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Decolorizing of seaweed extract by electrocoagulation

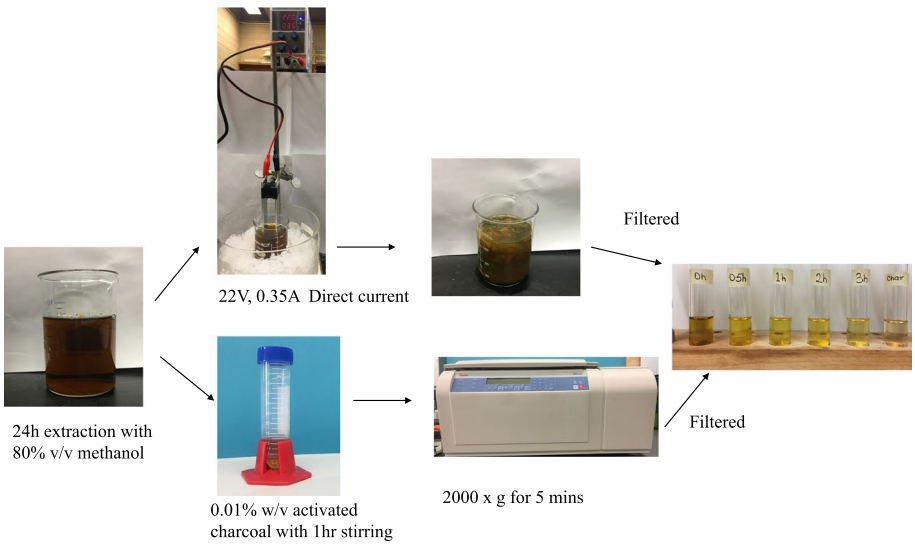
Winifred Akoetey[1], Simon Caton[1], Edward Lai[2] and Farah Hosseinian[1,2*]

Abstract

Electrocoagulation (EC) is a technique commonly used in wastewater treatment to remove biological and chemical contaminants, but the process has the potential to be used in clarifying plant extracts for the isolation and identification of secondary metabolites. Seaweed extracts contain copious amounts of chlorophyll and other pigments that obscure the characterization of secondary metabolites such as phenolic acids and flavonoids. In place of conventional methods that utilize solvents, EC can potentially be applied to clarify and fractionate extracts. In this research, an EC duration of 30 min (22 V, 0.3–0.5A) with aluminum electrodes resulted in a significant decrease, about 76%, of chlorophyll and 70% of carotenoids from seaweed extract measured at 666 nm and 410 nm. The decrease in extract green and yellow color intensity also mirrored a decrease in total phenolic content (TPC) of the extract from 54 ± 1.55 mg GAE/g DW to 3.2 ± 0.01 mg GAE/g DW after 30 min of EC. However, the phenolic acid profile of the extract after electrocoagulation via HPLC-RP indicated the removal of an interference probably caused by polymeric compounds from the extract, thus leaving the simple phenolic acids in solution for detection. The major phenolic acids detected in seaweed crude extract were p-coumaric, o-coumaric, ferulic and syringic acid. Flavonoids detected included catechin, epicatechin, quercetin-3-glucoside and rutin. The results of this study show the potential of replacing conventional plant extract purification methods with a green method that requires no additional solvent.

*Correspondence:
Farah Hosseinian
farah.hosseinian@carleton.ca
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Seaweeds are aquatic flora known to contain secondary metabolites identified as bioactive compounds, many of which possess antioxidative, anti-inflammatory, anti-carcinogenic, and anti-bacterial properties. Bioactive compounds identified in seaweed include bromophenols, phenolic acids, phlorotannins, flavonoids, phenolic terpenoids and other non-typical phenolic compounds (Cotas et al., 2020). These compounds can be extracted for food and drug applications. However, like all plants, seaweeds contain pigments such as chlorophyll and carotenoids (Aryee et al., 2018; Vimala & Poonghuzhali, 2013) which are present in the extracts and can impede the detection, isolation, or purification of the secondary metabolites (Jumpatong et al., 2006; Kouar et al. 2019). To facilitate the analyses of plant extracts for bioactive compounds, the extracts are more commonly subjected to solvent–solvent extraction using chemicals such as chloroform, which is not environmentally friendly. Other methods also employed are activated charcoal bleaching or the use of specialized filter cartridges manufactured for the purpose (Tzima et al., 2020). To mitigate some of the disadvantages of conventional methods, a procedure that is less solvent and material-intensive and thus "greener," is often sought. Opting for a greener method aligns with the UN Sustainable Development Goals (SDG) agenda of sustainable consumption and production, which includes a reduction in the generation of hazardous wastes ('The 17 goals', 2024). Electrocoagulation (EC) could reduce the quantity of extraction solvents used in solvent–solvent extractions for clarifying plant extracts.

