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Optimisation of Extraction Methods, Antioxidant Potential, and Molecular Docking Studies of *Dunaliella salina* and *Spirulina platensis* Extracts for Cosmetic Applications

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Abstract

Microalgae are valuable marine resources with bioactive compounds that can be used for health and industrial applications. This study investigated the optimisation of extraction methods and antioxidant properties *of Dunaliella salina* and *Spirulina platensis* to explore their potential as sources of natural compounds for cosmetic use. Extraction was performed using aqueous, methanol, and ethanol solvents, with methanol yielding the highest recovery in *D. salina* (21.67% ± 0.58) and aqueous extraction being most effective for *S. platensis* (24.00% ± 0.50). Phytochemical analysis revealed solvent-dependent variation, as methanolic *D. salina* extracts showed maximum phenolic content (32.15 ± 2.05 mg GAE/g DW), while aqueous extracts contained the highest flavonoids (94.90 ± 0.90 mg QE/g DW). In *S. platensis*, aqueous extracts exhibited superior phenolic (70.99 ± 1.03 mg GAE/g DW) and flavonoid content (65.39 ± 2.68 mg QE/g DW). Antioxidant capacity assessed by ABTS, DPPH, and FRAP assays confirmed strong radical scavenging and reducing activities, with methanolic *S. platensis* showing the highest ABTS activity (IC_{50} = 113.44 µg/mL) and aqueous extracts demonstrating the strongest reducing power (FRAP IC_{50} = 138.08 µg/mL). Molecular docking revealed strong binding of key phytochemicals with Keap1 protein, suggesting a role in oxidative stress modulation. These findings highlight the potential of *D. salina* and *S. platensis* as marine-derived sources of natural antioxidants for biotechnological and cosmetic applications.

Keywords: microalgae; *Dunaliella salina*; *Spirulina platensis*; total phenolic content (TPC); total flavonoid content (TFC); antioxidant activity; molecular docking; cosmetic applications

1. Introduction

The global market's shift towards natural and sustainable products has driven up demand for marine-derived bioactive components in cosmetic formulations [1,2]. Marine microalgae are particularly valuable resources because of their high metabolic diversity and capacity to produce a wide range of beneficial compounds, such as antioxidants, anti-inflammatory agents, and UV-protective substances [3,4]. Among the thousands of microalgal species, *Dunaliella salina* and *Spirulina platensis* are of particular interest due to their well-documented nutritional and pharmacological properties [5, 6]. *D. salina* is a halophilic microalga known for its high β-carotene content, which accumulates during stress circumstances. This potent carotenoid is a crucial antioxidant that protects skin from oxidative damage and photoaging, making it a popular ingredient in anti-aging and sun-

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care products [5,6,7]. Similarly, the cyanobacterium *S. platensis* is well-known for its high-quality proteins, vitamins, and phycobiliproteins, with multiple studies emphasising its phenolic compounds and flavonoids for their antioxidant and skin-rejuvenating properties [2,4].

The potential of these microalgae is well established; however, the scientific literature reveals several gaps that restrict their complete commercial and therapeutic application. Contemporary studies frequently emphasise general characterisation, failing to adopt a systematic method for optimising the extraction of target compounds. Conventional solvent extractions are foundational but often inefficient and lacking in selectivity, indicating a necessity for improved techniques that enhance yields while preserving compound stability [8,9]. Moreover, although the antioxidant activity of these microalgal extracts is extensively documented [9,10], a comprehensive understanding of their underlying mechanisms is frequently lacking. Connecting observed biological activity to specific molecular interactions offers important insights into efficacy and informs the development of more effective formulations.

This study employs three key methodologies to conduct a comprehensive analysis of *D. salina* and *S. platensis* extracts, thereby addressing existing gaps in the literature. A comparative analysis of various solvent systems was performed to determine the most effective extraction methods for optimising bioactive compound yield. Secondly, a series of in vitro antioxidant assays, including 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Ferric reducing antioxidant power (FRAP), were conducted to quantitatively assess the radical scavenging and reducing capacities of the extracts. Molecular docking studies were employed to understand the mechanistic basis of antioxidant effects by predicting the binding affinities of key phytochemicals with the Keap1 protein, a principal regulator of the cellular antioxidant response [11,12].

These findings contribute to the growing body of knowledge on marine-derived compounds and their potential as natural antioxidants for cosmetic and biotechnological applications. By providing a structured, data-driven approach, this research not only validates the potential of *D. salina* and *S. platensis* but also provides a path for developing effective and environmentally sustainable cosmetic products that meet the evolving demands of the consumer market.

2. Results and Discussion

2.1. Extraction Yields and Solvent-Dependent Efficiency

The quantitative assessment of extraction yields from *Dunaliella salina* and *Spirulina platensis* using aqueous, methanol, and ethanol solvents provided significant insights into their respective biochemical profiles (Table 1). Methanol proved to be the most effective solvent for *D. salina*, yielding the highest result of 21.67% ± 0.58. This suggests that *D. salina* is rich in methanol-soluble compounds, such as carotenoids and other non-polar antioxidants, which are vital for mitigating oxidative stress in cosmetic formulations [13,14]. In contrast, aqueous extraction yielded 18.84% ± 0.86, while ethanol extraction resulted in the lowest yield at 15.54% ± 0.54. Conversely, aqueous extraction was the most effective method for *S. platensis*, yielding the highest result of 24.00% ± 0.50. This indicates a high concentration of water-soluble compounds, such as polysaccharides and hydrophilic proteins, which are advantageous for skin hydration and soothing properties in cosmetic applications [15,16]. Methanol extraction of *S. platensis* was less efficient at 14.50% ± 0.58, and ethanol was the least effective solvent with a yield of 4.06% ± 0.06.

