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# Validation of Folin – Ciocalteu assay for phlorotannins analysis in fresh and storm-cast brown algae *Ascophyllum nodosum* (Phaeophyceae)

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**Abstract**

**Introduction.** Phlorotannins are secondary metabolites produced mainly by brown seaweeds and belong to the class of polyphenolic compounds with diverse bioactivities. Storm-cast brown algae, a problem for coastal biocenoses, may be a valuable source of polyphenols. The Folin-Ciocalteu reagent (FCR) is the most commonly used for the quantification of total polyphenols in natural samples. Different spectrophotometric methods with FCR for the determination of phlorotannins in algae have been described in the literature.

**Aim.** The primary aim of this study is to standardize and validate the spec-trophotometric determination of total phlorotannins using FCR and demonstrate its applicability to analysis of storm-cast and fresh algae.

**Materials and methods.** *A. nodosum* samples were collected in sheltered beach on the Olenitsa Bay (66°27'15.7"N 35°18'20.4" E), Kandalaksha Gulf (White Sea, Russia) on two tidal levels: one located at low tide at a depth of 0.6–1.0 m (fresh) and the second was located at the supralittoral in the zone of wave splashing (storm-cast). Field sampling was carried out between June and September. The cleaned seaweed were transported to the laboratory, washed accu-rately with clean water, freeze-dried, ground into powder. Functional groups present in the algae were identified using Fourier Transform Infrared (FT-IR) spectroscopy. Spectrophotometric determination of total phlorotannins content (TPhC) with FCR was used and validated according to national and international guidelines.

**Results and discussion.** The optimum conditions for analysis time, wave-length, and standard substance were 45 min, 750 nm, and phloroglucinol, respec-tively. Under these conditions, validation by UV/Vis spectrophotometry proved the method to be linear ($R^2 > 0.99$), specific, precise, accurate, reproducible, robust, and easy to perform. The limit of detection and limit of quantification were 0.005 and 0.02 mg/mL, respectively. For precision analysis, an intra-day test (RSD 2.16 %) and an inter-day test (RSD 2.84 %) were performed. Matrix effect assessment demonstrated that this had a negligible effect (1.9 %) on the phlorotannins quantification. TPhC in storm-cast algae ranged from 59 to 101 mg/g, while freshly collected algae were statistically significantly higher ($p < 0.01$) and ranged from 71 to 135 mg/g. Maximum accumulation of phlorotannins in *A. nodosum* was observed between July and August, after which a decrease was observed.

**Conclusion.** Results of current study could be utilised for routine analysis of TPhC in brown algae and storm-cast seaweed using optimized spectrophotometric method with FCR on readily available low-cost equipment in most laboratories to provide rapid. This methodology complies with the requirements for pharmaceutical analysis to ensure the reliability of results during pharmaceutical development and routine control both in fresh and storm-cast of *A. nodosum*.

**Keywords:** Brown algae, storm-cast, methodology optimization, total phlorotannins, UV/Vis spectrophotometric, validation, Folin-Ciocalteu

**Conflict of interest.** The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

**Contribution of the authors.** Ekaterina D. Obluchinskaya – study design, sample extraction and analysis, data processing. Olga N. Pozharitskaya – study design, analysis and interpretation of data, review of publications. Anna V. Daurtseva – sample extraction and analysis. Alexander N. Shikov – validation, data curation. All authors participated in writing the text of the article and discussing the results.

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# Валидация метода Фолина – Чокальтеу для анализа флоротаннинов в свежих и штормовых выбросах бурых водорослях *Ascophyllum nodosum* (Phaeophyceae)

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**Резюме**

**Введение.** Флоротанины, являющиеся вторичными метаболитами, в основном вырабатываются бурыми морскими водорослями и относятся к классу полифенольных соединений, обладающих разнообразной биологической активностью. Бурые водоросли, выбрасываемые на берег штормом и составляющие угрозу для прибрежных экосистем, могут стать ценным источником полифенолов. Для количественного определения общего содержания полифенолов в природных образцах чаще всего применяется реактив Фолина – Чокальтеу (FCR).

**Цель.** Основной целью данного исследования является стандартизация и валидация спектрофотометрического определения общего содержания флоротаннинов с использованием FCR и демонстрация его применимости для анализа выброшенных штормом и свежих водорослей.

**Материалы и методы.** Образцы *A. nodosum* были собраны на защищенном пляже губы Оленица (66°27'15.7" с.ш. 35°18'20.4" в.д.) Кандалакшского залива (Белое море, Россия) на двух приливных уровнях: один находился во время отлива на глубине 0,6–1,0 м (свежие водоросли), а второй – на супралиторали в зоне заплеска волн (водоросли из штормовых выбросов). Полевой отбор проб проводился в период с июня по сентябрь. Очищенные водоросли доставляли в лабораторию, тщательно промывали чистой водой, лиофильно высушивали и измельчались в порошок. Функциональные группы, присутствующие в водорослях, идентифицировались с помощью инфракрасной Фурье-спектроскопии (ИК-Фурье). Спектрофотометрический метод с использованием реактива Фолина – Чокальтеу оптимизирован для анализа свежих и выброшенных штормом бурых водорослей и валидирован в соответствии с национальными и международными рекомендациями.

