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Evaluation of Phenolic Compounds and Pigments in Freshwater *Cladophora glomerata* Biomass from Various Lithuanian Rivers as a Potential Future Raw Material for Biotechnology

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Abstract: Freshwater macroalgae produces a wide range of bioactive compounds, and interest in utilizing its biomass is growing rapidly. Meanwhile, exploiting renewable sources for biomass collection could lead to more sustainable biotechnological chains. The aim of this study was to investigate *Cladophora glomerata* biomass from Lithuanian rivers as a potential raw material for biotechnology. For this reason, phenolic compound profiles, antioxidant activity and pigment concentrations were determined in macroalgal biomass samples collected from the following four Lithuanian rivers: Dubysa (B1), Šventoji (B2), Nevėžis (B3) and Jūra (B4). The highest total phenolic compound content was determined in B3 (1.32 mg GAE/g). Three phenolic acids were identified, namely gallic (12.94–35.13 µg/g), *p*-hydroxybenzoic (23.97–29.05 µg/g) and *p*-coumaric (1.79–6.46 µg/g). The results indicate significant *C. glomerata* antioxidant activity; the highest reducing power reached 0.737 AU (B3), the total antioxidant content was 1.47 mg Trolox/g (B3), DPPH and ABTS radical scavenging was 11.09% (B3) and 97.86% (B1) and FRAP activity 20.86 µmol/L (B3). The content of pigments ranged from 0.56-0.74, 0.39–0.57, 0.17–0.23 to 0.11–0.17 mg/g in chlorophyll *a*, *b*, carotenoids, and lutein, respectively. To conclude, *C. glomerata* macroalgal biomass may have the potential to act as a functional raw material, as several groups of bioactive compounds and antioxidant activities were observed.

Keywords: green macroalgae; bioactive compounds; antioxidant activity; sustainability; renewable sources

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1. Introduction

Freshwater macroalgae are less diversified than marine seaweeds, yet they thrive in a variety of habitats and play an important ecological function in aquatic ecosystems. In freshwaters, macroalgal mats mainly consist of filamentous green algae species that may function as habitat-structuring elements within the benthic zone [1]. Cosmopolitan opportunistic macroalgae such as *Cladophora*, *Ulva* and *Enteromorpha* have rapid growth rates and form agglomerations under increased water fertility, reduced flow rates and good light conditions [1]. Additionally, environmental factors have an impact on the variation of macroalgal populations, the formation of their life strategies and the chemical composition of the biomass. Due to the synthesis of secondary metabolites (e.g., polyphenols, flavonoids, phenolic acids), algae are resistant to stress factors such as changes in environmental

variables [2,3]. Freshwater macroalgae produce a high variety of bioactive compounds; however, they are far less studied and underutilized as a feedstock [4–6] compared to the biocompounds of seaweeds [7].

Despite the fact that algae is being investigated as a potential feedstock or industrially relevant co-product extraction, there are numerous research barriers to the commercialization of algae-based products [8]. Regular algae are composed of three major compounds, namely proteins, carbohydrates, and lipids. Additionally, algal biomass also produces starch, pigments, antioxidants, vitamins and phytohormones, which can be used as pharmaceuticals, biofertilizers, natural colorants, or animal feed [9]. Biologically active compounds are prevalent in algae species and unique secondary metabolites are frequently confined to a small number of species within a phylogenetic group, which has a variety of health advantages [10]. Overall, there is scientific evidence that phenolic chemicals originate in algae species [10–14]. Macroalgae may be rich in bioactive compounds as well, such as antioxidants (phenols, pigments, etc.), which have significant therapeutic potential. Due to their various morphological and physiological characteristics, macroalgae synthesize these bioactive compounds, which have significant commercial relevance [12]. Secondary metabolites are critical components of defensive systems, and they are divided into several bioactive compound classes based on their structural arrangements [15]. Alkaloids, flavonoids, phenolics, tannins and terpenoids are the key classes, and they all have important pharmacological activities [16]. Pigments are organic compounds that preferentially absorb and reflect a particular spectrum of light. There are three major categories of pigments occurring in algae: chlorophylls, carotenoids and phycobiliproteins [17]. So, green macroalgae are also a valuable source of pigments since they can synthesize chlorophyll and carotenoids as photosynthetic organisms. These characteristics are critical when looking for feedstock and other manufacturing prospects that can be included into a sustainable supply chain.

Nowadays, a variety of strategies are being pursued to extract raw materials that not only exceed the highest quality requirements but that are also more sustainable and ecologically friendly. Therefore, there is a great deal of interest in considering the natural resources from which multifunctional raw materials can be extracted at a green rate. Considering the conception of the "bioeconomy" and its tactics today, not only must biomass production be environmentally beneficial, but the whole bioprocess must be sustainable, optimizing raw material valorisation [18]. For example, the macroalgal biomass *C. glomerata* could be used as a raw material and removing it from freshwater bodies would not only create a multifunctional raw material for various industries, but also clean up water bodies and increase biodiversity. Furthermore, *C. glomerata* is one of the most frequent macroalgae-forming mats in aquatic ecosystems and achieves high biomass in a short period of time [19]. Saturated and unsaturated fatty acids, sterols, terpenoids and phenolic compounds, pigments are among the physiologically active substances found in *C. glomerata* [19,20]. There has been a lot of study surrounding the subject of marine algae [21,22]. For example, with regard to research conducted in Lithuania, the biomass of marine *C. glomerata* collected at the northern beach of Klaipėda was converted to oil by pyrolysis as a potential feedstuff [23]. Other Lithuanian researchers, Baltrėnas and Misevičius [24], investigated the application of *C. glomerata* for biogas production, although these macroalgae were produced in bioreactors rather than being harvested from natural water sources. However, there is currently limited research available about freshwater *C. glomerata* from Lithuanian rivers. Our recently published research has provided a detailed analysis of this freshwater macroalgae's general nutritional value (chemical composition, element accumulation, fatty and amino acid profiles) [25]. Nevertheless, even more extensive and deeper research into chemical composition is required. As an outcome, the objective of this research was to investigate freshwater *C. glomerata* macroalgae biomass from various Lithuanian rivers as a potential raw material by analysing its comprehensive phenolic compound profile, antioxidant activity and most importantly, pigment concentration.

