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Microalgae for Anti-Aging Cosmeceuticals: Synergistic Bioactivities and Physicochemical Potential

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ABSTRACT

Microalgae are promising biofactories for cosmeceuticals owing to their ability to produce high-value metabolites with antioxidant and anti-aging potential. Yet, comparative studies across strains, extraction methods, and their application in skincare remain scarce. This study evaluated ethanol-water extracts (20%, 50%, 90%, 100% v/v) from *Thalassiosira weissflogii* TRG10-P105 (Tw), *Arthrospira platensis* (Sp), *Chlorella vulgaris* (Cv), and a 1:1 Sp-Cv mixture (SC). Extracts were initially screened for carotenoids and total phenolics, while selected Tw and SC extracts were further analysed for total flavonoids, phenolic profiles, antioxidant activity (DPPH), and collagenase inhibition as anti-ageing-related bioactivities. 90% ethanol was the most efficient for extracting both hydrophilic and lipophilic compounds. Tw had the highest carotenoid (2.97-8.63 mg g^{-1}) and flavonoid content (8.83 mg g^{-1}), whereas SC showed the highest phenolics (6.57-19.12 mg g^{-1}) with diverse compounds including catechin, quercetin, ferulic acid, and caffeic acid. SC also exhibited the highest radical-scavenging activity (23.96 mg TE g^{-1} DW) and collagenase inhibition (31.4-100%), supporting its anti-ageing-related potential. Based on these results, 90% ethanol extracts of Tw and SC were formulated into anti-aging creams, which showed acceptable pH, spreadability, stability,

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and rheology. These findings highlight Tw and SC as potential microalgal ingredients for further development in anti-ageing cosmeceutical formulations.

Keywords: Anti-aging, antioxidant, carotenoid, microalgae, phenolic

INTRODUCTION

The global cosmeceutical industry is shifting toward natural, sustainable, bioactive-rich ingredients, driven by consumer demand for multifunctional products that enhance appearance while providing therapeutic benefits (Ragusa et al., 2021). The global market for natural and organic cosmetics and personal care products was valued at USD 37 billion in 2022 and is projected to reach USD 58.6 billion by 2031 (Ruf et al., 2024). Cosmeceuticals, which combine the properties of cosmetics and pharmaceuticals, are widely used to manage photoaging, hyperpigmentation, oxidative stress, and inflammation. Growing concerns about the health and environmental risks of synthetic ingredients have further intensified the search for safe, eco-friendly alternatives. Algae have emerged as a promising bioresource for cosmeceutical applications (Table 1) because of their rich metabolic diversity, high biomass productivity, and low ecological footprint. The microalgae market in cosmetics is projected to exceed USD 1.3 billion by 2030, driven by growing interest in naturally derived bioactive ingredients (Chinjoo & Golzary, 2025). This interest is largely due to

Table 1
Various algae strains and their concentration in some formulation types. w/w: weight-to-weight ratio

Algae Species	Concentration	Purpose	Formulation Type	Citation
Laminaria japonica	10% (w/w)	Moisturizing	Cream	Choi et al. (2013)
Rhizoclonium hieroglyphicum	0.5%	Moisturizing	Cream	Leelapornpisid et al. (2014)
Sargassum muticum	0.15% (w/w)	Antioxidant	Cream	Balboa et al. (2017)
Fucus vesiculosus	0.1% (w/w)	Antioxidant	Cream	Hermund et al. (2018)
Spirulina sp.	5% (w/w)	Anti-aging and anti-acne	Cream	Józsa et al. (2020)
Chlorella vulgaris	5%	Anti-aging, antioxidant, and anti-wrinkles	Gel	Amrutkar et al. (2020)
Gracilaria sp.	3%	Psoriasis treatment	Cream	Shatalebi et al. (2020)
Gelidium spinosum	0.75%	Anti-aging	Cream	Poulose et al. (2020)
Sargassum spp.	0.5 g	Brightening and anti-aging	Cream	Al-Momani et al. (2022)
Fucus vesiculosus	28% (w/w)	UV protection and antioxidant	Lotion	Hermund et al. (2022)

their production of carotenoids, phenolics, flavonoids, polysaccharides, and polyunsaturated fatty acids (PUFAs), which exhibit antioxidant, anti-inflammatory, UV-protective, and antimicrobial activities. Their ability to thrive under extreme conditions also enables the synthesis of structurally unique and stable bioactives (Yarkent et al., 2020). Compared with terrestrial plants, microalgae grow year-round, do not compete with food crops for land, and can be cultivated using seawater or wastewater, aligning with the principles of the circular bioeconomy.

Oxidative stress is a major driver of skin aging, causing collagen degradation, reduced elasticity, wrinkles, and impaired skin barrier function. Microalgal bioactives may counter these processes through several mechanisms, including scavenging reactive oxygen species (ROS), limiting lipid peroxidation, modulating endogenous antioxidant defenses, and inhibiting collagen-degrading enzymes (Nascimento et al., 2019). In cellular systems, some microalgal compounds may also attenuate ROS-mediated matrix metalloproteinase expression, thereby helping to limit extracellular matrix degradation. For instance, *Dunaliella salina* is rich in β-carotene and vitamin F, protecting skin from oxidative damage (Tsai et al., 2012); *Haematococcus pluvialis* produces astaxanthin, which reduces blemishes, fine lines, and sagging (Budzianowska et al., 2025); and *Tetraselmis chuii* supports detoxification pathways, delaying aging (Cokdinleyen et al., 2024). Among the studied species, *Thalassiosira weissflogii* TRG10-P105 is notable for its fucoxanthin and eicosapentaenoic acid (EPA), both of which are linked to antioxidant and anti-aging effects (Katayama et al., 2022; Rahman et al., 2020). *Arthrospira platensis* (commonly known as *Spirulina*) is rich in carotenoids and phenolic acids (Uzlaşır et al., 2024), while *Chlorella vulgaris* contains β-carotene, lutein, and zeaxanthin, supporting oxidative stress protection (Serra et al., 2021). Other diatoms, including *Phaeodactylum tricornutum* and *Chaetoceros calcitrans*, also possess omega-3 fatty acids, fucoxanthin, and phenolics, which reduce inflammation and repair barrier function (Mourelle et al., 2024). Since diatoms are inherently rich in fucoxanthin, they may offer greater anti-aging potential compared to phenolic-rich species such as *Spirulina* and *Chlorella*.