**Результаты и обсуждение.** Оптимальными условиями для анализа были время анализа, длина волны и стандартное вещество: 45 мин, 750 нм и флороглюцин, соответственно. В этих условиях валидация методики спектрофотометрии показала, что метод линейный ($R^2 > 0,99$), специфичный, точный, достоверный, воспроизводимый, надежный и простой в применении. Предел обнаружения и предел количественного определения составили 0,005 и 0,02 мг/мл, соответственно. Внутридневная (RSD 2,16 %) и междневная (RSD 2,84 %) прецизионность анализа была рассчитана. Оценка влияния матрицы показала, что она оказывает незначительное влияние (1,9 %) на количественное определение флоротаннинов. Содержание флоротаннинов в водорослях, выброшенных штормом, варьировалось от 59 до 101 мг/г, в то время как в свежесобранных водорослях было статистически достоверно выше ($p < 0.01$) и варьировалось от 71 до 135 мг/г. Максимальное накопление флоротаннинов в *A. nodosum* наблюдалось в период с июля по август, после чего наблюдалось снижение.

**Заключение.** Полученные в ходе данного исследования результаты могут быть применены для регулярного анализа содержания флоротаннинов в бурых водорослях и водорослях, извлеченных из штормовых отходов. Это возможно благодаря оптимизированному спектрофотометрическому методу с использованием реактива Фолина – Чокальтеу, который основывается на применении доступного и недорогого оборудования, имеющегося в большинстве лабораторий, что обеспечивает оперативность анализа. Данная методология соответствует требованиям фармацевтического анализа, обеспечивая надежность результатов при разработке лекарственных препаратов и рутинном контроле как свежего, так и штормовых выбросов *A. nodosum*.

**Ключевые слова:** бурые водоросли, штормовые выбросы, оптимизация методологии, общие флоротаннины, УФ-видимая спектрофотометрия, валидация, метод Фолина – Чокальтеу

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**Вклад авторов.** Е. Д. Облучинская – дизайн исследования, отбор и анализ образцов, обработка данных. О. Н. Пожарицкая – дизайн исследования, анализ и интерпретация данных, обзор публикаций. А. В. Даурцева – отбор и анализ образцов. А. Н. Шиков – валидация, курирование данных. Все авторы принимали участие в написании текста статьи и обсуждении результатов.

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## INTRODUCTION

Brown algae (Phaeophyceae) represent a category of algae that are extensively found in polar and temperate regions, predominantly thriving at depths ranging from 6 to 30 meters, and they exhibit significant potential for growth and utilization[1]. These algae are rich in a range of bioactive compounds such as polysaccharides, proteins, amino acids, polyphenols, terpenes, mannitol, fucoxanthin, hormones, and other active substances. Notably, phlorotannins represent a distinct category of polyphenolic compounds that are primarily found in brown seaweeds [1]. Phlorotannins show medical, cosmetic, and biotechnological applications [2]. Significant amount of seaweed biomass accumulate along the coasts as a result of storms, winds, and ocean currents. The accumulation of storm-cast of seaweeds on the beaches causes unpleasant odors and greenhouse gas emissions. This seaweed holds the potential to be harnessed as biomass for the extraction of functional ingredients, which could facilitate the advancement of innovative biotechnological applications [3]. Although the relative phenolic composition of macroalgae changes significantly during aging and decay after they are washed ashore [4], is noted that most of the current information in the scientific literature on phenolic levels in algae is derived from live tissue analyses. Data on the polyphenol content of storm-cast algae are limited and no previous studies have been conducted in Arctic seas.

The extraction of phlorotannins has gained significant importance due to their biological activities and extensive range of applications. The literature indicates that there is no standardized or unified approach for the extraction of phlorotannin [5]. However, various methods have been employed to extract it from macroalgae, including the following: Solvent extraction, enzymatic extraction, hydrothermal extraction, supercritical fluid extraction, microwave-assisted extraction, ultrasonic-assisted extraction, and deep eutectic-based solvent extraction. Solvent extraction, also referred to as solid–liquid extraction, involves the removal of a solute from a solid matrix using a liquid solvent. This method is a well-established technique frequently employed to isolate various compounds from biological matrices or for quantification purposes. It is particularly regarded as the most widely used and efficient approach for extracting phlorotannins from macroalgae. A range of solvents, including ethanol, methanol, acetone, hexane, and their combinations, are utilized in this extraction process [6].

