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ORIGINAL RESEARCH

Comparative Biochemical Profiling and Industrial Application Potentials of *Dunaliella salina* and *Spirulina platensis*

Morufat A. LI-HAMMED[1*], Taofikat A. ADESALU[1], Nimisha TRIPATHI[2], Olayinka T. ASEKUN[3]

[1]*Department of Botany, Faculty of Science, University of Lagos, Nigeria*

[2]*Indo-UK Centre for Environment Research and Innovation, University of Greenwich, United Kingdom*

[3]*Department of Chemistry, Faculty of Science, University of Lagos, Nigeria*

***Correspondence**
Morufat Abimbola Li-Hammed, Department of Botany, Faculty of Science, University of Lagos, Nigeria.
Email: morufatlihammed@gmail.com

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Abstract:

Introduction: Microalgae, like *Dunaliella salina* and *Spirulina platensis*, hold immense promise for biotechnology due to their diverse biochemical profiles and ability to accumulate valuable compounds. This study explores the distinct biochemical profile of these two species.

Aims: To compare the protein, lipid, carbohydrate, and pigment content of *Dunaliella salina* and *Spirulina platensis* and evaluate their potential for various applications.

Materials and Methods: Proximate analysis was conducted on both microalgae, measuring protein, carbohydrate, ash, moisture, and pigment content. Statistical analysis (independent t-tests) was used to compare the means of each biochemical parameter.

Results: Independent t-tests revealed significant differences (p-values < .001) in all measured parameters except 9-cis carotenoids (data not available for *Spirulina platensis*). *Dunaliella salina* displayed significantly higher levels of β-carotene (6.90 ± 0.15 % vs. 0.66 ± 0.02 %) and protein (32.53 ± 0.49 % vs. 44.24 ± 0.02 %) compared to *Spirulina platensis*. However, *Spirulina platensis* had a slightly higher carbohydrate content (8.68 ± 0.68 % vs. 7.02 ± 0.049 %) and lower moisture content (3.25 ± 0.74 % vs. 6.54 ± 1.03 %).

Conclusion: This study highlights the unique biochemical properties of *Dunaliella salina* and *Spirulina platensis*, suggesting their potential in nutraceuticals (*Dunaliella salina*) and food/biofuel industries (*Spirulina platensis*).

Keywords: Microalgae, *Dunaliella salina, Spirulina platensis,* Biochemical composition, Biotechnology

1. INTRODUCTION

Microalgae, small photosynthetic organisms, are recognised for their capacity to synthesize a diversity of organic molecules, including carbohydrates, lipids, proteins, and vitamins, using carbon fixation [1]. Their numerous uses in industries including as biofuel, food, cosmetics, and medicines have positioned them as a sustainable and sought-after feedstock [1, 2, 3]. The unique biodiversity of microalgae, coupled with advancements in genetic engineering, further highlights their potential, with *Dunaliella* and *Spirulina* standing out as prominent examples [4, 5, 6]. Genetic engineering techniques have enhanced their potential by enabling the manipulation of their biochemical profiles, which include proteins, carbohydrates, lipids, vitamins, minerals, and other bioactive molecules [7]. The biochemical composition of each microalga species is crucial for determining their suitability for specific purposes [8, 9, 10].

Dunaliella, a green microalga genus, is notable for its ability to accumulate significant amounts of β-carotene, vitamin B12, and other beneficial compounds, making it an important source of nutraceutical biomass for human consumption [11, 12]. Its protein-rich biomass also positions it as a potential supplement for enhancing human health [13]. Similarly, *Spirulina*, a cyanobacteria genus, is well-known for its rich biochemical composition, which includes proteins, carbohydrates, lipids, and minerals, and its potential health benefits. These have garnered attention for their antioxidant, anti-inflammatory, and immunomodulatory effects, making *Spirulina* a valuable dietary supplement, particularly in the context of exercise and sport [14, 15]. Recognized by the United Nations for its ability to combat malnutrition, *Spirulina's* pharmacological properties, such as antiviral, anticancer, and hypocholesterolemic effects, are well-documented, with emerging studies suggesting its protective effects against heavy metal neurotoxicity [16, 17, 18].

