Acta Scientiarum

http://periodicos.uem.br/ojs
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v45i1.61471

SCIENCE, FOOD TECHNOLOGY AND FOOD ENGINEERING

Effects of solvent extraction on phenolic concentration and antioxidant capacity of the *Oedogonium* sp. (Chlorophyta) using a simplex-centroid mixture design

Rodolfo José Tófoli[1], Adriana Luiza Ferreira[1], Eutimio Gustavo Fernández Núñez[2], Charles Windson Isidoro Haminiuk[3], Ciro Cesar Zanini Branco[1] and Ivanise Guilherme Branco[1*]

[1]Departamento de Ciências Biológicas, Faculdade de Ciências e Letras, Universidade Estadual Paulista, Av. Dom Antônio, 2100, 19806-900, Assis, São Paulo, Brazil. [2]Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, São Paulo, São Paulo, Brazil. [3]Departamento Acadêmico de Química e Biologia, Universidade Tecnológica Federal do Paraná, Curitiba, Paraná, Brazil. *Author for correspondence. E-mail: ivanise.branco@unesp.br

ABSTRACT. Among the freshwater benthic macroalgae found in Brazilian rivers and streams, species of *Oedogonium* (Chlorophyta) have shown great ability to produce high biomass in several environmental conditions. Their resilience to eutrophic habitats provides an opportunity to assess the use of *Oedogonium* biomass for applications in different fields, especially those related to the extraction of biologically active compounds of interest for health, food, and cosmetic applications. Thus, this study assessed the effects of different organic solvents (acetone, ethanol, and methanol) and their mixtures when combined with water (20% v/v), on the extraction efficiency of total phenolic compounds (TPC), and their antioxidant capacity (AC), from *Oedogonium* sp. biomass, using simplex-centroid mixture design. TPC ranged from 90 to 150 mg of gallic acid equivalent per 100 g dry weight (DW), while AC ranged from 1 to 8 μM of Trolox equivalent per gram (DW). The highest TPC and AC were found for extracts using an ethanol/acetone (50:50) binary aqueous solvent mixture, while the lowest values for both TPC and AC were found for the methanolic aqueous solvent. Considering the relatively low-cost and less adverse environmental impacts of the ethanol and acetone aqueous mixture, our results suggest that the use of this specific mixture of organic solvents may have significant advantages in an eventual industrial process for the extraction of phenolic compounds from *Oedogonium* species.

Keywords: stream macroalgae; phenolic compounds; antioxidant; solvent extractor; mixture design method.

Received on November 8, 2021.
Accepted on May 30, 2022.

Introduction

Green algae can produce a vast array of biologically active compounds, including phenolic chemicals (Seal, Halder, Chaudhuri, & Sinha, 2014), photosynthetic and photoprotective pigments (Fabrowska, Ibañez, Łęska, & Herrero, 2016), as well as alkaloids, ketones, terpenes, hydrocarbons, esters, and sterols (Abdel-Aal, Haroon, & Mofeed, 2015). Chemical compounds produced by algae exhibit diverse biological properties, such as antimicrobial, antiviral, and anti-inflammatory activities (Shalaby, 2011), which has facilitated their application as ingredients in the cosmetic, food, and pharmaceutical industries (Fabrowska et al., 2016).

Among the freshwater green algal species, those belonging to the genus *Oedogonium* have recurrently been reported as essential components of the macroscopic primary producer assemblages in lotic and lentic habitats worldwide (Lawton, Cole, Roberts, Paul, & Nys, 2017). In Brazil, these filamentous green algae comprise approximately 65 species and have been classified as one of the most species-rich genera of Chlorophyta (Menezes et al., 2015). Species of the genus *Oedogonium* can be found in diverse freshwater habitats in different Brazilian ecoregions/biomes, and, commonly, they can produce huge amounts of algal biomass in these environments (Branco, Peres, Krupek, & Bertusso, 2009).

Some authors have suggested that species of *Oedogonium* may be a promising and relevant source of bioactive compounds, as observed for other freshwater green algae (e.g., *Scenedesmus* sp. in Bulut et al., 2019). Wang, Manabe, Sugawara, Paul, and Zhao (2018), for instance, identified seven major carotenoids from *Oedogonium intermedium*, including loroxanthin, which is a kind of carotenoid that is lacking in land plants. An additional reason to investigate *Oedogonium* species as potential sources of bioactive compounds is that species of this genus usually produce much larger amounts of biomass than microscopic algae, as well as being able to grow in a wide variety of polluted wastewater bodies (Lawton et al., 2017). In this context, some studies have shown the possibility of using these green algae species in wastewater treatment systems, replacing the pollutants (e.g., phosphorus and nitrogen compounds) with algal biomass. In this sense, species of *Oedogonium* may be used, among other applications, to treat various components of municipal, industrial, or agricultural wastewater and simultaneously deliver biomass that can be conveniently harvested and used in biotechnological processes (Lawton et al., 2017; Neveux et al., 2016).