Various methods are employed to assess the total polyphenolic content in algae, including nuclear magnetic resonance [7], high-performance liquid chromatography [8], and particularly optical techniques [9], etc. [10, 11]. Among these, the spectrophotometric method utilizing Folin-Ciocalteu reagent (FCR) is one of the most commonly used and simplest approaches for determining total phlorotannins [12]. The underlying principle involves the oxidation of phenolic compounds in an alkaline environment using molybdenum and tungsten phosphates, resulting in the formation of a

[1] Listing the World's Algae. Available at: http://www.algaebase.org. Accessed: 22.07.2025.

blue-colored complex. The intensity of this blue tungsten-molybdenum complex with polyphenols is quantitatively assessed spectrophotometrically at a wavelength of 750 nm. This method has undergone numerous modifications over time, with the approach introduced by Singleton and Rossi in 1965 being the most commonly employed for the analysis of total polyphenols [13]. Several other authors [12] reported and proposed modification of this method.

**The primary aim** of this study is to unify and validate the spectrophotometric determination of total phlorotannins using FCR and demonstrate its applicability to analysis of storm-cast and fresh algae.

## MATERIALS AND METHODS

### *Standards and Chemicals*

FCR, sodium carbonate anhydrous, and phloroglucinol were purchased from Sigma-Aldrich (USA). All other analytical-grade chemicals and solvents were received from local chemical suppliers. Ultrapure water was purified using a Milli-Q system (Millipore, USA).

### *Algal material*

*A. nodosum* samples were collected in sheltered beach on the Olenitsa Bay (66°27'15.7"N 35°18'20.4" E), Kandalaksha Gulf (White Sea, Russia) on two tidal levels: one located at low tide at a depth of 0.6–1.0 m (fresh) and the second was located at the supralittoral in the zone of wave splashing (storm-cast). Field sampling was carried out between June and September. The cleaned seaweed were transported to the laboratory, then cleaned of epiphytes, sediment particles, and other contaminants, washed with tap water, and freeze-dryied in Inei-4 freeze dryer (IBF RAS, Russia). The dried seaweed was ground using a non-metallic mill (CT 293 Cyclotec, Foss, Danmark) and sieved through 1.0 mm. All samples were stored at –25 °C until the experiment.

### *Characterization of the algae samples*

The Fourier–Transform Infrared Spectroscopy (FT–IR) spectra of dried and ground algal material without additional preparation were recorded using the Perkin–Elmer® Spectrum™ 3 spectrometer (Perkin Elmer Inc., USA) in mid-IR mode, equipped with a Universal ATR (attenuated total reflectance) sampling device containing diamond/ZnSe crystal. Triplicates of each sample were averaged.

### *Extraction of Phlorotannins*

0.5 grams of algae samples was suspended in 5 ml of 70 % acetone and subjected to continuous shaking in a 360-degree rotating shaker (Bio RS-24 BioSan, Latvia) for one hour et 4–6 °C. Following this, the mixture was centrifuged for 10 minutes at 3200 rpm and 6 °C (C1015R Centurion Scientific, United Kingdom). The supernatant was then collected, and an additional 5 ml of 70 % acetone was added to the remaining solid residue. This extraction process was repeated four times. After the fourth extraction, the supernatants from each step were combined, and the acetone was evaporated to dryness under a stream of nitrogen. The resultant extract with phlorotannins was freeze-dried and stored in a freezer [14].

### *Pre-Method Standardization for Determination of TPhC*

During the optimization and standardization of the spectrophotometric method utilizing the FCR, five critical parameters were evaluated: (1) reaction kinetics, (2) wavelength of maximum absorption, (3) the standard that best represents algal phlorotannins, (4) the amount of alkaline additive, and (5) the order in which reagents are added. The employed method was a colorimetric assay, adapted from a general protocol established by Singleton and Rossi [13], with minor modifications. A 2 % sodium carbonate solution (SCS) in 0.1 M NaOH was utilized. Spectra were recorded using a 1 cm quartz cell on a Shimadzu UV-1800 spectrophotometer (Japan) over a period of 5 to 120 minutes following the addition of the SCS, across a wavelength range of 400–850 nm. Ultrapure water was used as a blank.

The method was validated for specificity, linearity, analytical range, accuracy, precision, and limit of quantification (LOQ) as recommended[1, 2].

The fresh *A. nodosum* and its storm-cast were extracted with 70 % acetone according [14]. The freeze-dried extracts were dissolved and analyzed for phlorotannins content. The matrix effect (%ME) was calculated to determine the influence of the seaweed matrix on the extraction of phlorotannins [Eq. 1, 15]. The applicability of the validated methodology was assessed on fresh seaweed samples and storm-washed seaweed samples collected from the beach. All samples were analyzed in triplicate in all tests performed.

### *Statistical Analysis*

The analysis of the data was conducted using STATGRAPHICS Centurion XV (StatPoint Technologies Inc., Warrenton, VA, USA) through one-way analysis of variance (ANOVA) accompanied by Tukey's test, with a significance level set at $P < 0.05$. Results are presented as mean standard deviation (SD) [or relative standard deviation (RSD, %)].

