, & Vilares, 2023; Pattnaik, Mohanty, Sahoo, & Mohanty, 2023), aligning with the global movement

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towards sustainable and eco-friendly healthcare practices (Jab, Ciganovic, Jablan, & Marguí, 2021).

One promising herb that has garnered attention for its cosmeceutical and therapeutic potential in dermatological applications is *Polygonum minus*, commonly known as "kesum" or "Vietnamese coriander." This herb, rich in bioactive compounds, including polyphenols, quinones, tannins, terpenoids and flavonoids, has a long-standing tradition of use in traditional medicine (Christapher *et al.*, 2016; Vikram, Chiruvella, Ripain, & Arifullah, 2014). Recent reports have unveiled its diverse pharmacological properties, encompassing free radical scavenging abilities (Adli *et al.*, 2024; Ahmad *et al.*, 2014; George, Ng, O'Callaghan, Jensen, & Wong, 2014), inflammation inhibiting properties (George, Chinnappan, *et al.*, 2014), and function as an antimicrobial agent (Shen *et al.*, 2018). Phytochemical compounds, notably gallic acid, coumaric acid and quercetin, with aromatic rings in their molecular structures, play a pivotal role in free radical oxygen scavenging and protection against UV radiation damage, thereby contributing optical shielding capabilities (Ghazi, 2022). Quercetin in particular is a valuable ingredient in topical formulations, offering a range of benefits such as antioxidant and anti-inflammatory effects (Zaborowski, Długosz, Błaszak, Szulc, & Leis, 2024). As a potent antioxidant, quercetin neutralizes free radicals, reducing oxidative stress and protecting the skin from damage, in a way that is critical for skin health and preventing premature aging (Zaborowski *et al.*, 2024). Additionally, quercetin inhibits melanogenesis, the process of melanin production, which can help even out skin tone and reduce hyperpigmentation. Studies also show that quercetin shields the skin from UV-induced damage by absorbing UV radiation and minimizing its harmful effects, thereby preventing sunburn and other related issues (Nan *et al.*, 2018). Furthermore, quercetin-loaded formulations, such as chitosan nanoparticles, enhance stability and retention in the skin, allowing for prolonged and consistent delivery of this active ingredient (Nan Wenhao *et al.*, 2018). This plant extract holds a patent that asserts the extract's dual functionality, boasting both antioxidant and anti-collagenase properties (George and Ming, 2015). These unique phytochemical components of *P. minus* position it as a compelling candidate for incorporation into topical creams. These findings underscore quercetin's potential as a valuable addition to herbal creams and other topical skincare products. However, a comprehensive exploration of its formulation, characterization, and stability is imperative to unlock its full potential and address the existing gaps in the herbal skincare market.

Despite the increasing popularity of herbal-based topicals, challenges persist within the current market landscape. Many commercially available herbal formulations lack comprehensive scientific scrutiny, suffering from poor solubility and instability, which lead to variations in efficacy and quality (Cláudia *et al.*, 2019; Teja, Mithiya, Kate, Bairwa, & Chauthe, 2022). Moreover, the scarcity of systematic studies on the stability and formulation aspects of herbal creams often results in inconsistencies in their performance and shelf life. This research aims to contribute to the scientific understanding of herbal formulations by undertaking a detailed investigation into the formulation development, characterization, and stability studies of *P. minus* creams for cosmeceutical use. Through systematic exploration, we aim to bridge the current gaps in herbal skincare, providing a foundation for the development of effective, standardized, and scientifically validated botanical formulations.

2. Materials and Methods

2.1 Materials

Standardized *P. minus* extract was provided by Biotropics Malaysia Berhad. The quercetin standard was acquired from Sigma-Aldrich, USA. Other key ingredients included Olivem® 1000 (Handmade Soap, Malaysia), sodium lactate ($C_3H_5NaO_3$; Dawei Bio-Engineering Co. Ltd., China), phenoxyethanol (Hexza Corporation Berhad, Malaysia), xanthan gum (Earthen Chemicals Sdn Bhd, Malaysia), glycerin (Duro Kimi Sdn Bhd, Malaysia), and olive oil (YKL Multi Sdn Bhd, Malaysia). Deionized water (DI) was obtained from the Reservoir® Elga Water System (High Wycombe, UK). All other chemicals and solvents used were of analytical grade.

