ANTIOXIDANT EXTRACTION FROM *SPIRULINA PLATENSIS* MICROALGAE BY ULTRASONIC-ASSISTED EXTRACTION (UAE) TECHNOLOGY

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Abstract
This innovative research study highlights on the optimization of extraction process parameters of total phenolic content and antioxidant activity and caffeic acid extraction from *Spirulina platensis* using ultrasonication (probe) technology. Optimization of ultrasonication parameters was carried out employing Box-Behnken Design (BBD) and response surface methodology. The three levels of extraction parameters i.e. solvent volume, extraction time and frequency have been fixed. As responses, the total yield of extract, total phenolic contentand DPPH radical scavenging activity (IC_{50}) of the extracts were determined. The variability of each response variable was determined by developing multiple linear regressions. The statistical analysis (ANOVA) of developed mathematical models allowed the prediction of the behaviour of the responses, as a function of the variables involved in the process. The optimized extraction conditions were obtained at 58.76 kHz frequency, 10 mL of solvent volume for 6 min. This extract exhibited the highest content of total phenolic content (30.89 mg GAE/g powder), IC_{50} for DPPH activity (151.27 µg/mL). The extract exhibited an important antioxidant i.e. caffeic acid, which was quantified as 660.72±41.05 µg/g of dry algal powder using HPLC. In this way, this study offers an alternative method for obtaining natural antioxidants from algae for food and pharmaceutical applications.

Keywords: Microalgae; Probe sonication extraction; Total phenolic content; Antioxidant activity

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Practical Applications

Spirulina platensis and its extraction products are employed in agriculture, food industry. Spirulina has several pharmacological activities such as antimicrobial (including antiviral and antibacterial) as well as antioxidant effects due to its rich content of protein, polysaccharide, lipid, essential amino and fatty acids. This research object aids as an overview, introducing the basic biochemical composition of this algae and moves to its medical applications.

1. INTRODUCTION

There is an increasing trendin the food processing industry for producing natural sources of antioxidants preventing oxidative damage of food. Among several algae, *Spirulina platensis*, blue-green microalgae of the cyanobacteria family is widely consumed as a whole food or as a supplement in several countries. It is apotential source of high amount of bioactive compounds with functional properties such as antioxidants, phenolic compounds i.e. salicylic, trans-cinnamic, synaptic, chlorogenic, caffeic acid and polyunsaturated fatty acids [1, 2, 3].

Extraction of bioactive elements from microalgae species was successfully conducted by several researchers [4, 5, 6]. Caffeic acid (hydroxyl cinnamic acid consists of both phenolic and acrylic groups) is one of the most important antioxidants used as biopharmaceutical molecule. The various extraction factors such as nature of extraction solvent, frequency and extraction time would give an impact on the rate of extraction/extraction efficiency and amount of extracted antioxidative biomolecules [7]. The various conventional methods such as maceration and Soxhlet extraction has been conducted to obtain algal extract from biomass

[8, 9, 10]. On the other hand, these conventional extraction methods have demerits like; large amount of solvent usage, lengthy extraction time and lower extracted constituents [11].

For that reason, the above extraction methods are not applicable in food and pharmaceutical industries. Hence, the alternative extraction methodologies like ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) are employed to overcome these drawbacks [12]. Among these, UAE is an inexpensive, emerging and efficient technique that accelerates heat and mass transfer during extraction. UAE reduces extraction time, increases extraction yield, rate of extraction and antioxidant properties of extracts as compared to conventional methods [13,14]. Hence, it is an attractive alternative green technology.

The present study focuses on the optimization of the probe sonication extraction parameters such as solvent volume, extraction time and frequency using response surface methodology based on the best combination of phytochemical properties (total phenolic content and antioxidant activity) of the extracts. This study reports the extraction of caffeic acidby UAE as an antioxidant from *Spirulina platensis*. Thus the work endeavours to acquire a harmless 'green' extract from algae for favourable end-use of the same as a nutraceutical food supplement.

2. MATERIALS AND METHODS

Materials

The microorganism *Spirulina platensis* var. lonor used in this study was procured from Antenna Green Trust, Madurai, Tamil Nadu, India. The samples were stored under dry and dark conditions in amber colouredsealed plastic container until use.