[1] Bioanalytical Method Validation, Guidance for Industry. Available at: https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed: 22.07.2025.

[2] ICH guideline Q2 (R1) «Validation of analytical procedures: Text and methodology». Available at: https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf. Accessed: 22.07.2025.

## RESULTS AND DISCUSSION

### *Characterization of the algae samples by FT–IR*

Figure 1 shows the infrared absorbance spectra of the raw fresh and storm-cast algal biomass. They are very similar since no modification occurs on the type and amount of functional groups present on the algae surface.

The FT-IR spectrum provided a detailed fingerprint of the molecular components, revealing several characteristic peaks. A broad band observed at 3390 $cm^{-1}$ was attributed to O—H stretching vibrations, indicative of hydrogen-bonded alcohol and phenol groups [16]. This peak is significant as it confirms the presence of phenolic compounds, which are known for their antioxidant properties. The occurrence of two bands in the 1800–1600 $cm^{-1}$ range signifies the existence of a carbonyl group in two variants: as a carboxylic acid ester (C double bond O) at 1743 $cm^{-1}$ and as a carboxylate anion (—COO—) at 1611 $cm^{-1}$, thereby validating the presence of alginic acid [17]. Four weak peaks appeared between 1500 and 1562 $cm^{-1}$, and their presence is ascribed to bending vibrations of N—H in protein amide II and stretching vibrations of C—N. Likewise, a medium-intensity peak was observed at 1611 $cm^{-1}$, indicating the stretching vibrations of C═O from protein amide [18]. The peak at 1408 $cm^{-1}$ corresponds to the deformation of the C—OH vibration, along with the contribution from the O—C—O symmetric stretching vibration of the carboxylate group [18]. The peaks at 1229 $cm^{-1}$ (S=O stretching) and 814 $cm^{-1}$ (C—S—O) are attributed to sulphate groups, which are typically present in fucoidan and other sulphated polysaccharide elements of the cell wall in brown seaweeds [17]. Consequently, the primary groups of chemical compounds found in the cell wall of both fresh and storm-cast macro-algae *A. nodosum* are phenolic compounds, carbohydrates, and polysaccharides. The FT–IR analysis clearly shows that although the strength of absorption bands might differ, their positions stay the same.

### *Pre-Method Standardization for Determination of TPhC*

After the pre-method standardization step for the FC assay, all other experiments were performed by (1) setting the wavelength at 750 nm for analysis; (2) using phloroglucinol as the reference compound to be used as the analytical standard for TPhC quantification; (3) setting the reaction time in 45 min; (4) the initial introduction of SCS into the reaction medium; and (5) optimum amount of 2 % SCS in 0.1 M NaOH.

### *Methodology validation*

The specificity of the method was demonstrated by comparing the UV spectra (after the reaction with the FCR) of the test solution (0.2 mg/mL), the solution of the standard sample of phloroglucinol (1 mg/mL), the test solution with the addition of the standard (0.2 mg/mL + 5 µL 1 mg/mL) and the blank (Figure 2).

The calibration equation obtained for phloroglucinol presented a linear response for the analyzed con-