EC, as a clarification technique, has been explored extensively in the treatment of wastewater from various industries. The effects of EC have included the reduction of the biological oxygen demand (BOD) and chemical oxygen demand (COD), which indicate organic and chemical pollution in wastewater from food processing plants such as coffee (Ibarra-Taquez et al., 2017), olive mill waste, and brewery waste (Moreno-Casillas et al., 2007; Papadopoulos et al., 2020; Swain et al., 2020). The technique has also been useful in the removal of chlorophyll from plant extracts, dyes from textile wastewater, and phenolic compounds from olive mill wastewater (Dalvand et al., 2011; Fajardo et al., 2015; Jumpatong et al., 2006).

EC applied to plant extracts has shown the potential to be a cost-effective method of clarifying highly pigmented extracts (Chairungsi et al., 2006). It has also been used in sugar cane processing (Ogando et al., 2021), treatment of papermill effluents (Uğurlu et al., 2008), and removal of phenolics from olive mill wastewater (Adhoum & Monser, 2004). Additionally, the process has been applied for the isolation of alkaloids from plant leaves (Chalom et al., 2019), resulting in an increased alkaloid yield after the EC process.

The EC process involves generating coagulating species from ions released by sacrificial electrodes. Utilizing electrical current and metal plates that could be stainless steel, aluminum, iron or platinum, this technique utilizes

minimal solvent and is a cheaper option than conventional methods (Mollah et al., 2004; Shahedi et al., 2020). Commonly used electrodes are aluminum plates and a minimum of two are required; one acts as the anode and the other as the cathode. Electrical current from a direct current (DC) unit is passed through the electrodes placed in solution. A series of reactions occur at the electrodes and between the resulting electrode products and the molecules in solution. The anode is oxidized whiles the cathode is reduced, and the generation of electrons from these actions cause the hydrolysis of water to produce oxygen and hydrogen gas at the electrodes. The metal ion products at the electrodes form hydroxides and polyhydroxides, which then, either by complexation, electrostatic attraction, or co-precipitation with dispersed particles form coagulated materials known as "flocs." The gases formed aid in the flotation of the flocs by buoyancy (Cañizares et al., 2005; Comninellis et al., 2010; Mollah et al., 2004).

Although EC has extensively been studied in wastewater treatment and employed industrially for the same purpose, there are very few studies on its use in plant extracts as a means of purification or clarification. Moreover, the effects of EC on the removal of chlorophyll from seaweed extracts have not been studied so far. Owing to the many benefits of seaweed cultivation and its potential source for secondary metabolites, this study examined the effect of electrocoagulation on the depigmentation of seaweed extracts and the resultant impact on the characterization of secondary metabolites such as phenolic acids and flavonoids in the extract.

Materials and methodology

Seaweed flour, a blend of *Ascophylum Nodosum* and *Fucus*, was provided by Pro-algue marine in Quebec, Canada. Reagent grade methanol was purchased from Fisher Scientific (Ontario, Canada), Folin & Ciocalteu's phenol reagent, gallic acid, 4-hydroxybenzoic acid (p-OH-benzoic), chlorogenic, vanillic acid, p-coumaric acid, o-coumaric acid, protocatechuic acid, syringic acid, ferulic acid, rutin, quercetin-3-glucoside, catechin, epicatechin, activated charcoal and sodium carbonate monohydrate were from Sigma-Aldrich (Missouri, USA), sodium chloride from Bioshop (Ontario, Canada) and acetonitrile from Caledon Laboratory Chemicals (Ontario, Canada). Filter paper (P5) was purchased from Fisher scientific (Pennsylvania, USA) and aluminum plates (15×3×0.3 cm) were provided by the Carleton University Technology department (Ontario, Canada).

