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Original Research Article

Formulation and Stability Evaluation of Facial Lifting Serums Incorporating *Pelargonium graveolens* for 5-Lox and Hyaluronidase Activity Suppression

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ABSTRACT

Plant-based products offer promising avenues in anti-aging skincare. In this study, both *Pelargonium graveolens* flower extracts using different extraction methods, and lifting facial formulations incorporating the hydroethanolic extract were evaluated for their Dermocosmetic potential. The hydroethanolic sonicated extract demonstrated significant hyaluronidase inhibitory activity (IC_{50} = 17.86 ± 0.49 µg/mL) and effectively suppressed 5-lipoxygenase activity (IC_{50} = 18.65 ± 0.96 µg/mL). No statistically significant differences were observed in sun protection factor (SPF) (24.33 - 26.02) values across the extraction methods. Serum formulations enriched with the extract (2%), exhibited enhanced 5-lipoxygenase inhibition (IC_{50} = 13.9 - 17.5 µg/mL), and hyaluronidase inhibition (IC_{50} = 11.76 - 31.66 µg/mL). All samples showed interfacial tension-lowering capacity. F1 and F2 remained stable over 90 days; F3 showed phase separation. Minimal changes in physicochemical parameters confirming interfacial tension-lowering capacity, and antioxidant effects of the extract, with potential lifting and anti-inflammatory effects.

Keywords: Anti-aging, Formulation, 5-Lipoxygenase, *Pelargonium graveolens.*

Introduction

Extracellular matrix is a central pathway involved in structural and functional deterioration of the epidermis.[1]Among the enzymes involved, hyaluronidase plays a pivotal role by catalysing the hydrolysis of hyaluronic acid, a major glycosaminoglycan responsible for dermal hydration and viscoelasticity. This enzymatic activity leads to a progressive loss of moisture, elasticity, and volume within the dermis.[2] Notably, increased hyaluronidase expression has been reported in both intrinsic ageing and photoaging, underscoring its relevance as a therapeutic target in strategies aimed at preserving cutaneous integrity.[3, 4] While numerous small-molecule inhibitors have been described.[5] Recently, the emphasis has increasingly been placed on agents based on plants, which combine biocompatibility with beneficial security profiles.[6] For such candidates to progress from laboratory discovery to practical application, their performance within finished formulations must be established.[7]In the Dermocosmetic field, evaluation extends beyond biological activity to encompass physicochemical stability, rheological performance, and the capacity to preserve formulation homogeneity under thermal and mechanical stress.[8] Such assessments are essential to ensure that plant extracts retain their functional efficacy when incorporated into complex delivery systems such as serums or emulsions.[8] Stable incorporation at efficacious concentrations is regarded as a prerequisite for industrial adoption, as instability can lead to variable dosing, diminished shelf-life, and compromised consumer acceptance.[9]

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In fact, the combined requirements of potent enzymatic inhibition and formulation stability provide the framework for current research efforts, guiding the selection, optimisation and eventual commercial deployment of new natural active ingredients designed to reduce the visible signs of skin aging.

Plants are widely explored for their reservoir of bioactive compounds, including flavonoids, alkaloids, and tannins, which contribute to their therapeutmedicinal and economic importance.[10] *Pelargonium graveolens* (*P. graveolens*), a fragrant perennial herb of the Geraniaceae family, has been previously investigated for its pharmacological and biological properties; including anti-inflammatory, antimicrobial, and skin-regenerative effects.[11-13] In the present study, extracts *of P. graveolens* were explored not for their hyaluronidase inhibitory activity, photoprotective capacity and physicochemical behavior in emulsion systems. Notably, to the best of our knowledge, this is the first report to characterize *P. graveolens* extracts in terms of their ability to reduce surface and interfacial tension; key parameters influencing emulsion formation and stability. One extract was subsequently incorporated into facial serums, and the physical stability was monitored over a 90-day period under accelerated conditions.