However, despite the real potential for the biotechnological use of *Oedogonium* species, there are few studies on bioprospecting and/or extraction of biologically active compounds of interest for health, food, and cosmetic applications using these organisms as sources of biomass. Although other studies have shown that in addition to the type of solvent, factors such as temperature, solvent-solid ratio (Liu, Luo, Wang, & Yuan, 2019), time, pressure, and lighting condition (Qiu, Shen, Wu, Zhang, & Ge, 2021) affect the antioxidant properties of micro and macroalgae, to the best of our knowledge, no study has evaluated the effect of different solvents on the extraction of the biologically active compounds in this genus of green algae.

Phenolic compounds account for one of the largest and most widely distributed groups of phytochemicals showing significant antioxidant capacities. They are cyclic derivatives of benzene with an aromatic ring bearing one or more hydroxyl groups (Tan, Tan, & Ho, 2013). The recovery of total phenolic compounds from a given biological sample depends on the sample-solvent interactions and the efficiency of the solvent systems to extract the predominant forms of these compounds in the sample matrix (Handa, Lima, Guelfi, Georgetti, & Ida, 2016). Phenolic compounds may have different hydroxyl groups that can be conjugated to sugars, acids, or alkyl groups, and, consequently, their polarities can vary greatly. This wide variation in polarity makes it difficult to develop a unique and efficient extraction method with the ability to completely extract all phenolic compounds. Several methodological procedures have been proposed to extract phenolic compounds from algae, including those using enzymes (Sánchez-Camargo et al., 2016), pressurized liquids (Fabrowska et al., 2016; Kumar et al., 2020), microwaves, and ultrasound (Kadam, Tiwari, & O'donnell, 2013), as well as organic solvents (Abdel-Aal et al., 2015; Chernane, Mansori, Latique, & Kaoua, 2014; Goh, Yusoff, & Loh, 2010; Kumar et al., 2020; López, Rico, Rivero, & Tangil, 2011; Seal et al., 2014).

Secondary metabolite extraction yield using organic solvents depends on their chemical nature (Lonni, Longhini, Lopes, Mello, & Scarminio, 2012). Solvent extraction solutions can be fractionated since each fraction may contain different classes of substances with the ability to scavenge different kinds of free radicals (Goh et al., 2010). One class of these antioxidant compounds, the polyphenols, has a wide polarity range that exhibits highly polar to non-polar behaviors. In this context, a wide range of organic solvents has been evaluated as extracting agents in the process of extracting polyphenols from algal biomass. Among the solvents with the highest number of applications are methanol (Chernane et al., 2014; Lima et al., 2016), ethanol (Cho, Kang, Won, Lee, & You, 2010; Kumar et al., 2020), ethanol-water mixtures (Bulut et al., 2019; Kumar et al., 2020), acetone-methanol mixtures (Abdel-Aal et al., 2015; Seal et al., 2014), and ethanol-methanol mixtures (Goh et al., 2010; López et al., 2011). Although the literature reports numerous studies assessing the efficiency of aqueous mixtures of pure organic solvents in the extraction of algal phenolic compounds, there are still few investigations using statistical experimental design to optimize these extraction processes (e.g., Leyton et al., 2016; Liu et al., 2019). Specifically, in the case of green algae, the scarcity of such studies is even more evident. On the other hand, this methodological approach has been used to optimize other antioxidant extraction processes from vegetable sources. Lonni et al. (2012) used, for instance, statistical design mixtures of four solvents (water, methanol, acetone, and ethanol) to extract compounds with antioxidant activity and total phenolic content from *Trichilia catigua* (Meliaceae). Similarly, Marcheafave et al. (2019) applied an experimental mixture design to evaluate the effects of solvent on the antioxidant activity of *Coffea arabica* leaves, while Mendes et al. (2019) have developed an extraction method based on a multivariate analytical approach for phenolic compounds in cashew nut extracts. Alcântara et al. (2019) also used a mixture design to investigate the extraction efficiency of the phenolic compounds from chia seeds.

Thus, in this study, we evaluated the effects of three solvents (ethanol, methanol, and acetone), as well as their mixtures as part of aqueous solutions, on the extraction yield of total phenolic compounds (TPC) and antioxidant capacity (AC) of the green algae *Oedogonium* sp. using simplex-centroid mixture design.