Recent studies have further emphasized the importance and potential of microalgae in various fields. Yang et al. [19] explored the bioactive compounds of microalgae as antioxidants, uncovering research trends, key areas of study, and projecting future advancements in extraction methodologies. Dussably et al. [20] highlighted microalgae, including diatoms, for their pharmaceutical and cosmetic applications, emphasizing the need for valorising natural biomass in cosmetics to protect wild resources. Meanwhile, Martínez-Ruiz et al. [21] emphasized the cosmeceutical applications of their biomolecules. Focusing on specific strains, Wang & Miao [22] explored lipid dynamics in *Chlorella pyrenoidosa*, and Safi et al. [23] provided a detailed examination of *Chlorella vulgaris* and its potential in industrial applications.

In light of this extensive body of research, this study aims to deepen the comparative understanding of *Dunaliella salina* and *Spirulina platensis* by examining their biotechnological potential and uncovering novel insights into their specific biochemical metrics. While recognizing the critical influence of cultivation conditions and harvesting techniques on biomass composition, the objectives are to meticulously characterize and quantify the unique bioactive compounds of each species, focusing on their potential contributions to biofuel production, food industry, cosmetics, and pharmaceutical applications [24]. This comprehensive approach intends to fill knowledge gaps and enhance the application of these microalgae, thereby facilitating their refined exploitation in both established and emerging biotechnological sectors.

However, to fully leverage the biotechnological advantages of these microalga biomasses, detailed characterization of their compositions is indispensable. Factors like strain selection, cultivation conditions, and harvesting techniques invariably affect these parameters [25]. This study delves deep into the comparative evaluation of *Dunaliella salina* and *Spirulina platensis*. Specific metrics were characterized, including dry weight, moisture content, ash content, ash-free dry weight, organic matter, pigments, carotenoids, carbohydrates, and proteins, providing a holistic perspective on their potential biotechnological

applications. This comprehensive approach aims to demystify their unique advantages, thereby streamlining their use in industries like biofuel, food, cosmetics, and pharmaceuticals.

2. MATERIAL AND METHODS

2.1 Microalgae Biomass

The two microalgae species used for analysis in this study – *Dunaliella salina* and *Spirulina platensis*, were obtained in spray dried form. *Dunaliella salina* was obtained from the algae biotechnology of the University of Greenwich, while *Spirulina platensis* was purchased from The Soap Kitchen, Devon, United Kingdom. The biomass was stored at temperature of -20°C before further analysis and characterisation.

2.2 Dry Weight

Microalgae biomass samples weighed and dried in an oven (Fistream Int. Ltd., Cambridge, UK) for 24 hr at 105^{o}C. The samples were allowed to cool in a desiccator after drying and reweighed. Moisture content was calculated as the loss of water at 105°C [26].

2.3 Ash Content

Ash content which is the inorganic matter was measured by placing the weighed oven dried microalgae biomass in a muffle furnace (Vecstar Ltd., Chesterfield, UK) at 550°C for 4 hours and left to cool until constant weighed is achieved. The differences in weight afterwards, were gravimetrically determined [26]. Ash free dry weight (AFDW) was calculated as followed.

AFDW = Biomass dried weight – Ashed weight ---------- (I)

2.4 Pigment

Chlorophyll (Chl) content was determined with some modifications as described by Li et al. [27]. Microalgae biomass samples (10 mg) were mixed with 5 ml of 80 % acetone, vortexed for some minutes and centrifuged at 1500 ×g for 5 min in an Eppendorf Centrifuge 5810 R, and the supernatants were collected. Chl a and Chl b levels were measured in a Jenway 6305 UV spectrophotometer at wavelengths 663 and 645 nm respectively while acetone was used as blank.