2. Materials and Methods

2.1. Macroalgal Biomass Collection

Freshwater *C. glomerata* biomass was collected in August–September 2019 from the following four Lithuanian rivers: Dubysa (N55°12′25.07″, E23°30′30.44″; B1), Šventoji (N55°39′20.14″, E25°10′18.39″; B2), Nevėžis (N55°5′46.52″, E23°46′55.57″; B3) and Jūra (N55°27′19.58″, E22°2′14.72″; B4). More detailed information about the tested rivers is provided in Nutautaitė et al. [25].

The rivers were selected because of the presence of dense agglomerations of *C. glomerata*, which typically cover more than half of the river bottom area (Figure 1A). The macroalgal biomass was harvested manually from the rivers (Figure 1B). Subsamples of up to 1 kg of wet biomass were collected and blended from up to six distinct locations. To remove sand and mud particles, macroalgal biomass was washed numerous times with tap water. The biomass was manually cleaned of macrozoobenthos, macrophytes and other debris (Figure 1C). The samples were dried overnight in a 60 °C oven and stored in darkness at room temperature in closed plastic bags until the analysis. To avoid access humidity forming during storage, biomass was dried again at 105 °C until constant weight before each analysis. Each river's three subsamples (n = 3 from each river) were evaluated for each analysis.

Figure 1. (**A**) Agglomerations of macroalgal *C. glomerata* biomass in River Šventoji; (**B**) Collected *C. glomerata* biomass; (**C**) Washed and cleaned biomass of macroalgae.

2.2. Macroalgal Biomass Phenolic Compounds Profile Analysis

All the chemicals were of analytical grade and were used as received. Solvents methanol HPLC Chromasolv gradient grade, acetonitrile Chromasolv (Sigma Aldrich Chemie GmbH, Steinheim, Germany) and for high performance liquid chromatography (HPLC) gradient grade, ethanol absolute (Sigma Aldrich Chemie GmbH, Steinheim, Germany) were used. Acetic, formic, hydrochloric, orthophosphoric acids, sodium hydroxide, phenolic acid (*o*-coumaric, cinnamic, m-coumaric, vanillic, caffeic, salicylic, ferulic, sinapic, chlorogenic, 3,4-dihydroxybenzoic, gallic, *p*-hydroxybenzoic and *p*-coumaric) standards, flavonoid (quercetin, rutin trihydrate, kaempferol, myricetin, xantohumol) standards, cate-

chin (catechin hydrate, epicatechin, epigallocatechin gallate) standards and tertiary butylhydroxyquinoline (TBHQ) were purchased from Sigma-Aldrich (Sigma Aldrich Chemie GmbH, Steinheim, Germany). Ultrapure water (resistivity of 18.2 MΩ) was supplied by Elga purification system Purelab Ultra (Elga, Bucks, UK).

HPLC analyses were performed on a Shimadzu HPLC system (Shimadzu corp., Kyoto, Japan) consisting of system controller SCL-10A, solvent delivery module LC-10AT, auto injector SIL-10AD, UV-Vis detector SPD-10AV, column oven CTO-10AC and on-line degasser DGU-14A.

The retention periods of the test compounds were compared to the retention times of the corresponding standard solutions to identify each. Data collection and evaluation were performed by using the operating system Workstation LC solution (Shimadzu corp., Kyoto, Japan) and measuring the peak areas of the corresponding compounds in the sample and calculating their concentrations based on the calibration curves.

2.2.1. Phenolic Acids

The profile and content of phenolic acids was determined by extraction according to Kvasnička et al. [26]. One gram (±0.0001 g) of ground biomass sample was weighed and 25 mL of 0.1 M NaOH was added before shaking at 40 °C for 60 min in a water bath GFL 1083 (GFL GmbH, Burgwedel, Germany), cooling to room temperature, acidifying with 2 M HCl to pH 5–6and supplementing with 20 mL of methanol (99%). The flask was placed in an ultrasonic bath (Bandelin Electronic, Berlin, Germany) for 30 min, cooled to room temperature and made up to volume with methanol. The filtrate after filtration by a 0.22 µm membrane filter (Frisenette ApS, Knebel, Denmark) was analysed by HPLC. Phenolic acids in standard mixtures and extracts were separated by the method described by Amarowicz and Weidner [27] with some modifications (Table 1).

Table 1. High performance liquid chromatography (HPLC) conditions for phenolic compound determination.

Conditions	Phenolic Acids	Flavonoids	Catechins
Mobile phase (*v*/*v*/*v*)	A—methanol B—water/acetonitrile/acetic acid 88/10/2	A—methanol B—0.2% formic acid in water	A—0.1% orthophosphoric acid in water B—0.1% orthophosphoric acid in methanol
Elution	Gradient: 100% B (4 min), 0–100% A (15 min), holding 100% A 10 min, decrease to 0% A in 0.5 min holding 100% B 6.5 min	*Gradient:* 35–50% A at 0–25 min, 50–80% A at 25–30 min, 80–95% A at 30–35 min, 95-100% at 35–40 min, holding 100% A 5 min, decrease to 35% A in 5 min. After each run, the chromatographic system is set to 35% A for 5 min	*Gradient:* 0–5 min, 20% B; 5–7 min, linear gradient from 20 to 24% B; 7–10 min, holding 24% B; 10–20 min, linear gradient from 24 to 40% B; 20–25 min, linear gradient from 40 to 50% B; 25–30 min decrease to 20% B; holding 20% B 5 min
Flow rate (mL/min)	1.0	1.0	1.0
Column	LiChrospher 100 RP-18 250 × 4.6 mm, 5 µm (Alltech Associates Inc., Deerfield, IL, USA)	LiChrospher 100 RP-18 250 × 4.6 mm, 5 µm (Alltech Associates Inc., Deerfield, IL, USA)	LiChrospher 100 RP-18 150 × 4.6 mm, 5 µm (Alltech Associates Inc., Deerfield, IL, USA)
Guard column	LiChrospher 100 RP-18 7.5 × 4.6 mm, 5 µm (Alltech Associates Inc., Deerfield, IL, USA)	LiChrospher 100 RP-18 7.5 × 4.6 mm, 5 µm (Alltech Associates Inc., Deerfield, IL, USA)	LiChrospher 100 RP-18 7.5 × 4.6 mm, 5 µm (Alltech Associates Inc., Deerfield, IL, USA)
Column oven temperature (°C)	30	30	30
Injection volume (µL)	10	10	10
Detection (nm)	260 and 320	340 and 367	210 and 280

2.2.2. Flavonoids

Flavonoids were extracted according to Khuluk et al. [28]. One gram (±0.0001 g) of ground biomass was sonicated (Bandelin Electronic, Berlin, Germany) for one hour at room temperature in the presence of HCl 6M (5 mL) and TBHQ solutions (20 mL) in 62.5% methanol. The sample extracts obtained following sonication were filtered through a 0.22 µm membrane filter (Frisenette ApS, Knebel, Denmark) before being injected into the HPLC device. Flavonoids in standard mixtures and extracts were separated by the method described by Khuluk et al. [28] with some modifications (Table 1).