Materials and Methods

Plant Collection and Identification:
Plant material and extraction
The flowers of *P. graveolens* were harvested in May 2022 from Sahel Boutaher (Taounate, Fes-Meknes region, Morocco; 34°29'52.067" N, 4°48'17.397" W). The species was authenticated by Professor Hamid Khamar from the Department of Botany and Plant Ecology at the Scientific Institute, Mohamed V University of Rabat. A voucher specimen (RAB114770) was deposited in the herbarium of the same institute. The collected flowers were air-dried for two weeks, the dried flowers were crushed with an electric grinder (Fritsch, Industriestrasse 8 55743 Idar-Oberstein Germany.) until a fine powder was obtained (diameter ≤ 300 µm). for subsequent analyses.

Extraction of Plant materials:
Three extraction methods were then used to obtain the phytochemical compounds from the plant material: maceration (w/v: 10 g/100 mL), sonication (w/v: 10 g/100 mL) and infusion (w/v: 2.5 g/75 mL). The solvent used for maceration and sonication was ethanol (100%) and

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ethanol-water (70:30), while water (100%) was used for infusion. Following extraction, the liquid extracts were filtered and were then concentrated under reduced pressure using a rotary evaporator (Rotavapor R-300, Büchi Labortechnik AG, Switzerland). The remaining aqueous portions were subsequently frozen and lyophilized with a freeze-dryer (FreeZone 2.5 L, Labconco, USA).

Facial lifting serums formulation:
For each of the three formulations (F1, F2, and F3), the aqueous phase was prepared by combining distilled water, glycerine, and propylene glycol in a 1 L glass beaker under continuous stirring. Once homogenised, the emulsifier was gradually incorporated, followed by the sequential addition of all remaining components as detailed in Table 1. Each mixture was then homogenized with a mechanical stirrer (Philips GmbH HR2535/00 650W, Hamburg, Germany). The solutions were agitated until a uniform white aqueous mixture was obtained (approx. 3 minutes), cooled and collected in glass jars. The formulas were also pH-adjusted with triethanolamine. Xanthan gum, tragacanth gum or *Linum usitatissimum* seed extract was used to provide a thicker consistency.

Table 1: Liquid serum formulas composition

F1 (INCI)	%, w/w	F2 (INCI)	%, w/w	F3 (INCI)	%, w/w
Aqua	Q.S	AQUA	Q.S	AQUA	Q.S
Xhantan gum	0.2	Tragacanth Gum	0.2	*Linum usitatissimum* seed extract	0.2
Sodium Benzoate	0.3	Sodium Benzoate	0.3	Sodium Benzoate	0.3
Glycerin	7	Glycerin	7	Glycerin	7
Propylene glycol	4	Propylene glycol	4	Propylene glycol	4
Myritol	10	Myritol	6,5	Myritol	0.2
Polysorbate 80	0.5	Polysorbate 80	0.5	Carboxymethy cellulose	0.5
Triethanolamine (50%)	-	Triethanolamine (50%)	-	Triethanolamine (50%)	-
P.graveolens extract	2	*P.graveolens* extract	2	*P. graveolens* extract	2

Q. s: Quantum Satis, Values are expressed as mean ± standard error (n = 3). Distinct superscript letters within the same column denote statistically significant differences ($p < 0.05$). Ingredients were obtained from BASF Beauty Care Solutions, Lyon, France.

Stability
pH, conductivity, water activity, and lipid oxidation stability were evaluated at various intervals post-production (1, 15, 30, 60, and 90 days) to detect any changes over time. pH and conductivity were measured directly from the samples using a pH and conductivity meter (BOECO Bench-CT-676, Germany). Water activity was assessed by measuring the weight difference of initial and dried sample at 100°C for 48 hours. Lipid oxidation was evaluated using (CDR FoodLab® Junior, Perugia, Italy) and results were expressed quantitatively as meqO2/Kg. Physical stability was examined through centrifugation (Sigma 2-16P, Osterode am Harz, Germany) at 3000 rpm for 30 minutes, simulating accelerated conditions, and subjected to six temperature cycles between 4 °C and 40 °C, with each cycle consisting of 24 h at each temperature. Changes in visual appearance, including color and odor, were assessed and compared between the freshly prepared formulations.