Although individual microalgae have been widely investigated for skin-related bioactivities, the effects of combining different microalgal species remain poorly understood. The cosmeceutical potential of individual microalgal ingredients is supported by studies extending beyond chemical assays; for example, a fucoxanthin concentrate from *Phaeodactylum tricornutum* showed collagen-related effects in human fibroblasts and was subsequently evaluated in a topical cream, while a *Dunaliella salina* extract demonstrated antiglycation and anti-inflammatory activities in ex vivo skin models and improvements in selected skin-ageing parameters in a clinical study (Havas et al., 2022). However, comparable evidence for formulations combining multiple microalgal species remains scarce. Studies involving other biological combinations provide some rationale for

examining complementary extract mixtures. Hejna et al. (2024) reported complementary or synergistic antioxidant interactions in selected 1:1 mixtures of the seaweeds *Ascophyllum nodosum*, *Palmaria palmata*, and *Ulva lactuca*, whereas Abu-Serie et al. (2018) observed synergistic antioxidant activity when *C. vulgaris* extract was combined with the mushroom *Ganoderma lucidum*. Although neither study represents a multi-microalgal combination, both indicate that combining extracts with different biochemical profiles can alter antioxidant activity.

Extraction conditions can substantially alter the chemical profile of a microalgal extract because pigments, phenolics, flavonoids, lipids, and other metabolites differ in polarity and solubility. In *Spirulina*, water favoured phycocyanin recovery, whereas ethanol was more effective for chlorophylls and carotenoids, and 70% ethanol yielded the highest polyphenol content (Tavakoli et al., 2021). Ethanol concentration likewise influenced the recovery of carotenoids and lipids from *Heterochlorella luteoviridis* (Jaeschke et al., 2016). In *C. vulgaris*, 90% aqueous ethanol was effective for extracting carotenoids and chlorophylls, while changes in extraction conditions also affected phenolic content and antioxidant activity (Georgiopoulou et al., 2022). Hence, solvent composition influences not only extraction yield but also the relative abundance of bioactive constituents and, consequently, the activity measured in the resulting extract. Nevertheless, the suitability of an extract for cosmeceutical use cannot, however, be inferred from bioactivity alone. Once incorporated into a topical formulation, the extract is exposed to a different physicochemical environment that may influence its dispersion, release, stability, and overall product performance. In *Spirulina*-containing creams, the emulsifying system affected the release of the incorporated material and its antioxidant activity (Józsa et al., 2020). Similarly, creams containing *Spirulina*, *Tetraselmis* sp., or *Dunaliella* sp. showed species-dependent differences in texture and colour stability during storage, despite maintaining acceptable overall physicochemical stability (Dammak et al., 2022). These highlight the importance of evaluating microalgal extracts not only for chemical composition and bioactivity but also for their behavior after incorporation into a topical formulation.

This study therefore compared *T. weissflogii*, *Spirulina*, *C. vulgaris*, and a 1:1 *Spirulina* - *C. vulgaris* mixture extracted with different ethanol concentrations (20-100%). *T. weissflogii* TRG10-P105 was included as a previously identified high-performing strain with strong antioxidant capacity and a metabolite profile rich in carotenoids and n-3 PUFAs (Katayama et al., 2022; Rahman et al., 2020). *Spirulina* and *C. vulgaris*, by contrast, are well-characterised sources of antioxidant and cosmeceutically relevant metabolites and were examined both individually and as a 1:1 mixture to determine whether their combination could improve bioactivity compared with the corresponding single-species extracts. This targeted design allowed *T. weissflogii* to serve as a previously screened reference candidate while specifically testing the effect of combining two established microalgal sources. The

extracts were characterised for carotenoid, total phenolic and flavonoid contents, selected phenolic compounds, radical-scavenging activity, and collagenase inhibition as an anti-aging-related endpoint. Selected extracts were subsequently incorporated into cream formulations and evaluated for appearance, pH, spreadability, physical stability, particle size, and rheological behaviour. By integrating solvent-dependent extraction, individual and mixed-species comparison, bioactivity screening, and formulation characterisation, this study provides a comparative basis for selecting potential microalgal ingredients for further development in anti-aging cosmeceuticals.

MATERIALS AND METHODS

Microalgae and Growth Conditions

Microalgae species including *Thalassiosira weissflogii* TRG10-P105 (Rahman et al., 2020), *Arthrospira platensis* (*Spirulina*), and *Chlorella vulgaris* (Natrah et al., 2007) were maintained in the exponential growth phase using 250 mL Conway medium prepared with 30 ppt sterile seawater, following Tompkins et al. (1995). Cultures were grown under continuous aeration at 24 ± 1 °C, illuminated at 100 µmol photons m^{-2} s^{-1} with a 12:12 h light-dark cycle until sufficient biomass was obtained. On day 7 of cultivation, cells were harvested by centrifugation at 4500 rpm for 10 min, freeze-dried, and stored at -80 °C until analysis.

Extraction of Microalgae with Different Solvents

Prior to analysis, freeze-dried microalgal powders (0.25 mg mL^{-1}) were sonicated (Devey KQ-250DE, China) at 100% amplitude for 10 min in 20%, 50%, 90%, or 100% ethanol to disrupt the biomass and enhance solvent penetration. The suspensions were then incubated at 4 °C for 12-16 h to allow further diffusion and extraction of soluble metabolites before filtration. Alongside the individual preparations of *T. weissflogii* (Tw), *Spirulina* (Sp), and *C. vulgaris* (Cv), a *Spirulina* - *C. vulgaris* mixture (SC) was prepared at a 1:1 ratio by dry biomass weight. Equal amounts of Sp and Cv were combined before extraction and processed under the same conditions as the individual samples. All mixtures were filtered through 0.22 µm PTFE syringe filters to obtain clear supernatants, which were stored at -20 °C until further analysis.

For subsequent analyses, Tw, Sp, Cv, and SC were first screened across four ethanol concentrations for total carotenoid and phenolic contents. Tw and SC were then selected for further characterisation, representing the highest carotenoid and TPC responses, respectively, with SC also serving as the mixed-microalgal preparation of interest. Both were subsequently evaluated for phenolic composition, total flavonoid content, radical-scavenging activity, collagenase inhibition, and cream formulation.

Total Carotenoid Content

Total carotenoid content was determined following the method described by Lichtenthaler (1987). Algal extracts (0.25 mg mL^{-1}) were prepared in triplicate and analysed using a double-beam UV-Vis spectrophotometer (Jasco V-770, Japan). Absorbance readings were taken at 470 nm, 652.4 nm, 665.2 nm, and 750 nm. The absorbance at 750 nm was subtracted from the other readings to correct for turbidity.

Total Phenolic Content (TPC)

TPC was measured using the Folin-Ciocalteu method (Farahin et al., 2019). Extracts (25 µL, 0.25 mg mL^{-1}) were mixed with Folin-Ciocalteu reagent and sodium carbonate, incubated for 2 h in the dark, and measured at 765 nm. Gallic acid (0-100 µg mL^{-1}) was used as the standard, and results were expressed as mg GAE g^{-1} DW (mean ± SD).