Table 1. Percentage yield of *Dunaliella salina* and *Spirulina platensis* extracts.

Solvent	*Dunaliella salina* Yield (%) (Mean ± SD)	*Spirulina platensis* Yield (%) (Mean ± SD)
Aqueous	18.84 ± 0.86	24.0 ± 0.50
Methanol	21.67 ± 0.58	14.50 ± 0.50
Ethanol	15.54 ± 0.54	4.06 ± 0.06

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The results of a one-way ANOVA confirmed a statistically significant difference in extraction yields across the solvents ($F(2,6) = 9.35$, $p = 0.001654$). Post-hoc analysis showed that both aqueous and methanol extractions yielded significantly higher amounts compared to ethanol ($p < 0.05$). Specifically, the mean difference between aqueous and ethanol was -11.620 ($p = 0.0015$), and the difference between methanol and ethanol was 8.285 ($p = 0.0183$). However, there was no significant difference observed between aqueous and methanol extractions ($p = 0.4431$), suggesting a similar overall extraction efficiency between these two polar solvents, despite their different target compound classes. These findings highlight the critical importance of solvent selection, which must be tailored to the specific target species and desired cosmetic application. The high yield from methanol in *D. salina* supports its potential for anti-aging products rich in antioxidants, while the substantial yield from the aqueous extraction of *S. platensis* highlights its suitability for hydrating and calming cosmetic formulations. These findings are consistent with existing literature [17,18].

2.2. Total Phenolic Content (TPC)

The quantitative assessment of Total Phenolic Content (TPC) provided significant insights into the solvent-dependent extraction efficiency for *Dunaliella salina* and *Spirulina platensis* (Table 2). Methanol extraction of *D. salina* yielded the highest phenolic concentration, with a mean value of 32.153 ± 2.053 mg GAE/g DW. This value was significantly greater than both the aqueous (15.949 ± 1.254 mg GAE/g DW) and ethanol (4.215 ± 0.374 mg GAE/g DW) extractions. This finding aligns with previous studies that report the effectiveness of polar solvents like methanol in interacting with and extracting various phenolic structures [9,19,20]. Phenolic compounds are particularly valued in cosmetics for their ability to protect the skin from oxidative stress, a primary contributor to aging [21]. These findings align with previous research indicating that *D. salina* extracts, particularly those obtained using methanol, are rich in phenolic compounds and exhibit significant antioxidant properties, making them valuable for anti-aging and skin-rejuvenating cosmetic formulations [22].

Table 2. Phenolic Content of *Dunaliella salina* and *Spirulina platensis* extracts.

Microalgae/Extract	Aqueous (mg GAE/g)	Methanol (mg GAE/g)	Ethanol (mg GAE/g)
Dunaliella salina	15.95 ± 1.25a*	32.15 ± 2.05b	4.22 ± 0.37c
Spirulina platensis	70.99 ± 1.03d	1.98 ± 0.05e	27.12 ± 0.85f

* Different letters within a column indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test.

In contrast, *S. platensis* exhibited a starkly different profile, with aqueous extraction yielding the highest phenolic concentration at 70.99 ± 1.029 mg GAE/g DW. This was significantly higher than the methanol (1.98 ± 0.047 mg GAE/g DW) and ethanol (27.12 ± 0.850 mg GAE/g DW) extractions. This result implies that *S. platensis* contains phenolic chemicals that contribute significantly to its antioxidant activities [23,24]. Many of these phenolics are hydrophilic due to their numerous hydroxyl groups, which increases their solubility in water and allows them to interact well in aqueous cosmetic compositions. Such qualities are especially useful in skincare products because water-soluble antioxidants can increase skin hydration and give calming benefits [24]. The increased phenolic content noted in aqueous extracts of *S. platensis* further supports its use in formulations aimed for hydration and skin-calming benefits.

A two-way ANOVA revealed a statistically significant interaction between the microalgae species and the solvent used for extraction ($p < 0.001$), demonstrating that the efficacy of a solvent for phenolic extraction is highly species-dependent. *S. platensis* consistently exhibited higher overall phenolic levels than *D. salina*, emphasizing the importance of selecting both the appropriate microalgae species and solvent to optimize extraction for specific cosmetic benefits [25]. While Tukey's Honestly Significant Difference (HSD) test showed no significant difference in phenolic content between the ethanol extract of *D. salina* and the methanol extract of *S. platensis* ($p > 0.05$), these results further underscore that the choice of solvent should be tailored to the specific application and

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desired characteristics of the final product [26]. Our findings align with previous research indicating that both aqueous and methanol solvents can be optimized for extracting phenolic compounds from microalgae for various applications, including cosmetics [27,28].

2.3. Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of extracts from *Dunaliella salina* and *Spirulina platensis* exhibited significant variation across different solvents, highlighting their unique biochemical profiles and potential for cosmetic applications (Table 3). The flavonoid concentration in *D. salina* was consistently high across all solvents, with values of 94.90 ± 0.904 mg QE/g DW for aqueous, 94.23 ± 2.543 mg QE/g DW for ethanol, and 91.56 ± 1.296 mg QE/g DW for methanol. The consistent high yields of flavonoids in polar solvents indicate that *D. salina* is a valuable source of these bioactive compounds. Flavonoids from marine microalgae are known for their powerful antioxidant activities, which protect skin cells from oxidative stress and improve the efficacy of anti-aging and skin-protective cosmetic formulations [9]. This conclusion is similar with previous research on other marine-derived compounds, such as fish proteins and peptides, which are commonly utilised in cosmeceuticals due to their antioxidant, antibacterial, and skin-repairing properties [29].