2.2 Chromatographic profiling via reverse phase high performance liquid chromatography (RP-HPLC)

The experiment was performed using the Agilent Technologies version 1290 series, Infiniti Liquid Chromatography system with a Phenomenex Kinetex C18 100 Å column (4.6 mm × 150 mm, 2.6 µm) at 36°C and a wavelength of 366 nm. The mobile phase consisted of 0.1% formic acid in water (A), and 0.1% formic acid in acetonitrile (B), with gradient elution at 0.4 mL/min as follows: 0–3 min (90% A), 3–10 min (90%–60% A), 10–12 min (60% A), 12–18 min (60%–10% A), 18–21 min (10% A), 21–22 min (10%-90% A), and 22–25 min (90% A).

2.3 Preparation of cream

Oil-in-water (o/w) creams were prepared using the compositions listed in Table 1. Oil and aqueous phases were heated separately to 70°C and then mixed under continuous stirring. After cooling to 40°C, *P. minus* extract and excipients were added, followed by homogenization at 10,000 rpm for 5-10 minutes to form a uniform cream. The creams were stored in sealed containers at room temperature until further use.

Table 1. Compositions of creams

Name of the ingredient	Percentage (%, w/w)		
	Placebo	KC1	KC2
Oil phase*	11	11	11
Aqueous phase[#]	5	5	5
Other excipients phase[+]	4.5	4.5	4.5
P. minus extract	0	5	10
Distilled water	q.s.	q.s.	q.s.

*Oil phase ingredients: Olivem® 1000, olive oil, cetearyl alcohol
[#]Aqueous phase ingredients: Sodium lactate, glycerine
[+]Other excipients phase ingredients: Glycerine, phenoxyethanol, xanthan gum

2.4 Rheological properties

Rheological properties were evaluated using a Physica MCR301 rheometer (Anton Paar GmBH, Austria) with a serrated parallel plate geometry system (40 mm diameter, 1 mm gap) at 25°C. Measurements included storage modulus (G'), loss modulus (G") and plastic viscosity, recorded over a shear rate range of 1 to 100 s^{-1}. Data shown are averages of three replicates (n=3).

2.5 Texture analysis

Texture properties were assessed using a TA-XT Plus texture analyzer (USA). The back-extrusion test measured hardness, consistency, cohesiveness, and viscosity using a back-extrusion rig (A/BE), while the spreadability test evaluated stickiness, hardness, and work of shear with an HDP/SR* rig. All tests were performed in triplicate (n=3), and results were analyzed using the instrument's software.

2.6 Stability study

Stability testing followed ICH guidelines (ICH, 2003). Cream samples (n=3) were stored under three conditions: (1) 40±2°C and 75% RH in an oven (Venticell, Germany); (2) 5±3°C in a refrigerator (ESCO, Singapore); and (3) 25±2°C and 60% RH for six months. Physical properties, including organoleptic properties and pH, were evaluated monthly (Giradkar and Rode, 2014). Rheological properties (section 2.4) and HPLC analysis (section 2.2) were performed after six months to assess any changes in physical and chemical stability.

3. Results and Discussion

3.1 Chromatographic profiling of *P. minus* aqueous extracts

The aqueous extract of *P. minus* was quantitatively analyzed using RP-HPLC, specifically targeting quercetin in the extract. Figure 1(a) depicts well-separated chromatogram peaks within the retention time range of 1.5 to 12.5 minutes, with the major peak at 2.51 minutes, followed by a significant peak at 10.08 minutes. Hence, to target the presence of quercetin in the extract, the retention time of the principal peak in the extract's chromatogram was compared to that of reference standards (Figure 1(b)), confirming quercetin at 10.08 minutes. The standardized aqueous extract, containing 0.43% quercetin, demonstrates high phenolic and flavonoid contents, further corroborating the recent research by Adli *et al.*, (2024). Various antioxidant assays, including Oxygen Radical Absorbance Capacity (ORAC), Cellular Antioxidant Protection (CAP-e), DPPH, and Ferric Reducing Antioxidant Power (FRAP) assay (Adli *et al.*, 2024; George & Ming, 2015), support its therapeutic potential. The antioxidant mechanism is attributed to the interaction between hydroxyl and phenolic groups and free radicals, which facilitates electron transfer and stabilizes free radicals through resonance. These properties highlight the therapeutic potential in mitigating age-related oxidative stress (Alsabeelah, Arshad, Hashmi, Ahmed, & Khan, 2021) and further justify its incorporation into cosmeceutical formulations designed to protect and rejuvenate the skin, positioning the extract as a valuable ingredient in anti-aging and skin health products.