Chl a = 12.25 × $A_{663.6}$ – 2.55 × $A_{646.6}$ ---------- (II)

Chl b = 20.31 × $A_{646.6}$ – 4.91 × $A_{663.6}$ ---------- (III)

Total Chl = [Chl a + Chl b] = 17.76 × $A_{646.6}$ + 7.34 × $A_{663.6}$ ---------- (IV)

2.5 Carotenoids (HPLC)

The carotenoids were determined from the samples with methanol and MTBE (80: 20). Drops of water was added to wet the microalgae biomass before the addition of methanol and MTBE. The mixture was sonicated for about 1 min using ultrasonic processor UP50H (Sciemed, UK), and then centrifuged at 3000 rpm at room temperature for 5 min. the supernatant thereafter was filtered using 0.45 µm syringe into amber HPLC vials. Analysis was carried out on an YMC30 250 × 4.9 mm I.D 5 - 5µ HPLC column with a diode array detector (DAD) at 25°C, and isocratic elution with methanol and MTBE (80:20), flow/ml/min, pressure 78 bar. The relative quantities of 9-cis and all-trans β carotene standard in the biomass were estimated using a standard curve that was related to the peak area of the all-trans β carotene standard from Sigma Aldrich.

2.6 Carbohydrates Content

Carbohydrates was determined by the phenol-sulphuric acid method as described by Laurens et al. [28] with some modifications. About 10 mg microalgae biomass was reconstituted in 10 ml of water to make a known concentration of 1 mg/ml. Afterwards 1 ml of the sample was mixed with 3 ml of concentrated H_2SO_4 (72 % wt) and 1 ml of 5 % w/v phenol in a water bath. The mixture was incubated for 5 min at 90°C. The mixtures in triplicate were measured using spectrophotometer at a wavelength of 490 nm. The absorbance of the mixtures was afterwards compared to glucose standard curve. Carbohydrates content was expressed as percentage of dry weight.

2.7 Protein Content

To estimate the protein in the microalgae biomass, protein was first extracted as described by Tayebati et al. [29] with alkaline treatment. Approximately 10 mg of each microalgae biomass sample was weighed, and 10 ml of methanol was added into the test tube. Then after, 0.5 ml of 0.5 N KOH was added to the mixture. The mixture was allowed to sit for about 2 hours and centrifuged at 10,000 rpm for 10 min at 20°C. The protein concentration of the disrupted biomass was measured with a modified Lowry protein assay kit from Thermo Scientific TM Pierce TM [30, 31]. This kit is based on the reaction of protein with copper and Folin reagent, which produces a blue colour that can be quantified by spectrophotometry. The absorbance of all mixtures in triplicates was measured at 750 nm and compared to the standard curve of bovine serum albumin (BSA). Protein content was expressed as percentage of dry weight.

2.8 Statistical Analysis

The data were analysed using independent samples t-tests from the SciPy library to compare the means of each biochemical parameter between the two species. A significance level of $\alpha = .05$ was used for all tests. The assumptions of normality and homogeneity of variances were assessed prior to the t-tests. All analyses were performed using Python.

3. RESULTS AND DISCUSSION

The results of the t-tests revealed that there were statistically significant differences between *Dunaniella salina* and *Spirulina platensis* for all measured biochemical parameters. The p-values, which were extremely low p-values (< .001) indicate that the differences in means between the two species are extremely statistically significant for all tested parameters except for the 9-cis carotenoids, where data was not available for *Spirulina platensis*. An extensive proximate analysis was conducted on two well-known microalgal species, *Dunaliella salina* and *Spirulina platensis*. The results were compiled and are presented in Table 1.

3.1 Proximate Analysis

Compared to *Spirulina platensis*, which has a moisture content of 3.20 %, *Dunaliella salina* was discovered to have a moisture content of 6.37 %, which is comparatively greater. *Dunaliella salina* had an ash content of 10.05 %, which is a measure of the number of inorganic materials in the samples. *Spirulina platensis*, on the other hand, showed an ash level of 20.90 %, which is almost twice that of the former. *Dunaliella salina* obtained a value of 9.54 % for AFDW, which offers insights on the organic matter free of ash. *Spirulina platensis*, on the other hand, had a somewhat elevated AFDW of 12.41 %. *Dunaliella salina* was discovered to have an organic matter concentration of 92.58 %, whereas *Spirulina platensis* had a slightly higher organic matter content of 93.80 %.