Determination of Phenolic Compounds via Ultra-High Performance Liquid Chromatography (UHPLC)

Phenolic compounds in extracts of *Thalassiosira weissflogii* (Tw) and the *Spirulina-Chlorella vulgaris* (SC) mixture (10 mg mL^{-1}) were analysed using UHPLC (Waters Acquity Arc UPLC, USA) with an InfinityLab Poroshell 120-EC-C18 column (4.6 × 250 mm, 4 µm). Detection was performed at 280, 290, 310, 330, and 360 nm. Separation used gradient elution with methanol (phase A) and 1% acetic acid in water (phase B) as described in Table 2. Each run lasted 45 min, consuming ~12.2 mL A and 34 mL B. Compounds were identified by retention times and UV spectra against standards (Table 3).

Table 2
Composition of two different mobile phases for a 45-minute UHPLC

Time (min)	Composition (%)	
	Mobile Phase A	Mobile Phase B
0	4	96
4	4	96
24	22	78
30	60	40
34	90	10
40	20	80
45	4	96

Total Flavonoid Content (TFC)

TFC was measured spectrophotometrically following Srisuksomwong et al. (2023) with minor modifications. A quercetin stock (1 mg mL^{-1}) was used to prepare the calibration curve (0.2-2.0 mL). For each assay, 250 µL of sample (13.3 mg mL^{-1}) or blank was mixed with 50 µL of 10% $AlCl_3$, 750 µL methanol, 50 µL of 1 M potassium acetate, and 1400 µL water. After 30 min incubation in the dark at room temperature, absorbance was read at 415 nm (Jasco V-770, Japan). Results were expressed as mg quercetin equivalents (QE) per g dry weight.

Table 3
Absorbance and retention time of phenolic compounds (45-Minute UHPLC)

Compounds	Absorbance (nm)	Retention Time (min)
Gallic acid	280	7.4
4-hydroxybenzoic acid	280	20.4
Catechin	280	23.0
Protocatechuic acid	290	13.5
Vanillic acid	290	25.9
p-coumaric acid	310	30.9
Caffeine acid	320	27.2
Ferulic acid	320	31.6
Chlorogenic acid	330	25.2
Quercetin	360	34.6
Kaempferol	360	35.4

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Antioxidant activity was assessed with a DPPH Assay Kit (D678, Dojindo). Trolox (0-80 μg mL^{-1}) served as the standard. Samples (20 μL, 13.3 mg mL^{-1}), standards, or blanks were mixed with 80 μL assay buffer and 100 μL DPPH solution in 96-well plates, incubated at 25 °C in the dark for 30 min, and read at 517 nm (Tecan Spark). Results were expressed as mg Trolox equivalents (TE) g^{-1} DW.

Collagenase Inhibition Assay

Collagenase inhibition was measured with a fluorometric Collagenase Inhibitor Screening Kit (Abcam, ab211108). Algal extracts (0.01-0.10 mg mL^{-1}) or control (1,10-phenanthroline) were added to wells with diluted collagenase (5 μL) and fluorogenic substrate (50 μL). After 15 min incubation, fluorescence was recorded at 490/520 nm (Tecan Spark).

Formulation of Anti-Aging Creams

Cream formulation followed a modified protocol from Mawazi et al. (2022). Ingredients (Table 4) were grouped into oil, water, active, antimicrobial, and pH adjuster phases. The oil and water phases were separately heated to 75 °C, combined under stirring to form a stable emulsion, and then cooled to ~30 °C. Algal extracts were incorporated during cooling and mixed thoroughly. The final cream was stabilised at 25 °C for 24 h before physicochemical and biological evaluation.

Table 4
Composition and function of ingredients of the formulated anti-aging creams

Ingredient	Function	% w/w
Oil Phase		
Stearic Acid	Thickening agent and stabiliser	2%
Glyceryl Stearate SE	Emulsifier and improves spreadability	3%
Cetearyl Alcohol	Emulsifier, stabiliser, and enhances texture	2%
Squalane (Plant-derived)	Lightweight emollient and skin barrier repair	6%
Caprylic/Capric Triglyceride (MCT Oil)	Non-greasy moisturiser and enhances penetration	12%
Water Phase		
Distilled Water	Solvent	Q.S. to 100%
Glycerin	Humectant and prevents dehydration	4%
Active Phase		
Microalgae Extract	Anti-aging, antioxidant, and collagen booster	1% or 2.5%
Antimicrobial System		
Liquid Germall Plus	Antimicrobial	1%
Stabilisers & pH Adjusters		
Xanthan Gum	Natural stabiliser and improves consistency	0.3%
Citric Acid	Adjusts pH (4.5-5.5)	0.2%

Note. Change 1% to 0.5% and 2.5% to 1.25% of each microalgae extract for the combination cream. Q.S. (quantum satis or quantity sufficient): Replace microalgae with distilled water for the control formulation

Physicochemical Properties of the Formulated Creams

Appearance, Odour, and Homogeneity Test

The formulated cream was assessed for organoleptic properties, including colour, odour, and homogeneity. Colour was visually inspected under consistent lighting (Dąbrowska et al., 2015), while odour and homogeneity were evaluated as described by Chen et al. (2016). For homogeneity, a small amount of cream was pressed between two fingers to check texture uniformity.

pH, Stability and Spreadability

To determine the pH of the formulation, 0.5 g of the cream was dispersed in 50 mL of distilled water and left to equilibrate at room temperature for approximately two hours. The pH was then measured using a calibrated digital pH meter, following the procedure described by Miettinen et al. (2006). Meanwhile, the spreadability of the formulated cream was evaluated following the method of Chen et al. (2016), with minor modifications. Briefly, 0.5 g of the cream was placed between two glass slides, and a 57 g weight was applied to the upper slide to facilitate spreading. After one minute, the diameter of the resulting spread was measured to assess spreadability.

To determine stability, the creams were stored in 50 mL centrifuge tubes and subjected to six freeze-thaw cycles (-18 ± 2 °C for 24 h, then 40 ± 2 °C for 24 h), following Daudt et al. (2015). After each cycle, stability was checked by centrifuging 1 g of sample at 3,000 rpm for 30 min (Papaioanou et al., 2018; Tafuro et al., 2022). Samples were deemed stable if no creaming, phase separation, or sedimentation occurred (Chu & Nyam, 2020). Colour and odour were also recorded to monitor sensory stability.

Particle Size Test

Particle size distribution was measured using a dynamic image analyser (Litesizer DIA 500, Anton Paar, Austria). Cream samples were dispersed in distilled water, vortexed to avoid agglomeration, and transferred into a water-filled cuvette for analysis.