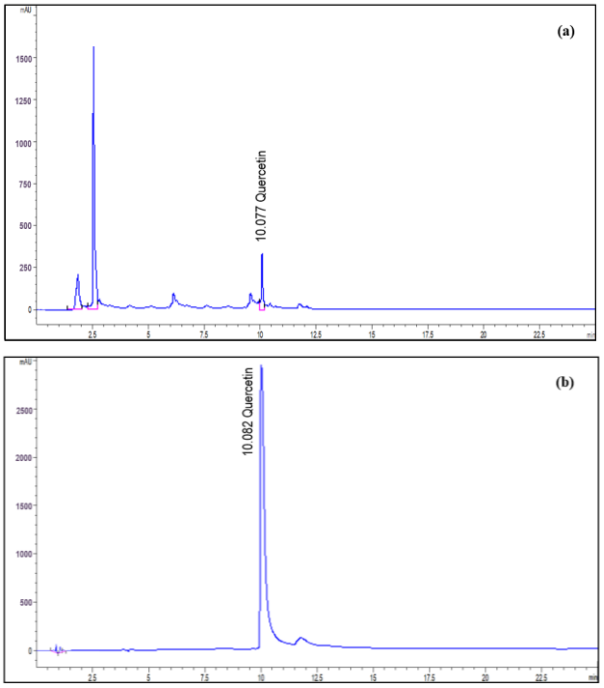


Figure 1. Chromatogram through HPLC at 366 nm. (a) *P. minus* aqueous extract, and (b) reference compound

3.2 Rheological properties

Rheology plays a crucial role in the quality of the cream formulations which will directly impact the characteristics of the final products. Frequency sweep tests, a valuable tool in rheological analysis, provide vital insights into the intermolecular interactions and mechanical spectra of the ingredients within the prepared formulation. In the context of cream formulation, understanding the rheology of creams through storage modulus (G'), loss modulus (G"), and complex viscosity (η*) measurements helps in optimizing the product's texture, spreadability, stability, and overall sensory experience. The desired rheological properties of a cream depend on its intended application, such as whether it should exhibit characteristics of thickness and stability or possess a lighter and easily spreadable consistency.

Figure 2 shows the variation of dynamic moduli (G', G", and η*) with angular frequency (ω) of the creams with different percentages (5% w/w and 10% w/w) of extract, compared to placebo and commercial vitamin E cream. In general, all the samples exhibit G'>G" throughout the ω range, describing stable creams with viscoelastic solid properties (Maccabi *et al.*, 2018) with a tendency towards more elastic behavior (Dabbaghi *et al.*, 2021). There was no point of intersection between the G' and G" of the tested creams from $1s^{-1}$ to $100s^{-1}$ (Figure 2), indicating that all samples predominantly displayed elastic behavior (Dabbaghi *et al.*, 2021) and did not undergo a transition from elastic to viscous behavior within the tested range (Markgraf, Watts, Whalley, Hrkac, & Horn, 2012).

The correlation between G' and G" for each cream's elasticity is further explained by Figure 3(a) of the damping factor (tan δ) versus ω. Initially, all the tested creams exhibit tan δ< 1, with KC1 having the highest value (0.778), followed by commercial Vitamin E cream (0.62), placebo (0.472), and KC2 (0.232). As the angular frequency increases, the damping factor decreases progressively. This indicates that the creams demonstrate elastic behavior and possess the ability to partially recover their initial state after the removal of applied force (Hu, Haruna, Gao, Nourafkan, & Wen, 2017; Wang *et al.*, 2023). This behavior can be attributed to the complex microstructure in cream formulations, which influences their rheological properties (Korhonen, Hellen, Hirvonen, & Yliruusi, 2001). Among the creams tested, the commercial cream demonstrates the most significant decrease in tan δ when the applied shear rate exceeds $1s^{-1}$. Moreover, this commercial cream exhibits similar or minimal damping changes with increasing angular frequency. A higher damping factor (tan δ) suggests predominantly viscous behavior, indicating greater energy dissipation capacity in the material. Conversely, a lower tan δ correspond to a more elastic behavior in the material (Tafuro, Costantini, Baratto, Francescato, & Semenzato, 2020).