2.2.3. Catechins

The content of catechins was determined by the method described by Wang et al. [29]. About 0.5 g of ground biomass was accurately weighed (±0.0001 g) and extracted for 20 min using sonication (Bandelin Electronic, Berlin, Germany) with 40 mL of a solution of ethanol and water (10:90, *v*/*v*). The extraction solution was filtered into a 50 mL volumetric flask, the flask and filter were rinsed with the solution of ethanol and water (10:90, *v*/*v*) and then made to volume with the same solvent. For HPLC analysis, approximately 1 mL of the sample solution was centrifuged at 13,000× *g* (Sanyo MSE, London, UK) for 10 min prior to HPLC analysis. Catechins in standard mixtures and extracts were separated by the method described by Wang et al. [29] with some modifications (Table 1).

2.2.4. The Total Phenolic Compound Content

From each river, 0.25 g of dried algal biomass was mixed with 25 mL of methanol solvent (70%). The extraction was carried out for 2 h at 40 by shaking in the dark using a benchtop shaker Certomat (Sartorius Stedim Biotech GmbH, Goettingen, Germany). The Folin–Ciocalteu method was used to determine the total phenolic content [30]. A total of 100 µL of *C. glomerata* extract, 1.8 mL of distilled water and 150 µL of the Folin–Ciocalteu reagent was added to the tubes. The tubes were mixed and 1.02 mL of 7.5% sodium carbonate (Na_2CO_3) was added after 1 min. The prepared samples were stored in the dark at 20 °C for 2 h. The absorbance of the samples was measured with a Schimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 765 nm.

The total phenolic compound content was expressed as gallic acid equivalent (GAE) from the calibration curves of the standard gallic acid solutions per gram of dry mass of *C. glomerata*.

2.3. Macroalgal Biomass Antioxidant Activity Analysis

2.3.1. Biomass Extracts Preparation

The dried *C. glomerata* biomass (0.1 g) was extracted for 0.5 h in methanol (5 mL; 99%) at 45 °C. It was then centrifuged for 10 min at 9000× *g* (Hettich Universal 320Randreas Hettich GmbH & Co., Tuttlingen, Germany) and used for antioxidant activity analysis.

2.3.2. Reducing Power (RP) Bioassay

A total of 0.5 mL of algal methanol extract (0.02 g/mL) was mixed with phosphate buffer (1.25 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1.25 mL, 1%). For 20 min, the mixture was incubated at 50 °C. To stop the reaction, a portion (1.25 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 9000× *g* for 10 min (Hettich Universal 320Randreas Hettich GmbH & Co., Tuttlingen, Germany). The absorbance was measured with a UV/Visible spectrophotometer (UV-1280, Shimadzu, Japan) at a 700 nm wavelength after the upper layer of solution (1.25 mL) was mixed with distilled water (1.25 mL) and $FeCl_3$ (0.25 mL, 0.1%). A rise in the reaction mixture's absorbance indicated an increase in the reducing power.

2.3.3. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The antioxidant activity and content of antioxidants in *C. glomerata* extracts were determined using the spectrophotometric DPPH radical scavenging method according to

Korzeniowska et al. [6]. A solution of DPPH (6.5×10^{-5} M) in methanol was prepared. The test samples were prepared by mixing 0.1 mL of *C. glomerata* extract and 1 mL of prepared DPPH solution. For the preparation of the control sample, 1 mL of DPPH solution was mixed with 0.1 mL of methanol. The prepared samples were incubated in the dark at 20 °C for 30 min. The absorbance of the samples at 515 nm was measured by a Schimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Additionally, the absorbance of the biomass extract without the reagent (background of the sample) was measured. Antioxidant activity is expressed as the radical scavenging activity calculated by the following formula:

$$\text{Radical scavenging activity } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

where A_c is the absorbance of the control sample and A_s is the absorbance of the test sample (value after subtracting the sample background).

The antioxidant content was calculated according to the calibration curve of the standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solutions and expressed in Trolox equivalents (TE) per gram of dry mass of *C. glomerata*.

2.3.4. 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid) (ABTS) Radical Scavenging Assay

The ABTS stock solution (2 mM) was prepared by reacting ABTS with 0.17 mM potassium persulfate in 20 mM phosphate buffer (pH = 7.4) at room temperature under dark conditions for 12 h. A working ABTS solution was then prepared by diluting the stock solution with 20 mM phosphate buffer (pH = 7.4). The working reagent was adjusted to obtain an absorbance of 0.80 ± 0.05 at 734 nm. Then, 0.5 mL of the algal methanol extract (0.02 g/mL) was reacted with 0.3 mL of the working ABTS solution and 1.7 mL of 20 mM phosphate buffer (pH = 7.4). The ABTS radical scavenging ability was measured at 734 nm wavelengths using the UV/Visible spectrophotometer (UV-1280, Shimadzu, Japan). The radical scavenging activity (%) was calculated using the same formula presented in Section 2.3.3.

2.3.5. Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The FRAP assay is a method for determining the antioxidant activity of a sample to reduce Fe^{3+} to Fe^{2+}. The FRAP reagent was freshly prepared by mixing 300 mM of acetate buffer (pH = 3.6) with 10 mM of TPTZ solution and 20 mM of ferric chloride solution.

The dried biomass (0.1 g) was extracted for 0.5 h in methanol (5 mL; 99%) at 45 °C. It was then centrifuged for 10 min at 9000× *g* (Hettich Universal 320Randreas Hettich GmbH & Co., Tuttlingen, Germany). Then, the FRAP reagent (3 mL) was reacted with 100 µL of the algal methanol extract (0.02 g/mL) for 5 min before measurement with a UV/Visible spectrophotometer (UV-1280, Shimadzu, Japan) at 593 nm. A linear regression was generated using $FeSO_4 \cdot 7H_2O$ at a final concentration of 5–25 µmol/L. FRAP activity (µmol/L) was calculated and compared with the $FeSO_4 \cdot 7H_2O$ calibration curve.