Table 3. Flavonoid Content of *Dunaliella salina* and *Spirulina platensis* extracts.

Microalgae/Extract	Aqueous (mg GAE/g)	Methanol (mg GAE/g)	Ethanol (mg GAE/g)
Dunaliella salina	94.90 ± 0.90a*	91.56 ± 1.30b	94.23 ± 2.54c
Spirulina platensis	65.39 ± 2.68d	52.36 ± 1.93e	60.47 ± 2.30f

* Different letters within a column indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test.

In contrast to *D. salina*, *S. platensis* showed a significant solvent-dependent variation in flavonoid content. Aqueous extraction yielded the highest concentration at 65.39 ± 2.678 mg QE/g DW, followed by ethanol (60.47 ± 2.296 mg QE/g DW), and methanol (52.36 ± 1.933 mg QE/g DW). This result highlights the efficacy of water-based solvents in extracting more polar flavonoids, which are advantageous for cosmetic formulations targeting skin hydration and soothing effects [30]. The high flavonoid concentration from aqueous extraction aligns with previous research demonstrating that phenolic compounds exhibit greater solubility in polar solvents like water and ethanol than in less polar solvents [30,31]. The presence of flavonoids in *S. platensis* enhances its antioxidant capacity and supports its potential for anti-inflammatory applications, as extracts from this species have been shown to inhibit pro-inflammatory cytokines [32].

A two-way ANOVA confirmed a significant interaction between the microalgae species and the solvent type ($p < 0.01$), indicating that the optimal solvent for flavonoid extraction is species-specific. *D. salina* proved to be a reliable source of flavonoids independent of the solvent used, as evidenced by the same flavonoid concentration observed throughout water, ethanol, and methanol extractions [9,33]. On the other hand, *S. platensis* higher aqueous extraction efficiency suggests that it could be used in cosmetic products that aim to hydrate and soothe the skin. According to studies, *S. platensis* aqueous extracts not only yield higher amounts of phenolic and flavonoid chemicals but also have positive effects on skin health, including promoting wound healing and offering antioxidant protection [34].

2.4. Antioxidant Activity

2.4.1. ABTS (2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation Decolourization Assay

The ABTS radical cation decolourization assay quantitatively assessed the antioxidant capacity of the microalgal extracts. The ethanolic extract of *D. salina* exhibited the highest antioxidant activity for this species, with an IC_{50} value of 142.32 μg/mL (Table 4a). The significant activity is likely linked to its high carotenoid content, especially β-carotene, known for its capacity to reduce oxidative stress

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and protect against photoaging [35,36]. This finding supports the incorporation of this extract in cosmetic formulations aimed at mitigating environmental stressors and improving skin health.

Table 4. a. Percentage inhibition of ABTS assay by *D. salina and S. platensis* extracts in aqueous, methanolic and ethanolic solvents at different concentrations (µg/mL).

Conc (µg/mL).	*D. salina* Aqueous (%)	*D. salina* Methanol (%)	*D. salina* Ethanol (%)	*S. platensis* Aqueous (%)	*S. platensis* Methanol (%)	*S. platensis* Ethanol (%)
50	18.310	25.34	25.55	24.66	28.96	21.17
100	21.040	45.49	46.11	33.13	45.56	31.97
150	34.970	56.76	53.01	50.48	65.1	49.59
200	59.150	60.31	60.79	58.33	79.03	63.80
250	62.300	68.03	72.95	74.86	83.27	70.36
IC_{50}	193.001	144.08	142.32	156.82	113.44	160.06

The methanolic extract of *S. platensis* demonstrated a lower IC_{50} of 113.44 µg/mL, signifying its enhanced radical scavenging activity compared to the *D. salina* extracts. This finding highlights the potential of *S. platensis* as a vital component in skincare formulations designed to improve skin elasticity and reduce the visibility of wrinkles [37]. The significant free-radical scavenging capacity demonstrated by microalgal extracts indicates their effectiveness in protecting skin cells from oxidative stress, crucial for preserving youthful and healthy skin.

The reference standards, ascorbic acid and Trolox, demonstrated concentration-dependent inhibition, confirming the efficacy of the ABTS assay (Table 4b). Ascorbic acid exhibited 94.13% inhibition at a concentration of 10 µg/mL, whereas Trolox displayed 94.67% inhibition at 2 mM, thereby validating the assay's effectiveness in evaluating the antioxidant potential of these extracts. The observed scavenging activities correspond with the growing consumer preference for natural ingredients and establish these microalgae as effective solutions for reducing oxidative stress and improving skin vitality in the cosmetic industry [37,38].

Table 4. a. Percentage inhibition of ABTS radical cation by Ascorbic acid and Trolox at different concentrations.

Ascorbic Acid Conc (µg/ml)	% Inhibition	Trolox Conc (mM)	% Inhibition
2	25.27	0.125	17.49
4	44.26	0.25	22.27
6	63.25	0.5	47.95
8	85.11	1	74.32
10	94.13	2	94.67
IC_{50}	4.79	IC_{50}	0.80

2.4.2. DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH radical scavenging activity of the microalgal extracts exhibited a positive, dose-dependent correlation with concentration (Table 5a). The methanolic extract of *D. salina* exhibited a moderate antioxidant capacity, with an IC_{50} value of 185.74 µg/mL. The observed activity can be attributed to the extract's high carotenoid content, recognised for its capacity to scavenge free radicals and reduce oxidative damage [39,40]. This finding supports the incorporation of *D. salina* in cosmetic formulations designed to enhance skin health.

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Table 5. a. Percentage inhibition of DPPH assay by *D. salina and S. platensis* extracts in aqueous, methanolic and ethanolic solvents at different concentrations (µg/mL).