Furthermore, the complex viscosity of all tested creams decreased as the frequency (ω) increased (Figure 3B). Initially, the commercial cream (722 Pa*s) had the highest viscosity, followed by placebo (456 Pa*s), KC1 (411 Pa*s), and KC2 (390 Pa*s). Throughout the experiments, however, these creams exhibited relatively similar viscosities, with all displaying shear thinning behavior (Mrokowska & Krztoń-Maziopa, 2019). The difference in viscosity between the commercial vitamin E cream and the formulated creams is likely due to the variations in the emulsifying agents used. The commercial cream incorporates glyceryl monostearate, whereas the formulation creams use Olivem 1000, a derivative of olive oil (Wojciechowska, Walczak, Rostowska, & Poleszak, 2021). Glyceryl monostearate exhibits stronger thickening properties and has the potential to yield higher viscosity than Olivem 1000 due to the formation of a gel-like network (Eccleston, 1997) within the cream. This network restricts the movement of the dispersed phase, resulting in increased viscosity.

3.3 Texture profile analysis

Texture profile analysis was conducted to investigate the textural properties of the creams under applied force (Bogdan, Moldovan, Man, & Crișan, 2016). This analysis provides valuable insights into replicating human sensory perception during topical application (Ain *et al.*, 2021; Kumar *et al.*, 2014; Mustaffa *et al.*, 2021). Key metrics evaluated include firmness, which quantifies the force needed to induce deformation in an elastic formulation (Tai, Bianchini, & Jachowicz, 2014), and cohesiveness, which represents the cohesive forces that contribute to the structural integrity of the prepared creams (Ain *et al.*, 2021). As shown in Figure 4, KC2 exhibits the least firmness (69.54±1.46 g), consistency (574.68±9.06 g.sec), cohesiveness (-42.54±0.54 g), and viscosity index (-367.8±6.28 g.sec), followed by the placebo cream and KC1. Conversely, the commercial

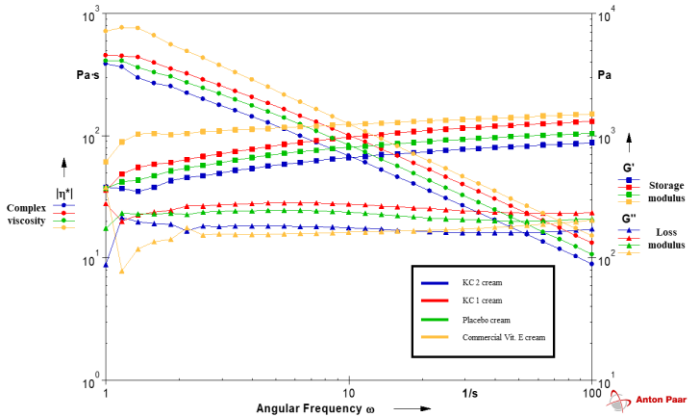


Figure 2. Frequency sweep test of each tested cream

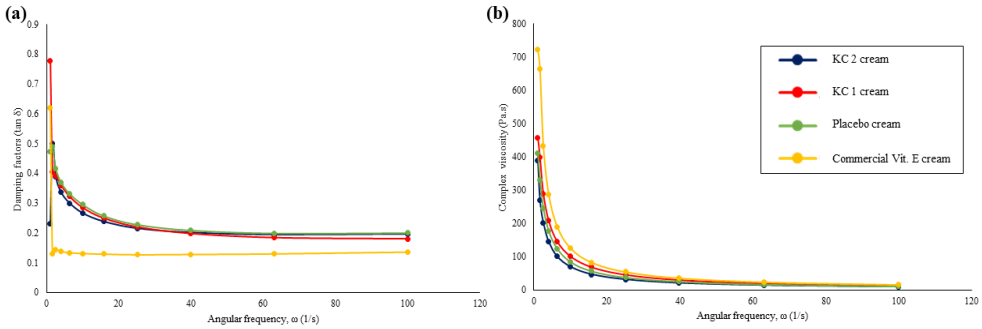


Figure 3. Effects of angular frequency (ω) on (a) the damping factor (tan δ), and (b) the complex viscosity of each tested cream