Chemical Reagents

Ethanol 99.9% (ChemSoln), Gallic acid (Merck, Mumbai, India), Sodium carbonate anhydrous (Fisher Scientific), Sodium nitrite (R&M Chemicals), Aluminium chloride (System), Folin & Ciocateu's Phenol Reagent (ChemSoln), Quercetin (Sigma-Aldrich), 2,2-Diphenyl-1-Picrylhdrazyl (DPPH) (Riendemann Schidt) and Sodium hydroxide (Riendemann Schidt) were of analytical grade.

Preparation of samples for UAE

The algal biomass was placed in a freezedryer for lyophilisation (Eyela, FDU 1200, Tokyo, Japan) and stored in dry and dark conditions in amber coloured airtight container prior to UAE. Then the lyophilized algal powder wassievedin a sieve shaker [15]. The algal powder samples with mean particle diameter (d_p) of 1 mmwere selected for extractions.

Optimization of UAE parameters to obtain antioxidant-rich extract from *Spirulina platensis*

An ultrasonic probe processor (UP50H, Hielscher Ultrasound Technology, Teltow, Germany) with a 2 mm titanium microprobe tip operating at the different frequency level of 20-100 kHz and 50 W input power was employed for treatments of extraction media. The entire extraction procedure was performed at a fixed duty cycle (ultrasonic pulse cycle ratio) of 1.0. Preliminary trials conducted with three different extraction solvents (ethanol, distilled water and hexane), revealed that ethanol gave the maximum total yield of the extract. This polar solvent has been known as a suitable solvent for recovering polyphenols[16]. UAE was carried out with 1 g of lyophilizedalgal samples at different frequency, solvent volume, and sonication times according to the experimental design. In the present study, the Box-Behnken design (BBD), a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial design [17] with three independent variables (solvent volume, frequency and time) and three dependent variables (total yield percentage, total phenolic content and IC_{50} values of DPPH radical scavenging activity of dried algal extract) was used for designing the experiment. Preliminary trials suggested that significant yields of algal extract with appreciable phytochemical properties were obtained within a sonication time of 2-10 min, solvent volume in a range of 10 to 25 mL and

frequency of 20 to 100 kHz. Based on the preliminary trials, the extraction time (2, 6, 10 min) solvent volume (10, 30, 50 mL) and frequency (20, 60, 100 kHz) were varied at three levels respectively. A total of 13 experiments were performed and four additional runs 0….at the star points were carried out in a randomized run order **[**18].

All algal extracts were then centrifuged for 15 min at 13500 g (SIGMA Laborzentrifugen 2-16 PK refrigerated centrifuge) to separate the liquid phase. The liquid supernatant was collected and dried using a rotary evaporator (Supervac-R/180; Superfit Continental Pvt. Ltd., Mumbai, India) under reduced pressure of 50 mbar Hg at vacuum temperature of 40-50 ^{o}C and finally dried by purging a gentle stream of nitrogen and their yields were determined gravimetrically [$yield$ (%) = "mass of concentrated extract " / "Initial mass of microalgae" *100]. The extracts were kept in nitrogen flushed amber coloured screw capped glass vials at 4°C for the subsequent analyses.

Evaluation of phytochemical properties of UAE algal extracts

Determination of total phenol content and DPPH radical scavenging activity of the UAE extract

Total phenolic content and free radical scavenging activity (DPPH) of the algal extracts were estimated in accordance with the method described by Singleton and Rossi (1965) and Ghosh et al., (2013) respectively [19,20].

Liquid chromatography–mass spectroscopy (LC-MS) analysisof the UAE extract obtained under optimized extraction conditions

The extract obtained under optimized UAE conditions was coded as UAE_{best}**.** The sample was subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the identification of compounds. The UAE extract was suspended in 0.1% formic acid, injected and captured in a C_{18} trapping cartridge. The trapped bioactive compounds were eluted onto a C18 analytical column. The elution gradient consisted of mobile phase A (water and 0.1% formic acid) and mobile phase B (methanol and 0.1%formic acid). The mass spectrum was obtained in the negative ESI ionisation mode over anm/z range of 100–1200. The temperature and voltage of the curved desolvation line were set to 230 °C; and +85 V, respectively. The probe voltage and nitrogen nebulizer gas flow were set at +4.5 kV and 4.5 l/min, respectively.