Conc (µg/mL)	*D. salina* Aqueous (%)	*D. salina* Methanol (%)	*D. salina* Ethanol (%)	*S. platensis* Aqueous (%)	*S. platensis* Methanol (%)	*S. platensis* Ethanol (%)
50	30.56	42.67	42.42	42.18	42.85	43.77
100	40.46	45.42	44.56	45.23	46.39	45.23
150	44.62	48.41	46.33	50.37	48.41	48.66
200	45.60	49.63	48.59	52.93	49.76	51.47
250	51.47	54.16	52.44	54.83	54.03	52.44
IC50	229.39	185.74	214.83	163.50	183.15	186.49

The aqueous extract of *S. platensis* demonstrated greater antioxidant capacity, evidenced by a lower IC50 of 163.50 µg/mL. The enhanced activity of *S. platensis* is attributed to its high levels of phycocyanin and other hydrophilic phenolic compounds, which demonstrate significant efficacy in neutralising DPPH radicals [30,41]. This finding indicates that aqueous extracts of *S. platensis* are beneficial for formulating skincare products that requires hydration and antioxidant protection [42,43].

In a comparative analysis, the reference standards ascorbic acid and Trolox showed considerably greater antioxidant potency (Table 5b). Ascorbic acid showed an IC_{50} of 4.84 µg/mL, while Trolox presented an IC_{50} of 0.92 mM. The benchmarks suggest that microalgal extracts exhibit notable antioxidant properties; however, their effectiveness is typically lower to that of conventional chemical antioxidants, a pattern consistent with previous comparative research [44]. Such comparative framework is crucial for accurately assessing the antioxidant potential of natural extracts and guiding their effective application in cosmetic formulations [45,46].

Table 5. b. Percentage inhibition of DPPH radical cation by Ascorbic acid and Trolox at different concentrations.

Ascorbic Acid Conc (µg/ml)	% Inhibition	Trolox Conc (mM)	% Inhibition
2	35.56	0.125	15.65
4	47.68	0.25	26.77
6	53.18	0.5	39.61
8	67.36	1	51.96
10	80.81	2	78.24
IC50	4.84	IC50	0.92

2.4.3. FRAP (Ferric Reducing Antioxidant Power) Assay

The results of the Ferric Reducing Antioxidant Power (FRAP) assay demonstrate that the methanolic extract of *D. salina* exhibits considerable reducing power, evidenced by an IC50 value of 159.841 µg/mL (Table 6a). This finding aligns with the literature that identifies methanol as an effective solvent for extracting various bioactive compounds, including polar and non-polar antioxidants such as carotenoids and phenolics, thus improving the overall antioxidant capacity of the extracts [44,47]. The reducing capacity of this extract indicates its potential application in anti-aging cosmetic formulations aimed at mitigating oxidative stress.

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Table 6. a. Percentage inhibition of FRAP assay by *D. salina and S. platensis* extracts in aqueous, methanolic and ethanolic solvents at different concentrations (µg/mL).

Conc (µg/mL)	*D. salina* Aqueous (%)	*D. salina* Methanol (%)	*D. salina* Ethanol (%)	*S. platensis* Aqueous (%)	*S. platensis* Methanol (%)	*S. platensis* Ethanol (%)
50	10.66	17.21	13.93	22.13	18.85	17.21
100	20.49	30.33	18.85	36.07	31.97	28.69
150	25.41	43.44	39.34	55.74	45.08	45.90
200	40.16	63.93	56.56	71.31	63.93	58.20
250	51.64	79.51	69.67	83.61	76.23	72.95
IC_{50}	249.97	159.84	184.59	138.08	181.31	185.44

The aqueous extract of *S. platensis* showed increased antioxidant activity, indicated by a lower IC_{50} of 138.080 µg/mL. This result highlights the important role of water-soluble antioxidants from this species, including phycobiliproteins, especially allophycocyanin, recognised for its potent radical scavenging properties. This finding corresponds with the increasing consumer preference for natural, water-soluble ingredients in skincare products [48,49].

In comparison to the reference standards, both ascorbic acid and Trolox demonstrated significantly greater reducing power. Ascorbic acid achieved a 100% reduction at a concentration of 10 µg/mL, whereas Trolox exhibited a maximum reduction of 80.328% at 2 mM (Table 6b). The results highlight the greater efficacy of traditional, pure antioxidants compared to microalgal extracts. This comparison is essential for establishing the potential of the extracts and highlights the necessity of assessing new antioxidant sources relative to established standards [50,51].

Table 6. b. Percentage inhibition of Ferric cations by Ascorbic acid and Trolox at different concentrations.

Ascorbic Acid Conc (µg/ml)	% Inhibition	Trolox Conc (mM)	% Inhibition
2	25.41	0.125	17.21
4	36.89	0.25	27.05
6	55.74	0.5	34.43
8	80.33	1	59.02
10	100.00	2	80.33
IC_{50}	5.028	IC_{50}	1.014

2.4.4. ANOVA Analysis of Antioxidant Activity

The analysis of variance (ANOVA) indicated that multiple factors significantly affected the inhibition percentages of antioxidant activity. The assay type significantly influenced the results ($p=0.0446$), indicating that the evaluation method is essential for assessing antioxidant potential. Assays such as ABTS, DPPH, and FRAP function through different mechanisms, resulting in variable outcomes for identical extracts [52]. The results align with current literature supporting the utilisation of multiple assays to achieve a thorough understanding of the antioxidant potential of natural extracts.