Material and methods

Collection and preparation of the *Oedogonium* sp. samples

Samples of the green algae *Oedogonium* sp. were collected from two streams: the Pari-Veado River (22°38’29” S; 50°12’13” W) and the Água da Fortuna River (22°40’11” S; 50°28’59” W), both located in the Cervo River Basin, central-west region of São Paulo State, southeastern Brazil. After sampling collections, specimens were cleaned and dried in an air circulation oven at 50°C for 3h. Then, the dried material was freeze-dried in liquid nitrogen, ground using a mortar into a powder, and stored in plastic bags wrapped in aluminum foil under light protection at room temperature.

Extract preparation procedures

The extracts were prepared according to López et al. (2011), with modifications. For each 0.5 g of algae power, 10 mL of solvent/water was added. The volumetric proportion of solvent and water (% v/v) for all tests (Table 2) were 80 and 20, respectively. Solvent extraction consisted of pure, binary, or ternary solvents aqueous mixtures (Table 2). The solutions containing algae powder and solvent/water (80:20) were kept under agitation at 800 rpm in an orbital shaker device (Tecnal, model TE-141, Brazil) for 24h. After that period, the samples were centrifuged (Quimis, model 022tm216, Brazil) for 30 min. at 3,000 rpm (1,006 xg), and the algal extracts for each solvent or solvent mixture were then recovered. From these extracts, assays for TPC and AC quantifications were performed. Extracts were prepared in duplicate, and the biochemical assays (i.e., TPC and AC) were performed in triplicate.

Determination of total phenolic content (TPC) and antioxidant capacity (AC)

According to Singleton and Rossi (1965), TPC was obtained by colorimetric assay, using the Folin-Ciocalteau reagent (Sigma-Aldrich). For the colorimetric reaction, Folin-Ciocalteau reagent (0.5 mL) was added to the algal extract (100 μL), followed by the addition of 15% Na_2CO_3 (2.0 mL). The absorbance of the samples was measured in a spectrophotometer (Biospectrum, model SP-220) at 765 nm. The standard curve was prepared with gallic acid, and TPC was expressed as mg Gallic Acid Equivalents (GAE) per 100 g dry algae (mg GAE 100 g^{-1} DW).

The AC of *Oedogonium* sp. extracts was determined using the ABTS radical cation (2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) assay, as described by Re et al. (1999). The ABTS (Sigma-Aldrich) radical solution was obtained by reacting ABTS solution (7 mM) with potassium persulfate solution (140 mM, Sigma-Aldrich, MO). Algal extract (30 μL), or Trolox standard, was mixed with ABTS solution (3 mL), and the absorbance at 734 nm was measured after 6 min. Results are expressed as μM Trolox Equivalent (TE) per g of dry weight (μM TE g^{-1} DW).

Results of TPC and AC were expressed as the mean values x standard deviation, using the software ORIGIN 8.5® (OriginLab Corporation) to compare data obtained from different solvents and their mixtures.

Experimental design and response modeling

To assess the effect of different extracting solvents on TPC and AC of *Oedogonium* sp. extracts, a simplex-centroid mixture design was used (Table 1). In this context, seven different mixtures were prepared using pure solvents (Methanol (M), Ethanol (E), and Acetone (A); runs 1-3, Table 1), binary mixtures (ME, MA, and EA; runs 4-6, Table 1) and ternary mixture (MEA; run 7, Table 1), in an 80:20 proportion of solvent (pure or combined) and water, respectively. As these solvent mixtures were prepared in duplicate, a total of 14 runs were performed.

The quadratic model (Equation 1) and special cubic polynomial model (Equation 2) were appropriately fitted to the AC and TPC data, respectively. The fitting of the models to the responses was evaluated with the coefficient of determination (R^2), and the statistical significance of the coefficients was checked by analysis of variance (ANOVA). Mixture response surfaces, data analysis, and model fitting approaches were performed using Design-Expert 6.0 software (Stat-Ease, Inc., Minneapolis, MN, USA), with a confidence level of 95%.

$$Y = \beta_1 M + \beta_2 E + \beta_3 A + \beta_{12} ME + +\beta_{13} MA + \beta_{23} EA \quad (1)$$

$$Y = \beta_1 M + \beta_2 E + \beta_3 A + \beta_{12} ME + +\beta_{13} MA + \beta_{23} EA + \beta_{123} MEA \quad (2)$$

where β_1, β_2, β_3, β_{12}, β_{13}, β_{23}, β_{123} are the regression coefficients of the models, which represent, respectively, the contribution of pure solvents, as well as binary and ternary mixtures of those solvents. In this context, M (methanol), E (ethanol), and A (acetone) are the independent variables, whereas Y represents the response variables studied, that is, TPC and AC.

Results and discussion

Total phenolic contents (TPC) and antioxidant capacity (AC) of extracts

TPC of the extracts ranged from 90-150 mg GAE 100 g^{-1} DW. In contrast, AC varied from 1 to 8 µM TE g^{-1} DW (Table 1), suggesting that the composition of the solvents system might have had a direct and relevant influence on the efficiency of the bioactive compound extraction process. The highest TPC and AC values were obtained for extractions using the ethanol-acetone (50:50) binary aqueous solvent mixture. On the other hand, the lowest values for both TPC and AC were found for extractions using the methanolic solvent.