Rheological Characterisation

Rheological properties were analysed using a rheometer (MCR 320e, Anton Paar, Austria) with a 50 mm CP50-1 plate. Cream samples were carefully loaded to avoid air entrapment, and flow ramp tests were performed by increasing shear rate from 0.1 to 100 s^{-1} at 37 ± 0.1 °C. Viscosity-shear rate plots were generated to evaluate flow behaviour.

Statistical Analysis

Statistical analyses were performed in SPSS v30.0 (IBM, Armonk, NY, USA). Data are presented as mean ± SD from triplicate measurements. Total carotenoid and phenolic contents were analysed by two-way ANOVA using microalgal preparation and ethanol concentration as factors, including their interaction. Collagenase inhibition was analysed similarly, using extract type and concentration as factors. Cream physicochemical properties were compared by one-way ANOVA. Tukey's HSD test was used for post hoc comparisons, and homogeneity of variance was assessed using Levene's test. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Total Carotenoids

Figure 1 shows the total carotenoids of microalgae extracted with varying ethanol concentrations (20-100%). Among these, 100% EtOH consistently yielded the highest ($p < 0.05$) carotenoid levels across all strains (Tw, Sp, SC, Cv), while 20% EtOH produced no detectable carotenoids, confirming its ineffectiveness. Moderate yields were obtained with 50% and 90% EtOH, though significantly lower ($p < 0.05$) than with 100% EtOH. Tw exhibited the highest carotenoid content, reaching 8.63 mg g^{-1} in 100% EtOH, followed

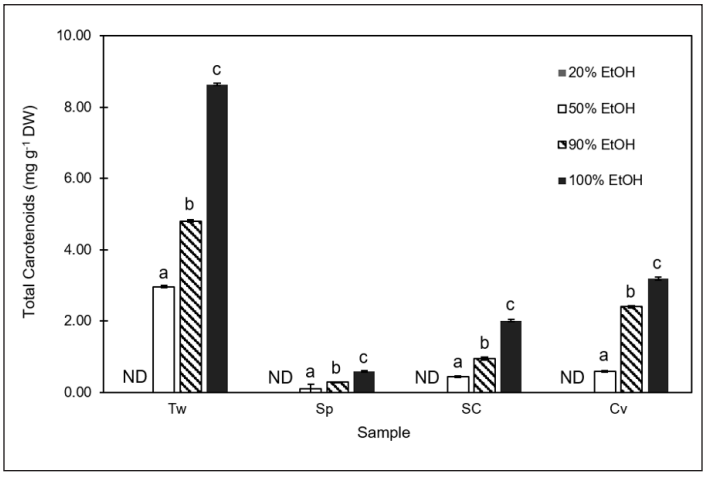

Figure 1. Total carotenoid content. Tw: *Thalassiosira weisslflogii*; Sp: *Spirulina platensis*; SC: combination of *Spirulina platensis* and *Chlorella vulgaris*; Cv: *Chlorella vulgaris*. ND: Not detected. Significant differences ($p < 0.05$) are indicated by different letters

by 4.81 and 2.97 mg g^{-1} in 90% and 50% EtOH, respectively. Cv and SC produced moderate yields, whereas Sp consistently had the lowest yield, at only 0.59 mg g^{-1} in 100% EtOH. Interestingly, the mixed extract SC showed higher carotenoid levels than Sp alone, suggesting a possible synergistic or complementary effect. Previous studies support these trends. *Thalassiosira weissflogii* TRG10-p105 contains high fucoxanthin (11.5 mg g^{-1} DW) and total carotenoids of 20.2 mg g^{-1} DW (Katayama et al., 2022), explaining the higher yields observed in Tw compared to *Spirulina* and *Chlorella*. While *Spirulina* is rich in β-carotene and zeaxanthin (7393 ± 2.76 and 6652 ± 3.69 μg mL^{-1}) (Maoka, 2011), *Chlorella* contains diverse carotenoids, including astaxanthin, lutein, β-cryptoxanthin, and canthaxanthin (Pantami et al., 2020).

Extraction efficiency depends on solvent polarity, solute-solvent interactions, and cell wall properties. Carotenoids are lipophilic; thus, less polar solvents enhance recovery. In this study, 100% ethanol was most effective, consistent with Jaeschke et al. (2016), who reported the highest carotenoid yield in *C. vulgaris* with 75% ethanol (1.21 ± 0.08 mg g^{-1}), compared to much lower yields with 50% and 20%. By contrast, Sp yielded less here than in acetone extraction (1.69 ± 0.07 mg g^{-1}) (Rahim et al., 2021), likely because acetone penetrates *Spirulina*'s protein-polysaccharide cell wall more effectively. Similarly, Katayama et al. (2022) reported higher carotenoids from Tw in 95% methanol (20.2 mg g^{-1} DW) compared to ethanol (8.63 mg g^{-1} DW), reflecting methanol's higher polarity and stronger disruption of pigment-protein complexes. Although methanol can provide efficient pigment extraction, its toxicity limits its suitability for cosmeceutical applications (Mojica et al., 2020). Ethanol therefore represents a more appropriate food- and cosmetic-grade alternative while maintaining effective carotenoid recovery.

Total Phenolic Content (TPC)

Figure 2 presents the total phenolic content (TPC) of microalgae strains extracted with ethanol at concentrations ranging from 20% to 100%. No significant differences ($p > 0.05$) were observed between 50% and 100% ethanol, both of which yielded lower TPC than 20% and 90% ethanol ($p < 0.05$). SC showed the highest TPC with 20% ethanol (19.12 mg g^{-1}), followed by 90% ethanol (12.74 mg g^{-1}), 100% ethanol (7.62 mg g^{-1}), and 50% ethanol (6.57 mg g^{-1}). Sp similarly produced its maximum in 20% ethanol (15.75 mg g^{-1}), whereas Cv and Tw showed higher values with 90% ethanol (15.48 mg g^{-1} and 7.38 mg g^{-1}, respectively).

Solvent polarity plays a key role in phenolic extraction, as the moderate polarity of ethanol dissolves both hydrophilic and lipophilic phenolics while maintaining safety for food and cosmetic use. In *Thalassiosira rotula*, 70% ethanol yielded nearly double the TPC of 50% ethanol (Matas et al., 2023), aligning with the present findings where Tw performed better at higher ethanol concentrations. Similarly, *Chlorella vulgaris* showed higher TPC in 96% ethanol than in water (Garcia et al., 2024), consistent with the elevated values for Cv and Tw in 90% ethanol. By contrast, Sp yielded significantly higher TPC in 20% ethanol, in line with Kumar et al. (2022a), who reported greater phenolic recovery from aqueous extracts. This reflects the hydrophilic nature of many cyanobacterial phenolics, such as simple phenolic acids and water-soluble flavonoids. Notably, the TPC obtained here (15.75 ± 1.30 mg g^{-1}) was higher than previous aqueous reports, suggesting 20% ethanol enhances solubilisation beyond water alone.