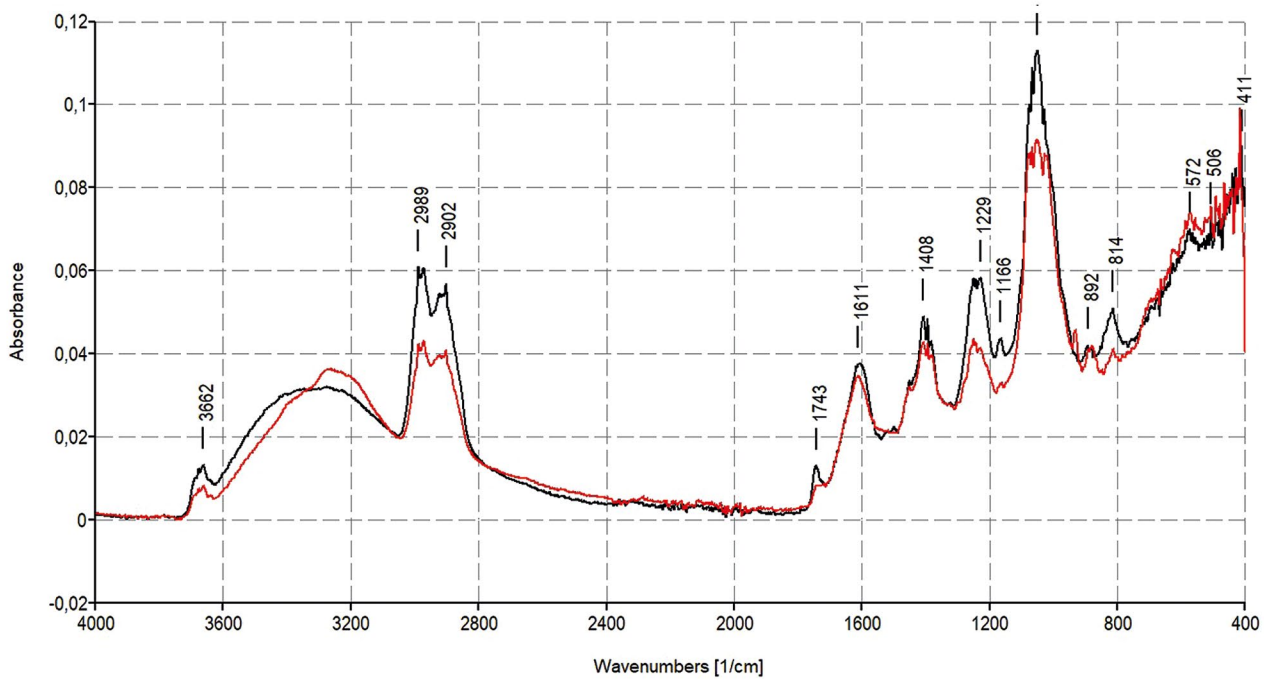


**Figure 1. FT–IR spectra of the fresh and storm-cast algal biomass *A. nodosum*: black line – fresh algae; red line – storm-cast algae**

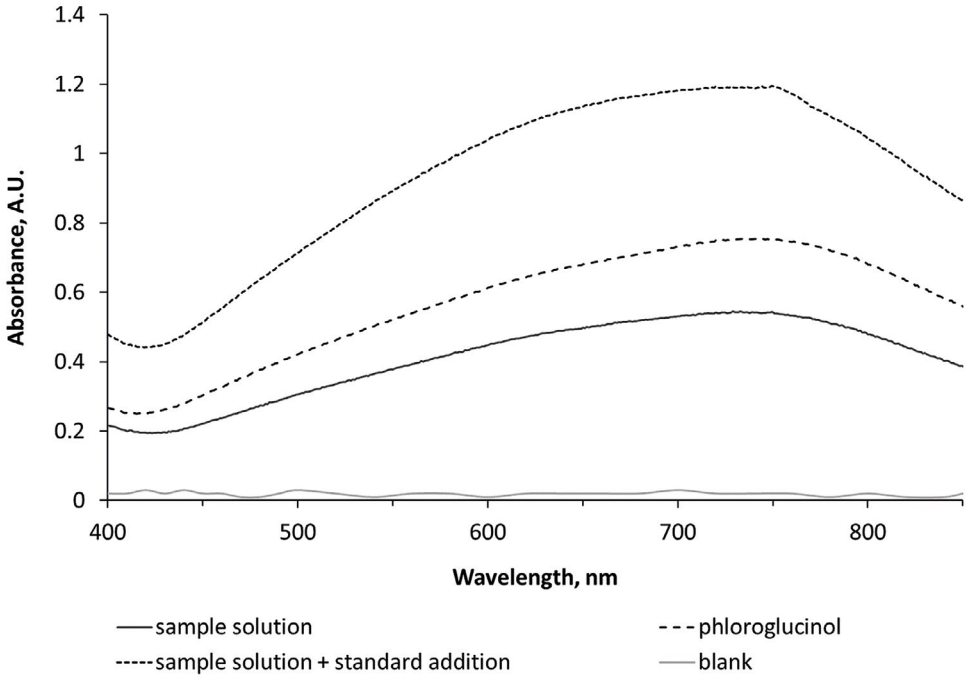


**Figure 2. The typical absorption profile of reference compound phloroglucinol, sample solution *A. nodosum* extract (with and without additives) and the blank in the wavelength range of 400–850 nm**

centration range with RSD lower than 1.5 % for all points (Figure 3, A). Also, the coefficient of determination $R^2$ 0.9992 satisfies the requirement, which stipulates that the $R^2$ has to be more than 0.99.

To determine the linearity, aliquots of the stock solution (algae extract) were diluted to the final concentrations of 0.005–0.25 mg/mL. The linearity curve, shown in Fig. 3B, was plotted with five consecutive concentration points (0.04–0.16 mg/mL) chosen to result in an absorption range between 0.4 and 0.8 A.U. The value of $R^2$ (0.9926) assures the linearity of the adapted method for the evaluated range of concentrations.

The effect of the matrix within algal extracts can be assessed by analyzing the linearity and selectivity curves, as the intricate nature of the matrix complicates the separation of analytes from the raw material. If the linearity and selectivity curves run parallel to one another, the method is deemed to be selective [19, 20].