Sample preparation and electrocoagulation

Seaweed flour was extracted in 80% methanol with a 1:40 w/v ratio while stirring for 24 h, covered with aluminum foil, and following extraction protocols established in our laboratory. Extraction was conducted in triplicate and the extracts were filtered with P5 filter paper and 120 mL aliquots were used for the electrocoagulation process. Two aluminum plates (15×3×0.3 cm), 1.5 cm apart were placed to a depth of 6 cm in the extract. The beaker of extract was placed in an ice bath in lieu of a jacketed vessel as used by Jumpatong et al. (2006). Sodium chloride at a concentration of 0.1% w/v was added to improve electrolysis. Direct current 22 V, 0.3–0.5 A, supplied by a direct current (DC) power supply was passed into the solution through the aluminum electrodes for different durations. At the end of electrocoagulation, the solution was filtered through the filter paper and spectrophotometric analyses of pigments in the supernatant was conducted immediately. Remaining supernatants were then stored under refrigeration (4 °C) prior to further analysis. In comparison to the EC process, activated charcoal (AC) decolorization was conducted. At 0.01% w/v, the activated charcoal was added to 20 mL seaweed extract and stirred for 30 and 60 min at room temperature. The extract was centrifuged at 2000×g for 5 min and filtered for subsequent analysis.

Spectrophotometric analyses of pigments

300 µL aliquots of the seaweed extract before and after electrocoagulation were placed in a microplate for absorbance measurements at 666 nm and 410 nm, corresponding to green pigments (chlorophyll) and yellow pigments (carotenoids) respectively, on a Biotek Cytation 5 Imaging Reader (Biotek Instruments, Vermont, USA). Readings were conducted in triplicate.

Total phenolic content

Total phenolic content was measured following the Folin-Ciocalteu method as modified by Gunenc et al. (2015). In foil covered test tubes, 1.9 mL of tenfold diluted Folin's reagent was added to 200 µL of sample. The mixtures were kept at room temperature for 8 min and then 1.9 mL of 60 g/L Sodium carbonate was added. The mixture was incubated in the dark for 2 h after which the absorbance was read at 725 nm on a Biotek Cytation 5 Imaging Reader (Biotek Instruments, Vermont USA). The concentration of total phenolics was calculated as Gallic Acid Equivalent (GAE) based on standard curves, 0.0312 to 0.5 mg/mL. Readings were conducted in triplicate.

HPLC identification of phenolic compounds

The phenolic compounds present in extracts and the effect of electrocoagulation on the removal of polyphenols was analyzed via RP-HPLC following a modified method by Chait et al. (2020). Analyses was conducted on a Waters e2695 system equipped with 2998 PDA

detector and Empower software 3 (Waters, Milford, MA, USA). Separation was conducted on an Atlantis®T3 column (4.6×150 mm, 0.3 µm) (Waters, Milford, MA, USA) at 32 °C with a solvent flow of 1 mL/min using solvent A, 0.5% Formic acid in water and B, 100% acetonitrile with a gradient system; 0–35 min (95% A: 5% B to 50% A:50% B), 35–36 min (50% A:50% B), 36–37 min (95% A: 5% B). Detection and quantification of phenolic acids and flavonoids were conducted at 280 nm using calibration curves obtained from standard concentrations (0.313 mg/L- 10 mg/L) of each phenolic acid and of flavonoids (5 mg/L—50 mg/L). The R-squared values of standard phenolic acids ranged from 0.9937 to 0.9998, and that of flavonoids ranged from 0.9923 and 0.9995.

Statistical analysis

All experiments were conducted in triplicate and results presented as the average. Data was analyzed with SAS windows Version 9.4 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) at $p < 0.05$, and Duncan's multiple range test also at $p < 0.05$ was used to for significantly different means.

Results and discussion

Visually, electrocoagulation resulted in flocs that were filtered from the extract to obtain a clear supernatant. The supernatant color was more translucent and slightly yellow after the EC treatment compared to the crude extract which had a greenish hue. Decolorization with activated charcoal also resulted in a color that had the same hue as the original but was slightly lighter lighter (Fig. 1). The lighter colors in the EC-treated samples suggest the removal of darker pigments, such as chlorophyll, from the extracts. The lightness in color of the extract was also noted by Jumpatong et al. (2006) who observed a color change in ethanolic extracts of plant leaves after 2 h of EC. Adhoum and Monser (2004), while investigating olive oil mill wastewater treatment, noted that approximately 95% of the dark color was removed with aluminum electrodes, compared to about 45% with iron electrode. Similarly, a study by Azarian et al. (2007) on the removal of chlorophyll from lagoon water showed clear, colorless water after 15 min of EC.