Interfacial and surface tension characteristics
Interfacial and surface tension were measured by the pendant drop method using a fully automatic tensiometer (DataPhysics Instruments GmbH - Filderstadt, Germany). Briefly, the extract (1%, w/w) was placed in glass syringe and injected through a 22-gauge stainless steel needle into air (surface tension), or soybean oil placed inside a glass cell (interfacial tension). The drop of extract solution was incubated for approximately 10 min inside the continuous phase, during which a high-resolution camera captured its dimensions to measure the interfacial or surface tension using the Young-Laplace equation and calculated automatically by dpiMAX software.

Hyaluronidase inhibition assay
The inhibitory activity against hyaluronidase was evaluated using a previously described method.[14] with some modifications. Briefly, the reaction mixture contains 25 µL $CaCl_2$ (12.5 mM), 12.5 µL of *P. graveolens* extracts or the facial lifting serums containing the hydroethanolic sonicated extract, 12.5 µL hyaluronidase enzyme (1.5 mg/mL in 0.1 M acetate buffer, pH = 3.5, Thermo Fisher Scientific, Waltham, USA), and 100 µL hyaluronic acid (1 mg/mL in 0.1 M acetate buffer, pH 3.5, Carbosynth Ltd, Compton, UK). Following incubation, the mixtures were heated in a water bath at 100°C for 3 minutes, then cooled at room temperature. A volume of 25 µL potassium tetraborate (0.8 M) was added, followed by 800 µl 4-(dimethylamino)benzaldehyde. Once the 20-minute incubation period had elapsed, the absorbance was recorded at 600 nm (FLUOstar® Omega BMG Labtech, USA). Inhibition percentage was calculated using the equation (1). Results are presented as mean ± SEM of IC50 (µg/mL) values. Retinol was tested as a positive control.

$$I\% = \frac{(A_{c-}\, A_{Bc}) - (A_S - A_{BS})}{(A_C - A_{BC})} \times 100 \quad (1)$$

A_c : Hyaluronic acid, hyaluronidase and acetate buffer.
A_{Bc} : Includes Hyaluronic acid and acetate buffer.
A_S : Extract, Hyaluronic acid and tyrosinase.
A_{BS} : Extract and Hyaluronic acid.

5-Lox inhibition assay
The *P. graveolens* extracts and the formulated products were evaluated for their inhibitory activity against 5-LOX following the established protocol developed by Wisastra and collaborators.[15] The reaction mixture was composed of 4 µL of *P. graveolens* extracts or facial lifting serum containing the hydroethanolic sonicated extract, 4 µL of 5-LOX from Glycine max (100 U/mL, Cayman Chemical, Ann Arbor, USA), and 40 µL of phosphate buffer (0.1 M, pH = 9.0). After pre-incubation at room temperature for 5 minutes, 4 µL of linolenic acid (4.18 mM in ethanol, Sigma-Aldrich, St. Louis, USA) was added. The formation of conjugated dienes was monitored by measuring absorbance at 234 nm (FLUOstar® Omega (BMG Labtech, USA). Results were expressed as IC_{50} values (µg/mL) and reported as mean ± SEM from three independent experiments (n = 3). Quercetin was used as the positive control.

