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SINGLE-RESPONSE OPTIMIZATION OF BIOACTIVE COMPOUND CONTENT AND ANTIOXIDANT ACTIVITY OF *Haematococcus pluvialis* EXTRACT THROUGH TAGUCHI ORTHOGONAL ARRAY

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ABSTRACT

The present research aimed to optimize the extraction conditions for maximum yield of bioactive compounds and antioxidant activity of *Haematococcus pluvialis* by using the Taguchi method (TM). A Taguchi L9 (3^2) orthogonal array experiment was carried out, examining two factors-methanol concentration (50%, 75%, 100%) and extraction time (60, 120, 180 minutes). Single-response optimization was performed to examine the effect of these variables on total phenolic content (TPC), total flavonoid content (TFC), pigment yield (carotenoids, β-carotene, and astaxanthin), and antioxidant activities determined by DPPH, H_2O_2, and TEAC assays, as well as CIELAB colorimetric parameters. The signal-to-noise (S/N) ratio was determined to find the best conditions for each respective response. ANOVA results indicated that methanol concentration had a significant influence on most of the responses (p<0.05), whereas extraction time was insignificant (p>0.05). The optimal conditions for bioactive recovery and antioxidant activity were found to be 75% methanol and 120 min extraction time, yielding 18.40 mgGAE g^{-1}TPC, 84.26 mgQE g^{-1} TFC, 15.47 µg/mL carotenoids, and maximum antioxidant activities (51% DPPH, 53% H_2O_2, and 109.24 mgTE g^{-1} TEAC). These findings highlight the effectiveness of the Taguchi method for optimizing solvent extraction in microalgae and demonstrate the potential of *H. pluvialis* as a source of high-value bioactives for nutraceutical and functional foods applications.

Keywords: antioxidant, Haematococcus pluvialis, nutraceutical, functional foods, Taguchi method.

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INTRODUCTION

Antioxidants are molecules that inhibit oxidation and protect the body against aging and diseases (Rahaman *et al*., 2023). They constantly scavenge free radicals, supporting immunity, while inhibiting inflammation and oxidative damage (Abdel-Hameed *et al*., 2023). Natural antioxidants can be obtained from plants, fruits, and algae (Flieger *et al*., 2021). Microalgal biomass is a nutrient-rich source of carbohydrates, proteins, lipids, and polyunsaturated fatty acids. Its pigments, phenolic antioxidants, vitamins, and minerals confer potential health benefits. In addition to the antioxidant activity of phenolic compounds, the pigments of microalgal biomass exhibit a certain degree of antioxidant activity (Almendinger *et al*., 2021).

Microalgae, particularly *Haematococcus pluvialis*, are at the top of microalgae because of the bioactive compounds they produce, mainly antioxidant compounds (Ahmad Kamal *et al*., 2024). *H. pluvialis* has a high content of carotenoids, chiefly astaxanthin (Astx) at high levels (Bian *et al*., 2023); this pigment has been classified as a strong-acting carotenoid considering its high antioxidant effectiveness (Patel *et al*., 2022). In addition, its content of phenolic compounds is high (Castillo *et al*., 2022), which are promising candidates for functional food, pharmaceutical, and nutraceutical industries (Sirotiya *et al*., 2023).

Therefore, effective extraction methods for bioactive compounds are required. Different approaches have been used to identify the most efficient cell disruption method for bioactive compounds extraction from microalgae (Vasistha *et al*., 2021). The extraction method is crucial because it significantly affects the quantity and quality of bioactive compounds obtained from the microalgal biomass (Sousa *et al*., 2023). The higher yield resulted from the polarity of the solvent in the medium, such as methanol or water, which improved the bioactive compounds yield (de Jesus *et al*., 2019). The polar solvent pulls out the polar compounds in microalgal tissue (Zarrinmehr *et al*., 2022). The chemical composition of microalgae generally contains a high percentage of medium and highly polar compounds (Stirk

et al., 2022). Furthermore, recent advancements in solvent proportion optimization techniques have markedly refined the extraction process. A study by Smedes (Smedes and Askland 1999) indicated the positive effect of methanol on extraction yield, even with changes in phase volumes.
Methanol solubilizes polar compounds, whereas extraction time affects solvent penetration and dissolution. This study employed optimization to address the challenges of extracting bioactive compounds from *H. pluvialis*, aiming to explore the effects of different methanol concentrations and extraction time on the recovery and antioxidant activity of bioactive compounds of *H. pluvialis*. This study focused on optimization by varying the methanol concentration (50, 75, and 100%) and extraction time (60, 120, and 180 min) to obtain the optimum parameters to achieve maximum antioxidant activity and recovery of bioactive compounds.