Quantification of caffeic acid in the UAE extract obtained under optimized extraction conditions

UAE_{best} was subjected to HPLC for quantification of caffeic acid [21]. UAE_{best} was filtered through a 0.22 µm polytetrafluorethylene syringe filter prior to HPLC injection at an injection volume of 20 µL into an Agilent ZORBAX SB C-18 column (150 × 4.6 mm, 5 µm) 1300 series HPLC (Agilent Technologies Inc., Alpharetta, GA, USA). The flow rate of the mobile phase was set at 0.8 mL/min with a gradient elution of water–acetic acid (95:5, v/v) (A) and methanol–acetonitrile–acetic acid (95:5:1, v/v/v) (B), starting from 0% to 40% B in 10 min, 40%–100% B in 10 min, 100% B in 5 min, and 100%–5% B in 5 min. The authentic standard of caffeic acid was solubilized in mobile phase B and concentration prepared at 100, 80, 60, 40,20 mg/mL in order to plot standard curve. Each solution was injected three times and the mean peak areas were considered for the preparation of standard curve and the results were expressed in µg/g of algal powder.

Analysis of amino acids profile of UAE_{best}

The nutritional quality of a protein is mostly determined by the proportion, content and availability of its amino acids [22]. In order to understand the nature of toxic peptides, the amino acid composition of $UAE_{best}$was determined [23]. The UAE_{best} filtrate was subjected to quantitative HPLC analysis for the determination of amino acids profile. A Licrosper 100 RP 18 column (4mm × 125 mL) was used to separate the compound. The mobile phase solution consisted of methanol and mercaptoethanol solvents.The fluorescence Shimadzu RF-138 detector was used and the

excitation wavelength and an emission wavelength were 360 nm and 460 nm respectively.

Statistical analysis

The results of all experiments were conducted in triplicateand data are reported as mean ± SD of three independent experimental runs. Statistical analysis of the data was conducted by one-way analysis of variance (ANOVA), response surface analysis (RSM) and regression modelling. The significance differences between variables were tested by ANOVA and significant differences between meanswere calculated with post hoc Duncan's test at 5% level of significance. In the present study, STATISTICA 8.0 (Statsoft, Oklahoma, USA) and Design Expert 7.0 (Stat-Ease Inc., Minneapolis, MN, USA) softwares were used to assess the experimental results.

3. RESULTS AND DISCUSSION

Total yield of Spirulina extracts and its corresponding total phenol content (TPC) and IC_{50} value under different conditions of UAE

Effect of UAE time on total yield and its corresponding TPC and IC_{50} value of UAE extract

The extraction time is very important to deduce the energy and cost of the extraction process. UAE time has insignificant effect on total phenol content (p = 0.11) but has a significant effect on the extract yield (p = 0.01) and IC_{50} value (p = 0.001). A significant interaction between extraction time and solvent volume on TPC (p = 0.01) and IC $_{50}$ value (p= 0.007) was obtained. The Figure 1 showed that the yield increased quickly with increasing time. The yield of extract during 2 to 6 min interval and at 10 min was low and highest respectively. The maximum concentration of phenolic compounds was achieved at extraction time of 6 mins. The values of phenolic content after 6 mins of extraction time were decreased gradually and insignificantly ($p<0.05$). An excessive sonication time causes heating of the extraction solvent, results intothe degradation of extracted bioactive compounds [24]. It can also be ascertained that the DPPH in term of antioxidant capacity decreased after reaching a maximum value at 10 mins because of prolonged extraction that would increase the chances of phenolic compounds.

Effect of UAE frequencies on total yield and its corresponding TPC and IC_{50} value of algal extract

The Figure 1, 2 and 3 represented that the extract yield, TPC and IC_{50} were significantly affected by UAE frequencies. The yield of extract increased as frequency was increased. The IC_{50} value was affected by independent parameters viz., frequency and time. UAE frequency has insignificant effect on total phenol content (p= 0.62) but has a significant effect on the extract yield (p = 0.007) and IC_{50} value (p = 0.000). It showed that with an increase in frequency from 20 to 60 kHz, the yield increased, but; beyond this the yield decreased. A significant interaction between solvent volume and frequency on TPC (p = 0.02) was obtained. At frequency up to 60 kHz, with increase in solvent volume, TPC decreased from 26.97 to 23.8 mg GAE/g algal powder, whereas; at higher frequency, TPC remained constant at 26.97 mg GAE/g algal powder.