Table 1. Total phenolic content (TPC) and Antioxidant capacity (AC) (mean ± standard deviation; n = 3) in the *Oedogonium* sp. extracts by simplex-centroid mixture design.

	Mixture variables (volumetric percentage, %)			Responses	
Runs	Methanol (M)	Ethanol (E)	Acetone (A)	TPC (mg GAE 100 g^{-1})	AC (µM TE g^{-1})
1	1	0	0	91.96 ± 3.05	2.37 ± 0.008
2	0	1	0	104.09 ± 2.29	5.25 ± 0.017
3	0	0	1	99.39 ± 9.99	5.31 ± 0.039
4	0.5	0.5	0	102.17 ± 11.00	3.41 ± 0.010
5	0.5	0	0.5	96.39 ± 13.56	1.27 ± 0.004
6	0	0.5	0.5	134.45 ± 6.90	8.05 ± 0.016
7	0.33	0.33	0.33	90.03 ± 5.77	6.12 ± 0.011
8	1	0	0	103.28 ± 5.28	3.48 ± 0.010
9	0	1	0	93.96 ± 1.40	5.41 ± 0.016
10	0	0	1	119.59 ± 9.83	5.22 ± 0.042
11	0.5	0.5	0	119.70 ± 9.98	4.70 ± 0.013
12	0.5	0	0.5	83.15 ± 5.64	2.42 ± 0.006
13	0	0.5	0.5	150.03 ± 4.12	7.70± 0.021
14	0.33	0.33	0.33	93.28 ± 3.03	5.25 ± 0.009

The antioxidant potential of phenolic compounds is strongly affected by the polarity (Tan et al., 2013) and solubility (Abdel-Aal et al., 2015) of the solvent system used in the extraction process. Considering the polarity of the solvents used in the present study (following Mokrani & Madani, 2016), and consequently, the solubility of phenolic compounds in each of them, we can order them as follows: methanol (0.762) x ethanol (0.654) x acetone (0.355). Based on this polarity scale, our results showed that higher TPC and AC were obtained when less-polar solvents were used in a combined mixture (Table 1). In line with our findings, many previous studies have shown that less polar solvents, such as acetone and ethanol, are the best solvents for extracting TPC, including several classes of anthocyanins, tannins, and flavonoids (Bulut et al., 2019; Mokrani & Madani, 2016; Tan et al., 2013).

Higher values of AC from algal extracts have also been reported when less polar solvents were used in the solvent systems (Bulut et al., 2019; Seal et al., 2014). However, divergent results are also found in studies that investigated other algal species. Seal et al. (2014), for instance, found the highest amounts of phenolic content in methanolic extracts of *Oedogonium globossum*, while Raja, Hemaiswarya, Arunkumar, and Carvalho (2016) observed high phenolic and flavonoid contents with higher antioxidant activities in methanolic extracts from *Eisenia arborea* (Phaeophyta). López et al. (2011) reported a higher efficiency in polyphenol extraction and antioxidant activity from extracts of *Stypocaulon scoparium*, a marine brown alga, using an aqueous methanolic solution. One possible reason for these contradictory results may be related to the amount of water in the solvent system used in the extraction processes.

In the present study, the addition of water during the preparation of the solvent system may have eventually led to an increase in the polarity of the solvent system, which might be the underlying reason for enhanced polyphenol extraction. A mixture of water and organic solvents may have created a moderate-polarity solvent system, which, in turn, may have contributed to the improvement of polyphenol extraction efficiency, as the extraction of these substances is most favored in polar media (Alcântara et al., 2019). Kumar et al. (2020) also reported that 40% ethanol was the most effective in extracting total polyphenolic compounds in brown seaweeds than 60% ethanol or even pure ethanol in the aqueous mixture.

Our results indicated that the compounds extracted from *Oedogonium* sp. had an affinity for solvents with low polarity, suggesting the prevalence of non-polar phenolic compounds in this freshwater macroalgae. In this context, ethanol and acetone were the solvents with higher extraction efficiencies for total phenolic compounds. In addition, our results pointed out that extracts obtained with these same solvents, ethanol, and acetone showed higher antioxidant capacity.