MATERIALS AND METHODS

Materials

Hamaetoccocus pluvialis AstaLuxe 3% Biomass Cracked (ABC) obtained from Evergen (Kendal, Central Java, Indonesia). Methanol, chloroform, and ethanol (analytical grade, purity ≥ 99%) were purchased from Merck (Merck KGaA, Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl, quercetin, gallic acid, aluminum chloride ($AlCl_3$), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), and potassium tartrate (purity ≥ 98%) ($K_2C_4H_4O_6$) purchased from Millipore Sigma (Burlington, Massachusetts, United States).

Haematococcus Pluvialis Extraction

Approximately 0.3 g of *H. pluvialis* biomass was soaked in mixed solvent with a proportion of chloroform: methanol: water 1:1:0.9; v/v/v). As a treatment, MeOH was used at different percentages at 50, 75, and 100% of this proportion. The samples were homogenized and sonicated for 5 min at 69 kHz. Macerations were performed for 60, 120, and 180 min. The solvent and time formulations are listed in Table-1. The filtrate was then filtered and evaporated for analysis.

Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method as described by Abduh *et al*. (Abduh *et al*., 2023), with slight modifications. Herein, 0.5 mL of the extract was mixed with 2.5 mL of the Folin-Ciocalteu reagent, followed by the addition of 2 mL of Na_2CO_3 solution. The mixture was homogenized and incubated in the dark at room temperature (30±1°C) for 60 min. After incubation, the absorbance was read using a UV-VIS spectrophotometer at 765 nm. The calibration curve was obtained by diluting the standard solution containing 0–100 mg L^{-1} of gallic acid. The results were expressed as milligrams of gallic acid equivalent per gram of sample on a dry weight basis (mgGAE g^{-1}).

Total Flavonoid Content

The total flavonoid content (TFC) in *H. pluvialis* extract was measured using the $AlCl_3$ colorimeter method described by Sarker *et al*. (Sarker *et al*., 2020). A tube containing 0.5 mL of extract was added to a mixture of 1.5 mL of methanol, 0.1 mL of 1M CH_3COOK, 0.1 mL of 10% $AlCl_3$, and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min before counting using a UV-VIS spectrophotometer at a wavelength of 415 nm. The calibration curve was obtained by dilution of the standard solution with 0-100 mg L^{-1} of quercetin. TFC was expressed in mg of quercetin equivalents per gram of dry weight (mgQE g^{-1}).

Measurements of Carotenoids

Additionally, 1 µg mL^{-1} of *H. pluvialis* extract was dissolved in acetone and read using a UV-VIS spectrophotometer at 663.2, 646.8, 470, 479, 645, and 663 nm. The absorbance values were entered into the pigment formula as follows:

a. Carotenoid (Koopmann *et al*., 2022)

$$Ca\ (\mu g.mL^{-1}) = (12{,}2 \times A663{,}2) - (2{,}79 \times A646{,}8) \quad (1)$$

$$Cb\ (\mu g.mL^{-1}) = (21{,}5 \times A646{,}8) - (5{,}1 \times A663{,}2) \quad (2)$$

$$\text{Carotenoid} = \frac{(1000 \times A470) - (1{,}82 \times \text{Chl a}) - (85{,}02 \times \text{Chl b})}{198} \quad (3)$$

b. *Astx* (Koopmann *et al*., 2022)

$$\text{Astx} = 0{,}8 \text{ x karotenoid} \quad (4)$$

c. β-Carotene (Nagata, 2009)

$$\beta\text{-Carotene}\ (\mu g/mL) = 0.854 \text{ x } A479 - 0.312 \text{ x } A645 + 0.039 \text{ x } A663 - 0.005. \quad (5)$$

Measurements of Antioxidant Activity

DPPH Assay

DPPH was carried out according to the method of Luis *et al*. (Luís *et al*., 2018) with the modification that 0.4 mL of *H. pluvialis* extract was added to 3.6 mL of 0.1 mM DPPH in a methanol solution. The samples were homogenized and stored in the dark at room temperature for 60 min. Subsequently, the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. The radical potential of the sample was determined based on the visible absorbance value of DPPH, according to the following equation:

$$\text{DPPH}(\%): \text{blankControl} - \text{Abs.} \times 100 \quad (6)$$

Where *Asample* is the absorbance of the sample after reaction with DPPH, *Ablank* is the absorbance of the methanolic solution added to DPPH, and *Acontrol* is the absorbance of the control sample.