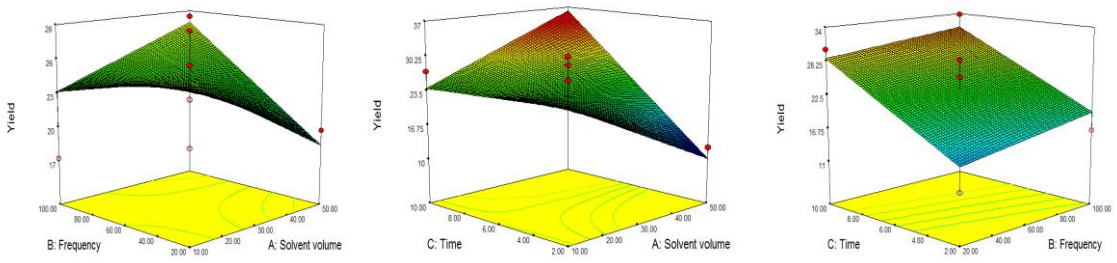

Figure 1: Response surface plot for Total yield of extract with respect to frequency (kHz), solvent volume (mL) and extraction time (min) and their mutual interactions

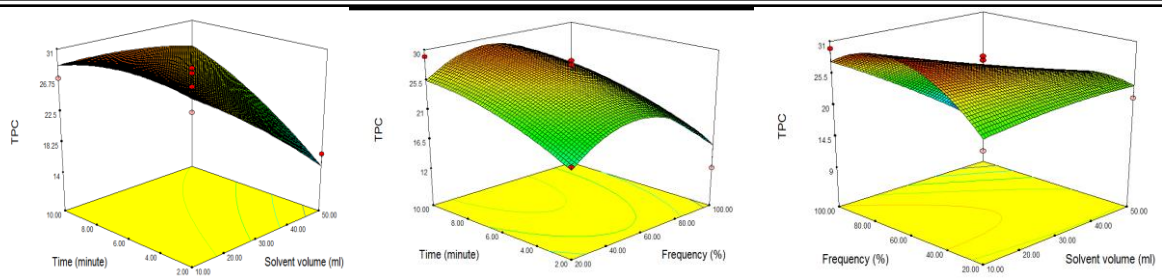

Figure 2: Response surface plot for Total phenolic content (TPC) with respect to solvent volume (mL), extraction time (min) and frequency (kHz) and their mutual interactions

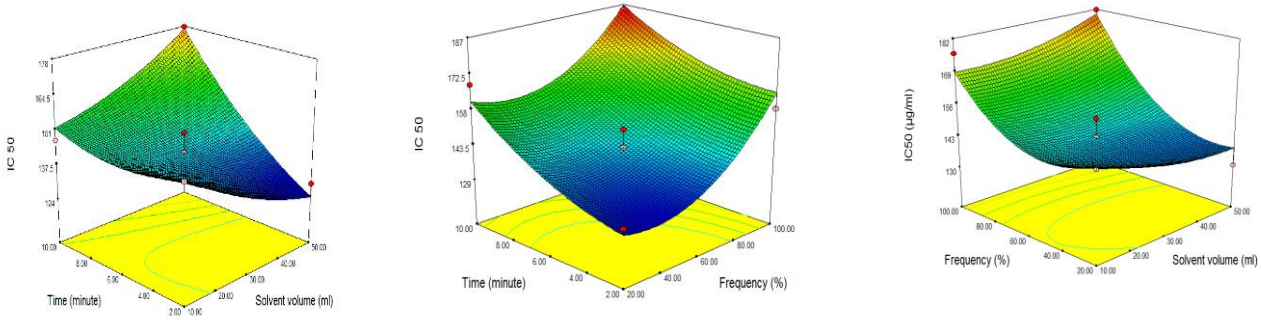

Figure 3: Response surface plot for DPPH scavenging activity (IC_{50}) with respect to solvent volume (mL), extraction time (mL) and frequency (kHz) and their mutual interactions on DPPH scavenging activity (IC_{50})

Effect of extraction solvent volume on total yield and its corresponding TPC and IC_{50} value of algal extract