2.4. Macroalgal Biomass Pigments Contents Analysis

2.4.1. Chlorophyll *a*, *b* and Carotenoids Contents

Dried algal material (0.1 g) was mixed with 10 mL of 96.3% ethanol for the determination of chlorophyll *a*, *b* and the total carotenoids amount [31]. The extract was centrifuged at 9000× *g* for 10 min (Hettich Universal 320Randreas Hettich GmbH & Co., Tuttlingen, Germany). Optical density readings were recorded at wavelengths of 662 nm (chlorophyll *a*), 644 nm (chlorophyll *b*) and 441 nm (carotenoids). Ethanol (96.3%) was used as a blank. The measurements of chlorophyll *a*, *b* and carotenoids were determined by the UV/Visible

spectrophotometer (UV-1280, Shimadzu, Kyoto, Japan). The concentrations of chlorophyll *a*, *b* and carotenoids were calculated by:

Chlorophyll *a* concentration (mg/L):

$$C_a = 9.784\ OD_{662} - 0.990\ D_{644}$$

Chlorophyll *b* concentration (mg/L):

$$C_b = 21.426\ OD_{644} - 4.650\ D_{622}$$

$$C_a + C_b = 5.134\ OD_{622} + 20.436\ OD_{644}$$

Carotenoid concentration (mg/L):

$$C_{car} = 4.695\ OD_{441} - 0.268\ (C_a + C_b)$$

The concentration of separate pigments per gram of DM (mg/g DM) was calculated by:

$$C_{pig} = \frac{C \times V \times V_2}{n \times V_1 \times 1000}$$

where:

OD—optical density according to the wavelengths of the pigments.
Coefficients—the absorption coefficients of the pigments according to the wavelengths.
C—chlorophyll *a*, *b*, carotenoids concentration, mg/L.
V—initial volume of the algal extract, mL.
V_1—initial volume of the algal extract for dilution, mL.
V_2—diluted volume of the algal extract, mL.
n—weight of the algal material, g.

2.4.2. Lutein Content

The lutein content, with a few modifications to the method, was determined according to Jajali Jivan and Abbasi [32]. In this analysis, 100 mg of dried algal biomass was mixed with 20 mL of acetone and left on the environmental shaker-incubator (ES-20, Grant Instruments, Shepreth, UK) for 3 h at 300× *g* at room temperature (25 °C) in dim light. The suspension was then allowed to stand for 5 min before we collected the supernatant, but the pellet was mixed (1 h) again with acetone (10 mL) under the same circumstances and the former process was repeated two more times until the pellet remained colorless. Finally, the supernatants were pooled and kept at 4 °C for lutein content analyses.

The molar extinction coefficient of lutein was used to quantify it [33]. The extract (2 mL) was centrifuged at 9000× *g* and 25 °C during 15 min (Hettich Universal 320Randreas Hettich GmbH & Co, Germany). Optical density (OD) was measured at 446 nm against acetone as a blank (UV/Visible Spectrophotometer UV-1280, Shimadzu, Japan), as the λmax of lutein had the least interference with other carotenoids. The lutein content in biomass samples was then calculated using the following formula:

$$C = A_{466}/(14.45 \times 10^4) \times (1/b) \times 568.88 \times V/M \times 1\ L/10^3\ mL \times 10^3\ mg/g \times Kg/10^3\ g$$

where C is the lutein content (mg/g), A_{446} is the absorbance wavelength, b is the path length (cm), 568.88 is the molecular weight of lutein (g mol^{-1}), V is the volume of algal extract (mL), M is the weight of the consumed algal biomass (kg)and 14.45×10^4 is the molar extinction coefficient of lutein in acetone (L mol^{-1} cm^{-1}).

2.5. Statistical Analysis

The study used four algal biomass samples, each with three duplicates (n = 3 duplicates/river). Data analysis was performed by SPSS for Windows, version 25.0 (IBM Corp., Released 2017, Armonk, NY, USA). A one-way analysis of variance (ANOVA) test post

hoc (Fisher's least significant difference test) was conducted to detect differences among *C. glomerata* biomass from various Lithuanian rivers. A calculated p value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results

3.1. Phenolic Compounds

In order to achieve a comprehensive profile of the phenolic compounds in biomass of *C. glomerata*, separate phenolic acids, flavonoids and catechins were identified (Table 2). The following three phenolic acids: gallic, *p*-hydroxybenzoic and *p*-coumaric of the thirteen analysed were identified. Gallic acid was mainly found in B3 biomass (35.13 μg/g DM) and was lower by 13.82, 21.39 and 22.19 μg/g DM, respectively in B2, B4and B1 ($p < 0.05$). Another phenolic acid, *p*-hydroxybenzoic, was also found in high amounts (29.05 μg/g DM) in B3 and B4 (28.31 μg/g DM) *C. glomerata* biomass ($p < 0.05$). However, in these cases, the differences between the various alga biomasses were found to be slightly smaller, as a difference of 5.08 and 3.62 μg/g DM was found in the biomass of B1 and B2 compared to the biomass of B3 ($p < 0.05$). *P*-coumaric acid was identified in only three out of the four biomasses analysed (unidentified in B3). The highest concentration of the mentioned phenolic acid was found in B4 biomass (6.46 μg/g DM; $p < 0.05$); compared to the concentrations found in B1 and B2, B4 biomass contained two and almost four times more p-coumaric acid than B1 and B2, respectively ($p < 0.05$). The highest total phenolic compounds content (1.32 mg GAE/g DM; $p < 0.05$) was determined for B3. The results obtained in B3 did not significantly differ from B4 as similar concentrations were found ($p > 0.05$). However, significant differences were obtained when the total phenolic content of B1 and B2 was 0.37 and 0.22 mg GAE/g DM lower than in B3, respectively ($p < 0.05$).

The analysis of phenolic compounds also sought to identify the following five flavonoids: quercetin, myricetin, kaempferol, rutin and xantohumol. However, when analysing the chromatograms and comparing the resulting peaks with standards of different flavonoids and catechins (catechin, epicatechin, epigallocatechin gallate), none of these phenolic compounds were identified.

Table 2. Phenolic compounds profile of *C. glomerata* biomass from various rivers in Lithuania.