The observed patterns also correspond with known phenolic diversity: Sp contains flavonoids such as acacetin, pinocembrin, and sakuranetin, while Cv contains ferulic,

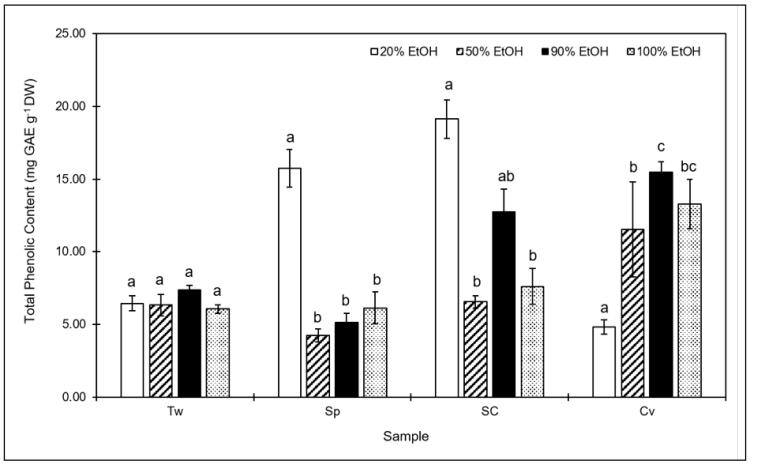

Figure 2. Total phenolic content of *Thalassiosira weissflogii* (Tw), *Spirulina* (Sp), *Chlorella vulgaris* (Cv), and the 1:1 *Spirulina* - *C. vulgaris* mixture (SC) extracted with different ethanol concentrations. Different letters indicate significant differences among treatments ($p < 0.05$)

caffeic, and p-coumaric acids (Guldas et al., 2020; Zakaria et al., 2020). Ferulic acid has limited water solubility but dissolves readily in ethanol, while caffeic and p-coumaric acids are ethanol-soluble (Tuzen & Ozdemir, 2003). These solubility characteristics explain why Sp was optimal at 20% ethanol, whereas Cv and Tw peaked at 90% ethanol. The high TPC in SC at both 20% and 90% ethanol further suggests synergistic effects from combining Sp and Cv. Overall, 90% ethanol emerged as the most versatile solvent, providing strong extraction for both phenolics and carotenoids, especially in Tw and SC. Its intermediate polarity enables recovery of hydrophilic and lipophilic compounds, making it well-suited for multiphase formulations such as emulsions (Cubero-Cardoso et al., 2024). Notably, 90% ethanol extracts from Tw yielded the highest levels of carotenoids, while SC extracts yielded the highest levels of phenolics. This dual enrichment highlights their potential as bioactive-rich candidates for anti-aging cosmeceutical development.

UHPLC Determination of Phenolic Compounds

The phenolic profiles of *Thalassiosira weissflogii* (Tw) and the *Spirulina-Chlorella* combination (SC) were analysed by UHPLC (Table 5). SC exhibited a significantly higher total phenolic content (1156.02 µg g^{-1} DW) than Tw (519.10 µg g^{-1} DW), indicating its richer polyphenolic composition. Both extracts contained gallic acid, 4-hydroxybenzoic acid, vanillic acid, and kaempferol at comparable levels ($p > 0.05$). However, SC demonstrated a broader spectrum, with additional phenolics detected exclusively in this extract: catechin (390.72 µg g^{-1}), quercetin (103.43 µg g^{-1}), chlorogenic acid (58.03 µg g^{-1}),

Table 5
List of major phenolic composition via UHPLC

Phenolic Composition	TW (µg g-1 DW)	SC (µg g-1 DW)
Gallic acid	169.05 ± 0.27a	174.37 ± 2.56a
4-Hydroxybenzoic acid	68.69 ± 2.42a	75.57 ± 5.45a
Catechin	ND	390.72 ± 2.81
Protocatechuic acid	ND	ND
Vanillic acid	208.29 ± 0.42a	208.89 ± 0.77a
p-Coumaric acid	ND	53.85 ± 0.01
Caffeic acid	ND	ND
Ferulic acid	ND	19.69 ± 0.10
Chlorogenic acid	ND	58.03 ± 0.12
Quercetin	ND	103.43 ± 0.26
Kaempferol	73.07 ± 0.15a	72.47 ± 0.27a
Total	519.10 ± 2.47a	1156.02 ± 6.70b

Note. Tw: *Thalassiosira weisslflogii*; SC: combination of *Spirulina platensis* and *Chlorella vulgaris*. Significant differences ($p < 0.05$) are indicated by different letters

p-coumaric acid (53.85 µg g^{-1}), and ferulic acid (19.69 µg g^{-1}). In contrast, Tw was limited to only four detectable compounds, all shared with SC. The broader profile of SC suggests stronger antioxidant potential, as many of these compounds are known to be bioactive. Ferulic and caffeic acids exhibit potent radical scavenging capacity and protect against oxidative stress (Tutun & Yipel, 2024), while quercetin inhibits free radical propagation. These phenolics are also widely used in cosmeceuticals; ferulic acid, quercetin, and gallic acid are common in skincare formulations at 0.1-0.5%, while ferulic and caffeic acids provide photoprotection in sunscreen and after-sun products (Jesus et al., 2025). The high levels of catechin and quercetin in SC thus support its stronger antioxidant and anti-aging potential compared to Tw.

Although studies on Tw remain limited, related diatoms such as *Phaeodactylum tricornutum* contain diverse phenolics including gallic acid, protocatechuic acid, catechin, and vanillic acid, with catechin and sinapic acid as major constituents (Rico et al., 2012). However, catechin was absent in Tw here, suggesting species-specific variation among diatoms. In contrast, previous reports on Sp and Cv align with the present findings. Sp has been shown to contain chlorogenic acid, caffeic acid, quercetin, gallic acid, and kaempferol (Guldas et al., 2020), though at much lower levels than those observed in this study. Arrari et al. (2023) also identified catechin as the predominant phenolic in Sp, consistent with the high catechin content of SC. Similarly, Cv extracts have been reported to contain gallic, chlorogenic, caffeic, p-coumaric, and ferulic acids (Zakaria et al., 2020). The detected phenolics further support the cosmeceutical potential of both extracts. Gallic acid, present in Tw and SC, has been shown to suppress UVB-induced ROS and MMP-1 while increasing type I procollagen in human dermal fibroblasts (Hwang et al., 2014). SC additionally contains chlorogenic acid, which increased collagen I and reduced MMP-1 and MMP-3 in UVA-exposed human dermal fibroblasts (Xue et al., 2022). Ferulic acid has demonstrated topical photoprotective activity in an experimental skin model, while quercetin reduced UV-induced inflammatory signalling in primary human keratinocytes (Lin et al., 2005). These compound-specific activities provide a stronger biological basis for the anti-aging potential of Tw and, in particular, the more phenolics-diverse SC extract.