H_2O_2 Assay

Approximately 1.0 mL of *H. pluvialis* extract was mixed with 1.0 mL of $FeSO_4$, 0.3 mL of sodium salicylate, and 0.7 mL of H_2O_2. The resulting mixture was incubated in the dark at room temperature for 60 min before measurement with a UV-VIS spectrophotometer at 510 nm (Kathiravan S *et al*., 2021). The radical scavenging activities of the samples were calculated using the following formula.

$$\% \text{ inhibition: } \frac{Abs.control - Abs.Sampel}{Abs.control} \; x \; 100\% \quad (7)$$

Where Where *Asample* and *Acontrol* are the absorbances of the sample and control, respectively.

Trolox equivalent antioxidant (TEAC)

Based on Picos-Salas *et al*. (Picos-Salas *et al*., 2024) with modifications, radial ABTS was formed by mixing 7.4 mM ABTS with 2.4 mM potassium persulfate in a 1:1 ratio and incubating at room temperature in the dark for 12-16 h before use. The 0.4 mL extract was added to 3.6 mL of ABTS and incubated in the dark for 1 min, and the absorbance was measured at 734 nm using a UV-VIS spectrometer. The ABTS radical was used as the blank. The results were expressed as milligrams of Trolox equivalents per gram of extract (mgTE g^{-1})

CIELAB Color measurement

The color of the extract was measured using a chromatograph (CR-400; Minolta Co., Ltd.. Osaka, Japan) to quantify lightness. The L* (lightness: 100 light to 0 dark), a * (+ red to green), and b * (+ yellow to blue) values were measured at the center of the extract sample plate. (Ikeura *et al.*, 2023).

Identification of factor and response variables

Identification was based on a modified version of the study by Wang *et al*. (Wang *et al*., 2014). These factors were the methanol concentration and extraction time. Methanol is a polar solvent that can be used to extract polyphenols from plant matrices (Valle-Sánchez *et al*., 2025). Extraction time will determine the yield of the extracted bioactive compounds. If not properly determined, the extraction time can cause structural damage to the solute and reduce the extraction yield (Ratanasongtham *et al*., 2024). Each process variable has a three-factor level. The methanol concentrations were 50, 75, and 100%. The extraction times were 60, 120, and 180 min.

Single-objective optimization of taguchi ANOVA

The Taguchi method, developed by Genichi Taguchi, is a statistical method of experimental design used to find the optimum parameters and ranking factors; it is only applied in the context of one response of the control factors (M. A. Ben Taher, U. Pelay, S. Russeil, 2023). Two parameters with three levels were selected as control factors, as presented in Table-1. These factors are organized according to the L9 Taguchi orthogonal arrays, as presented in Table-2.

The Taguchi orthogonal array determines the optimal signal-to-noise (S/N) ratio. The highest S/N ratio reveals the optimal point for each response, transforming the response variables to a constant value (decibel, dB) using the formula "Larger is better" for TPC, TFC, pigment, antioxidant activity, a* and b *, while "Smaller is better" was applied for the L* value.

Table-1. Process parameters and their levels for the extraction.

Variables	Level 1	Level 2	Level 3
Methanol concentration (%)	50	75	100
Extraction time (minutes)	60	120	180

Table-2. Taguchi L9 orthogonal array.

Run	Factor	
	Methanol concentration (%)	Extraction time (minutes)
1	50	60
2	50	120
3	50	180
4	75	60
5	75	120
6	75	180
7	100	60
8	100	120
9	100	180

Table-3. Response variable results.