The enrichment of the extraction yield of polyphenols can occur when a higher solvent volume can dissolve ingredients more effectively [25]. In such context, different solvent volume was tested in order to express the effect of solvent concentration in extracting the natural antioxidants. Solvent volume has insignificant effect on extract yield (p= 0.75) and IC_{50} value (p = 0.86), however; has a significant effect on TPC (p = 0.00) due to sonothermal degradation of bioactive compounds [26]. A significant interaction between solvent volume and extraction time on TPC (p = 0.01) and IC_{50} value (p= 0.007) was obtained. The effect of ethanol solvent volume on antioxidant capacities and phenolic contents of crude extract is shown in Figure 2 and 3. It was observed that at the lower time, with an increase in solvent volume, IC_{50} value decreased from 142.26 to 133.57 µg/mL, whereas; with an increase in both solvent volume and time, there was maximum IC_{50} 184.58 µg/mL (Table 1).

Optimization of UAE parameters for maximizing total yield, TPC and IC_{50} value of the extract

Generation of response curves

The effects of extraction time, solvent volume and frequency on the total yield percentage, TPC and IC_{50}values of extracts are shown in Figures 1 (A-C) respectively. Regression modeling was used for the characterization of the response surfaces.

Regression Modelling

Regression modelling was conducted by generating second order polynomial equations for response as a function of extraction time and frequency. Based on a designed set of experimental data using RSM, the model parameters of Eqn. 1 were statistically determined. In order to be evaluated in RSM, the real value of independent variables must be transformed into the coded variables. In this study, three independent variables were used and Y was set as response variables (total yield percentage, TPC and IC_{50}) of dried algalextract.

Table 1: Experimental design of three-level independent variables and its corresponding responses

Run	A: Solvent volume (mL)	B: Frequency (kHz)	C: Time (min)	Yield % of algal extract	Total phenolic content (mg gallic acid equivalent/g dry algae)	IC_{50} value of DPPH radical scavenging activity (µg/ml)
1	10	20	6	20.86 ± 0.006^{i}	20.16 ± 0.22^{i}	149.22 ± 0.04^{h}
2	50	20	6	19.72 ± 0.03^{j}	21.35 ± 0.06^{h}	130.78 ± 0.09^{l}
3	10	100	6	27.17 ± 0.05^{g}	29.75 ± 0.09^{b}	176.14 ± 0.05^{d}
4	50	100	6	28.44 ± 0.02^{f}	11.75 ± 0.03^{l}	181.63 ± 0.03^{b}
5	10	60	2	34.02 ± 0.13^{c}	30.89 ± 0.07^{a}	150.98 ± 0.06^{g}
6	50	60	2	32.2 ± 0.05^{d}	16.59 ± 0.07^{k}	129.87 ± 0.0^{m}
7	10	60	10	37.17 ± 0.07^{b}	27.11 ± 0.04^{e}	147.02 ± 0.11^{i}
8	50	60	10	41.91 ± 0.05^{a}	25.92 ± 0.15^{f}	177.67 ± 0.02^{c}
9	30	20	2	11.72 ± 0.07^{m}	19.19 ± 0.04^{j}	132.87 ± 0.06^{k}
10	30	100	2	16.35 ± 0.11^{k}	25.12 ± 0.03^{g}	158.56 ± 0.07^{f}
11	30	20	10	14.22 ± 0.03^{l}	29.01 ± 0.06^{c}	168.12 ± 0.02^{e}
12	30	100	10	29.81 ± 0.10^{e}	20.12 ± 0.02^{i}	184.58 ± 0.07^{a}
13	30	60	6	22.43 ± 0.08^{h}	28.46 ± 0.02^{d}	140.12 ± 0.03^{j}

All data are the mean ± SD of three replicates. SD followed by different subscripts in the same column differs significantly ($p\leq0.05$)

A second-order polynomial regression model for the dependent variable was established to fit the experimental data for each response as per the following equation:

$$Y= \beta_o+\sum\beta_i X_i+ \sum\beta_{ii}X_i^2+ \sum\beta_{ij}X_iXj \quad (1)$$