Additionally, the type of solvent had a significant impact on the inhibition percentages ($p= 0.0256$), suggesting that the selection of solvent is essential for optimising the extraction of bioactive compounds. This corroborates earlier research indicating that polar solvents, including methanol and aqueous solutions, are particularly effective in extracting antioxidants from microalgae, thus improving their efficacy [53,54]. Methanol's capacity to solubilise various polar compounds, such as phenolic acids and carotenoids, significantly contributes to the increased antioxidant capacity noted in the microalgal extracts [55].

The findings are consistent with existing literature emphasising the significance of assay selection and solvent choice in antioxidant research [56]. The variability observed in assays highlights the complexity of antioxidant mechanisms and the necessity for a comprehensive approach to

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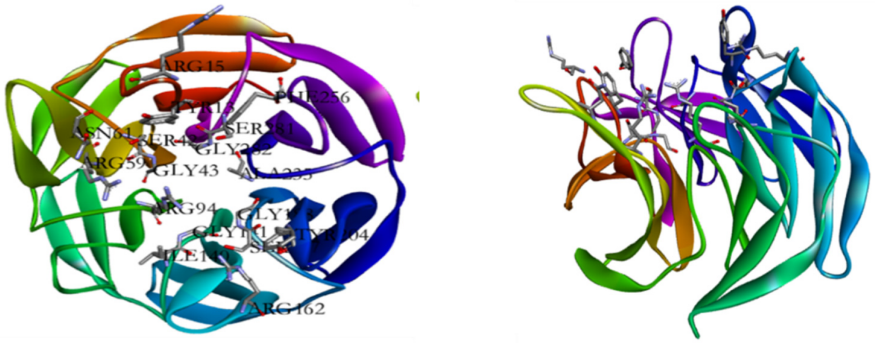
effectively evaluate the potential of microalgal extracts. In line with current market trends for natural ingredients with functional benefits [57], this approach not only improves understanding of the bioactive compounds but also promotes the development of effective formulations in the nutraceutical and cosmetic industries [58].

2.5. Molecular Docking Analysis

The virtual screening and molecular docking results provided insights into the interactions between phytochemicals and the Keap1 protein, which is involved in cellular defense mechanisms against oxidative stress (Figure 1). Keap1 regulates the activity of the transcription factor NRF2, crucial for the antioxidant response pathway [59]. By preventing Keap1 from binding to NRF2, the selected inhibitors promote NRF2 accumulation and nuclear translocation, leading to the upregulation of antioxidant and cytoprotective genes. The validation of docking calculations by redocking the native ligand, fuu, and observing similar interactions and binding affinity confirmed the reliability of the molecular docking process. The binding energies of the selected phytochemicals, all lower than that of fuu, indicate stronger interactions with the Keap1 protein (Table 7). The presence of specific amino acid residues in the binding pocket, such as SER 42, TYR 13, and ARG 59, highlights potential targets for inhibition.



(**a**) (**b**)

Figure 1. (**a**) a top view of the Keap1 with labeled amino acid residues; (**b**) side view of the Keap1; the amino acid residues are displayed with line model.

Table 7. Binding affinity and molecular interaction of selected ligands with KEAP-1.

	Phytochemicals	Binding Affinity (ΔG), kcal/mol	Amino Acids of keap-1 Receptor Forming h-Bond with Ligand	Other Interactions Involved (Electrostatic/Hydrophobic)
1	Alpha-carotene	-10.1		ALA 45, ALA 235, ARG 94, TYR 204
2	Beta-carotene	-9.9		ARG 94, TYR 204, ALA 235, ALA 45, VAL 285, TYR 251
3	Beta-cryptoxanthin	-10	GLY 46	TYR 204, ARG 94, ALA 235
4	Canthaxanthin	-10	GLY 46	TYR 204, ARG 94, ALA 235
5	Lutein	-9.7	GLY 46, VAL 285	ARG 94, ALA 235, TYR 204
6	Neoxanthin	-10.1	VAL 97	ALA 25, ARG 94, TYR 204
7	Violaxanthin	-11.2	GLY 46, VAL 285	ARG 94, ALA 235, TYR 13
8	Zeaxanthin	-10.1	VAL 191	ALA 235, ARG 94, TYR 204, TYR 251
9	Lariciresinol4-O-glucoside	-9.8	VAL144	ALA 235, ARG 94 , TYR 13, PHE 256
10	FolateCID_1	-10.1	ALA 189, GLY 46, SER 187, SER 234	ALA 45, VAL 97, ARG 94, ALA 235