According to, the extract concentration of 0.02 mg/mL, corresponding to the lowest point on the calibration curve (Figure 3, B), was adopted as the limit of quantification (LOQ). Detection limit is the minimum content of the substance being determined in the sample, the signal from which can be reliably distinguished from the background. The experimentally obtained detection limit for the algae extract in the reaction with the FCR was 0.005 mg/mL**.**

Precision was assessed at two levels: repeatability (intra-day) and intermediate precision (inter-day). The results, expressed in percentage of TPhC in the algae sample (±SD [RSD(%)]), were 59.2 ± 1.28 (2.16) and 58.7 ± 1.67 (2.84) for repeatability and intermediate precision, respectively. These values were tested and found statistically equal since the obtained F value (1.69) is lower than the F critical (5.05). The matrix effect was calculated to determine the interaction that seaweed matrix has on TPhC using FC assessment, and showed that its variability did not exceed 1.9 %.

Reproducibility. The same sample of *A. nodosum* storm-cast seaweeds was extracted and analyzed six times, and the measured absorbance values were recorded. The calculated RSD (CV) was 2.28 %, which indicated that the experiment had high reproducibility.

To assess the accuracy of the FC assay, samples with different raw material weights were used. According to the results, comparable results are obtained at all three concentration levels of the analyzed solution, and the relative standard deviation does not exceed 4 %, which corresponds to the RSD value optimal for quantitative determination.

To confirm the effectiveness of the modified FC assay protocol in minimizing the interference of the algae matrix, additional analyses were also conducted using a standard additional method (SAM), where the actual samples are used to construct the calibration curve individually. The principal advantage of this method is that it allows a matrix effect correction, since the same matrix is present in the calibration standards and the sample [21].

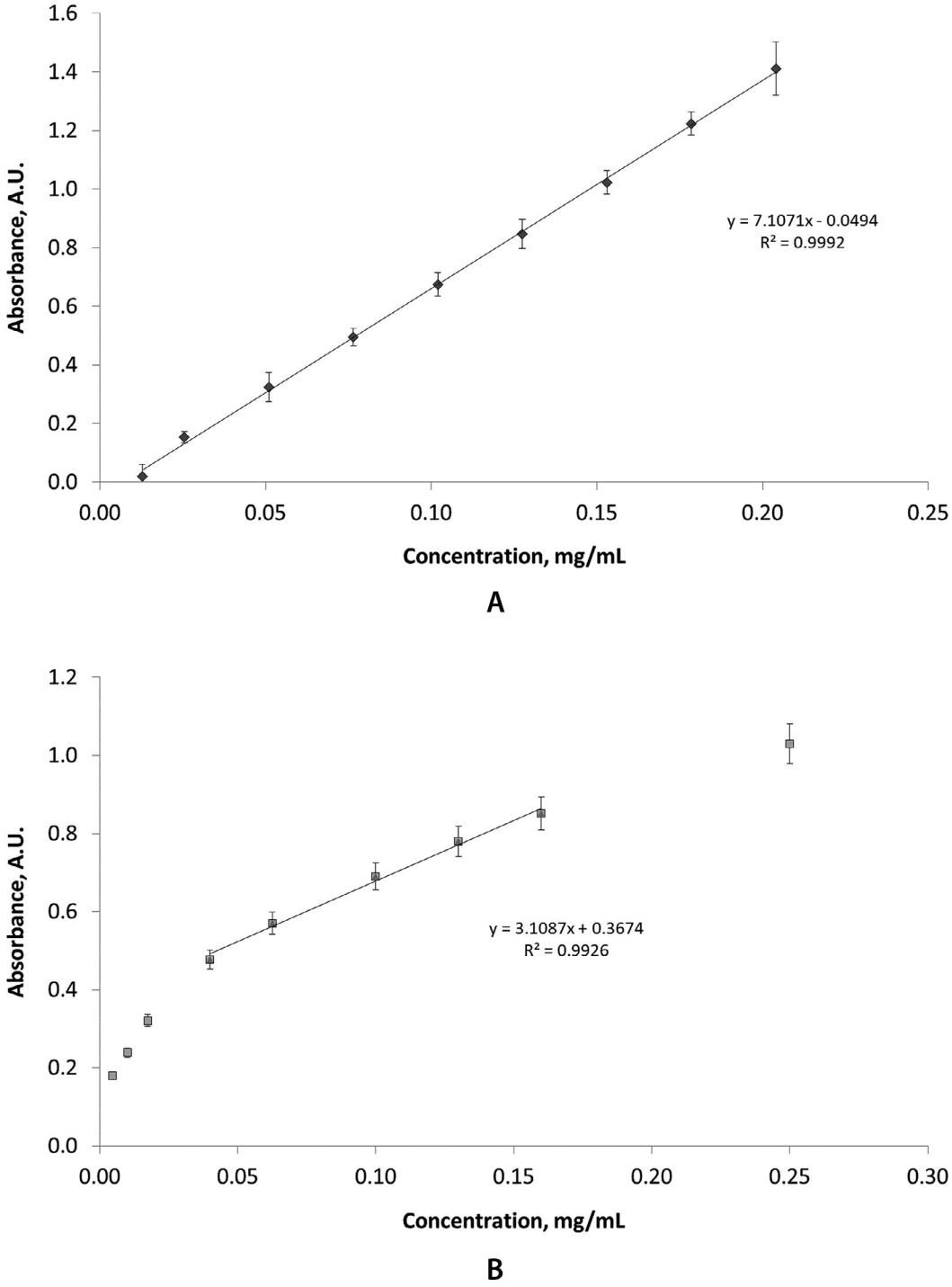


**Figure 3.** **(A) Calibration curve obtained with the phloroglucinol standard for the analysis of TPhC; (B) Linearity curve of *A. nodosum* extract from storm-cast seaweeds**