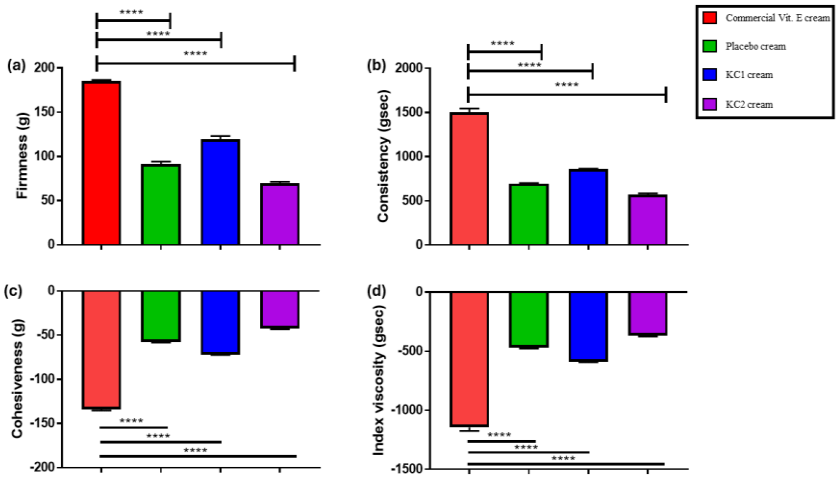


Figure 4. Back extrusion test for commercial vitamin E, placebo, KC1 & KC2 cream. The test is represented by (a) firmness, (b) consistency, (c) cohesiveness, and (d) viscosity index. Data are presented as mean ± SD, n=3. Differences were calculated using one-way ANOVA, followed by Tukey's post hoc test, and deemed significant at $p < 0.05$.; n.s = not statistically significant.

vitamin E cream demonstrates significantly higher ($p<0.05$) firmness (185.11±1.20 g), consistency (1505.75±32.62 g.sec), cohesiveness (-133.86±1.1g), and viscosity index (-1143.79±25.08 g.sec). These findings indicate that the prepared creams (KC1, KC2 and placebo) show significantly reduced resistance to deformation and weaker internal structural integrity. The observed characteristics can be attributed to the presence of *P. minus* aqueous extract and the use of Olivem 1000 in the formulation, as previously discussed. Notably, the formulated creams require less shear force to spread on the skin (Estanqueiro, Amaral, and Sousa Lobo, 2016; Kryscio *et al.*, 2008). This characteristic allows for consistent application of active ingredients, potentially enhancing the cream's effectiveness and desired outcomes.

These findings were corroborated by the spreadability test (Figure 5), where the commercial vitamin E cream displayed significantly ($p<0.05$) higher firmness (397.41±2.42 g) and work of shear (381.35±2.26 g.sec) compared to the formulated creams. These were followed in rank order by KC1 (193.13±4.51 g; 184.35±6.09 g.sec), placebo (173.63±0.83 g; 157.22±2.42 g.sec), and KC2 (144.77±0.74 g; 125.86±1.02 g.sec), respectively. The work of shear in the spreadability test measures the energy or work required to shear or spread the cream over a given surface area (Garcia-Fontanals, Llorente, Valderrama, Bravo, & Talens, 2023). In simpler terms, it quantifies the effort needed to apply the cream and distribute it evenly. Both firmness and work of shear from texture analysis contribute to understanding a cream's spreading behavior. The results indicate that KC1, placebo, and KC2 exhibited greater spreadability due to the lower work of shear needed to overcome the forces between the cream and the applied surface. On the other hand, the commercial cream displayed the lowest spreadability among all evaluated creams. Generally, higher firmness translates to less ease of spreading. These differences can be attributed to variations in cream compositions, as discussed previously. Achieving good spreadability while maintaining a consistent texture on the skin are crucial factors for consumer acceptance and successful product commercialization (Simpson *et al.*, 2015; Tai *et al.*, 2014).