Spectrophotometric analyses

The absorbance of the extracts after EC at 666 nm and 410 nm was used to determine the removal of green pigments (chlorophyll) and yellow pigments (carotenoids), respectively (Jumpatong et al., 2006). Results (Fig. 2) showed a decline in absorbance of green and yellow pigments after 30 min of EC with similar values after 60, 120 or 180 min. The average pigment removal was about 76% for green pigments and 70% for yellow pigments within 30 min. Kouar et al. (2019) also reported lower absorbance at 665–666 nm and 408–410 nm of ethanolic plant extracts after 2.5 h of EC compared to solvent dechlorophyllation using chloroform followed by filtration with charcoal. Chairungsi et al. (2006), noted the decrease in absorbance of ethanolic chlorophyll solutions with increasing EC duration. The results of this study suggest that at the power supplied (22 V), 30 min of EC was sufficient to clarify the seaweed extract. In this study, the use of activated charcoal (AC) resulted in approximately 92% removal of green and yellow pigments. Although absorbance values decreased in AC-treated samples, the visual appearance differed in comparison to EC-treated samples, possibly due to the different mechanisms of pigment removal.

Total phenolic content and phenolic acid profile

The Total phenolic content (TPC) of the crude SW extract was approximately 54 ± 0.92 mg GAE/g DW comparable to the TPC of commercial and harvested Kelp, *Ascophyllum nodosum*, which was 44.3 mg GAE/g DW and 59 mg GAE/g DW after extraction with acidified aqueous acetone (Tibbetts et al., 2016). After 30 min of EC, there was a reduction of about 91% in TPC, signaling the removal of some phenolic compounds from the

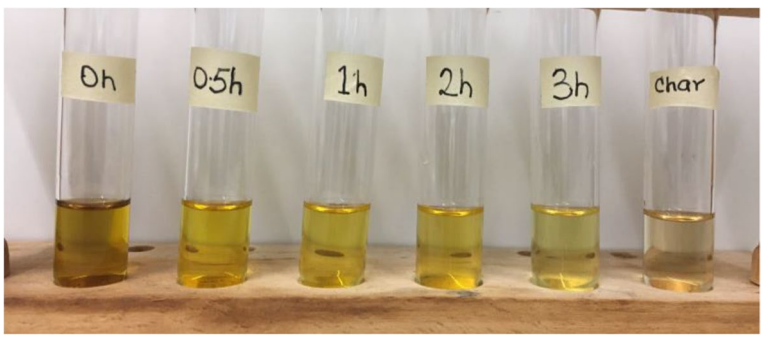


Fig. 1 Visual observation of extract color after electrocoagulation and activated charcoal