### *Method applicability*

The completeness of extraction was estimated using multiple extractions [14, 22]. The effect of the number of consecutive extractions on the phlorotannins content of the algae is demonstrated in Figure 4. Exhaustive extraction of TPhC is provided after seven consecutive cycles.

Notable that after four cycles the yield of TPhC is 97.8 and 98.8 % for seaweeds and storm-cast seaweeds, respectively (Figure 4). Therefore, we suggest to use four consecutive extractions for quantitative analysis of TPhC.

Despite the growing interest in the use of storm-cast seaweeds, there are no studies on their phlorotannin content compared to freshly collected seaweed at low

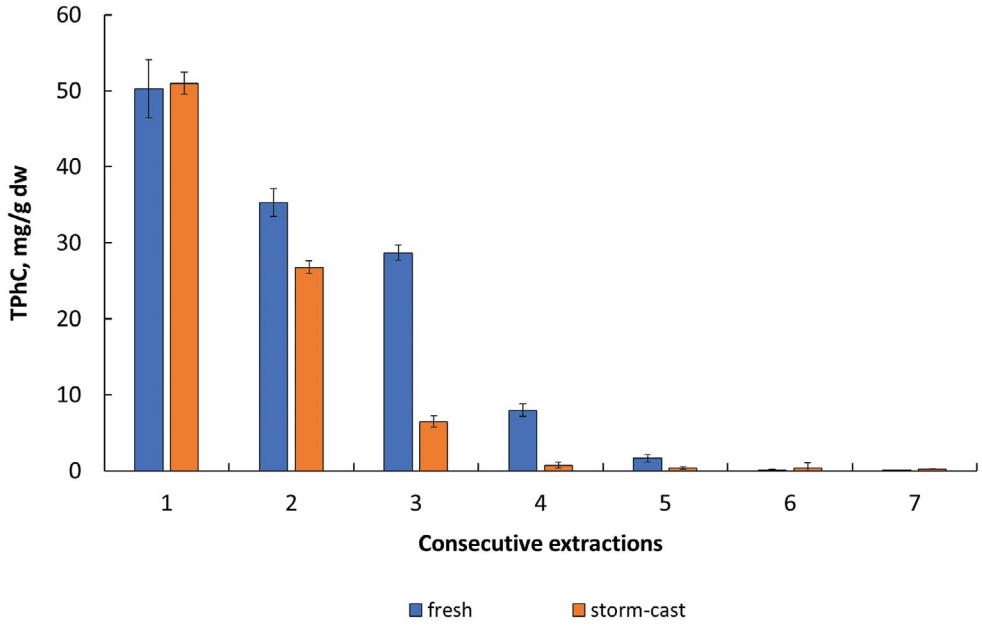


**Figure 4.** **Consecutive extractions of brown algae and storm-cast seaweed in 70 % acetone and 4–6 °C**

tide. In this study, we determined the TPhC in samples collected from the White Sea coast between June and September. The results are presented in Figure 5. The TPhC of storm-cast seaweeds ranged from 59 to 101 mg/g. TPhC of freshly collected seaweed was statistically higher and ranged from 71 to 135 mg/g. The maximum accumulation of phlorotannins was observed in fresh samples during the period July-August, followed by a decrease (Figure 5).

In current experiments, a significantly high amount of phlorotannins was found in freshly collected algae compared with storm-blown algae. According to literature data, the maximum content of polyphenols in species from this geographic zone does not exceed 12 % of dw [23]. An increase in polyphenols is noted in July–August. TPhC of *A. nodosum* from storm-cast in our studies reached 10 % dw in September and did not differ from that in fresh algae. The dynamics of the phlo-