Table 1: Proximate analyses results of *Dunaliella salina and Spirulina platensis*

Parameter	*Dunaniella salina* (Mean ± SD)	*Spirulina platensis* (Mean ± SD)
Moisture Content (%)	6.540 ± 1.025	3.247 ± 0.741
Ash (%)	10.050 ± 0.397	20.900 ± 1.884
Ash-Free Dry Weight (%)	9.400 ± 0.556	12.410 ± 1.352
Organic Matter (%)	92.458 ± 0.252	93.799 ± 0.940

**SD - Standard deviation*

This study provides a comprehensive biochemical profiling of *Dunaliella salina* and *Spirulina platensis*, demonstrating notable differences in moisture content, ash, organic matter, and pigment composition. These findings are crucial for understanding the biotechnological potential and quality of these microalgae species [9, 32].

The moisture content, a key factor in biomass stability and shelf life, was found to be relatively low in both *Dunaliella* and *Spirulina*. This suggests a significant level of dehydration, potentially enhancing preservation and reducing microbial contamination risks. However, it also raises the possibility of losing some bioactive compounds or altering the cellular structure and function of the microalgae. Various studies have emphasized the impact of drying methods and conditions on the quality of microalgae biomass, particularly how different techniques like freeze-drying and oven drying affect the retention of vital components such as chlorophyll, proteins, and lipids [33, 34, 35, 36, 37, 38]. Comparatively, the moisture content in *Dunaliella* and *Spirulina* is lower than in other microalgae like *Chlorella* and *Haematococcus*, suggesting a higher degree of drying and dehydration. This aspect of moisture content is crucial in the characterization of microalgae biomass, as it affects stability, storage, and processing.

Ash content, indicative of the mineral content in biomass, varies with species, growth conditions, and processing methods. The ash content observed in this study falls within the typical range for microalgae, reflecting a standard mineral profile that can affect colour, texture, and stability. High ash content can be undesirable for certain biotechnological applications, such as cosmetics, and can also influence thermochemical conversion processes [39, 40, 41].

The ash-free dry weight (AFDW) metric, representing the organic matter content in microalgae, showed that *Dunaliella* and *Spirulina's* AFDW values are within the expected range for microalgae species. AFDW is crucial for determining biomass productivity and is essential in biofuel and nutraceutical production. It also plays a role in calculating the lipid content for biodiesel production, highlighting its importance in bioenergy research [32, 38, 42, 41].

The organic matter content (OMC) ratio to total dry weight in microalgae is key to assessing their quality and suitability for biotechnological applications. A high OMC, as observed in our study, indicates a low concentration of inorganic matter or ash. This aspect of microalgae is inversely related to their ash content and varies with species, culture conditions, and harvesting methods, making it a vital parameter in biomass characterization, especially for biofuel production [38, 41].

3.2 Chlorophylls and Carotenoids Content

The pigment composition of the two microalgal species was carefully evaluated in an attempt to comprehend their pigmentary profiles. Table 2 presents the results, which provide an overview of the pigment concentrations in *Dunaliella salina* and *Spirulina platensis* biomass. Important findings from the investigation of pigment content are highlighted:

Table 2: Pigment composition of *Dunaliella* and *Spirulina*

Pigment	Dunaniella salina (mg/g dry weight) Mean ± SD	*Spirulina platensis* (mg/g dry weight) Mean ± SD
Chlorophyll a	1.153 ± 0.154	3.472 ± 0.054
Chlorophyll b	0.750 ± 0.047	1.306 ± 0.216
Total Chlorophyll	1.614 ± 0.075	3.946 ± 0.076
Carotenoids (Total)	6.901 ± 0.153	0.656 ± 0.020
HPLC (Carotenoids		
all-trans[a]	1.195 ± 0.152	0.075 ± 0.011
9-cis[a]	0.163 ± 0.009	ND[b]

[a] *values are expressed as percentage of spray dried samples;* [b] *not detected*