Run.	TPC (mgGAE/g)	TFC (mgQE/g)	Carotenoids (µg/mL)			Antioxidant			Color		
			Carotenoid	β-carotene	*Astaxanthin*	DPPH (%)	H_2O_2 (%)	TEAC (mgTE/g)	L*	a*	b*
1	11.20	53.18	13.90	3.23	11.12	32	33	93.71	16.09	4.33	4.90
2	13.12	57.06	14.31	3.35	11.44	37	38	96.53	16.98	5.23	6.65
3	11.82	64.67	14.73	3.17	11.78	36	34	97.47	16.38	4.62	5.91
4	15.04	84.26	15.44	3.43	12.35	51	52	106.57	17.57	3.08	2.99
5	18.40	81.28	15.47	3.51	12.38	51	53	109.24	17.23	3.02	2.85
6	14.36	77.02	17.18	3.62	13.74	47	49	108.00	15.77	3.38	3.53
7	12.50	55.73	10.62	3.14	8.49	30	30	76.78	18.80	2.62	3.67
8	10.53	42.93	14.11	3.01	11.29	30	32	76.20	19.70	2.77	2.79
9	13.38	67.28	13.59	3.17	10.87	38	38	88.08	17.18	3.67	4.26

Larger is better:

$$\text{S/N ratio} = -10 \log\left(\frac{1}{n}\sum_{i=1}^{i=n}\frac{1}{yi^2}\right) \quad (8)$$

Smaller is better:

$$\text{S/N ratio} = -10 \log\left(\frac{1}{n}\sum_{i=1}^{i=n} yi^2\right) \quad (9)$$

Description:
n = number of data
yi = measurement response data

ANOVA determines the importance of the process characteristics that affect system performance. Taguchi uses ANOVA with an S/N ratio to optimize the factor independence for each response variable. This allows ANOVA to determine the contribution of each parameter for which the S/N ratio alone could not be determined.

RESULTS AND DISCUSSIONS

This research has the objective of optimizing extraction conditions to improve bioactive compounds of *H. pluvialis* using a "larger-the-better" objective function to monitor response variables. The results, including mean responses and S/N ratios, were in a systematic arrangement using a mixed-level orthogonal array design (L9: 3^2), as shown in Table-1 Factor levels (1-3) represent different experimental conditions. Table-4 presents S/N ratios, delta values, and ranks to identify the most affecting factors. ANOVA analyzed the statistical significance of each factor and interaction using DF, Adj SS, Adj MS, F-values, and P-values. ANOVA for pigments, phenolics, colours, and RSA is given in Table-5.

Table-4. S/N ratio values of L9.

Exp No.	S/N Ratios of Responses										
	TPC	TFC	Carotenoid	β-caroten	Astaxanthin	DPPH	H2O2	TEAC	L*	a*	b*
1	20.98	34.51	22.86	10.18	20.92	30.10	30.37	39.44	-24.13	12.73	13.80
2	22.36	35.13	23.11	10.50	21.17	31.36	31.60	39.69	-24.60	14.37	16.46
3	21.45	36.21	23.36	10.02	21.42	31.13	30.63	39.78	-24.29	13.29	15.43
4	23.54	38.51	23.77	10.71	21.83	34.15	34.32	40.55	-24.90	9.77	9.51
5	25.30	38.20	23.79	10.91	21.85	34.15	34.49	40.77	-24.73	9.60	9.10
6	23.14	37.73	24.70	11.17	22.76	33.44	33.80	40.67	-23.96	10.58	10.96
7	21.94	34.92	20.52	9.94	18.58	29.54	29.54	37.70	-25.48	8.37	11.29
8	20.45	32.66	22.99	9.57	21.05	29.54	30.10	37.64	-25.89	8.85	8.91
9	22.53	36.56	22.66	10.02	20.72	31.60	31.60	38.90	-24.70	11.29	12.59

Table-5. ANOVA for individual responses.