Item [1,2]	*C. glomerata* Biomass [3,4,5]				SEM [6]	*p* Value
	B1	B2	B3	B4		
DM (% of dried samples)	94.95 [a]	92.63 [a]	95.19 [a]	91.12 [b]	0.35	0.000
			Phenolic acids (μg/g DM)			
o-Coumaric	n.d.	n.d.	n.d.	n.d.	-	-
Cinnamic	n.d.	n.d.	n.d.	n.d.	-	-
m-Coumaric	n.d.	n.d.	n.d.	n.d.	-	-
Vanillic	n.d.	n.d.	n.d.	n.d.	-	-
Caffeic	n.d.	n.d.	n.d.	n.d.	-	-
Salicylic	n.d.	n.d.	n.d.	n.d.	-	-
Ferulic	n.d.	n.d.	n.d.	n.d.	-	-
Sinapic	n.d.	n.d.	n.d.	n.d.	-	-
Chlorogenic	n.d.	n.d.	n.d.	n.d.	-	-
3,4-Dihydroxybenzoic	n.d.	n.d.	n.d.	n.d.	-	-
Gallic	12.94 [a]	21.31 [b]	35.13 [c]	13.92 [d]	0.16	0.000
p-Hydroxybenzoic	23.97 [a]	25.43 [b]	29.05 [c]	28.31 [c]	0.31	0.000
p-Coumaric	3.16 [a]	1.79 [b]	n.d.	6.46 [c]	1.71	0.000

Table 2. *Cont.*

Item [1,2]	*C. glomerata* Biomass [3,4,5] B1	B2	B3	B4	SEM [6]	*p* Value
Flavonoids (μg/g DM)						
Quercetin	n.d	n.d	n.d	n.d	-	-
Myricetin	n.d.	n.d.	n.d.	n.d.	-	-
Kaempferol	n.d.	n.d.	n.d.	n.d.	-	-
Rutin	n.d.	n.d.	n.d.	n.d.	-	-
Xantohumol	n.d.	n.d.	n.d.	n.d.	-	-
Catechins (μg/g DM)						
Catechin	n.d.	n.d.	n.d.	n.d.	-	-
Epicatechin	n.d.	n.d.	n.d.	n.d.	-	-
Epigallocatechin gallate	n.d.	n.d.	n.d.	n.d.	-	-
The total phenolic content (mg GAE/g DM)	0.95 [a]	1.10 [b]	1.32 [c]	1.22 [c]	0.09	0.003

Note: [1] DM, dry matter. [2] GAE, gallic acid equivalent. [3] *C. glomerata* biomass from Lithuanian rivers, Dubysa (B1); Šventoji (B2); Nevėžis (B3); Jūra (B4). [4] Means with different superscript letters (a–d) in a row were significantly different ($p < 0.05$). [5] n.d., not defined. [6] SEM, standard error of the means.

3.2. Antioxidant Activity

The higher value of absorbance of the reaction mixture indicated a greater reducing power. Our research showed that the highest reducing power according to absorption units (AU) at 700 nm was in *C. glomerata* macroalgal biomass (B3) from the Nevėžis River; it reached 0.737 AU ($p < 0.05$; Table 3). Compared to the other groups, the B3 biomass reduction power was 0.341, 0.436and 0.152 AU higher, respectively, than in the B1, B2and B4 macroalgal biomass samples ($p < 0.05$).

Table 3. Reducing power (RP), DPPH scavenging activity, antioxidant content, the relative ability of antioxidants to scavenge (ABTS) and ferric reducing-antioxidant power (FRAP) of *C. glomerata* biomass from various rivers in Lithuania.

Item [1,2]	*C. glomerata* Biomass [3,4] B1	B2	B3	B4	SEM [5]	*p*-Value [6]
RP (AU)	0.396 [a]	0.301 [a]	0.737 [b]	0.585 [c]	0.05	0.000
DPPH (%)	10.10	8.22	11.09	8.85	1.38	n.s.
Antioxidant content (mg Trolox/g DM)	0.55 [a]	0.39 [a]	1.47 [b]	1.31 [b]	0.11	0.000
ABTS (%)	97.68 [a]	96.80 [a]	97.09 [a]	93.76 [b]	1.18	0.011
FRAP (μmol/L)	15.04 [a]	16.61 [a]	20.86 [b]	19.93 [b]	1.54	0.005

Note: [1] AU, absorption units. [2] DM, dry matter. [3] *C. glomerata* biomass from Lithuanian rivers, Dubysa (B1); Šventoji (B2); Nevėžis (B3); Jūra (B4). [4] Means with different superscript letters (a–c) in a row were significantly different ($p < 0.05$). [5] SEM, standard error of the means. [6] n.s., not significant ($p > 0.05$).

The DPPH, ABTS and FRAP tests were used to assess freshwater *C. glomerata* macroalgal biomass biological activity (Table 3). The DPPH scavenging activity ranged from 8.22% to 11.09% and no significant differences between the *C. glomerata* biomasses collected from different rivers were observed ($p > 0.05$). The antioxidant content of macroalgae *C. glomerata* biomass from various Lithuanian rivers is presented in Table 3. Greater levels of antioxidants were found in B3 (1.47 mg Trolox/g DM; $p < 0.05$) and B4 (1.31 mg Trolox/g DM; $p < 0.05$). Compared to B1 and B2, the content of antioxidants was found to be higher by 2.7 and 3.8 times in B3 ($p < 0.05$) and in B4 by 2.4 and 3.4 times ($p < 0.05$), respectively.

Nevertheless, after ABTS assay, the highest scavenging was determined in B1 biomass samples (97.68%; Table 3). The ABTS assay also revealed no significant differences between groups B1, B2and B3, as the values were similar and differed minimally ($p > 0.05$). However, considering the significant differences between the mentioned groups and B4, the

scavenging of the biomass samples B1, B2 and B3 was 3.92%, 3.04% and 3.33% higher than in the B4 samples, respectively ($p < 0.05$).

The ferric reducing-antioxidant power (FRAP) analysis showed that the biomass of B3 and B4 macroalga had the highest power (20.86 and 19.93 µmol/L, respectively), with values calculated to be 5.82 and 4.25 µmol/L higher than B1 and 4.89 and 3.32 µmol/L higher than B2 ($p < 0.05$) (Table 3).