Compared to *Dunaliella salina*, which had a Chl a concentration of 1.15 %, *Spirulina platensis* had a concentration of 3.47 %. *Spirulina platensis* was the dominant species once more, with a Chl b concentration of 1.31 % compared to *Dunaliella salina*'s 0.75 %. *Spirulina platensis* has a higher total chlorophyll content (Chl a + Chl b) at 3.95 % than *Dunaliella salina* (1.61%). *Dunaliella salina* has a significantly higher concentration of carotenoids (6.90 %) than *Spirulina platensis* (0.66 %). *Dunaliella salina* showed a greater amount of 1.19 % of all-trans carotenoids, whilst *Spirulina platensis* showed a more moderate 0.08 %. In *Dunaliella salina*, 9-cis carotenoid was found at 0.16 %, but was not detected in *Spirulina platensis*.

Pigment composition, particularly chlorophylls and carotenoids, displayed notable variations between the species. Chlorophylls are involved in photosynthesis and possess properties beneficial to health, such as antioxidant and anti-inflammatory effects. Carotenoids, on the other hand, are known for their antioxidant, anti-inflammatory, immunomodulatory, and photoprotective properties, playing a crucial role in human health. The higher carotenoid content in *Dunaliella*, especially β-carotene, is indicative of its exceptional ability to produce these pigments, which is significant for applications in human health and nutrition. This finding aligns with previous research emphasizing *Dunaliella's* capability in carotenoid synthesis [43, 44, 45, 6].

The synthesis of carotenoids by microalgae, such as *Dunaliella*, is critical for their potential biotechnological applications. Carotenoids like all-trans and 9-cis isomers are particularly important due to their antioxidant and provitamin A activities. *Dunaliella salina* has been shown to accumulate significant amounts of β-carotene, existing in two geometric isomers: all-trans and 9-cis. This trait underlines *Dunaliella's* adaptability to stressful environmental conditions and efficient carotenoid biosynthetic pathways. Comparatively,

Spirulina produces lower amounts of carotenoids, focusing mainly on phycocyanin and allophycocyanin, which have been extensively researched for their pharmaceutical and food product potential [46, 47, 48, 49, 50, 51, 52, 53, 54].

3.3 Carbohydrates

From the absorbance of glucose at different concentrations (Table 3), Equation Y = 0.0059x – 0.1237 from Figure 1 was determined. The data was used to extrapolate the carbohydrates concentration of both *Dunaliella salina* and *Spirulina platensis* (Figure 2).

Table 3: Absorbance of glucose at different concentrations

Glucose concentration (µg/ml)	Absorbance at 490nm
-40	0.157
80	0.240
120	0.672
160	0.832
200	1.050