However, in general, the values of TPC and AC from *Oedogonium* sp. extracts proved to be relatively low. Although it is challenging to make comparisons given the methodological differences (e.g., extraction method and solvents used), some studies showed higher values of TCP from freshwater and marine algal extracts than those observed in the present study. Fabrowska et al. (2016), for instance, reported for *Chara fragilis*, a freshwater charophycean algae, and for *Ulva flexuosa*, a marine green algae, TCP values of 3,020 mg GAE 100 g^{-1} and 1,218 mg GAE 100 g^{-1}, respectively. Seal et al. (2014), in turn, showed values of TPC from *Oedogonium globossum* varying from 726–1,151 mg GAE 100 g^{-1}, while Raja et al. (2016) found 80.11 mg GAE g^{-1} from *Eisenia arborea*. Thus, considering that all extracts also revealed low scavenging potential (as observed by the ABTS radical scavenging assay; see AC values in Table 1), it seems likely that phenolic compounds may not be a major contributor to the antioxidant capacity of *Oedogonium* sp. extracts assessed in the present study. Li et al. (2007), evaluating antioxidant capacity and total phenolic content of 23 microalgae using the ABTS assay, found a low correlation between antioxidant capacity and phenolic content, suggesting that phenolic compounds were not a relevant contributor to the antioxidant capacity of these species. A similar observation was verified by Heo, Cha, Lee, Cho, and Jeon (2005) studying antioxidant activities of Chlorophyta and Phaeophyta species.

Establishment of response prediction models

A special cubic model was obtained to predict the extraction of total phenolic contents from *Oedogonium* sp., where the coefficients associated with linear (M, E, or A), binary (EA) and ternary (MEA) interactions were significant ($p \leqslant 0.05$) (Table 2). A synergic effect was registered for binary solvent mixtures comprising ethanol and acetone, based on the highest positive coefficient observed (143.52). This result suggested that the solvent mixture containing ethanol and acetone might significantly increase the yield of TPC extraction from *Oedogonium* sp. On the other hand, ternary mixtures of methanol, ethanol, and acetone showed an antagonistic effect on the yield of TPC extraction, which can be identified by the high and negative coefficient values observed for MEA (−596.12). From a theoretical point of view, the negative sign of a coefficient in the fitted model indicates the ability of its associated factor to decrease the analyzed response.

Table 2. Special cubic and quadratic equation obtained for the total phenolic contents (TPC) and antioxidant capacity (AC), respectively.

Y	M	E	A	ME	MA	EA	MEA	R^2
TPC	101.82* ± 6.84	103.23* ± 6.84	109.49* ± 7.50		-63.55 ± 36.21	143.52* ± 36.21	-596.12* ± 241.56	0.804
AC	2.85* ± 0.5	5.26* ± 0.5	5.19* ± 0.5	1.16 ± 2.27	−7.54* ± 2.28	11.76* ± 2.28		0.920

M: methanol; E: ethanol; A: Acetone; * significant model terms ($p < 0.05$).

Similar antagonistic effects for solvent ternary mixtures have been reported on the extraction yield of phenolic compounds from other biological materials (e.g., Mendes et al. (2019) for cashew nuts). The response surface of the cubic model for TPC as a function of ethanol, methanol, and acetone is represented in Figure 1A. The highest phenolic content was observed in the extract with equivalent proportions of ethanol and acetone. Antagonistic interaction among the three solvents fell in the center of the triangle, indicating the poor contribution of the ternary mixture to the yield of TPC extraction from *Oedogonium* sp. (Figure 1A).

Regarding the AC response, it was better described by a quadratic equation (Table 2), where the linear terms (M, E, A) and the binary interaction coefficients (MA and EA) were significant ($p \leqslant 0.05$). Although linear mixture components are important terms, E and A (5.2) values were almost double the M (2.8), indicating a greater effect on AC extraction. The highest AC was estimated with the binary mixture of ethanol and acetone (EA), as this mixture showed the highest model coefficient (11.76). This result suggests a clear synergistic interaction between these solvents. In the opposite direction, an antagonistic effect was observed between methanol and acetone when these solvents were in a binary mixture, given the low and negative value of the model coefficient (−7.54) observed for this specific mixture.

The response surface contour plot of the AC showed a synergistic effect for ethanol and acetone (Figure 1B). A comparison of response surfaces (Figures 1A and 1B) revealed that methanol had a greater effect on AC than TPC. This difference between these two response surfaces, TPC and AC, may be attributed to the possible presence of other bioactive compounds that act as antioxidants, such as the flavonoids, a group of substances that can have a lipophilic or polar character in algae (Aslan, Aksu, Korkmaz, Taskin, & Caglar, 2019).