UV-B absorption: photoprotective effect
P. graveolens extracts were investigated for their protective activity against UV-B rays according to Mansur's method.[16] The extracts absorbance (2 mg/mL) was measured at a range of wavelengths between 290 and 320 nm, with 5 nm intervals and a UV-VIS spectrometer (RoHS Uv-1800pc, Macy, China) was used. The Sun Protection Factor (SPF) was then calculated by applying the equation (2), where EE x I values are constants as set by Sayre et al. [17] Zinc oxide was used as the positive control.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times (\lambda) \times Abs\,(\lambda) \quad (2)$$

Where:
EE: Erythemogenic effect.
I: The radiation intensity.
Abs: Absorbance of extract.
CF: Correction factor.

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Data analysis

Experiments were carried out in triplicate (n = 3). Analysis of data was performed using Microsoft Excel, with results presented as mean ± standard deviation (SD). An ANOVA test followed by a Tukey's multiple comparisons test were undertaken using OriginPro 2024 (OriginLab Corporation, Massachusetts, USA). Different results were considered significantly different at $p < 0.05$.

Results and Discussion

Inhibition of 5-Lipoxygenase (5-LOX)

Inflammatory skin conditions are in many cases triggered by external stimuli such as UVB exposure, which induce oxidative stress and subsequent inflammatory processes. Cutaneous inflammation is a key factor in skin ageing, inducing loss of elasticity and irregular skin tone.[18] Therefore, reducing skin inflammation represents a promising strategy to prevent or reduce age-related skin damage. Increasingly, research and industry are turning their attention to natural anti-inflammatory agents. Integrating natural ingredients in cosmetic formulations offers significant added value by effectively reducing inflammation and improving skin texture, thereby achieving a therapeutic effect that is also cosmetically beneficial.[19] In this study, both the crude extracts and the formulations based on the extract were tested for their potential to suppress 5-LOX enzyme, the key one involved in fatty acid oxidation and in the biosynthesis of leukotrienes, major mediators of inflammation. Thereby various extraction techniques and solvents were used to assess their influence on bioactivity (Table 2).

Table 2: SPF and IC_{50} (µg /mL) values against 5-LOX and Hyaluronidase enzymes.

Extract/ Formula	SPF	5-LOX	Hyaluronidase
Sonication EtOH- H_2O	26.02 ± 0.020[b]	20.86 ± 1.66 [d, e]	31.46 ± 1.36 [b]
Sonication EtOH	24.33 ± 0.15[b]	18.65 ± 0.96 [d, e]	17.86 ± 0.49 [d]
Maceration EtOH-H_2O	24.78 ± 0.127[b]	17.5 ± 1.80 [e, f]	22.03 ± 0.87 [c]
Maceration EtOH	25.49 ± 0.016 [b]	62.16 ± 2.46 [b]	32.13 ± 0.15[b]
Infusion H_2O	25.56 ± 0.059[b]	82.23 ± 1.15 [a]	43.23 ± 1.59 [a]
F1	n.d	17.50 ± 1.13 [e, f]	11.76 ± 0.68 [e, f]
F2	n.d	13.9 ± 1.82 [c]	14.26 ± 0.20 [b]
F3	n.d	30.73 ± 2.28 [f]	31.66 ± 1.52 [e]
Standards	11.88 ± 2.08 [1, a]	22.83 ± 0.66 [2, d]	10.7 ± 0.65 [3, f]

1: Zinc oxyde, 2: Quercitin, 3: Retinol, n.d: not defined. Values are expressed as mean ± standard error (n = 3). Distinct superscript letters within the same column denote statistically significant differences ($p < 0.05$).

The IC_{50} values for 5-LOX inhibition varied markedly across the tested samples, ranging from 13.9 ± 1.82 µg/mL for F2 to 82.23 ± 1.15 µg/mL for the aqueous infusion. F2 exhibited the most potent inhibitory effect, significantly surpassing the standard reference (22.83 ± 0.66 µg/mL) ($p < 0.05$). Comparable activity was observed for Formula 1 and the hydroalcoholic macerate (17.50 ± 1.13 and 17.5 ± 1.80 µg/mL, respectively) ($p < 0.05$), while sonicated ethanolic and hydroalcoholic extracts showed moderate inhibition (18.65 ± 0.96 and 20.86 ± 1.66 µg/mL, respectively). In contrast, both the ethanolic macerate and aqueous infusion displayed considerably weaker activity, with higher IC_{50} values of 62.16 ± 2.46 and 82.23 ± 1.15 µg/mL, respectively ($p < 0.05$).