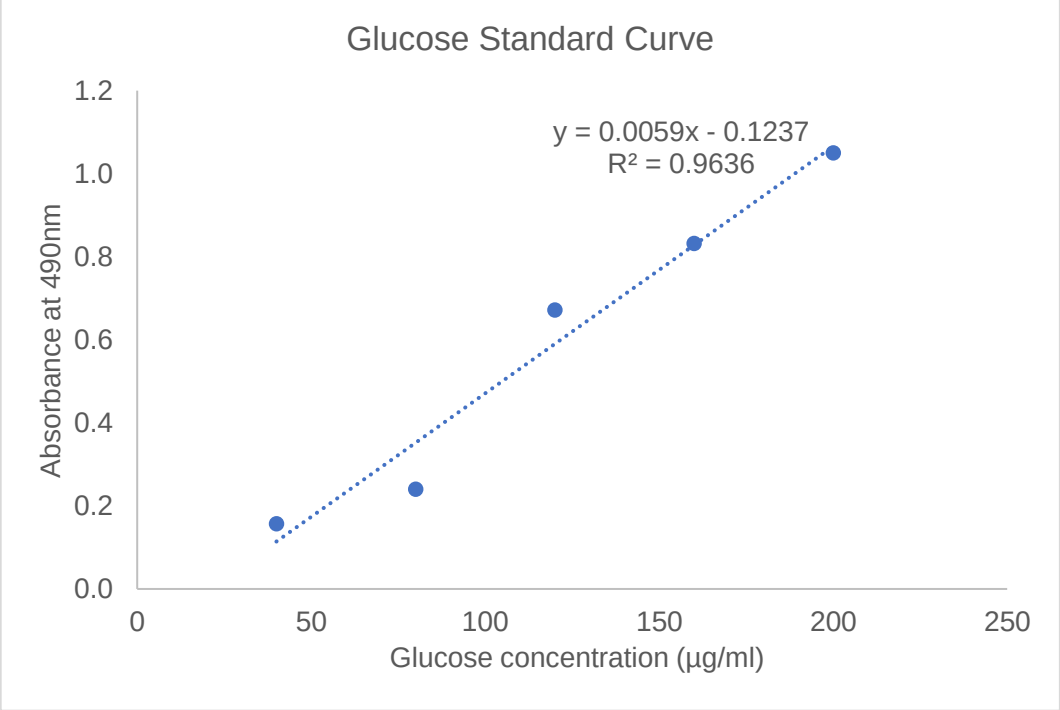

Figure 1: Concentration response curve for glucose at different concentrations

The carbohydrate content in *Spirulina platensis* constituted 8.68 % of its dry biomass. *Dunaliella salina*, on the other hand, contained a slightly lower percentage of carbohydrates, amounting to 7.02 % of its dry biomass.

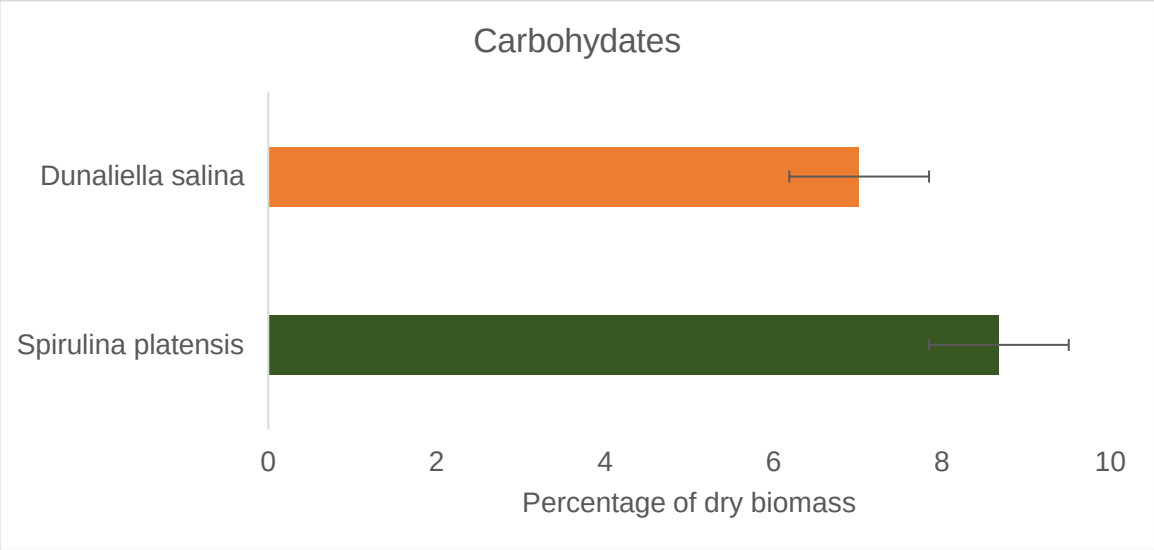

Figure 2: Carbohydrates content of *Dunaliella salina* and *Spirulina platensis* expressed as % of its dry biomass

The carbohydrate content comparison between *Dunaliella* and *Spirulina* revealed a slightly higher level in *Spirulina*. This is in line with previous studies, indicating that carbohydrate accumulation in microalgae is influenced by environmental factors and cultivation conditions. The higher carbohydrate content in *Spirulina* suggests its potential utility in applications where energy content is a priority, such as biofuel production. Conversely, the balanced composition of proteins, lipids, and carbohydrates in *Dunaliella* may render it more suitable for other applications like the nutraceutical industry. These variations in carbohydrate content are critical for understanding the specific applications of each microalgae species [55, 56, 57].