Where,Y represents the experimental responses [total yield of extract (Eqn. 2), TPC value of extract (Eqn. 3) and IC_{50} value of extract (Eqn. 4)]; X_i, Xj are two independent variables in coded forms; β_o, β_i,$\beta_{ii}$and β_{ij} are constants and regression coefficients of the model. The expanded model includes linear, quadratic, and cross-product terms as shown below (with intercept):

$$Yi=+36.01-1.79X_1+0.56X_2-3.43X_3+0.002X_1X_2+0.02X_1X_3+ 0.017X_2X_3 + 0.0261X_1^2+5.038E\text{-}003X_2^2+ 0.215X_3^2 \quad (2)$$

$$Yii=+3.54 + 0.347X_1+ 0.6597X_2 + 0.7045X_3 - 5.996E\text{-}003X_1X_2+0.0409\ X_1X_3- 0.023\ X_2X_3- 7.252E\text{-}003X_1^2-2.917E\text{-}003X_2^2-.0183X_3^2 \quad (3)$$

$$Yiii=+187.71-1.97X_1-0.75X_2-4.54X_3+7.47E\text{-}003X_1X_2+0.161X_1X_3-0.014X_2X_3+8.84E003X_1^2+8.24E\text{-}003X_2^2+0.32X_3^2 \quad (4)$$

Where, X_1, X_2 and X_3 are extraction solvent volume, frequency and time respectively.The above equations (2, 3, and 4) explain the effects of X_1, X_2 and X_3 on the response Yi, Yii and Yiii. The effects of the above parameters and their interactions were evaluated. The ANOVA table (Table 2) was used to study the effect of each independent variable constructing a model that maximized extraction yield, total phenolic content and antioxidant activity of *Spirulina sp*. This analysis also gave values of the model term tested for adequacy and fitness. The statistical analysis of F-value and probability value indicated that the model was actually significant. The fit of the model was checked by the coefficient of determination (R^2) which was 0.99, 0.93 and 0.93 and the p-value (>0.05) for the lack of fit analysis were 0.11, 0.08 and 0.07 for yield, TPC and IC_{50} respectively. The values of lack of fit were not significant, indicating that the model equations were adequate for predicting the yield percentage, TPC and IC_{50} under any combinations of variable factors.

The significance of each variable was determined by its respective p-value and F-value at a specified level of confidence. In fact, the smaller p-value is more important with respect to coefficient of the response variable. The p-value for each response was greater than 0.5, which suggests that the effect ofindependent variables on the response model was not statistically significant at 95% confidence level.

Table 2: Analysis of variance (ANOVA) for fitted response surface quadratic model of total yield of extract, total phenolic content (TPC) and DPPH radical scavenging activity (IC_{50})

Total Yield of extract

Source	Sum of squares	df	Mean square	F-value	Probability (P)>F	
Model	1102.33	9	122.47	233.81	0.0001	Significant
Lack of fit	2.73	3	0.91	3.91	0.1106	Non-significant
Pure error	0.93	4	0.23			
Corrected total	1105.89	16				
R^2	0.9967					
R^2_{adj}	0.9924					

Total phenolic content (TPC)

Source	Sum of squares	df	Mean square	F-value	Probability (P)>F	
Model	470.63	9	52.29	208.8	0.0001	Significant
Lack of fit	1.37	3	0.46	4.80	0.0818	Non-significant
Pure error	0.38	4	0.095			
Corrected total	472.38	16				
R^2	0.9372					
R^2_{adj}	0.8565					

IC_{50}

Source	Sum of squares	df	Mean square	F-value	Probability (P)>F	
Model	4977.23	9	553.03	11.75	0.0019	Significant
Lack of fit	259.80	3	86.60	4.98	0.0776	Non-significant
Pure error	69.62	4	17.41			
Corrected total	5306.65	16				
R^2	0.9379					
R^2_{adj}	0.8581					

Optimal Processing Conditions

The optimal values of X_1, X_2 and X_3 were determined to obtain the optimal processing conditions for total yield, TPC and IC_{50} of probe sonicated extract from *S. platensis* .The first partial derivatives of the regression equation were exhibited with respect to X_1, X_2, and X_3 and set to zero by putting the second-order regression equation in matrix form [27]. Thus the obtained points are known as the stationary point: X_{1S} = 10 mL, X_{2S}=58.76kHz, X_{3S}=6min. Under these conditions the maximum predicted yield, TPC and IC_{50} of the extractwere about 33.94%, 30.89 mg GAE/g of algal powder and 151.27 µg/mL respectively; whereas, the actual experimental values obtained were 34.02%, 29.52 mg GAE/g of powder and 150.98 µg/mL respectively; suggesting a close fit model.