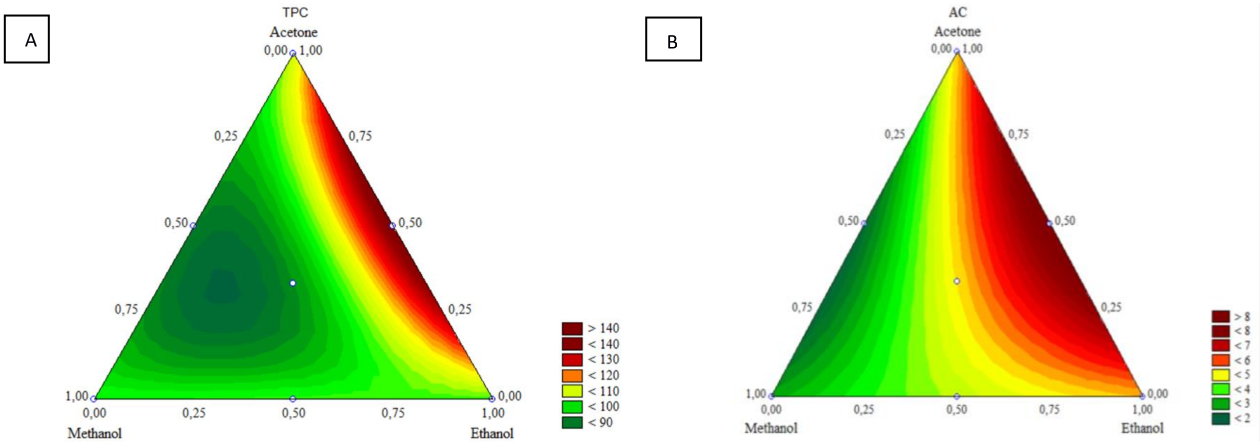

Figure 1. Response surface contour plots for the effect of different solvents on the (A) total phenolic contents and (B) antioxidant capacity extracted from *Oedogonium* sp. biomass.

The results of ANOVA associated with the two models are presented in Table 3. The performance of the model for describing the experimental data was evaluated by the coefficients of determination (R^2), which were higher than 0.8; therefore, the models can be considered mathematically reasonable to describe the influence of independent variables on the dependent variables. Both fitted models for TPC and AC were significant ($p \leqslant 0.05$), implying that they were suitable for factor-response descriptions, with 95% confidence. In addition, the models showed no significant lack of fit.

Table 3. Analysis of variance (ANOVA) for the total phenolic concentration (TPC) and antioxidant capacity (AC) for *Oedogonium* sp.

Response variables	Source	SS	DF	Mean Square	F Value	P Value
TPC	Cubic	3663.32	5	732.66	6.52	0.0105
	Total Error	899.45	8	112.43		
	Lack of fit	212.10	1	212.10	2.16	0.1851
	Pure error	687.35	7	98.19		
	Total	4562.77	13	350.98		
AC	Quadratic	45.57	5	9.11	18.42	0.0003
	Total error	3,96	8	0,49		
	Lack of fit	1.39	1	1.39	3.79	0.0923
	Pure error	2.56	7	0.37		
	Total	49.53	13	3.81		

SS = sum of squares; DF = degrees of freedom.

Conclusion

Total phenolic compounds and antioxidant capacity of extracts derived from *Oedogonium* sp. were affected by the solvent system used in the extraction process. The simplex-centroid design was effective for optimizing extraction procedures of both TPC and AC. The solvent combination that led to the highest yield of TPC extraction and AC was the 50:50 v/v ethanol:acetone binary mixture. These results are relevant since ethanol and acetone are relatively low-cost solvents and have low adverse effects on the environment, which would make them suitable and recommended for use in an eventual industrial process for the extraction of phenolic compounds from *Oedogonium* sp.

Acknowledgements

This work was supported by the São Paulo Research Foundation (FAPESP–Grant 2014/22952-6 to CCZB) and the National Council of Scientific and Technological Development (CNPq–Grants 306567/2014-8, 432172/2016-5, and 302993/2017-7 to CCZB).

References

Abdel-Aal, E. I., Haroon, A. M., & Mofeed, J. (2015). Successive solvent extraction and GC–MS analysis for the evaluation of the phytochemical constituents of the filamentous green alga Spirogyra longata. *The Egyptian Journal of Aquatic Research*, *41*(3), 233-246. DOI: https://doi.org/10.1016/j.ejar.2015.06.001

Alcântara, M. A., Polari, I. L. B., Meireles, B. R. L. A., Lima, A. E. A., Silva Junior, J. C., Vieira, E. A., ... Cordeiro, A. M. T. M. (2019). Effect of the solvent composition on the profile of phenolic compounds extracted from chia seeds. *Food Chemistry*, *275*, 489-496. DOI: https://doi.org/10.1016/j.foodchem.2018.09.133

Aslan, E., Aksu, A., Korkmaz, N. E., Taskin, O. S., & Caglar, N. B. (2019). Monitoring the antioxidant activities by extracting the polyphenolic contents of algae collected from the Bosphorus. *Marine Pollution Bulletin*, *141*, 313-317. DOI: https://doi.org/10.1016/j.marpolbul.2019.02.059