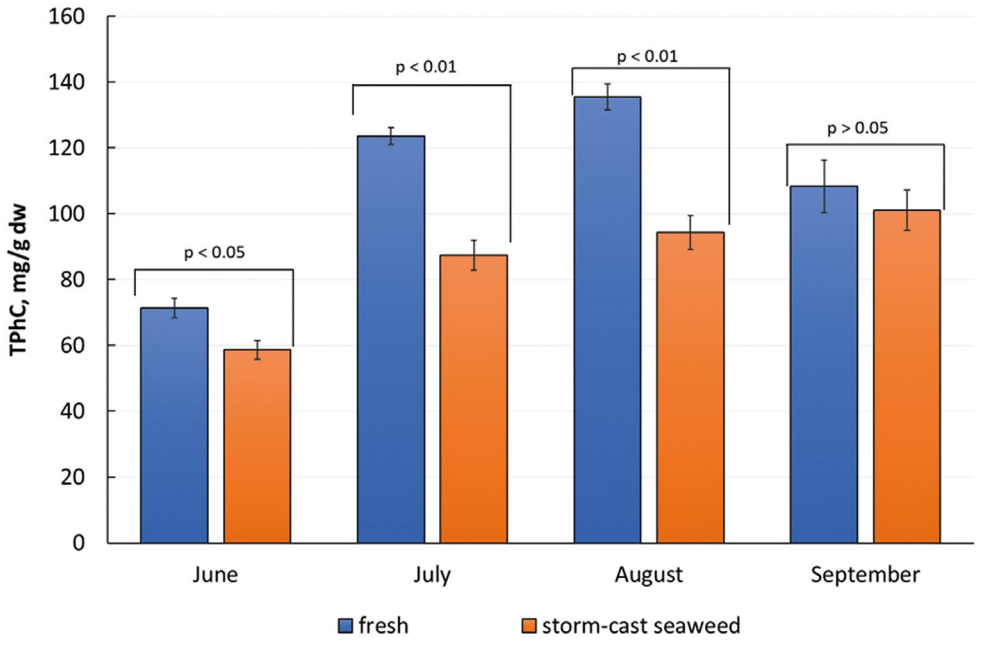


**Figure 5.** **Dynamics of TPhC in fresh and storm-cast algal biomass *A. nodosum***

rotannins content in storm-cast algae is not so pronounced. Polyphenolic compounds serve as protective agents against various biotic and abiotic stressors, including grazing, bacterial infection, UV radiation, and metal contamination [24]. In perennial algae like *A. nodosum*, seasonal fluctuations in chemical composition are frequently observed and correlate with physiological changes in vegetative thalli [25]. Given the potential applications of both freshly harvested and storm-cast *A. nodosum* in the food, cosmetic, and pharmaceutical industries, it is essential to identify the optimal times for algal material collection. Research conducted by Parys et al. [26], Apostolidis et al. [27], and Tabassum et al. [28] has highlighted notable seasonal variations in the polyphenol content of *A. nodosum*. Specifically, Apostolidis et al. [27] and Bogolitsyn et al. [23] documented analogous seasonal trends in phenolic compounds, indicating peak concentrations in June and July, while May exhibited the lowest levels. The variations in polyphenol content can be influenced by factors such as geographical location, light intensity, temperature, salinity, and the availability of surrounding nutrients [26].

A study on *A. nodosum* highlighted a significant interaction between UVB radiation and the emersion or immersion of plants: phenol levels were higher in thalli alternately emerged and immersed, and, thus, directly exposed to UV radiation for a few hours [29]. Changes in polyphenol content also correlate with the reproductive stage of *A. nodosum* from the Arctic [30]. The results of this work showed for the first time that *A. nodosum* collected from storm discharges showed a certain tendency to preserve the phlorotannin content compared to fresh seaweed. The average phenolic content of seaweeds' sediments from the beaches of the NW coast of Spain was around ~2 % dw, which is below the results for *A. nodosum* from White sea in 10 times lower [31]. These average values were similar to those measured in previous studies [22, 32]. Similar studies support the feasibility of processing Arctic seaweed storm emissions to produce polyphenols. This study confirms the feasibility of collecting and recycling Arctic algae from storm-cast seaweeds for polyphenol production.

## CONCLUSION

The results showed that the spectrophotometric method remains a reliable method for the rapid determination of phlorotannins in algae samples. The Folin – Ciocalteu method has been optimized for the first time for the analysis of polyphenols in brown algae and the storm seaweed *Ascophyllum nodosum*. The 45-minute reaction time was shown to be similar to the 120-minute reaction time in the original method, reducing analysis time and increasing throughput. Furthermore, the optimized Folin–Ciocalteu method demonstrated good linearity, accuracy, and stability, confirming its suitability for the determination of total phlorotannins in algae. The matrix effect of both fresh and discarded algae was found to be negligible (1.9 %) and did not significantly affect the quantification of phlorotannins. External validation, supported by SAM, proved extremely useful in determining the accuracy of various pretreatment methods for PP quantification. Application of the optimized method to simultaneously collected fresh and storm seaweed *A. nodosum* allowed us to evaluate the characteristic polyphenol content and its seasonal variability. Our data suggest that July–August may be the optimal period for collecting *A. nodosum* material with high phlorotannin content (more than 10 %). Ascophyllum from storm seaweed from the White Sea demonstrated significantly higher polyphenol levels than other previously analyzed seaweeds from other seas. The results of this study can be used for routine analysis of polyphenol content in both brown and storm seaweed using the optimized Folin – Ciocalteu assay.

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