3.4 Stability study

The stability of formulated products is a critical criterion for any cosmeceutical dosage form. Accelerated stability studies are employed to assess formulations and address stability concerns during the transportation or storage of products at room temperature. As shown in Table 2, the formulated creams exhibited minimal changes (or specify the observed changes) in organoleptic properties. The pH of the formulated creams remained within a range close to the skin's physiological pH (4.5 – 6.4) (Alzomor, Moharram, & Al Absi, 2014; Lukić, Pantelić, & Savić, 2021) and displayed stable variations throughout the 6-month study period. These findings suggest that the active ingredients in the creams remained stable and did not undergo significant degradation. Stability testing for the conventional vitamin E cream was not repeated, as it had already undergone a comprehensive stability study confirming its suitability for market release and consumer use.

3.4.1 Rheological properties after a six-month stability study

The stability of *P. minus* cream formulations (KC1 and KC2) was assessed through rheological studies over a 6-month period under various temperatures (5°C, 25°C, and 40°C) as illustrated in Figures 6 (a-c). Rheological properties, including the loss modulus (G") and storage modulus (G'), are reliable predictors of formulation stability and guide manufacturers in product development (Nogaeva *et al.*, 2022). The analysis of loss modulus (G") and storage modulus (G') revealed that there was no intersection between these values, indicating that the creams maintained their viscoelastic properties, which is essential for consistent application and absorption. The absence of an intersection further suggests that the creams retained their structural integrity, crucial for

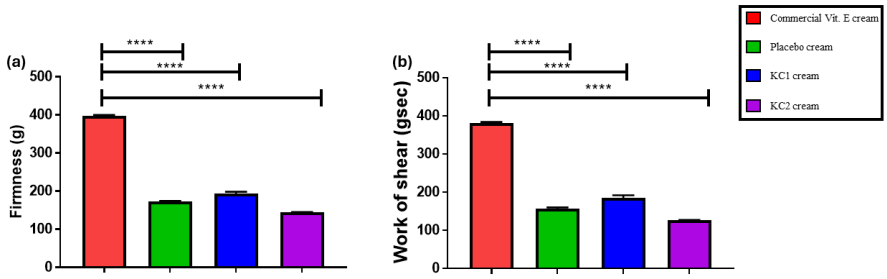


Figure 5. Spreadability test for commercial vitamin E, placebo, KC1 & KC2 cream. The test is represented by (a) firmness, and (b) work of shear. Data are presented as mean ± SD, n=3. Differences were calculated using one-way ANOVA, followed by Tukey's post hoc test, and deemed significant at $p < 0.05$. n.s = not statistically significant.

Table 2. Organoleptic properties and pH of formulated creams after 6-month stability study

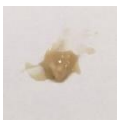
(a) at 5±3 °C

Formulation	KC1				KC2			
Time (months)	0-month	1-month	3-month	6-month	0-month	1-month	3-month	6-month
Appearance								
Color	Brown	Brown	Brown	Brown	Dark brown	Brown	Brown	Brown
Odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour
pH	5.58	5.52	5.53	5.55	5.58	5.54	5.52	5.53

(b) at 25°C ± 2°C / 60% RH ± 5%

Formulation	KC1				KC2			
Time (months)	0-month	1-month	3-month	6-month	0-month	1-month	3-month	6-month
Appearance								
Color	Brown	Brown	Brown	Brown	Dark brown	Dark brown	Dark brown	Dark brown
Odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour
pH	5.58	5.54	5.52	5.53	5.75	5.77	5.77	5.73

(c) at 40°C± 2°C/75% RH ± 5%

Formulation	KC1				KC2			
Time (months)	0-month	1-month	3-month	6-month	0-month	1-month	3-month	6-month
Appearance								
Color	Brown	Brown	Brown	Brown	Dark brown	Dark brown	Dark brown	Dark brown
Odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour	Herby odour
pH	5.58	5.54	5.52	5.44	5.75	5.70	5.62	5.45

topical applications (Chiarentin, Cardoso, Miranda, & Vitorino, 2023). Both KC1 and KC2 formulations demonstrated adequate resilience to varying storage conditions (5°C, 25°C, 40°C), which is vital for maintaining product quality over time (Jin *et al.*, 2023).