The inhibitory effects of *P. graveolens* flower extracts on 5-LOX are likely explained by their high phenolic compound content, as previously demonstrated in our phytochemical study.[11] Among the major compounds previously identified are quercetin, kaempferol, myricetin, catechin and epigallocatechin, as well as their glycosidic derivatives. Significant levels of phenolic acids, including gallic, caffeic, ferulic and rosmarinic acids have also been identified.[20] It was demonstrated that quercetin inhibited LOX-dependent oxygenation of linoleic acid in a concentration and time dependent manner, with 50% inhibition observed at 3 µM and complete suppression above 100 µM. Kinetic studies revealed a non-competitive inhibition, attributed to the reduction of peroxidation intermediates such as linoleic acid radicals. Quercetin oxidation, confirmed by a decrease in absorbance at 380 nm and an increase at 335 nm, was dependent on substrate concentration, with a Michaelis constant (K_m) of approximately 5 µM. While ascorbate at 0.1 mM had no direct effect on LOX-mediated oxygen uptake, it markedly inhibited quercetin oxidation by reducing its oxidised forms, without acting through superoxide scavenging or enzyme inhibition.[21] Other flavonoids, notably cirsiliol (3′,4′,5-trihydroxy-6,7-dimethoxyflavone), demonstrated to exhibit strong inhibition of 5-LOX. Cirsiliol inhibited 5-LOX by 97% at 10 µM (IC_{50} = 0.1 µM) and significantly reduced the release of anaphylactic mediators in sensitized guinea pig lung tissue, while showing minimal effect on cyclooxygenase activity.[21] In another study, Among the flavonoids tested, sophoraflavanone G was the most potent dual inhibitor of COX-1 (IC_{50} = 1.9 µM) and 5-LOX (IC_{50} = 2.2 µM), comparable to indomethacin and NDGA. Kuraridin and kurarinone selectively inhibited COX-1 and 5-LOX. Papyriflavonol A and kenusanone A were selective for 5- and 12-LOX, while morusin, kuwanon C, and sanggenon D showed broader but weaker inhibition. However, quercetin was moderately active.[22] Gavzan et al. developed a nanoemulsion of *P. graveolens* essential oil using Tween 80, producing spherical droplets (553 nm, PDI 0.113). At doses of 50-100 mg/kg, this formulation significantly attenuated formalin-induced nociceptive behaviours, reduced writhing responses, and increased latency in the hot plate test; these effects were reversed by opioid antagonists. Unlike *P. graveolens* oil alone (10 mg/kg), the nanoemulsion (100 mg/kg) also significantly decreased paw oedema.[23] In our previous study, topical application of 4% *P. graveolens* flower essential oil gels in mousse models demonstrated strong, dose-dependent anti-inflammatory effects, with oedema inhibition reaching 56.39% at 4 hours and 80.78% at 6 hours. This efficacy is likely attributed to esters and sesquiterpenes enhancing skin penetration, alongside key constituents such as β-citronellol and geraniol modulating inflammatory mediators including nitric oxide, prostaglandin E_2, and cyclooxygenase. [12]