Respons	Source	DF	Adj SS	Adj MS	F-Value	P-Value	% Contribution
Total Phenolic Content (TPC)	Methanol (%)	2	11.30	5.65	4.15	0.106	65.70
	Extraction (min)	2	0.45	0.23	0.17	0.853	2.63
	Error	4	5.45	1.36			31.67
	Total	8	17.19				100.00
Total Flavonoid Content (TFC)	Methanol (%)	2	20.33	10.17	6.73	0.052	68.22
	Extraction (min)	2	3.43	1.71	1.13	0.407	11.50
	Error	4	6.04	1.51			20.28
	Total	8	29.81				100.00
Carotenoid	Methanol (%)	2	6.18	3.09	6.31	0.058	59.04
	Extraction (min)	2	2.33	1.16	2.38	0.208	22.26
	Error	4	1.96	0.49			18.70
	Total	8	10.46				100.00
β-Karoten	Methanol (%)	2	1.81	0.91	11.37	0.022	84.04
	Extraction (min)	2	0.03	0.01	0.16	0.857	1.18
	Error	4	0.32	0.08			14.78
	Total	8	2.16				100.00
Astaxanthin	Methanol (%)	2	6.19	3.09	6.29	0.058	58.99
	Extraction (min)	2	2.33	1.17	2.37	0.209	22.24
	Error	4	1.97	0.49			18.77
	Total	8	10.49				100.00
Antioxidant Activity (DPPH)	Methanol (%)	2	23.31	11.66	15.00	0.014	85.22
	Extraction (min)	2	0.94	0.47	0.60	0.591	3.42
	Error	4	3.11	0.78			11.36
	Total	8	27.36				100.00
Antioxidant Activity (H2O2)	Methanol (%)	2	25.71	12.85	20.12	0.008	88.50
	Extraction (min)	2	0.78	0.39	0.61	0.585	2.70
	Error	4	2.56	0.64			8.80
	Total	8	29.05				100.00
Antioxidant Activity (TEAC)	Methanol (%)	2	10.14	5.07	33.96	0.003	90.30
	Extraction (min)	2	0.49	0.25	1.65	0.300	4.39
	Error	4	0.60	0.15			5.32
	Total	8	11.23				100.00
L* values	Methanol (%)	2	1.77	0.88	7.95	0.040	56.76
	Extraction (min)	2	0.90	0.45	4.06	0.109	28.96
	Error	4	0.44	0.11			14.28
	Total	8	3.11				100.00
a* values	Methanol (%)	2	28.04	14.02	14.86	0.014	80.34
	Extraction (min)	2	3.09	1.54	1.64	0.303	8.85
	Error	4	3.77	0.94			10.81
	Total	8	34.90				100.00
b* values	Methanol (%)	2	48.54	24.27	12.05	0.020	79.61
	Extraction (min)	2	4.38	2.19	1.09	0.420	7.18
	Error	4	8.06	2.01			13.21
	Total	8	60.97				100.00

Phenolics Compounds Content

The Folin-Ciocalteu TPC assay is based on the electron transfer mechanism, which accumulates antioxidant capacity by donating electrons to reduce free radicals (Chiu *et al*., 2025). TFC and TPC are crucial for improving the antioxidant capacity of algae (Esim *et al*., 2024). Methanol solvents are polar and effectively dissolve polar compounds, such as phenolics and flavonoids (Rajhard *et al*., 2021). A concentration of 75% facilitated the diffusion of these bioactive components. Flavonoid extraction requires a longer maceration time than phenolic extraction because of the more complex flavonoid structure (Osorio-Tobón, 2020).

Both TPC and TFC extracts of *H. pluvialis* were not significantly affected (p>0.05) by the following factors: percentages of methanol (A) and extraction time

(B) (Table-5.). A higher S/N ratio indicates better quality as the signal is more dominant over the noise. As shown in Figure-1, the combination of parameter levels that gives the optimal TPC value (18.40 mgGAE g^{-1}) is 75% methanol concentration with an extraction time of 120 minutes, while the combination that gives the optimal TFC value (84.26 mgQE g^{-1}) is 75% methanol concentration with an extraction time of 60 minutes. This may be attributed to the presence of phenolic compounds or groups that are more soluble in polar solvents such as methanol. Methanol, being a polar solvent, is particularly effective at extracting phenolic groups from biological samples. Additionally, longer extraction times increase the amount of phenolic compounds detected in *H. pluvialis*. This is likely due to improved diffusion of the compounds and enhanced mass transfer rates, which are facilitated by higher temperatures and prolonged contact between the solvent and the sample matrix (Osorio-Tobón, 2020). This study was corroborated with previous studies that different polar solvent concentrations affect the phenolic compounds content in the extract (Lohvina *et al*., 2022). Total phenolic content in this study was higher than *H. pluvialis* study by (Ben Hamouda *et al*., 2022) and several microalgae extracted with methanol studied by (Andriopoulos *et al*., 2022).