3.3. Pigments Content

The concentration of individual pigments was determined in *C. glomerata* biomass samples from various Lithuanian rivers (Table 4). In general, the total chlorophyll *a* content in the tested macroalgal biomass groups ranged from 0.56 mg/g to 0.74 mg/g DM and was distributed as follows: B2 (0.56 mg/g DM) < B4 (0.57 mg/g DM) < B1 (0.65 mg/g DM) < B3 (0.74 mg/g DM) ($p < 0.05$). Significant differences in chlorophyll *b* concentrations were obtained only between groups B1 and B4, when the biomass from the Dubysa River (B1) was found to be 0.18 mg/g DM higher in the mentioned pigment than the macroalgal biomass from the Jūra River (B4) ($p < 0.05$). The same trend was observed when calculating the total carotenoids concentration, when this pigments group was detected to be higher by 0.06 mg/g DM in B1 *C. glomerata* biomass compared to B4 ($p < 0.05$).

Table 4. Content of individual pigments (chlorophyll *a* and *b*, carotenoids, lutein) of *C. glomerata* biomass from various rivers in Lithuania.

	***C. glomerata* Biomass** [2,3,4]					
Pigment (mg/g DM) [1]	**B1**	**B2**	**B3**	**B4**	**SEM** [5]	***p*-Value**
Chlorophyll *a*	0.65 [a]	0.56 [b]	0.74 [c]	0.57 [b]	0.03	0.003
Chlorophyll *b*	0.57 [a]	0.42 [ab]	0.51 [ab]	0.39 [b]	0.06	0.042
Carotenoids	0.23 [a]	0.18 [ab]	0.20 [ab]	0.17 [b]	0.02	0.037
Lutein	0.12 [ab]	0.17 [a]	0.13 [ab]	0.11 [b]	0.03	0.044

Note: [1] DM, dry matter. [2] *C. glomerata* biomass from Lithuanian rivers, Dubysa (B1); Šventoji (B2); Nevėžis (B3); Jūra (B4). [3] Means with different superscript letters (a–c) in a row were significantly different ($p < 0.05$). [4] Means with ab superscript letters in a row did not have significant differences between groups ($p > 0.05$). [5] SEM, standard error of the means.

Lutein was calculated in the biomass of macroalgae *C. glomerata* from various Lithuanian rivers (Table 4). The most significant differences were found between the rivers Šventoji (B2) and Jūra (B4), with an almost 1.5 higher concentration of lutein obtained in B2 biomass (0.17 mg/g DM) compared to the results obtained in B4 samples (0.11 mg/g DM) ($p < 0.05$). No significant differences were found between the remaining groups ($p > 0.05$).

4. Discussion

4.1. Phenolic Compounds

Numerous factors determine the chemical composition of algal biomass and the level of biologically active compounds, including taxa, habitat, climatic conditions, environmental stressors, biomass collection time and techniques [34,35]. The quantity of bioactive compounds in macroalga is a result of the difficult, harsh, and competitive circumstances in which they thrive. In general, algae are a well-known natural raw material that contains phenolic compounds. One of the most significant and most discussed features of phenolic compounds is their antioxidant activity [6]. Its primary function is to bind, stabilize and inactivate free radicals [6,34,36].

Phenols, as bioactive components, possess a variety of chemical, physical and biological properties [6] that may contribute to the antioxidant activity of pharmaceutical and food products [12]. Many of them display not only antioxidant effects, but also antibacterial and antiviral activities, which are critical for protecting algal cells from stress. For example, one of the groups of phenolic compounds is flavonoids. Badshah et al. [37] recently demonstrated that flavonoids have strong antiviral activity against a variety of viruses,

with the tested flavonoids exhibiting strong antiviral activity, of more than 90% without impacting cell viability. However, we identified no flavonoids in freshwater *C. glomerata* biomass during a recent study. In terms of total phenolic compound content, the highest total phenolic compound content was determined in *C. glomerata* biomass from the Nevėžis River (1.32 mg GAE/g DM). While applying the same determination method and extractant on freshwater *C. glomerata* biomass, Korzeniowska et al. [6] discovered that the phenol content in biomass reaches only 0.27 mg GAE/g DM. Decreased or lower phenolic concentrations (0.30–20 mg GAE/g DM) in algal extracts may be linked to other chemicals, such as carotenoids, fatty acids, sterols, vitamins, and others that may be responsible for the indicated activities [6,38–40].

Only three phenolic acids (gallic, *p*-coumaric, *p*-hydroxybenzoic) were found in macroalgal biomass during the current study out of the 13 assessed. However, using HPLC qualitative examination, Korzeniowska et al. [6] were able to identify nine phenolic compounds (gallic, chlorogenic, syringic, *p*-coumaric, myricetin, 3,4-dihydroxybenzoic, vanillic, 4-hydroxybenzoicand rutin acids) in freshwater *C. glomerata* that belonged to both the phenolic acids and flavonoids [6]. These results are in line with our study as we identified gallic acid, which varied from 12.94 to 35.13% of DM and *p*-coumaric acid, which varied from 1.79 to 6.46% of DM, in all the analysed macroalgal biomass samples from various Lithuanian rivers. The other significant phenolic acid identified in our research was *p*-hydroxybenzoic, which varied from 23.97 to 29.05% of DM and prevailed in almost all biomass samples.

All three phenolic acids found in the recent study's macroalgal biomass present health benefits. Hydroxybenzoic acids, gallic and *p*-hydroxybenzoic, lead to dyslipidaemia treatment and glucose metabolism regulation [41]. They are considered as antihyperlipidemic, antihyperglycemic, cardioprotective [42] and anti-cancerous [43]. *P*-coumaric acid, a member of the hydroxycinnamic acid group, possesses anti-cancer properties as well [44].

4.2. Antioxidant Activity

The antioxidant activity of *C. glomerata* biomass is generally measured in terms of total phenolic and antioxidant content, reducing power, DPPH, ABTS radical scavenging activity and FRAP. Antioxidant activity is crucial in fighting free radicals and reactive oxygen species, which both cause oxidative stress. Several studies have found that it is the primary cause of several diseases, including human ailments [45–47]. In general, the accurate identification of antioxidant compounds can lead to their application in food, feed, cosmetics and medicine manufacturing.