Total Flavonoid Content (TFC)

As shown in Figure 3, the total flavonoid content (TFC) of *Thalassiosira weissflogii* (Tw) was significantly higher ($p < 0.05$) than that of the *Spirulina-Chlorella* combination (SC), with values of 8.83 and 7.28 mg g^{-1} DW, respectively. This suggests that Tw contributes a relatively flavonoid-rich antioxidant potential. Flavonoids are well recognised for their roles in mitigating oxidative stress, scavenging reactive oxygen species (ROS), inhibiting lipid peroxidation, and modulating signalling pathways associated with inflammation and aging (Matin et al., 2024). Their relevance to skincare is particularly significant, as

oxidative damage from UV exposure and environmental pollutants is a primary driver of skin aging. While data on the TFC of Tw remain limited, Ashashalini et al. (2019) reported a TFC of 2.06 mg g-1 DW using methanolic extraction, significantly lower than the value observed in this study (8.83 ± 0.34 mg g-1 DW using ethanol). This finding suggests that ethanol is more efficient for extracting flavonoids from Tw, likely due to its ability to solubilise both hydrophilic and moderately lipophilic compounds. For SC, the moderately high TFC (7.28 mg g^{-1} DW) may reflect the synergistic contribution of *Spirulina* and *Chlorella*, which together broaden the flavonoid spectrum and enhance the overall antioxidant potential.

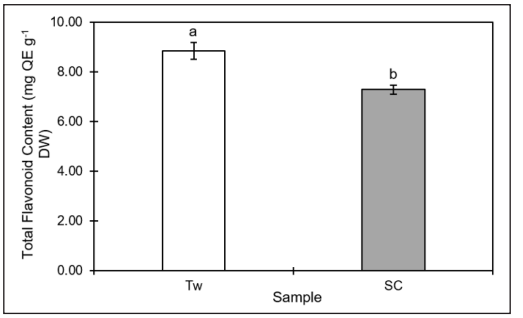

Figure 3. Total flavonoid content of *Thalassiosira weisslflogii* (Tw) and the combination of *Spirulina platensis* and *Chlorella vulgaris* (SC). Significant differences ($p < 0.05$) are indicated by different letters

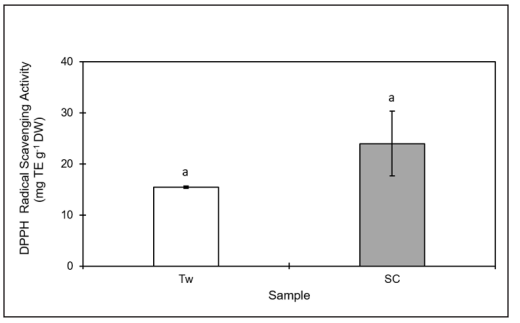

Figure 4. DPPH radical scavenging activity of the samples via a microplate reader. Tw: *Thalassiosira weisslflogii*; SC: combination of *Spirulina platensis* and *Chlorella vulgaris*. A significant difference ($p < 0.05$) is indicated by different letters

DPPH Radical Scavenging Activity

As shown in Figure 4, the DPPH radical scavenging activity of SC (23.96 mg TE g^{-1}) was higher than that of Tw (15.43 mg TE g^{-1}), although the difference was not statistically significant ($p > 0.05$). This trend suggests that SC may have comparatively stronger antioxidant potential. This activity is relevant to anti-aging cosmeceutical applications because mitigating oxidative stress may help limit processes associated with collagen degradation, inflammation, and wrinkle formation.

Tw also exhibited substantial radical-scavenging activity, likely linked to its carotenoid and phenolic contents. Previous studies have reported that Tw contains fucoxanthin and phenolic compounds with antioxidant, anti-inflammatory, and photoprotective properties (Katayama et al., 2022; Rahman et al., 2020), supporting its potential as a cosmeceutical ingredient for skin protection. In contrast, the higher activity observed in SC may reflect its richer, more diverse phenolic profile identified by UHPLC, consistent with previous findings showing stronger antioxidant activity in *Spirulina* than in *C. vulgaris,* which is associated with its higher polyphenolic content (Agregán et al., 2018). *C. vulgaris* may nevertheless contribute complementary phenolics, including ferulic acid, caffeic acid, and

quercetin, which can participate in radical scavenging, metal-ion chelation, and modulation of oxidative processes (Tutun & Yipel, 2024). Thus, combining Sp and Cv may broaden the antioxidant compound profile of SC and contribute to the higher antioxidant trend observed here. Although from a cosmetic-development perspective, no established minimum DPPH activity or bioactive-compound concentration currently defines the suitability of a microalgal extract as a cosmetic ingredient. Accordingly, the measurable antioxidant activity of Tw and SC, together with their quantified carotenoid and phenolic contents, provides functional evidence supporting their further evaluation as cosmeceutical active ingredients.

Collagenase Inhibition Activity

As shown in Table 6, both Tw and SC extracts exhibited a dose-dependent increase in collagenase inhibition. At all tested concentrations, SC demonstrated significantly higher activity than Tw. At 1 µg mL^{-1}, inhibition was 31.4% for SC versus 11.1% for Tw; at 3 µg mL^{-1}, 40.4% versus 23.1%; and at 5 µg mL^{-1}, 67.7% versus 43.7%. At the highest concentration (10 µg mL^{-1}), SC achieved complete inhibition (100%), while Tw reached 86.7%. This was reflected in the IC_{50} values, with SC (3.552 µg mL^{-1}) exhibiting stronger potency than Tw (5.728 µg mL^{-1}).