Branco, C. C. Z., Peres, C. K., Krupek, R. A., & Bertusso, F. R. (2009). Macroalgas de ambientes lóticos da região Centro-Oeste do Paraná, região Sul do Brasil. *Biota Neotropica*, *9*(2), 1-9. DOI: http://dx.doi.org/10.1590/S1676-06032009000200022

Bulut, O., Akın, D., Sönmez, Ç., Öktem, A., Yücel, M., & Öktem, H. A. (2019). Phenolic compounds, carotenoids, and antioxidant capacities of a thermo-tolerant *Scenedesmus* sp. (Chlorophyta) extracted with different solvents. *Journal of Applied Phychology*, *31*, 1573-1576. DOI: https://doi.org/10.1007/s10811-018-1726-5

Chernane, H., Mansori, M., Latique, S., & Kaoua, M. E. (2014). Evaluation of antioxidant capacity of methanol extract and its solvent fractions obtained from four maroccan macro algae species. *European Scientific Journal*, *10*(15), 35-48. DOI: https://doi.org/10.19044/esj.2014.v10n15p%25p

Cho, M. L., Kang, I-J., Won, M-H., Lee, H-S., & You, S.G. (2010). The antioxidant properties of ethanol extracts and their solvent-partitioned fractions from various green seaweeds. *Journal of Medicinal Food*, *13*(5), 1232-1239. DOI: https://doi.org/10.1089/jmf.2010.1124

Fabrowska, J., Ibañez, E., Łęska, B., & Herrero, M. (2016). Supercritical fluid extraction as a tool to valorize underexploited freshwater green algae. *Algal Research*, *19*, 237-245. DOI: https://doi.org/10.1016/j.algal.2016.09.008

Goh, S., Yusoff, F. M., & Loh, S. (2010). A comparison of the antioxidant properties and total phenolic content in a diatom, Chaetoceros sp. and a green microalga, Nannochloropsis sp. *Journal of Agricultural Science*, *2*(3), 123-130. DOI: https://doi.org/10.5539/jas.v2n3p123

Handa, C. L., Lima, F. S., Guelfi, M. F. G., Georgetti, S. R., & Ida, E. I. (2016). Multi-response optimization of the extraction solvent system for phenolics and antioxidant activities from fermented soy flour using a simplex centroid design. *Food Chemistry*, *197*(Part), 175-184. DOI: https://doi.org/10.1016/j.foodchem.2015.10.124

Heo, S-J., Cha, S-H., Lee, K-W., Cho, S.K., & Jeon, Y-J. (2005). Antioxidant activities of chlorophyta and phaeophyta from Jeju Island. *Algae*, *20*(3), 251-260. DOI: https://doi.org/10.4490/algae.2005.20.3.251

Kadam, S. U., Tiwari, B. K., & O'donnell, C. P. (2013). Application of novel extraction technologies for bioactives from marine algae. *Journal of Agricultural and Food Chemistry, 61*(20), 4667-4675. DOI: https://doi.org/10.1021/jf400819p

Kumar, L. R. G., Paul, P. T., Anas, K. K., Tejpal, C. S., Chatterjee, N. S., Anupama, T. K., ... Jayarani, R. (2020). Screening of effective solvents for obtaining antioxidant-rich seaweed extracts using principal component analysis. *Journal of Food Processing and Preservation*, *44*(9), 1-9. DOI: https://doi.org/10.1111/jfpp.14716

Lawton, R. J., Cole, A. J., Roberts, D. A., Paul, N. A., & Nys, R. (2017). The industrial ecology of freshwater macroalgae for biomass applications. *Algal Research, 24*(Part 3), 486-491. DOI: https://doi.org/10.1016/j.algal.2016.08.019

Leyton, A., Pezoa-Conte, R., Barriga, A., Buschmann, A. H., Mäki-Arvela, P., Mikkola, J. P., & Lienqueo, M. E. (2016). Identification and efficient extraction method of phlorotannins from the brown seaweed *Macrocystis pyrifera* using an orthogonal experimental design. *Algal Research*, *16*, 201-208. DOI: https://doi.org/10.1016/j.algal.2016.03.019

Li, H-B., Cheng, K-W., Wong, C-C., Fan, K-W., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, *102*(3), 771-776. DOI: https://doi.org/10.1016/j.foodchem.2006.06.022

Lima, R. L., Pires-Cavalcante, K. M. S., Alencar, D. B., Viana, F. A., Sampaio, A. H., & Saker-Sampaio, S. (2016). In vitro evaluation of antioxidant activity of methanolic extracts obtained from seaweeds endemic to the coast of Ceará, Brazil. *Acta Scientiarum*. *Technology*, *38*(2), 247-255. DOI: https://doi.org/10.4025/actascitechnol.v38i2.27275