Characterizing the Response Surfaces

The response curve was characterized by determining whether the stationary point obtained in the curve is a point of maximum response, minimum response, or a saddle point. For this purpose, the regression equation was transformed into the canonical forms and the eigen values in accordance with the method described by Chatterjee et al., (2013). Since the eigen values for extract yield (0.289266, 0.026627, 0.0047781) and IC_{50} value (0.320469, 0.008844, 0.00824) were positive, X_S was a point of minima [15]. The eigen

values obtained in case of TPC were -0.00044, -0.00325, -0.051156, since; the eigen values obtained were negative, the optimum point obtained was a point of maximum response.This extraction condition was considered as the optimized condition as the extract exhibited the highest yield percentage, total phenol content and antioxidant activity.

Yield of caffeic acid in UAE_{best}

From the above discussion, it was found that the algal extract obtained at 60 kHz in 10 mL of solvent for 6 min has the best combination of phenolic content and antioxidant/ DPPH radical scavenging activity. The amount of caffeic acid obtained in this extraction condition was 69.32 µg/g dry algae and identified as one of the active antioxidant.

Phenolic acidsprofile of the UAE_{best}

All food groups have phenolic acid abundantly found in vegetables, and fruits even in oilseeds and legumes. Several phenolic acids possess high antioxidant activity due to uptake hydroxyl radical, singlet oxygen, peroxyl radical, superoxide anion etc. Phenolic acid exists as hydroxyl cinnamic acids which include caffeic acid (CA), ferulic acid (FA), chlorogenic acid (CGA), and sinapic acids (SA) and hydroxyl benzoic acids which include gallic acid (GA), salicylic acid (SA) or occur as conjugated forms. However, these compounds are more energetic molecules than carbohydrate and protein. They can be used in different applications such as biofuels, food, etc. The phenolic components of UAE_{best} have been presented in Table 3. From Figure 4a and 4b it was found that ferulic acid, caffeic acid, trans caffeic acid hexoside, chlorogenic acidthat are the extracted components, all of whichreportedly have nutraceutical properties [28].

Table 3: Retention times, MS and MS^2values of the major bioactive constituents present in $SC\text{-}CO_2$ extract of *Spirulina platensis* identified by LC-MS

Retention time (RT)	Molecular ion peak $(M\text{-}H)^-$	MS^2 fragment ions intensity	Tentative Compounds Identified
8.62	341	161, 179	Trans caffeic acid hexoside
9.52	195	178,179	Ferulic acid
14.48	195	177, 178,195	Ferulic acid
21.16	340	179, 340	Caffeic acid derivative