Table 6
Collagenase inhibition (%) of Tw and SC

Concentration (µg mL-1)	Inhibition Percentage (%)	
	Tw	SC
1	11.1 ± 0.23a	31.4 ± 0.29a
3	23.1 ± 0.91b	40.4 ± 0.01b
5	43.7 ± 0.14c	67.7 ± 0.23c
10	86.7 ± 0.24d	100.0 ± 0.08d
IC50 value (µg mL-1)	5.728a	3.552b

Note. Tw: *Thalassiosira weisslflogii*; SC: combination of *Spirulina platensis* and *Chlorella vulgaris*. Significant differences ($p < 0.05$) are indicated by different letters

Collagenase, a matrix metalloproteinase (MMP), is a key enzyme that degrades dermal collagen and accelerates skin aging (Pittayapruek et al., 2016). Inhibiting its activity is therefore an important strategy in anti-aging skincare. According to Findrianny et al. (2015), IC_{50} values below 50 µg mL^{-1} indicate extremely strong activity, while 50-100 µg mL^{-1} represent strong activity. Based on this classification, SC demonstrated strong-to-extremely strong activity, whereas Tw showed strong activity. Compared with earlier studies, SC displayed higher inhibitory efficiency at lower concentrations. For example, Masoumifeshani et al. (2025) reported 86.21% and 90.52% inhibition for *Spirulina* and *Chlorella vulgaris*, respectively, but only at 80 µg mL^{-1}. Similarly, peptide-rich fractions from *Arthrospira maxima* achieved 92.5% inhibition at 75 µg mL^{-1} (Montalvo et al., 2019), while *Chlamydomonas reinhardtii* reached 83% inhibition at 1 mg mL^{-1} (Falcao et al., 2020). By contrast, brown macroalgae such as *Sargassum plagyophyllum* and *Carpomitra costata* exhibited substantially lower inhibition rates, even at much higher concentrations (Susano et al., 2021). The enhanced inhibition observed in SC may be attributed to the

synergistic contribution of its abundant phenolics, particularly catechin, caffeic acid, and quercetin. These compounds are recognised as MMP inhibitors, acting through zinc chelation at the catalytic site of collagenase, as well as by modulating signalling pathways such as NF-κB and MAPKs that regulate MMP expression (Staniforth et al., 2012). This mechanistic explanation is consistent with the strong radical scavenging capacity observed in the DPPH assay, suggesting overlapping antioxidant and anti-collagenase mechanisms.

In contrast, Tw showed comparatively lower inhibition, which may reflect its bioactivity being driven mainly by carotenoids rather than phenolics. Although carotenoids such as fucoxanthin have demonstrated antioxidant and potential anti-MMP effects (Lee et al., 2024), their inhibitory efficiency against collagenase may be weaker, mechanistically distinct from that of phenolics and flavonoids, or require higher concentrations to achieve comparable effects. Overall, both extracts exhibited strong anti-collagenase activity, but SC consistently outperformed Tw due to its higher phenolic diversity and content. These highlight the value of combining microalgal extracts with complementary phytochemical profiles, as in SC, to achieve enhanced antioxidant and anti-collagenase activity. Such properties reinforce the potential of both extracts, particularly SC, as functional ingredients in dermocosmetic formulations targeting oxidative stress, collagen degradation, and photoaging.

Physicochemical Properties of the Formulated Creams

Five creams were formulated using different microalgal strains and concentrations: a control without microalgae; 1% Tw (Tw1); 2.5% Tw (Tw2); 1% SC (SC1); and 2.5% SC (SC2). Distinct visual differences were observed among formulations (Figure 5). The control cream remained white, while Tw1 appeared yellow and SC1 pale green. At higher concentrations, Tw2 and SC2 developed a darker green colouration, reflecting the higher pigment load. After 24 h incubation at 4 °C, all creams exhibited uniform odour and homogeneity, with smooth textures and pleasant, acceptable scents (Table 7).

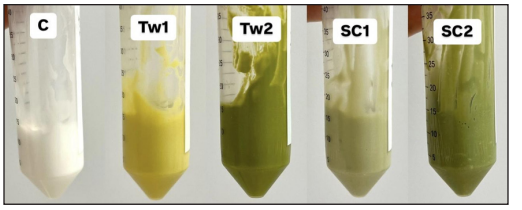

Figure 5. Colour differences of the formulated creams after 24-hour incubation. C: control cream (without algae); Tw1: 1% *Thalassiosira weisslflogii*; Tw2: 2.5% *Thalassiosira weisslflogii*; SC1: 1% combination of *Spirulina platensis* and *Chlorella vulgaris*; SC2: 2.5% combination of *Spirulina platensis* and *Chlorella vulgaris*

The physicochemical properties were strongly influenced by both algal type and concentration. Pigmentation was consistent with the known profiles of the respective species: *T. weissflogii* contains fucoxanthin and chlorophyll, producing yellow-brown hues, while *Spirulina* and *Chlorella vulgaris* are rich in phycocyanin and chlorophyll, respectively.

Table 7
Various physicochemical properties of the formulated creams after 24-hour incubation

Parameter	C	Tw1	Tw2	SC1	SC2
Colour	White	Yellow	Dark green	Pale green	Dark green
Odour	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6.47 ± 0.03a	6.53 ± 0.03a	6.03 ± 0.07b	6.47 ± 0.03a	6.07 ± 0.09b
Spreadability (cm)	3.63 ± 0.15a	3.07 ± 0.15b	2.43 ± 0.12c	3.47 ± 0.03 ab	3.03 ± 0.03b
Stability	No colour and odour changes; stable and no phase separation	No colour and odour changes; stable and no phase separation	No colour and odour changes; stable and no phase separation	No colour and odour changes; stable and no phase separation	No colour and odour changes; stable and no phase separation
Particle size (µm)	36.66 ± 0.89a	22.64 ± 1.01b	15.47 ± 0.39c	9.36 ± 0.02d	50.36 ± 1.46e

Note. C: control cream (without microalgae); Tw1: 1% *Thalassiosira weisslflogii*; Tw2: 2.5% *Thalassiosira weisslflogii*; SC1: 1% combination of *Spirulina platensis* and *Chlorella vulgaris*; SC2: 2.5% combination of *Spirulina platensis* and *Chlorella vulgaris*. Significant differences ($p < 0.05$) are indicated by different letters

Consequently, Tw-based creams (Tw1, Tw2) ranged from yellow to dark green, whereas SC-based creams (SC1, SC2) displayed pale to deep green shades. Increased extract concentration further intensified colouration, whereas the control cream remained pigment-free. Beyond colour, the formulations retained smooth and homogeneous textures, facilitating even distribution during application. The mild, naturally derived aroma from microalgae extracts, achieved without added fragrances, offers an advantage for users sensitive to synthetic scents, thereby supporting the broad acceptability of these formulations (Biswas et al., 2016).

As shown in Table 7, Tw2 (6.03) and SC2 (6.07) exhibited significantly lower pH values ($p < 0.05$) compared to other samples. In contrast, Control (6.47), Tw1 (6.53), and SC1 (6.47) did not differ significantly ($p > 0.05$) and showed higher pH values. A concentration-dependent trend was evident, with higher microalgal content lowering pH. Importantly, all formulations remained within the reported skin-compatible range (4.5-5.75), which is critical for maintaining barrier function, hydration, and microbial balance (Paramanya et al., 2024). Spreadability also declined with higher extract concentrations. Tw2 showed the lowest value (2.43 ± 0.12 cm), while the control exhibited the highest (3.63 ± 0.15 cm). This reduction is likely attributed to increased viscosity from the extracts. Nonetheless, all formulations remained within the acceptable cosmetic range of 18.33-32.33 mm (Fatima et al., 2017), ensuring ease of application. Physical stability was further confirmed by freeze-thaw and centrifugation tests. None of the formulations showed changes in colour, odour, or phase separation after stress cycles. This stability under thermal and mechanical stress highlights the structural robustness and suitability of the creams for topical application.