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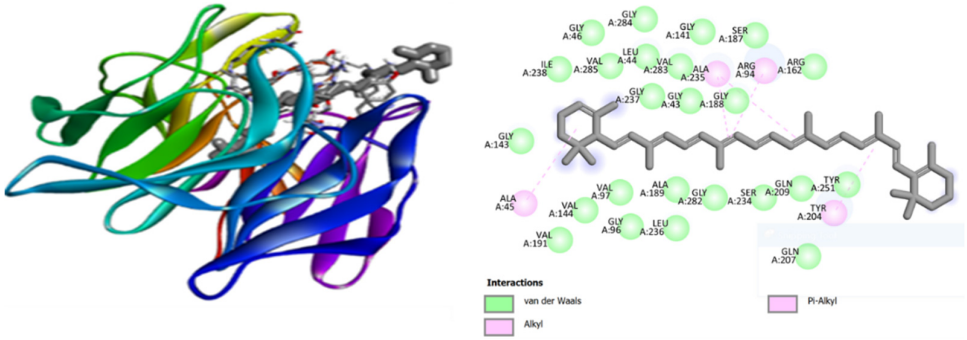
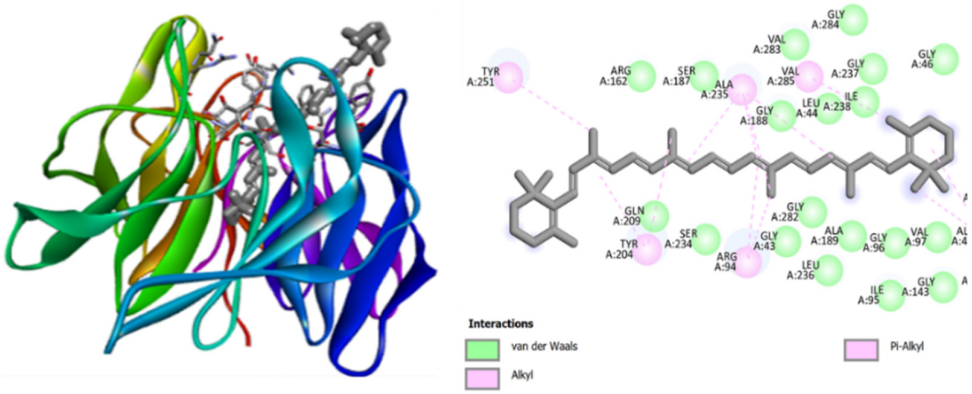
11	VitaminB2_Riboflavin	-10.2	VAL 97, VAL 144, GLY 43, VAL 281	SER 42, GLY 282 (C-H bond), ARG 94, ALA 235, SER 281
12	Phycoerythrin	-7.7	SER 42, ARG 94, SER 234	ALA 235, TYR 13, PHE 256, TYR 251, SER 281, GLY 282, GLY 188
13	Phytol	-9.8	ALA 189, VAL 142, ILE 95, ILE 238, VAL 285, SER 187	GLY 141, ARG 94, ARG 162, GLY 188, VAL 97, LEU 236, VAL 283

Carotenoids, which abundant in the two microalgae species studied, showed significant hydrophobic interactions within the binding pocket (Figures 2 and 3). The lack of hydrogen bonds in alpha-carotene and beta-carotene, due to the absence of electronegative atoms, suggested that their binding is predominantly driven by hydrophobic forces. This aligned with the understanding that hydrophobic interactions are crucial for protein folding and stability [60]. The involvement of hydrogen bonding in other carotenoids, such as beta-cryptoxanthin and lutein, further supported their potential as effective Keap1 inhibitors. The binding affinities of these compounds suggested their strong interaction with the target protein, which is essential for disrupting the Keap1-NRF2 interaction and enhancing cellular antioxidant responses.



(**a**) (**b**)

Figure 2. The (**a**) 3D docked pose of and; (**b**) side view of the Keap1; the amino acid residues are displayed with line model..



(**a**) (**b**)

Figure 3. The (**a**) 3D docked pose of and; (**b**) 2D interaction between Beta-Carotene and Keap1 protein target.

2.6. Toxicity and Safety Profile

The safety and pharmacokinetic profiles of the selected phytochemicals were evaluated using the ADMETlab2.0 platform (Table 8), emphasising their potential application in cosmetic formulations. Toxicological assessments revealed that most evaluated carotenoids exhibited a significant likelihood of causing skin sensitisation (probability ≥0.96). Phytol demonstrated a

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significant risk of eye irritation (0.95), whereas violaxanthin (0.91) and neoxanthin (0.52) showed increased probabilities of carcinogenicity. Neoxanthin (0.86), violaxanthin (0.70), and zeaxanthin (0.66) demonstrated relatively elevated risks of respiratory toxicity. The findings emphasise the importance of thorough safety evaluations, especially given that dermal application typically leads to minimal systemic exposure. Predictive toxicological modelling is a valuable tool for preliminary risk assessment; however, it may overestimate certain toxicity endpoints [61,62].

Table 8. Predicted toxicity profile of the selected phytochemicals from virtual screening.

Phytochemicals	BBB	hERG	SkinSen	Carcin.	EC	EI	Resp	LogS	Fu%	H-HT
Alpha-carotene	0.00	0.78	0.99	0.05	0.00	0.10	0.23	-7.98	2.03	0.44
Beta-carotene	0.00	0.85	0.99	0.04	0.00	0.23	0.31	-7.97	2.13	0.28
Beta-cryptoxanthin	0.00	0.85	0.99	0.04	0.00	0.04	0.49	-7.58	2.31	0.22
Canthaxanthin	0.00	0.58	0.99	0.05	0.00	0.19	0.36	-7.28	3.30	0.33
Folate	0.05	0.08	0.07	0.53	0.00	0.01	0.66	-3.90	34.40	0.99
Lariciresinol4-O-glucoside	0.40	0.08	0.04	0.38	0.00	0.01	0.01	-2.88	19.52	0.19
Lutein	0.01	0.63	0.97	0.04	0.00	0.01	0.36	-6.75	2.43	0.16
Neoxanthin	0.37	0.80	0.99	0.52	0.00	0.02	0.86	-5.86	3.13	0.70
Phytol	0.23	0.01	0.96	0.11	0.84	0.95	0.06	-6.64	2.40	0.10
Violaxanthin	0.17	0.76	0.98	0.91	0.00	0.04	0.70	-6.54	2.94	0.87
VitaminB2_Riboflavin	0.44	0.03	0.01	0.03	0.00	0.01	0.22	-3.66	21.69	0.12
Zeaxanthin	0.01	0.84	0.98	0.05	0.00	0.01	0.66	-7.12	2.56	0.19

Abbreviations: BBB, Blood–Brain Barrier penetration; hERG, cardiac toxicity risk; SkinSen, skin sensitisation potential; Carcin., carcinogenicity; EC, eye corrosion; EI, eye irritation; Resp, respiratory toxicity; LogS, solubility; Fu%, plasma protein unbound fraction; H-HT, human hepatotoxicity probability.