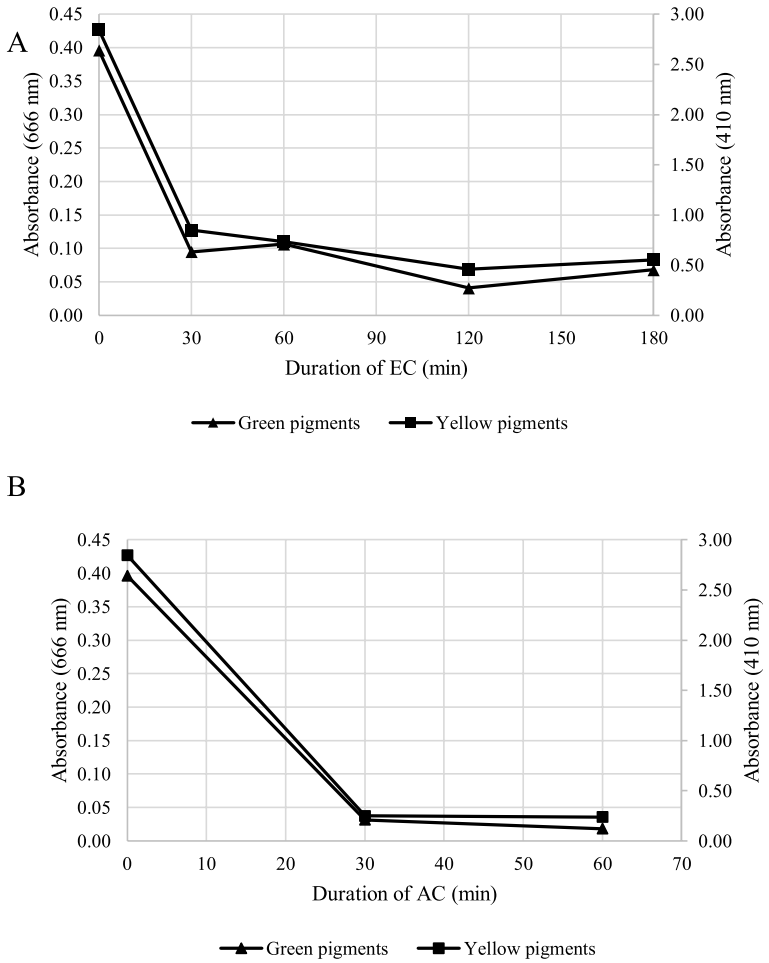


Fig. 2 Trend of absorbance of SW extract **A** at different durations of EC and **B** different durations of AC, at 666 nm and 410 nm corresponding to green and yellow pigments, respectively

extract (Fig. 3). Total phenolic compounds include polymeric phenolic compounds, which could have been removed from the extract along with chlorophyll. The extent of phenolic compounds removal during EC is comparable to observations from Adhoum et al. (2004) who reported approximately 80% reduction of polyphenols in olive mill wastewater using aluminum electrodes. Hanafi et al. (2010) reported 72% and 80% reduction of polyphenols and dark colour, respectively, in olive mill wastewater. Kouar et al. (2019), noted the removal of tannins alongside chlorophyll using aluminum electrodes in plant extracts, and Fajardo et al. (2015) reported about a 50% TPC reduction after 30 min of EC using Zinc and stainless-steel electrodes in olive mill wastewater. The removal of phenolic compounds could be attributed to their adsorption to the surface of the aluminum hydroxides formed during EC or a direct reaction of the hydroxyl groups with the aluminum ions generated at the anode to form an insoluble salt (Phutdhawong et al., 2000). The effect of the EC process on the retention of phenolic acids in the SW extract is presented in Table 1.

The major phenolic acids in the crude sample were protocatechuic, gallic, p-OH-benzoic, ferulic, and chlorogenic acid. Some of these phenolic acids such as ferulic, syringic, and coumaric acids, have also been identified as trace levels in certain seaweed species. Concentrations reported include 0.006 mg/g DW to 0.8 mg/g extract of syringic acid, 0.89 mg/g DW to 60 mg/g extract of protocatechuic acid, comparable to values of 0.009 mg/g DW and 0.227 mg/g DW, respectively (Farvin & Jacobsen, 2013; Tanna et al., 2019). Limited data on the phenolic and flavonoid content of seaweeds indicate that the types and levels of these compounds vary between species and locations (Cotas et al., 2020). It is noteworthy that the phenolic acid profile of the crude extract when compared to that of the 30 min EC, showed that most phenolic acids were retained. The outcome was different after 60 min of treatment with AC. Ogando et al. (2021) observed

Table 1 Phenolic acid (PA) profile of crude seaweed extract, 30 min EC and 60 min AC in mg/g DW of seaweed ± standard error of mean (SEM)

Phenolic	Crude SW	30 min EC	60 min AC
Gallic	0.030 ± 0.025^{a}	0.030 ± 0.003^{a}	nd
Proto Catechuic	0.227 ± 0.104^{a}	0.013 ± 0.003^{b}	0.001 ± 0.000^{b}
P-OH-Benzoic	0.073 ± 0.050^{a}	nd	0.001 ± 0.000^{a}
Chlorogenic	0.028 ± 0.014^{a}	0.002 ± 0.001^{a}	0.001 ± 0.000^{a}
Vanillic	0.004 ± 0.002^{a}	0.018 ± 0.007^{b}	nd
Syringic	0.009 ± 0.001^{a}	0.002 ± 0.000^{b}	nd
P-Coumaric	0.008 ± 0.003^{a}	nd	0.0003 ± 0.000^{b}
Ferulic	0.027 ± 0.008^{a}	0.005 ± 0.003^{b}	0.001 ± 0.005^{b}
O-Coumaric	0.006 ± 0.001	nd	nd

*Means with different letters (a and b) in the same row are significantly different at $p < 0.05$ (using Duncan's multiple range test) and "nd" is undetected

a similar phenomenon; although there was significant reduction in color and turbidity of sugar cane juice with increasing electrical voltage and EC time, simple phenolic acid profile was not equally affected. The quantities of the major phenolic acids as detailed in Table 1, demonstrate that EC retained the phenolic acids more effectively than 60 min of AC treatment.