Based on Table 7, different microalgae strains and concentrations show no direct correlation with the resulting particle size. The higher concentration of Tw resulted in a reduced particle size, while the higher concentration of SC resulted in an increased particle size. Subsequently, SC1 exhibited the lowest ($p < 0.05$) particle size (9.36 μm) among the other samples, suggesting it can be most easily absorbed by the skin. In contrast, SC2 showed the highest ($p < 0.05$) particle size (50.36 μm), indicating that more time is required for absorption by the skin. Moreover, Tw2, Tw1, and Control exhibited moderate ($p < 0.05$) particle sizes, which were 15.47, 22.64, and 36.66 μm, respectively, suggesting they are less effective at being absorbed by the skin than SC1, even though they are more optimal than SC2. Although optimal transdermal penetration is often associated with nanoscale particle sizes (~100 nm), which facilitate absorption through hair follicles (Adib et al., 2016), the creams formulated in this study exceeded that threshold. Larger particle sizes may reduce penetration efficiency and slow down active delivery; however, they can still provide significant topical benefits. These include forming an occlusive layer that reduces transepidermal water loss and releasing bioactive compounds at the stratum corneum, where antioxidant and anti-aging effects remain highly relevant (Hoang et al., 2021). Thus, even without deep skin penetration, the formulations may effectively deliver surface-level efficacy. Nonetheless, future optimisation using advanced technologies such as high-pressure homogenisation, spray drying, or supercritical anti-solvent processing (Kumar et al., 2022b) could further reduce particle size and improve skin absorption.

At low shear rates (~0.1 s^{-1}), Control exhibited the highest viscosity (~240,000 cP; $p < 0.05$), followed by Tw2 (~180,000-200,000 cP). Tw1, SC1, and SC2 showed slightly lower values (~120,000-150,000 cP; $p > 0.05$). At moderate shear rates (1-10 s^{-1}), viscosities narrowed, but Control and Tw2 remained higher, while Tw1, SC1, and SC2 were consistently lower. Under high shear (≈100 s^{-1}), all formulations converged within 500-2,000 cP, indicating comparable performance during application (Figure 6). The lower viscosity values suggest higher water content, contributing to faster skin absorption, though no direct correlation between microalgal strain/concentration and viscosity was observed. Nonetheless, all formulations demonstrated pseudoplastic (shear-thinning) behaviour, with viscosity decreasing as shear rate increased. This flow profile is advantageous for topical products: high viscosity at rest ensures storage stability, while reduced viscosity under stress (e.g., spreading) enhances spreadability and even skin distribution (Deuschle et al., 2015). Differences in viscosity likely reflect extract composition. The higher values in TW may be linked to polysaccharide and protein content, which strengthen emulsion structure via water-binding and thickening effects. In contrast, SC1 and SC2 (Sp-Cv combination) maintained more balanced viscosities, possibly due to phycobiliproteins and chlorophyll derivatives that stabilise emulsions without excessive thickening (Morone et al., 2022). Importantly, all creams maintained high-shear viscosities within the acceptable cosmetic

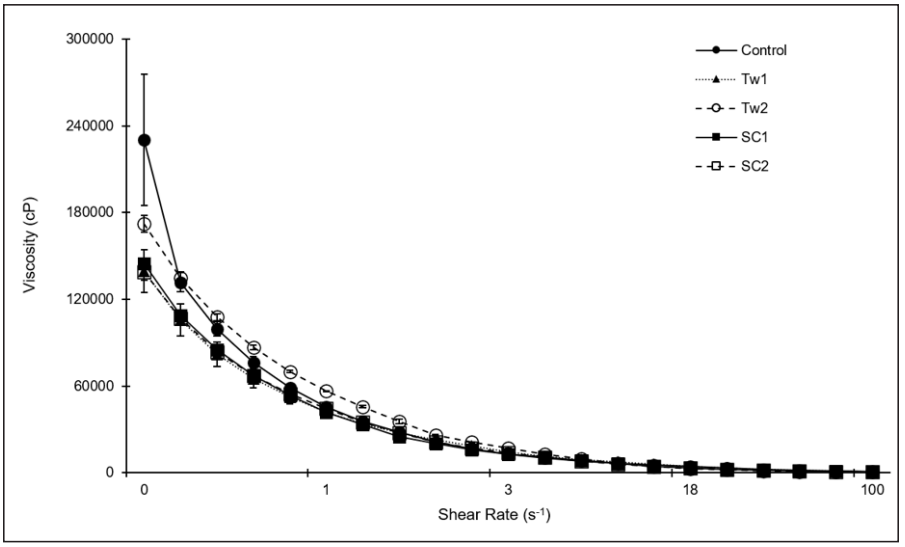

Figure 6. Viscosity of the formulated creams. Control cream (without algae); Tw1: 1% *Thalassiosira weisslflogii*; Tw2: 2.5% *Thalassiosira weisslflogii*; SC1: 1% combination of *Spirulina platensis* and *Chlorella vulgaris*; SC2: 2.5% combination of *Spirulina platensis* and *Chlorella vulgaris*

range (500-5,000 cP), ensuring consumer-friendly flow and effective skin adherence (Stabrauskiene et al., 2025). Overall, the rheological profiles confirm the creams' suitability for cosmeceutical use, balancing structural integrity with ease of application.

CONCLUSION

This study demonstrated the antioxidant and anti-aging potential of the diatom *Thalassiosira weissflogii* (Tw) and a mixed extract *of Spirulina* and *Chlorella vulgaris* (SC) through detailed phytochemical and bioactivity analyses. Tw was particularly rich in carotenoids, whereas SC contained higher levels of phenolics, with strong radical-scavenging and collagenase-inhibiting activities, indicating considerable potential for anti-aging efficacy. Notably, the SC extract exhibited a distinct, enriched bioactive profile, highlighting the potential of mixed-species approaches to enhance the functional properties of microalgal cosmeceutical ingredients. Both extracts were successfully incorporated into stable cream formulations with acceptable physicochemical properties, demonstrating their feasibility for topical use. Overall, these findings position Tw and SC as promising bioactive sources for developing natural cosmeceutical formulations targeting skin aging-related processes.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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