In the reducing power assay, the antioxidant activity in the sample leads to the reduction of Fe^{3+} to Fe^{2+} by donating one electron [48]. Previously, researchers discovered a link between antioxidant activity and the reducing power of particular plant extracts [49,50]. Reductones, which have been shown to exert an antioxidant effect by breaking the free radical chain by donating a hydrogen atom, are frequently related to the occurrence of reducing abilities [50]. The basis of this method is an increase in the absorbance of the reaction mixtures. More absorption suggests increased antioxidant activity [51]. Our data on the reducing power of freshwater *C. glomerata* biomass from various Lithuanian rivers was found to be in the order of B3 (Nevėžis) > B4 (Jūra) > B1 (Dubysa) > B2 (Šventoji). Nonetheless, the highest reducing power observed in the River Nevėžis, considering all macroalgal biomass results, suggests that it is likely to contribute significantly towards the observed antioxidant effect.

Algae are a natural material with a diverse and complicated matrix constitution. As a result, the methods for assessing antioxidant activity have proven not only the existence of several phenolic compounds in *C. glomerata* biomass, but also the presence of additional antioxidants. In this case, DPPH, ABTS and FRAP procedures are rapid and easy ways by which to assess antioxidant activity [6]. The high quantity of phenols in algal extracts demonstrates substantial DPPH radical scavenging activity, allowing them to neutralize free radicals that can activate cancer cells [52]. The molecule of 2-diphenyl-1-picrylhydrazyl

(DPPH) is classified as a stable free radical due to the delocalization of the spare electron over the molecule, preventing dimerization, as most other free radicals do [51]. The deep violet colour is also induced by electron delocalization, which is described by an absorption band in methanol solution at around 517 nm. When a DPPH solution is treated with a substance capable of donating a hydrogen atom, a reduced form is created, but the violet colour disappears. During our study, the DPPH in different *C. glomerata* biomass varied from 8.22% to 11.09%. Compared to the results of other researchers [6,53], the DPPH activity obtained in our experiment is relatively low. By determining DPPH by the same method and using the same concentration of methanol for the preparation of extracts, Korzeniowska et al. [6] found this indicator as being almost three times higher (32.67 ± 0.40%). However, on the contrary and in line with our findings, is the research of Laungsuwon and Chulalaksananukul [54], who revealed that antioxidant activity measured by DPPH was 16.7% in freshwater *C. glomerata* biomass [54]. Nonetheless, the findings of the mentioned researchers were only slightly higher than ours (by approximately 5%). It should be noted that the biomass samples in their study were freeze-dried, which may have affected the final test substance. Furthermore, to explain the disparities in the researchers' findings, Korzeniowska et al. [6] reported that antioxidant activity in freshwater *C. glomerata* is dependent on extraction procedures and solvents. It is also important to note that there were no significant differences identified between Lithuanian rivers following a DPPH scavenging activity assessment of *C. glomerata* biomass in our study.

The total antioxidant content found in algae varies depending on the season and geographic region [53]. In the recent study, the total antioxidant content was calculated using the Trolox standard curve and expressed according to the DPPH method. The number of antioxidants in the macroalgal biomass from different Lithuanian rivers varied from 0.39 to 1.47 mg Trolox/g DM. Freshwater *C. glomerata* biomass from two water reservoirs in the Wielkopolska region (Poland) was tested using the same method [6]. It was in line with our findings when we assessed antioxidant content in biomass from the Nevėžis (B3; 1.47 mg Trolox/g DM) and Jūra (B4; 1.31 mg Trolox/g DM) rivers. However, the antioxidant content of biomass from the Lithuanian rivers Dubysa (B1) and Šventoji (B2) was only 0.55 and 0.39 Trolox/g DM, respectively, whereas the same index in biomass from Polish water reservoirs reached a content of 2–3 times higher. Differences between our and Polish scientists' findings could simply be due to differences in macroalgae habitats.

The antioxidant activity of four macroalgal extracts was determined using the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) test, which is based on antioxidants' ability to scavenge the radical anion ABTS*+. The ABTS assay measures an antioxidant's capacity to prevent the absorption of ABTS radicals, which have a unique blue colour and maximum absorption of 734 nm [55]. The ABTS scavenging activity in macroalgal biomass collected from four different Lithuanian rivers ranged from 93.76 to 97.68% and was about 3 times higher compared with *C. glomerata* from freshwaters in Poland [6]. According to prior findings, carotenoids derived from algae often have a better scavenging activity for ABTS radicals, but with a lower reduction power [55], which in general explains our findings. In a recent study, the inhibitory activity against ABTS radicals was found to be greater than that against DPPH radicals in macroalgae biomass. This can be linked to the fact that ABTS is more sensitive in detecting antioxidant activity and has a stronger response to antioxidants due to faster reaction kinetics [56].

The ferric ion reducing antioxidant power (FRAP) method assesses antioxidants' capacity to decrease ferric iron [51]. The combination of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) is converted to ferrous form at low pH. This reduction is recognized by a shift in absorbance at 593 nm. The antioxidants in the biomass samples are treated as reductants in a redox-linked colorimetric process in the FRAP assay, and the value indicates the antioxidants' reducing power. Along with phenolic contents, molecular weight, and structural arrangement, FRAP activity is more strongly aligned to phlorotannin content than to radical scavenging capacity [57]. In our case, FRAP activity varied from 15.04 to 20.86 μmol/L.

The antioxidant activity of macroalgal extracts is mostly attributed to phenolic compounds. Carotenoids, on the other hand, assist in DPPH radical scavenging, ferric ion reduction antioxidant power (FRAP)and ABTS radical scavenging capacity activities [58]. The researchers furthermore provide data showing a strong link between phenolic compounds and FRAP with DPPH values in algal biomass [3,59]. As relatively few phenolic compounds were identified in our study and the levels of FRAP and DPPH were not high, we can assume and confirm that this dependence is also reflected in our results.