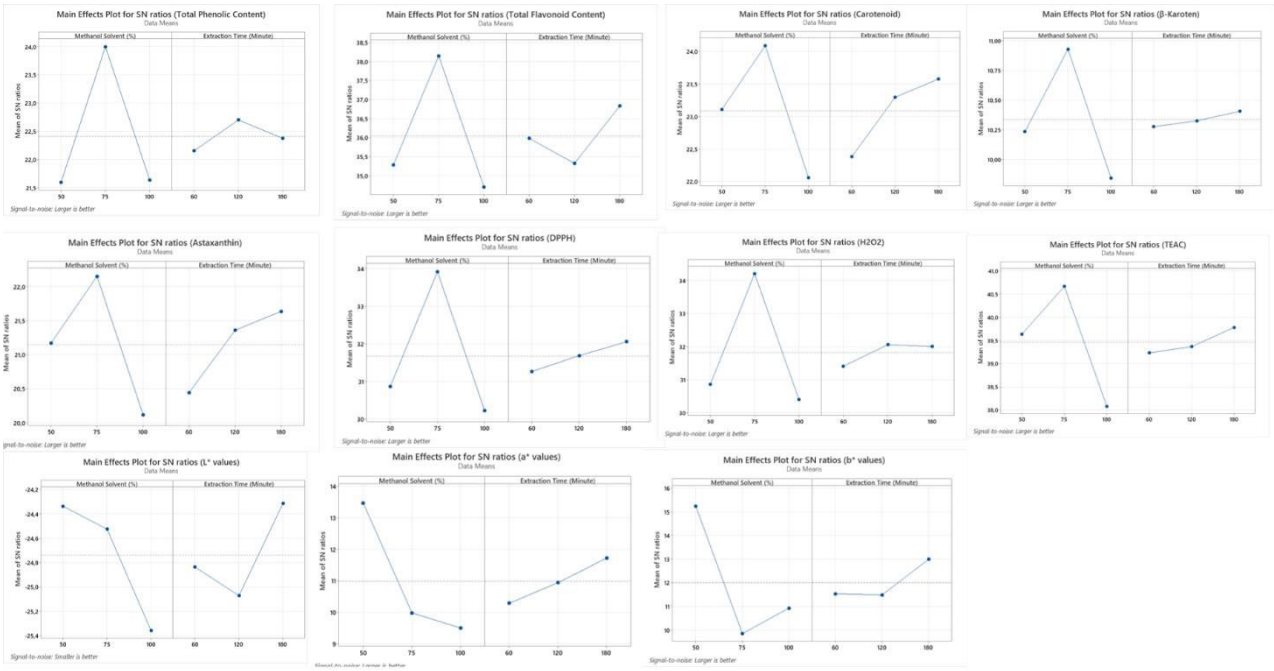

Figure-1. Main effect plot for S/N ratios for the response variable.

Pigments Content

The pigments identified in *H. pluvialis* include carotenoid pigments, which are a major class of pigments in microalgae, together with chlorophyll and phycobiliproteins (Chini Zittelli *et al*., 2023). *H. pluvialis* contains highly contained primary carotenoids, such as β-carotene and lutein, and secondary carotenoids such as Ast, which protects chlorophyll by wrapping it around microalgae cells (Rammuni *et al*., 2019).

The analysis of variance on the pigment content in *H. pluvialis* (Table-5) demonstrated that methanol concentration was significantly different from the pigment content (p<0.05). In addition to this, the extraction time was not found to be influential (p>0.05). According to the single objective Taguchi Method, the best combination of parameters for the highest carotenoids and Ast pigment was a methanol solvent with a concentration of 75% coupled ($CHCl_3$ 25%) with an extraction time of 180 min (Figure 1). The extraction efficiency of Ast depends on the polarity and solubility in the chosen solvent. A combination of $CHCl_3$ and MeOH demonstrated the best solvent combination to extract Ast from *H. pluvialis*. The type of Ast by using this solvent is dominated by all trans Ast, 13-cis Ast, and 9-cis Ast (Molino *et al*., 2018).