In terms of solubility and distribution, folate (−3.90), riboflavin (−3.66), and lariciresinol 4-O-glucoside (−2.88) were classified as soluble, with LogS values ranging from 4 to 0.5. The physicochemical properties, demonstrated by Chaiyasut et al. [63], are beneficial for inclusion in cosmetic formulations. The carotenoids demonstrated significant lipophilicity and low solubility in water, with LogS values ranging from −5.86 to −7.98. Advanced delivery systems, including lipid-based carriers and emulsions, have been proposed to improve bioavailability and efficacy in response to formulation challenges [64]. Furthermore, the examination of blood-brain barrier (BBB) penetration indicated that the majority of the phytochemicals assessed demonstrated limited BBB permeability, implying a decreased likelihood of central nervous system exposure. This is an important factor in assessing the safety profile of topical agents [65]. The findings highlight the necessity of combining predictive ADMET modelling with targeted formulation strategies to enhance the efficacy and safety of marine-derived phytochemicals in cosmetic applications.

Significant variations were noted among the phytochemicals in terms of their pharmacokinetic profiles, particularly for the fraction unbound in plasma. Riboflavin exhibited a higher percentage of unbound fraction, indicating relatively greater systemic bioavailability, as reported by Marczyński et al. [66]. Carotenoids exhibit low unbound fractions, reflecting their significant lipophilicity. This characteristic influences their systemic availability, which in turn affects formulation strategies, dosing, and the anticipated efficacy profiles of cosmetic products [67]. Despite potential toxicity concerns associated with high systemic absorption, the minimal systemic uptake resulting from dermal application allows for the safe inclusion of these compounds in cosmetic formulations, provided appropriate risk management measures are implemented [68]. These findings are consistent with earlier studies that have examined the safety and bioavailability of carotenoids in topical applications [69, 70].

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3. Materials and Methods

3.1. Microalgae Biomass

Spray-dried biomass of two microalgal species, *Dunaliella salina* and *Spirulina platensis*, was used in this study. The *D. salina* biomass was obtained from the Algae Biotechnology Laboratory, University of Greenwich (London, UK), while *S. platensis* biomass was purchased from The Soap Kitchen (Devon, UK). All biomass samples were stored at –20 °C prior to analysis and characterisation to preserve their biochemical integrity.

3.2. Extraction Yield

Metabolites were extracted from *Dunaliella salina* and *Spirulina platensis* using a modified protocol adapted from previous studies [23,71,72]. 100 mg samples of each microalga were processed in triplicate. Water, methanol, and ethanol were employed as solvents. The samples were sonicated using a Scimed UP50H ultrasonic processor to enhance metabolite extraction. After sonication, the samples were centrifuged, and the supernatants were filtered to remove particulate matter. Ethanol and methanol extracts were concentrated using a Genevac EZ 3835 evaporator, while aqueous extracts were freeze-dried. All extracts were stored at –20 °C for future analysis.

The extraction yield was calculated using the following formula:

$$Y\ \% = (\text{weight used for extraction} / \text{total amount of extract}) \times 100 \quad (1)$$

3.3. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, with modifications based on previous studies of Shirazi et al. [73]. Gallic acid was used as a standard for calibration, with concentrations ranging from 5 to 100 µg/mL prepared in distilled water. Samples (100 µL) were mixed with 500 µL of distilled water and 100 µL of Folin-Ciocalteu reagent. This mixture was incubated in darkness for 6 minutes, followed by the addition of 1 mL of a 7% Na_2CO_3 solution. The samples were then incubated for 90 minutes at room temperature (22 °C). The absorbance was measured at 760 nm using a Jenway 6305 UV spectrophotometer. TPC was calculated and expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (dry weight).

3.4. Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method, adapted from existing protocols of Shirazi et al. [73]. Quercetin served as the reference standard, with concentrations ranging from 5 to 100 µg/mL. Each test involved mixing 100 µL of quercetin dilution with 500 µL of distilled water and 100 µL of 5% sodium nitrate solution. After a 6-minute incubation, 150 µL of 10% $AlCl_3$ was added, followed by an additional 5-minute incubation. Finally, 200 µL of 1 M NaOH was added, and absorbance was measured at 510 nm using a Jenway 6305 UV spectrophotometer. TFC was expressed as milligrams of quercetin equivalents (QE) per gram of extract (dry weight).

3.5. Evaluation of Antioxidant Activity

3.5.1. ABTS Radical Scavenging Activity

ABTS radical scavenging activity (RSA) was measured based on a modified method from Brand-Williams et al. [74] and Asekunowo et al. [75]. ABTS reagent was prepared and diluted to an initial absorbance of approximately 0.7 at 734 nm. In a 96-well plate, 190 µL of diluted ABTS reagent was combined with 10 µL of microalgae extracts or standard antioxidants (ascorbic acid at 2-10 µg/mL and Trolox at 0.125-2 mM). Samples were tested in triplicate and incubated in the dark at room

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temperature for 30 minutes. Absorbance at 734 nm was measured using a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

ABTS scavenging activity (%) was calculated using the formula:

$$\text{ABTS scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (2)$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound.

3.5.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated following a method modified from Brand-Williams et al. [74]. A methanolic solution of DPPH (0.1 mM) was mixed with 50 µL of microalgae extracts (50–250 µg/mL) and incubated for 30 minutes in the dark. Absorbance at 517 nm was measured using a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

DPPH scavenging activity (%) was calculated:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (3)$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound.