4.3. Pigments

Algae has been utilized as a food source for a long time due to its high nutritional value. Chlorophyll, a green pigment found in plants, algae and cyanobacteria that is necessary for photosynthesis, is one of its bioactive components. According to the correlation between habitat conditions and Cladophora species diversity, water depth, chloride, orthophosphate, nitrate, total dissolved salts, and chlorophyll *a* content in water are essential criteria that define it [19]. For example, Messyasz et al. [4] demonstrated that the chlorophyll *a* content in freshwater *C. glomerata* extract was 0.30 mg/g of dry algal biomass. In our study, *C. glomerata* biomass from various Lithuanian rivers contained 0.57 to 0.74 mg/g DM chlorophyll *a*, which is nearly two times higher than Messyasz et al. determined. This demonstrates that the content of this compound is influenced by the algae habitat. For example, disadvantageous environmental conditions trigger disruptions in the morphometric structure of thalli, the seasonality of their occurrence and the formation of vast quantities of filamentous algal biomass [1]. Even changes in biomass collection sites, such as the surface, middle and bottom habitats of a dense *C. glomerata* mat, impact nutrients, underwater light environment, and temperature. Green algae also contain chlorophyll *b*, in addition to chlorophyll *a*. In our study, this compound reached 0.57 mg/g DM in biomass from the river Dubysa. Pikosz et al. [60] conducted a study comparing two freshwater species of *Cladophora* (*C. glomerata* and *C. fracta*) from lakes in Poland. These researchers discovered that *C. glomerata* had larger quantities of pigments, especially chlorophyll *b* (6.36 ± 0.11 μg mL^{-1}), compared to *C. fracta* (2.95 ± 0.07 μg mL^{-1}). However, compared to our recent study, we obtained much higher concentrations of chlorophyll *b*. These results may be influenced primarily by the environmental conditions described above. Additionally, biomass from Poland was collected in June, from a lake, whereas our biomass was collected in August-September and from rivers.

Carotenoids are a rich category of compounds that include over a hundred compounds found exclusively in algae, and they exhibit a broad range of chemical structure by alterations of end groups or chains, as well as isomerization [17]. Carotenoids come in a variety of colours, ranging from yellow to red, and they are in a range of algal species. They have significant antioxidant properties due to their ability to quench singlet oxygen and scavenge free radicals [61]. In our study, carotenoids in *C. glomerata* biomass accounted for the smallest proportion of pigments tested, ranging from 0.17 to 0.23 mg/g DM. Khuantrairong and Traichaiyaporn [62] discovered higher but still relatively low concentrations of carotenoids in freshwater *C. glomerata* (0.89 mg/g DM). In contrast to our results, Fabrowska et al.'s [63] analysis of *C. glomerata* was considered to have significant total carotenoids and phenol content, as well as antioxidant activity. For example, the mentioned researchers reported exceedingly higher total carotenoid levels (16.59 mg fucoxanthin equivalents/g). Nevertheless, results obtained by Messyasz et al. [4] were found to be two times lower in total carotenoids (0.08 mg/g) in *C. glomerata* dry algal biomass compared to ours (0.17–0.23 mg/g DM). However, differences found between *C. glomerata* harvested and analysed in different experimental investigations may be due to biodiversity as well as the effect of growth and environmental circumstances. Lower temperatures and less sunlight, for example, slow down metabolic processes, so algae require fewer chlorophyll for photosynthesis. As a result, algae produce less chlorophyll while producing more carotenoids [30].

Lutein is a prominent xanthophyll carotenoid prevalent in green algae that is strongly associated with the light-harvesting complexes of the photosynthetic apparatus. In addition to capturing light and acting as a structural component in the photosynthetic apparatus, lutein defends the photosynthetic system from oxidative damage under high light conditions via a mechanism known as non-photochemical quenching [64]. Prazukin et al. [65] analysed filamentous green algae *Cladophora* spp. from a hypersaline lake in Crimea, Russia. Prazukin et al. results showed *Cladophora* spp. contained lutein 0.55 mg/g DM, which shows its mat's potential use as a cheap raw material for pharmaceutical and food production purposes. In our case, lutein content in freshwater *C. glomerata* biomass from Lithuanian rivers varied from 0.11 mg/g in biomass from the River Jūra to even 1.5 times higher (0.17 mg/g) in biomass from the Šventoji river. This pigment promotes cognitive performance, lowers the risk of cancer [66] and exhibits anti-inflammatory effects [67]. It is a great natural protector against retinal, macular, and crystalline eye lens damage due to its antioxidant characteristics [68,69]. Individuals must consume at least 5 mg of lutein daily [70]. Because it is not synthesized in the human body it must be consumed with food [69]. Overall, the lutein market is divided into the following four sectors: pharmaceuticals, dietary supplements, food and animal and fish feed [71].

Aside from limiting free radical production, pigments are utilized to improve the coloration of animal origin products, making them more appealing and desirable to customers. The inclusion of algae, which are a source of colour in animal feed, has positive benefits as well [72]. Natural pigments, such as polyphenols, can be used in the prevention and treatment of many diseases because they inhibit the formation of free radicals [73]. Algal dyes can also be used to enhance the colour of animal products, such as egg yolks. Consumers find these products more appealing and desirable [73]. Algal extracts used in animal feeding research could help to biofortify animal origin with biologically active compounds [74]. Shah et al. [75] specifically investigated and outlined the potential uses of freshwater *C. glomerata* for the following purposes: the development of traditional medicines, nutraceuticals, food, and agricultural industries; as separate compounds that can be used as pesticides and insecticides; and the production of biofuels. To summarize, macroalgae have numerous applications. It can be used as a natural colorant in food pigments, such as dairy products or beverages, as well as a feed supplement in aquaculture, livestock, and animals and as a component in cosmetics and pharmaceuticals. It is worth noting that macroalgae of *Cladophora* species are edible and can be found in many people's diets all around the world [76].

5. Conclusions

Among the materials of natural origin, the macroalgal biomass of *C. glomerata* from various Lithuanian rivers (Dubysa, Šventoji, Nevėžis, Jūra) may play an important role and has the potential as a functional raw material in biotechnology. In a recent study, several groups of bioactive compounds, such as phenols and pigments, were identified, as well as some antioxidant activities. To summarise:

- Identified phenolic acids (gallic, *p*-coumaric, *p*-hydroxybenzoic) have health benefits (antihyperlipidemic, antihyperglycemic, cardioprotective and anticancer features) which can be manipulated in the development of pharmaceuticals or functional foods and animal feed supplements.
- Almost all the macroalgal biomass results indicate that *C. glomerata* is likely to have a significant role in the observed antioxidant effect, and the highest reducing power reached 0.737 AU; the total antioxidant content reached 1.47 mg Trolox/g DM; DPPH and ABTS radical scavenging reached 11.09% and 97.86%, respectively; and FRAP activity reached 20.86 μmol/L.
- The observed content of main pigments (chlorophyll *a* and *b*, carotenoids, lutein) shows a wide range of applications for *C. glomerata*, including natural colorants used as food pigments, feed supplements and components of cosmetics and pharmaceuticals due to their potential health benefits.

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