Figure 4 illustrates the flavonoids present in the crude SW extract and the extracts after EC and AC treatment. Some flavonoids, such as rutin and quercetin were detected in the 30 min EC sample but not in the crude sample. Conversely, quercetin-3-glucoside was detected in the crude sample but not in the 30 min EC extract. The concentrations of catechin, epicatechin and quercetin-3-glucoside in the crude extract were 0.08 ± 0.02 mg/g DW, 0.1 ± 0.01 mg/g DW, 0.1 ± 0.01 mg/g mg/g DW, respectively. The values of catechin and epicatechin decreased in the 30 min EC extract to 0.007 ± 0.002 mg/g DW and 0.006 ± 0.001 mg/g DW. The catechin value for the 60 min AC was 0.003 ± 0.001 mg/g DW. Rutin, quercetin and catechin have been reported in some green and brown algae species with average values higher than noted in this study with rutin levels at 3.93 mg/g DW, catechin levels ranging from 3.5 to 11.4 mg/g DW, and epicatechin at 0.024 mg/g DW (Cotas et al., 2020; Tanna et al., 2019). Both phenolic and flavonoid profiles support the proposal that EC has the potential to be used as a means of fractionating plant extracts. Factors such as salt concentration, voltage, and duration of EC can be modified to isolate specific compounds within the extract (Chindaphan et al., 2021). Furthermore, the compounds and pigments in the flocculates removed can be recovered by dissolving the precipitate in dilute acid, resulting in a process that utilizes the entire extract (Phutdhawong et al., 2000). Due to the hydroxyl groups on phenolic compounds, the pH of the solution can be modified to affect the charge on the molecules and hence their adsorption onto the coagulates or interaction with the aluminum ion from the electrode (Robić and Miranda 2010; Phutdhawong et al., 2000). A change in solution pH would also influence the reactions at the anode, which may complement interactions with the phenolic compounds (Cañizares et al., 2005) and was found to effect coagulation and subsequently the removal of phenolic acids in olive mill wastewater (Farjado et al., 2015). In another study, the biosorption of phenolic compounds on pine bark powder increased with rising pH up till pH 6 and decreased till pH 10 (Kumar et al., 2014). In EC involving proteins and phenolic compounds,

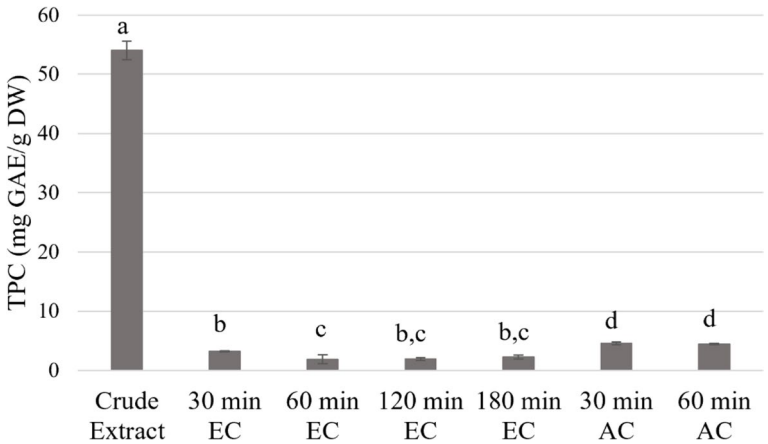


Fig. 3 Total phenolic content of extracts before and after EC or AC. Means with different letters (a,b,c and d) are significantly different at $p \leq 0.05$ (using Duncan's multiple range test)