Hyaluronidase activity suppression

Systemic skin aging is a multifactorial process influenced by intrinsic factors such as metabolic, cellular and hormonal changes, as well as extrinsic factors such as prolonged exposure to environmental pollutants, harmful chemicals and ionizing radiation, which progressively alter the structure and function of the skin.[24] In this context, our study assessed the dermoprotectrice potential of *P. graveolens* flowers in facial cosmetic serum formulations by examining their inhibitory activity against hyaluronidase. Hyaluronidase plays a critical role in the degradation of hyaluronic acid in connective tissues; thus, its inhibition is associated with the preservation of skin integrity and reduced tissue damage.[25] The findings in Table 2 demonstrated IC_{50} values ranged from 10.7 ± 0.65 µg/mL (standard) to 43.23 ± 1.59 µg/mL (infusion H_2O). The most potent formula was F1 (11.76 ± 0.68 µg/mL), showing comparable inhibition to the standard and suggesting strong anti-hyaluronidase activity. F2 followed closely with an IC_{50} of 14.26 ± 0.20 µg/mL, whereas Formula 3 exhibited weaker activity (31.66 ± 1.52 µg/mL), aligning with the range observed for macerated or sonicated ethanolic extracts. Sonication in EtOH-H_2O (31.46 ± 1.36 µg/mL) and maceration in EtOH (32.13 ± 0.15 µg/mL) produced moderate inhibition, while infusion (H_2O) again showed the lowest activity (43.23 ± 1.59 µg/mL). In addition, F1 (IC_{50} = 11.76 ± 0.68 µg/mL) shows promising potential for the management of skin aging and inflammation. Combined with its confirmed photoprotective properties

and stability during storage, these results suggest that the formula can effectively serve as a facial lifting serum, offering protection against sunburn and inflammation while simultaneously providing benefits in delaying the ageing process of the skin. In line with this, recently El Wafa et al [26] reported that polyphenolic extracts from *P. graveolens* significantly attenuated hyaluronidase activity (IC50 = 15.75 ± 0.8 µg/mL), reinforcing the current findings. Plant metabolites, particularly polyphenols, are diverse secondary compounds characterized by multiple hydroxyl groups that confer antioxidant and various biological activities, including antitumor, antiviral, and anti-inflammatory effects.[27] Serval Studies suggested that certain flavonoids effectively inhibit hyaluronidase enzymes involved in extracellular matrix metabolism. This inhibition is structure-dependent: the presence of a C2–C3 double bond, specific hydroxyl groups, and the absence of glycosylation enhance inhibitory potency, while glycoside conjugation generally reduces it. Additionally, low-molecular-weight compound aggregation via acidic groups may contribute to hyaluronidase inhibition, though further research is required.[6] Bralley and his group investigated the effects of various phenolic acids, flavonoids, and condensed tannins from *Sorghum bicolor* on hyaluronidase activity. However, only condensed tannin, apigenin, luteolin, and kaempferol showed significant inhibition. It is likely that condensed tannins inhibit hyaluronidase both by enzyme denaturation and through interactions with its hydrophobic channel.[28]

Photoprotective activity

The Sun Protection Factor (SPF), also referred to as the Protection Index (PI), quantifies a product's capacity to shield the skin from UV radiation.[29] Ingredients with high SPF are indispensable in cosmetic and pharmaceutical preparation of anti-pigmentation and lightening products.[30] *P. graveolens* extracts have been evaluated for their effect in providing medium to high levels of absorption. Among these, the extract obtained by hydroethanolic sonication, and the extract obtained by infusion exhibited the highest protective effects (26.02 ± 0.02 and 25.56 ± 0.059, respectively), while the extracts obtained by hydroethanolic maceration, hydroethanolic or ethanolic sonication provided close values (24.78 ± 0.127, 24.00 ± 4.39 and 24.33 ± 0.15, respectively) (Table 2). All extracts scored higher SPF than the positive control, zinc oxide (11.88 ± 2.088) ($p < 0.05$). A correlation between UVB exposure and collagen expression has been established in previous reports,[31] indicating that prolonged exposure to UVB could lead to collagen degradation, and therefore contribute to skin aging. Polyphenols are extensively proved as natural photoprotective agents, thereby mitigating UV-induced skin damage.[32, 33]