This stability suggests that the formulations are resilient to temperature-induced stress over time, highlighting the suitability of P. minus based creams for extended storage Understanding these properties is essential for ensuring the quality and efficacy of topical creams (Gräbner & Hoffmann, 2017).

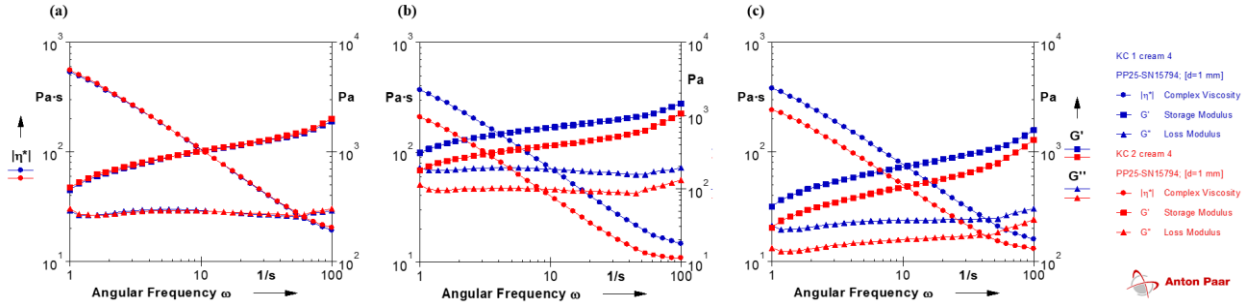


Figure 6. Frequency sweep test of each tested cream after 6-month storage at (a) 5ºC, (b) room temperature (25ºC), and (c) 40ºC.

3.4.2 Stability of quercetin in *P. minus* cream formulation

The stability of quercetin in the *P. minus* cream formulation was confirmed through HPLC analysis conducted after six months of storage (Figure 7). The analysis revealed a consistent peak at a retention time of 10.06 minutes, closely matching the initial profiling at 10.08 minutes (Figure 1(a)), with a similar peak area observed between the initial and six-month analyses, indicating no significant degradation of quercetin within the cream formulation over the storage period. This finding demonstrates the stability of quercetin, which is essential for maintaining the antioxidant efficacy and therapeutic potential of the cream as a cosmeceutical product. The sustained presence and stability of quercetin reinforce the product's suitability for long-term use in skin health applications, offering reliable antioxidant protection and promoting skin rejuvenation.

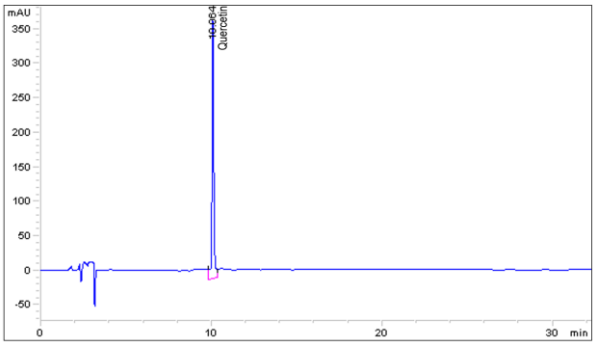


Figure 7. HPLC chromatogram of *P. minus* cream formulation after six months of storage

4. Conclusions

This study successfully formulated *P. minus* creams (KC1 and KC2) and compared them to a placebo and a commercial vitamin E cream. The formulated creams exhibited better spreadability, requiring less work for application on the skin compared to the commercial cream. They also maintained stability after six months of storage at different temperatures (5°C, 25°C, and 40°C), as shown by rheological analysis. The loss modulus (G") and storage modulus (G') exhibited no intersection, confirming the creams' retention of viscoelastic properties, which are crucial for consistent application and skin absorption. Additionally, HPLC analysis confirmed the stability of the bioactive compound quercetin, with no significant degradation observed over the storage period. This stability is vital for preserving the antioxidant efficacy and therapeutic benefits of the cream for topical use. Among the formulated creams, KC1, containing 5% (w/w) *P. minus* aqueous extract, emerged as the optimal choice due to its favorable characteristics compared to the 10% (w/w) concentration in KC2. This work suggests the potential of KC1 as a vehicle to deliver the bioactive ingredients of *P. minus* to the skin, opening doors for further exploration as a topical application in cosmeceuticals or other skin disease studies.

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