3.4 Proteins

Equation Y = 0.0009x – 0.2612 from Figure 3 was calculated using the absorbance of bovine serum albumin (BSA) at various values (Table 4). The protein concentrations of *Spirulina platensis* and *Dunaliella salina* were extrapolated using the data (Figure 4). From the data presented, it is evident that *Spirulina* surpasses *Dunaliella* in terms of protein concentration. *Spirulina platensis* had a high content of protein, accounting for 44.24 % of its dry biomass. *Dunaliella salina*, on the other hand, has a protein concentration of 32.53 % of its dry weight.

Table 4: Absorbance of bovine serum albumin (BSA) at different concentrations

Protein concentration (µg/ml)	Absorbance at 750nm
1	0.20

5	0.23
25	0.31
125	0.34
250	0.59
500	0.67
750	1.01
1000	1.21
1500	1.56

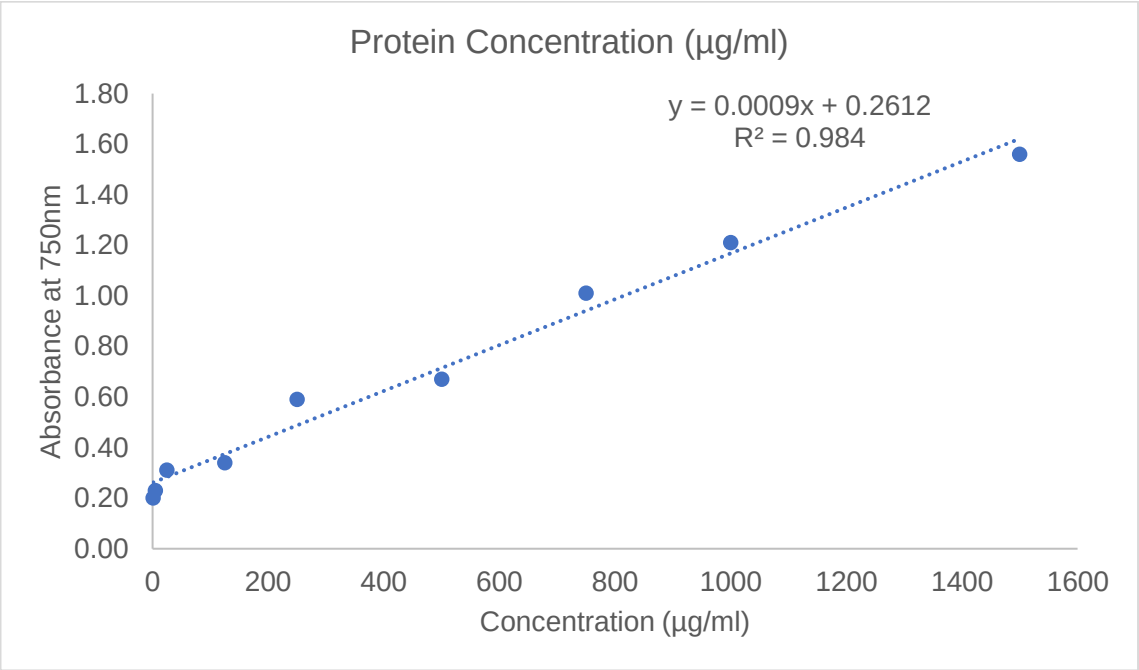

Figure 3: Concentration response curve for BSA at different concentrations

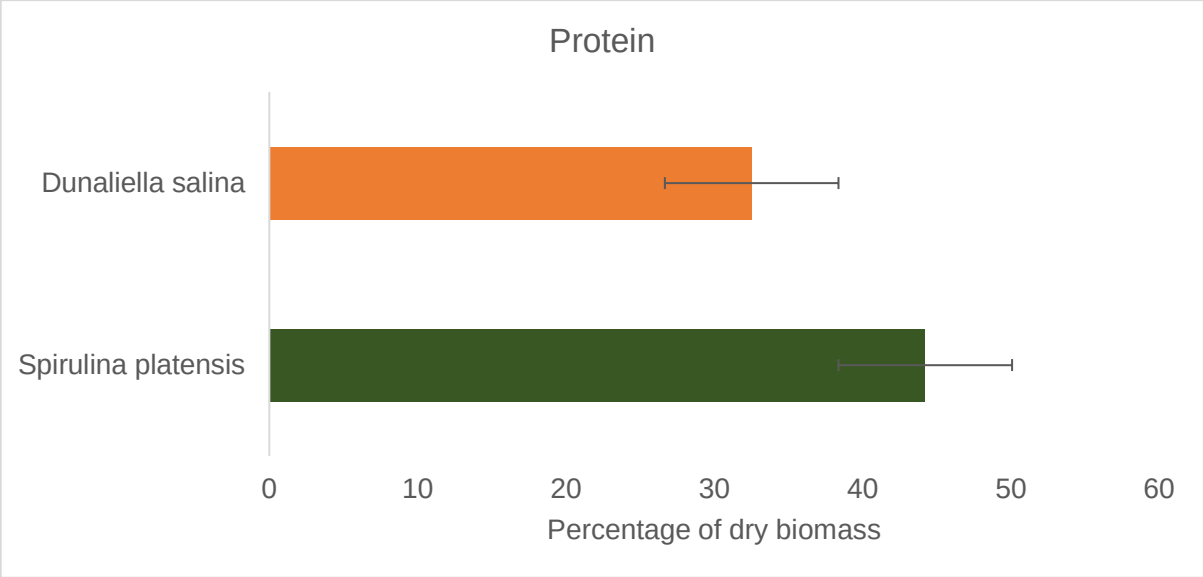

Fig. 4. Protein content of *Dunaliella salina* and *Spirulina platensis* expressed as its % of dry biomass

In the context of protein content, *Spirulina's* higher protein levels, as compared to *Dunaliella*, corroborate findings from previous research. This high protein content, along with the presence of essential amino acids, highlights the potential of *Spirulina* as a sustainable and rich protein source for food, feed, and biotechnological applications. The protein content in microalgae varies significantly among different species, with *Dunaliella* and *Spirulina* ranking among the higher protein-containing microalgae. This richness in proteins supports their use as feed additives in animal nutrition and in the production of biologically active components for medical and preventive nutrition [58, 59, 60, 61, 62, 63, 64, 65, 66].

To summarize, this study confirms that both *Dunaliella* and *Spirulina* possess distinct biochemical profiles with significant potential for varied biotechnological applications. The detailed analysis of their moisture content, ash content, organic matter, and pigment composition provides a deeper understanding of their properties and possible uses. As the demand for sustainable and versatile bioresources continues to grow, the insights gained from this study contribute valuable knowledge towards the effective utilization of these microalgae species in various sectors.