Interfacial and surface tension characteristics

The reduction of surface or interfacial tension plays a key role in preventing droplet coalescence and phase separation in a formulation. [34, 35] However, the surface-active properties of *P. graveolens* flower extracts have not been previously reported. Their dynamic surface tension and interfacial tension were assessed, and are shown in Figure 1. The surface tension and interfacial tension of air-water and soybean oil-water interfaces were between 72 and 25 mN/m at time 0s and reduced to 70 and 19 mN/m at the end of the measurement. As shown in Figure 2, all extracts showed valuable activity in reducing interfacial tension and surface tension. For instance, sonication extracts, reduced the surface tension to approximately 48 mN/m at time 0s and 42 mN/m after 600 s. In addition, extracts obtained by maceration and ethanolic sonication reduced the interfacial tension to approximately 11 mN/m at time 0s and 8 mN/m after 600s. Infusion extract showed a lower reduction of interfacial tension and surface tension, possibly due to the milder conditions of the infusion process, which may not enhance the release of surface-active active compounds. The standard polysorbate 20, which is often used as emulsifier in dermocosmetic formulations, lowered the interfacial and surface tension to 6.93 mN/m and 43.22 mN/m, respectively. Thereby, this finding indicates, that *P. graveolens* flower extracts could be used as natural co-emulsifier in emulsions.

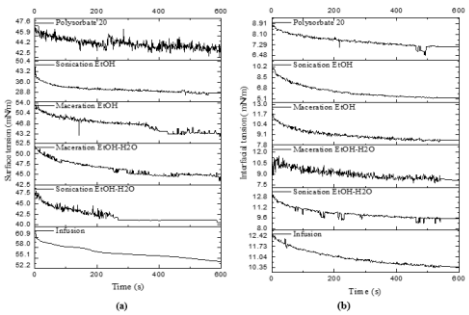

Figure 1: Over time, variations in interfacial tension (a) and surface tension (b) of *P. graveolens* flower extracts.

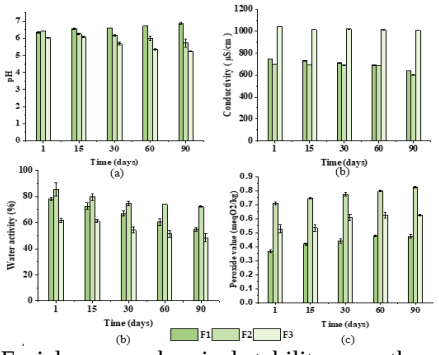

Figure 2: Facial serums chemical stability, over three months: (a) pH values, (b) conductivity, (c) water activity, and (d) peroxide values.

Emulsifiers are used to create stable emulsions with uniform droplet sizes. Their quality depends on several factors, including their ability to reduce interfacial tension, their adsorption rate at the oil-water interface, and their ability to generate sufficient repulsive forces during homogenization.[36] Due to their amphiphilic nature and relatively low molecular weight, saponins can rapidly adsorb to the surface of oil droplets formed during emulsification.[37] The carboxylic acid groups therefore promote the formation of a negatively charged interface around the droplets, which prevents coalescence and improves the stability of the emulsion[38]. It has been demonstrated that the total saponin content is significantly influenced by the solvent system. The hydroethanolic extract was found to have the highest saponin content. Previous reports demonstrated that hydroethanolic solvent improved the extraction of polar saponins. [39]

Stability evaluation

Following the formulation of facial lifting serums (F1, F2, and F3), their stability was evaluated over a period of three months. Accelerated stability tests were performed to assess the formulations under extreme stress conditions. Upon centrifugation, formulations F1 and F2 retained their homogeneity, with no evidence of phase separation, suggesting a robust physical stability under mechanical stress. In contrast, formulation F3 underwent visible phase separation, which may be attributed to the absence of an effective co-stabilizer. The interfacial activity of *P. graveolens* extract alone may have been insufficient to maintain emulsion integrity in this formulation.