Extract Colour

The L *, a *, and b* coordinates of the CIELAB color system quantify the brightness, redness, and yellowness of a sample, respectively. The colour of the extract physically represents how much pigment is contained in the *H. pluvialis* extract. The ANOVA analysis exhibited that the extraction time was not non-significantly affected (p>005) in the extract colour, while the methanol concentration significantly influenced (p<0.05) the colour of *H. pluvialis* extract. As a result, the optimum coordinate values of L *, a *, and b * are specified as follows: the concentration of methanol at 50% and the extraction time of 180 min (Figure-1). Moreover, the best CIELAB L* value of 15.77, whereas the second run 2nd experiment provided the best a* and b* values of

5.23 and 6.65, respectively. The a* and b* values indicate the colour intensity of the extract. This indicated that the *H. pluvialis* extract had a bright orange coloration (Bassijeh *et al*., 2020). Thus, it affects the reading of the LAB color: The L* value describes a brightness scale where increasing the concentration of the extracted pigments will cause a decrease in the level of L*, turning darker and more concentrated. Increasing the concentration of the extracted pigments increased the a *and b *values. The run 3rd experiment resulted in a lower L* value, with the a* and the b* values tending toward red and yellow, respectively. This is related to the carotenoid content in the extract; the Ast colour is pink-red, while β-carotene is yellow-orange (Benbelkhir *et al*., 2022). The higher concentration of those pigments resulted in a dark colour of the extract, resulting in a lower L* value and higher a* and b* values.

Antioxidants Activities

The antioxidant activity was determined using three methodologies: DPPH, H_2O_2, and TEAC. The principles behind these three techniques differ. DPPH acts as a hydrogen or electron acceptor for bioactive molecules, resulting in a reduction in its absorption. The reduction in the rate of absorption is used as an index of antioxidant activity (Jabbari *et al*., 2016). Hydroxyl radicals (OH) are biologically oxidized using the H_2O_2 method and then polymerized. The identification of these compounds exposes them to high hydroxyl atom scavenging due to oxidative stress. Bioactive compounds are potent • OH radical scavengers (Kathiravan S *et al*., 2021). The equivalent antioxidant capacity of Trolox was established when the redox reaction of each compound reached the steady state (Muñoz-Acevedo *et al*., 2011).

The analysis of variance on the antioxidant activity (Table-5) demonstrated that the main effects of methanol composition were affected by the percentage inhibition of DPPH, H_2O_2, and TEAC (p<0.05). In addition to this, the observed factors of extraction time were not found to be affected (p>0.05). The methanol concentration was found to be the most effective factor on the percentage inhibition of DPPH, H_2O_2, and TEAC in comparison with Table-3. According to the single objective Taguchi Method, the optimum antioxidant activities (DPPH, H_2O_2, and TEAC) of run 5th experiment showed a maximum value of 51%, 53%, and 109.24 mgTE/g, respectively, at a concentration of 75% methanol and an extraction time of 120 min (Figure-1). The best combination of parameters for all antioxidant activities is obtained when the methanol concentration and extraction time are 75% and 120 min, respectively. The DPPH and TEAC assays measure the ability of antioxidants to neutralize stable free radicals by donating electrons or hydrogen. The results obtained from the DPPH and TEAC tests were correlated, in accordance with what has been reported in several other studies on the methanol extracts of different fruits and vegetables (Sadef *et al*., 2022). Moreover, H_2O_2 assays bind to ROS that generate hydroxyl radicals via the decomposition of hydrogen peroxide (Kumar *et al*., 2023). Bioactive compounds, such as Ast, carotenoids, and phenolic compounds, can react differently with hydroxyl radicals and stable free radicals. Different principles of action affect the optimal point in the determination of the antioxidant activity of *H. pluvialis* extract.

CONCLUSIONS

The Taguchi single-response optimization method was effectively used in this investigation to identify the ideal extraction parameters for maximizing the antioxidant activity and bioactive component content of *H. pluvialis*. Methanol concentration was the most prevalent variable among those examined, having a significant impact on extract colour characteristics, pigment yields, antioxidant responses, and phenolic and flavonoid contents. The maximum concentration of total phenolics, flavonoids, carotenoids, and antioxidant activity was obtained under ideal extraction conditions, which included 75% methanol and 120 minutes. The results indicate that *H. pluvialis* is a viable source of natural antioxidants for use in pharmaceutical, nutraceutical, and functional food applications. They also support the usefulness of employing a systematic Taguchi technique to maximize extraction efficiency. Multi-response optimization (GRA) may be the subject of future study.

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