4. CONCLUSION

The current research has illuminated the significant roles of microalgae, particularly *Dunaliella salina* and *Spirulina platensis*, within the rapidly evolving biotechnology sector. This study comprehensive comparative analysis has verified the advantages of each microalga, contributing new insights into their distinctive characteristics and potential applications. *Dunaliella salina*, with its substantial protein content and impressive β-carotene accumulation, emerges as a formidable player in the nutraceutical market, poised to revolutionize the health supplement industry with its organic benefits. *Spirulina platensis*, with its superior protein profile, reaffirms its essential status as a nutritional supplement, bolstering its growing significance in global health discussions. Additionally, this study's discovery of minor differences in carbohydrate levels suggests *spirulina's* untapped potential in energy-demanding industries such as biofuel production, underscoring its extensive industrial applicability. The inclusion of parameters like moisture, ash, and organic matter in the proximate analysis has provided invaluable insights into the microalgae's versatility for biotechnological uses. These insights are vital for the microalgae's conservation, processing, and storage, influencing the industry's overall economic success. These findings underscore the importance of selecting

optimal cultivation, harvesting, and extraction methods to maximize the potential of any species. As the world grapples with sustainability and health challenges, *Spirulina platensis* and *Dunaliella salina* stand out as promising contenders. Their natural and sustainable solutions to diverse industrial needs align with the goals of green biotechnology. The study's contribution to the scientific community is clear: it underscores the need for continuous research and investment to ensure the full potential of these microalgae is harnessed in the swiftly shifting landscape of green biotechnology.

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