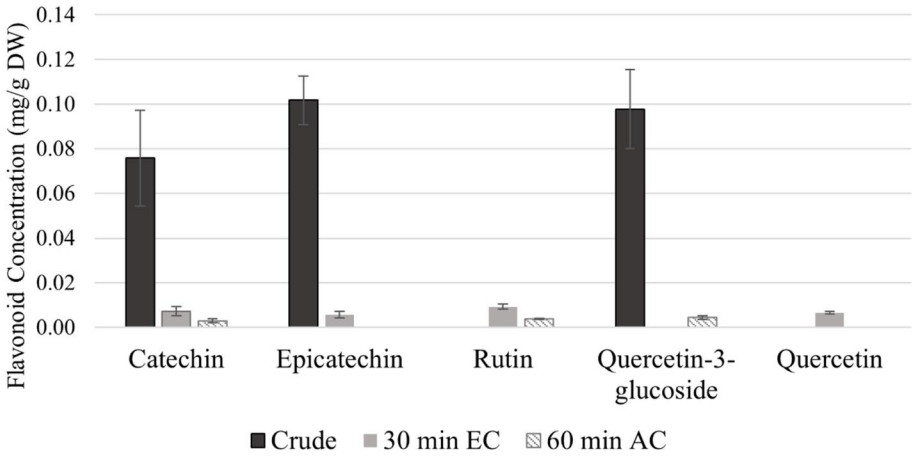


Fig. 4 Concentrations (mg/g DW) of major flavonoids after EC and AC in mg/g DW presented as means with error bars of SEM

Bovine Serum Albumin (BSA) and catechin molecules were found to interact with aluminum hydroxides differently at various pH levels, allowing for efficient separation. This interaction suggests the potential to separate proteins from phenolic compounds by modifying the pH during EC (Robić and Miranda 2010).

Figure 5 presents the HPLC profile of the crude SW extract before and after 30 min of EC and 60 min of AC treatment. The broad peak, observed in the chromatogram of the crude extract, was eliminated within 30 min of EC. This broad peak, from unresolved compounds likely to be polymeric phenolic acids and chlorophyll, were removed during EC. Chindaphan et al. (2021) observed similar improvement in compound peaks and the removal of the broad peak interferences from wine, attributed to polymeric pigments such as anthocyanins. Thus, the EC process enhances the identification and quantification of compounds that might otherwise be obscured by larger molecules.

Conclusion

Electrocoagulation was successfully used in the depigmentation of seaweed extract with a removal of chlorophyll and polymeric compounds which resulted in a lighter color of the seaweed extract. After 30 min of electrocoagulation, approximately 70% of the chlorophyll was removed resulting in a clear yellow solution. The effect of EC on the secondary metabolites of the seaweed extract was evident in the HPLC profile, which saw the removal of a broad peak in the crude extract. EC also offered better peak identification of phenolic acids and flavonoids. Although there was a significant ($p < 0.05$) reduction in the total phenolic content of the extract, the profile of simple phenolic acids was maintained. This study showed that the EC process can be utilized in removing pigments from seaweed extracts and for isolating and identifying secondary metabolites.

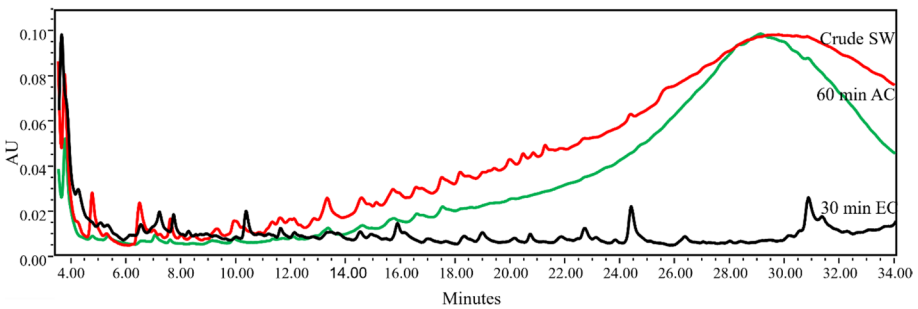


Fig. 5 HPLC chromatograms of Crude extract (No EC), 30 min EC and 60 min AC showing the removal of the broad peak in the crude extract after 30 min of electrocoagulation

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Authors' contributions
W.A: Investigation, Concept, Methodology, resources, formal analysis, writing-original draft, writing-review, and editing. S.C: Assisted with the investigation. E.L: Review, supervise, and editing. F.H.: Supervise the whole project, concept, methodology, writing –review and editing, Resource. The author(s) read and approved the final manuscript.

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Availability of data and materials
Data obtained during this study is available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate
N/A.

Consent for publication
N/A.

Competing interests
Prof. Farah Hosseinian is a member of Editorial Board of Food Production, Processing and Nutrition and she was not involved in the journal's review of, or decisions related to this manuscript.

Author details
[1]Food Science, Chemistry Department, Carleton University, 1125 Colonel By Dr, Ottawa, ON K1S 5B6, Canada. [2]Chemistry Department, Carleton University, 1125 Colonel By Dr, Ottawa, ON K1S 5B6, Canada.

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