3.5.3. Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay, based on Benzie and Strain [76]), was used to measure antioxidant reducing power by converting ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. The FRAP reagent was prepared by mixing acetate buffer, TPTZ solution and ferric chloride solution in a 10:1:1 ratio. After incubation at 37 °C, 150 µL of FRAP reagent was added to 50 µL of sample or standard antioxidants (ascorbic acid and Trolox). Absorbance was measured at 700 nm using a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer.

Reducing power was calculated using:

$$\% RP = [1- ((1-As))/Ac)] \times 100 \quad (4)$$

Where % RP is the percentage reducing power, *As* is absorbance of the sample while *Ac* is the absorbance of the standard at maximum concentration tested.

3.6. Molecular Docking for Toxicity Assessment

The 3D structures of various phytochemicals from the microalgae, including 2-Fluoropalmitic acid (CID_1560), Alpha-carotene (CID_6419725), Alpha-ionone (CID_5282108), Alpha-linolenic Acid (CID_5280934), ascorbyl-palmitate (CID_54680660), Beta-carotene (CID_5280489), Beta-cryptoxanthin (CID_5281235), Beta-Cyclocitral (CID_9895), Beta-ionone (CID_638014), Caffeic acid (CID_689043), Canthaxanthin (CID_5281227), Chlorogenic acid (CID_1794427), Folate (CID_135398658), Gamma-linolenic Acid (CID_5280933), Glycerol (CID_753), Lariciresinol 4-O-glucoside (CID_11972394), Lutein (CID_5281243), Mycosporine (CID_442866), Mycosporine-glycine (CID_14444485), Myxoxanthophyll (CID_102601544), Neophytadiene (CID_10446), Neoxanthin (CID_5282217), Niacin (CID_938), Oleic acid (CID_4456), Ostruthin (CID_5281420), Palmitic Acid (CID_985), Phycoerythrin (CID_238), Phytol (CID_5280435), Porphyra-334 (CID_6857486), Pyrogallol (CID_1057), Shinorine (CID_122706103), Spiculisporic acid (CID_316426), Stearidonic Acid (CID_5312508), Syringic acid (CID_459276651), Violaxanthin (CID_448438), Vitamin B1 (Thiamine) (CID_6042), Vitamin B2 (Riboflavin) (CID_493570), Vitamin B6 (Pyridoxine) (CID_1054), and Zeaxanthin (CID_5280899) were sourced from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and carotenoids analysis from previous publication [77–80]. These structures were minimised to avoid steric clashes using Gaussian 09W software [81] at the semi-empirical level of theory, which is appropriate for the type of ligands involved.

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The Keap1 protein, with PDB ID 3vnh and a resolution of 2.10 Å, served as the target protein in this study. The crystal structure of the protein was obtained from the Protein Data Bank (www.rcsb.org) [82] and was prepared by removing water molecules and bound ligands using BIOVIA Discovery Studio (BIOVIA, Dassault Systèmes, Discovery Studio, 2018). For virtual screening, the optimized phytochemical structures were converted to .mol2 format using GaussView [83] and subsequently to .pdbqt format with Geisteiger charges using raccoon.py. Amino acids in the binding site were identified using the LigPlot server [84]. Virtual screening was conducted with AutoDock Vina 1.7 [85] installed on a Windows operating system. The dimensions of the binding center were set to -9.659 × -26.081 × 4.199. The toxicity profiles of the top phytochemicals were assessed using the ADMETlab 2.0 server (https://admetmesh.scbdd.com/) [86].

3.7. Statistical Analysis

Statistical analyses were performed to evaluate both metabolite extraction and antioxidant activity. For extraction yield, total phenolic content (TPC), and total flavonoid content (TFC), one-way ANOVA was used to assess differences between solvents (water, methanol, ethanol) within *D. salina* and *S. platensis* samples. Two-way ANOVA was utilised to examine interactions between microalgal species and solvent type for phenolic and flavonoid contents. When ANOVA indicated significant differences, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to identify pairwise differences between group means.

For antioxidant activity, one-way ANOVA was applied to compare ABTS, DPPH, and FRAP assay results across different factors, including assay type, solvent (aqueous, methanol, ethanol), microalgal species, and concentration. Linear regression models were further used to evaluate main effects and interactions. Tukey's HSD post hoc test was performed to determine specific differences between means when significant variations were observed. All statistical analyses and data visualisations were performed using Python (version X.X) with the libraries Pandas, SciPy, Statsmodels, and Matplotlib. A p-value < 0.05 was considered statistically significant.

4. Conclusions

This study adds to the growing body of literature on the potential of *Dunaliella salina* and *Spirulina platensis* as viable sources of bioactive compounds for cosmetic applications. The optimisation of extraction techniques revealed that methanol and aqueous solvents are especially effective for maximising the yield of phenolic and flavonoid compounds. The high concentrations of these bioactive compounds, along with the significant antioxidant activity demonstrated, highlight their efficiency in mitigating oxidative stress, a primary contributor to skin ageing and damage. Furthermore, the molecular docking analyses demonstrated significant binding affinities of essential phytochemicals with the Keap1 protein, indicating their potential effectiveness in improving skin health. The ADMET analysis further buttresses the overall safety of the extracts for topical use, although it highlights specific toxicological risks for certain isolated compounds, such as potential skin sensitization and eye irritation. The results support the inclusion of these microalgal extracts in cosmetic formulations designed to enhance skin hydration, elasticity, and protection from environmental stressors. The incorporation of advanced extraction techniques, thorough evaluations of antioxidant properties, and comprehensive safety analysis not only validates the potential of *D. salina* and *S. platensis* but also aligns with the increasing consumer demand for natural and effective cosmetic components. Ongoing research in this area could lead to the development of innovative, effective, and environmentally sustainable cosmetic products that leverage the unique properties of these microalgae.

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