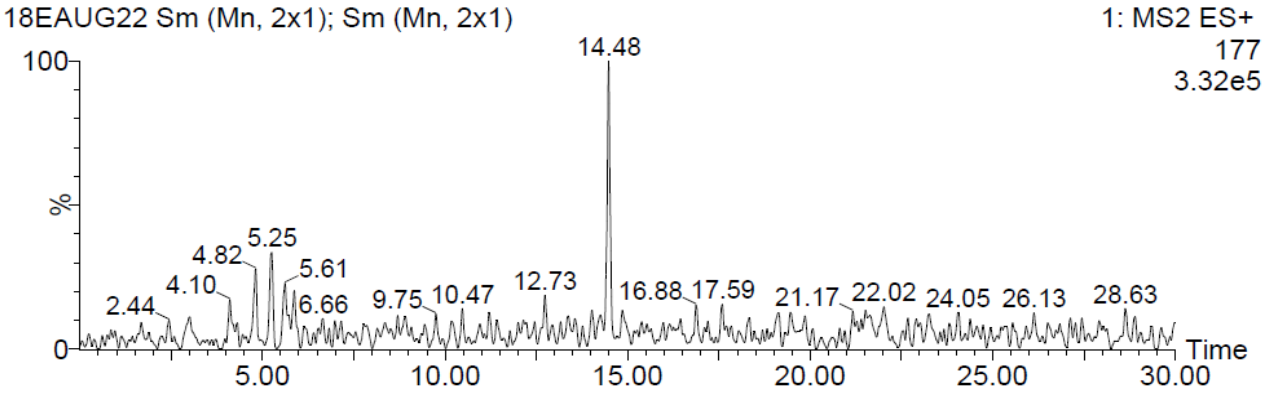

Figure 4a: Total chromatogram of UAE_{best} extract

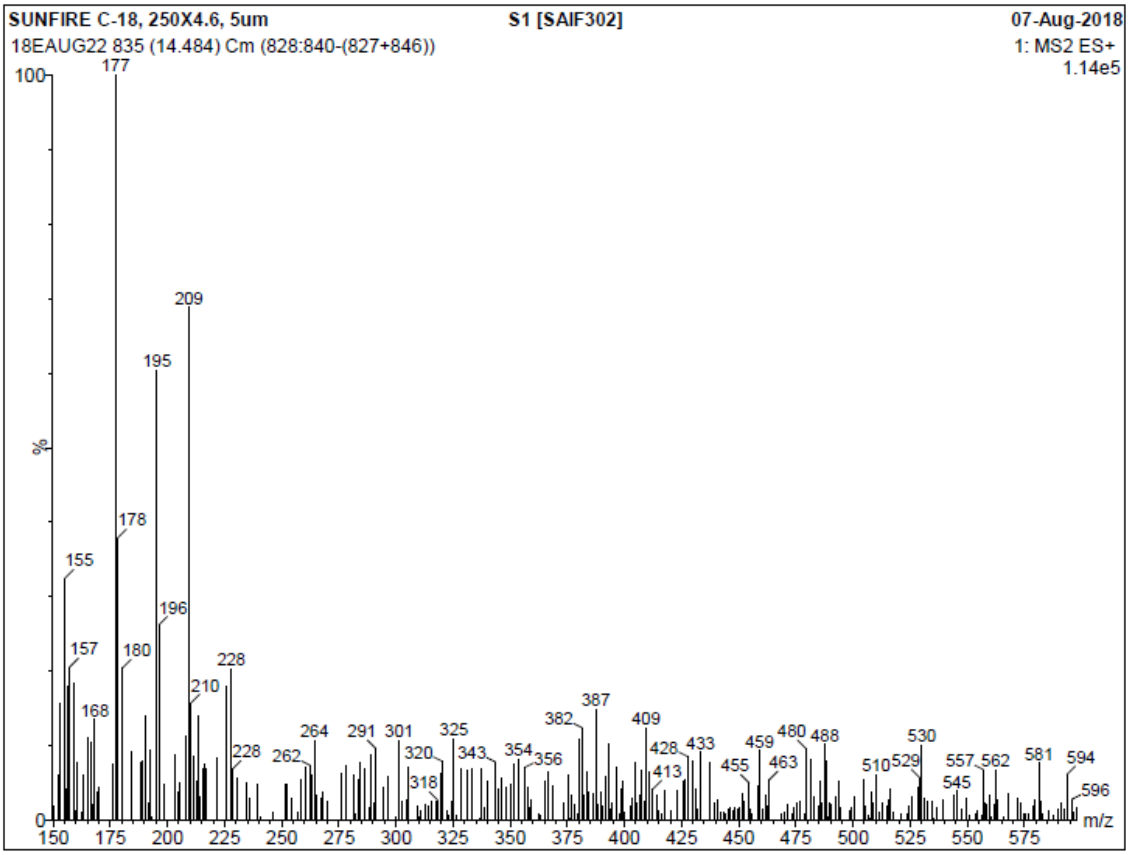

Figure 4b: LC-MS chromatogram of *Spirulina platensis*of UAE_{best} extract

4. CONCLUSIONS

The solvent volume, ultrasound frequency and extraction time had prominently influenced the yield, total phenolic content and antioxidant activity of the extract. The extraction yield was 33.94% and IC_{50} of DPPH radical scavenging activity was 151.27 µg/mL with caffeic acid content of 69.32 µg/g dry algae under the optimal conditions for UAE_{best}. Consequently, we envisage that caffeic acid is a safe and antioxidant-rich fraction obtained from *Spirulina platensis* by UAE green technology would have promising application in food and pharmaceutical industries. Additionally, there is considerable future scope for research in developing various types of food products with extract of microalgae.

Conflict of Interest:

Authors have no any conflict of interest and none financial involvement in this work.

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