Liu, X., Luo, G., Wang, L., & Yuan, W. (2019). Optimization of antioxidant extraction from edible brown algae *Ascophyllum nodosum* using response surface methodology. *Food and Bioproducts Processing*, *114*, 205-215. DOI: https://doi.org/10.1016/j.fbp.2019.01.003

Lonni, A. A. S. G., Longhini, R., Lopes, G. C., Mello, J. C. P., & Scarminio, I. S. (2012). Statistical mixture design selective extraction of compounds with antioxidant activity and total polyphenol content from *Trichilia catiguá*. *Analytica Chimica Acta*, *719*, 57-60. DOI: https://doi.org/10.1016/j.aca.2011.12.053

López, A., Rico, M., Rivero, A., & Tangil, M. S. (2011). The effects of solvents on the phenolic contents and antioxidant activity of *Stypocaulon scoparium* algae extracts. *Food Chemistry*, *125*(3), 1104-1109. DOI: https://doi.org/10.1016/j.foodchem.2010.09.101

Marcheafave, G. G., Tormena, C. D., Pauli, E. D., Rakocevic, M., Bruns, R. E., & Scarminio, I. S. (2019). Experimental mixture e design solvent effects on pigment extraction and antioxidant activity from *Coffea arabica* L. leaves. *Microchemical Journal*, *146*, 713-721. DOI: https://doi.org/10.1016/j.microc.2019.01.073

Mendes, M. K. A., Oliveira, C. B. S., Veras, M. D. A., Araújo, B. Q., Dantas, C., Chaves, M. H., ... Vieira, E. C. (2019). Application of multivariate optimization for the selective extraction of phenolic compounds in cashew nuts (*Anacardium occidentale* L.). *Talanta*, *205*, 1-7. DOI: https://doi.org/10.1016/j.talanta.2019.06.100

Menezes, M., Bicudo, C. E. M., Moura, C. W. N., Alves, A. M., Santos, A. A., Pedrini, A. G., ... Silva, W. J. (2015). Update of the Brazilian floristic list of Algae and Cyanobacteria. *Rodriguésia*, *66*(4), 1047-1062. DOI: https://doi.org/10.1590/2175-7860201566408

Mokrani, A., & Madani, K. (2016). Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. *Separation and Purification Technology*, *162*, 68-76. DOI: https://doi.org/10.1016/j.seppur.2016.01.043

Neveux, N., Magnusson, M., Mata, L., Whelan, A., Nys, R., & Paul, N. A. (2016). The treatment of municipal wastewater by the macroalga *Oedogonium* sp. and its potential for the production of biocrude. *Algal Research*, *13*, 284-292. DOI: https://doi.org/10.1016/j.algal.2015.12.010

Re, R., Pellegrine, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237. DOI: https://doi.org/10.1016/S0891-5849(98)00315-3

Qiu, S., Shen, Y., Wu, Z., Zhang, X., & Ge, S. (2021). Effects of algae subtype and extraction condition on extracted fuxoxanthin antioxidant property: A20-year meta-analysis. *Alga Research*, *53*, 1-13. DOI: https://doi.org/10.1016/j.algal.2020.102161

Raja, R., Hemaiswarya, S., Arunkumar, K., & Carvalho, I. S. (2016). Antioxidant activity and lipid profile of three seaweeds of Faro, Portugal. *Brazilian Journal of Botany*, *39*, 9-17. DOI: 10.1007/s40415-015-0200-8

Sánchez-Camargo, A. P., Montero, L., Stiger-Pouvreau, V., Tanniou, A., Cifuentes, A., Herrero, M., & Ibáñez, E. (2016). Considerations on the use of enzyme-assisted extraction in combination with pressurized liquids to recover bioactive compounds from algae. *Food Chemistry*, 192, 67–74. DOI: https://doi.org/10.1016/j.foodchem.2015.06.098

Seal, T., Halder, N., Chaudhuri, K., & Sinha, S. N. (2014). Effect of solvent extraction system on the antioxidant activities of algae. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*, 242-245.

Shalaby, E. A. (2011). Algae as promising organisms for environment and health. *Plant Signaling & Behavior*, *6*, 1338-1350. DOI: https://doi.org/10.4161/psb.6.9.16779

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144-158. DOI: http://doi.org/garfield.library.upenn.edu/classics1985/A1985AUG6900001.pdf

Tan, M. C., Tan, C. P., & Ho, C. W. (2013). Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems. *International Food Research Journal,* 20, 3117-3123.

Wang, N., Manabe, Y., Sugawara, T., Paul, N. A., & Zhao, J. (2018). Identification and biological activities of carotenoids from the freshwater alga *Oedogonium intermedium*. *Food Chemistry*, *242*, 247-255. DOI: https://doi.org/10.1016/j.foodchem.2017.09.075