Upon thermal stress testing, F1 and F2 maintained consistent organoleptic properties, with no alterations in colour, texture, and homogeneity, further confirming their resistance to temperature-induced instability. In the case of F3, a noticeable browning was observed following thermal cycling, accompanied by a visually more fluid consistency, indicating compromised physical integrity under thermal stress conditions. The formulation F3 primarily composed of carboxymethyl cellulose and *Linum usitatissimum* seed extract as thickening and structuring agents, exhibited notable physical instability over time. The lack of strong emulsifying agents in this formula, likely resulted in insufficient surface coverage of oil droplets, weakening

colloidal interactions and favouring coalescence.[40] Furthermore, exceeding the critical micelle concentration with an appropriate surfactant could potentially improve the integrity of the formulation by promoting finer droplet size distribution and suppressing gravitational separation phenomena.[41] Therefore, optimization of both the type and concentration of emulsifiers remains critical for achieving durable emulsion stability, particularly in formulations involving complex plant-based matrices.[41]

The serums chemical stability; in terms of water activity, conductivity, peroxide value and pH variations over a 90-day period are shown in Figure 2. Overall, fluctuations in the water activity, pH and conductivity of the samples remained minimal over the tested period. For instance, F1 exhibited conductivity values ranging from 747.60 ± 1.53 µS/cm at the time of preparation to 640.3 ± 1.53 µS/cm after 90 days. F2 started at 700.50 ± 601.50 µS/cm and decreased to 601.56 ± 1.25 µS/cm after 90 days. Similarly, F3 initially registered a conductivity of 1044.00 ± 1.00 µS/cm, showing a slight decrease to 1009.00 ± 1.15 µS/cm after 90 days. These values showed significatively minimal variation over time ($p < 0.05$). A correlation between conductivity and stability was previously demonstrated.[42]

The analysis of peroxide value shown in Figure 2 (d), revealed consistently low peroxide values across all formulations. After 90 days, the highest value ($p < 0.05$) was observed in F2 (0.82 ± 0.01 mEq/kg), followed by F3 (0.62 ± 0.01 mEq/kg) and F1 (0.50 ± 0.01 mEq/kg). However, there were statistically no significant variations observed from preparation through 15, 30, 60, and 90 days ($p > 0.05$). The minimal peroxide values may be attributed to the aqueous composition devoid of significant fatty acids. However, the addition of extracts exhibiting lipid peroxidation inhibitory activity may be beneficial in improving the shelf-life and oxidative stability of the formulas. [43] In fact, the physicochemical environment surrounding emulsion droplets has been shown to play a critical role in determining the long-term stability of the system. [44] Parameters such as pH, ionic strength, temperature, and the rheological characteristics of the continuous phase have been identified as major factors influencing interfacial behaviour and droplet interaction dynamics.[45] This influence is particularly pronounced in emulsions stabilized by ionic surfactants, where electrostatic repulsion serves as the primary stabilizing mechanism. [46] Alterations in pH or ionic composition have been observed to significantly affect the surface charge density of dispersed droplets by modulating the adsorption patterns of charged species at the interface.[47] For instance, carboxylate groups confer negative charges under physiological pH conditions, whereas cationic polymers, bearing protonated amine groups, tend to introduce positive surface charges in mildly acidic environments.[48]

Conclusion

The present findings underscore the strong potential of *P. graveolens* extracts as effective bioactive agents for Dermocosmetic applications. The hydroethanolic extract demonstrated notable inhibitory activity against both hyaluronidase and 5-LOX. These results lay a solid foundation for the development of stable, plant-derived topical formulations with lifting and anti-inflammatory benefits. However, to ensure the effective translation of formulas F1 and F2 into clinical use, comprehensive safety assessments, including dermatological tolerance studies, are essential in future development stages.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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