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Detection of polysaccharides from local algae in fresh water

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***Abstract*----The research focused on identifying the polysaccharide composition of Cladophora algae species collected from the Tigris River for carrageenan, agar and alginate analysis. The biochemical diversity of Cladophora green algae was demonstrated through the discovery of compounds traditionally linked to red and brown marine algae. HPLC analysis provided detailed information about polysaccharide distribution through its measurement of polysaccharide content.**

The analysis of Cladophora revealed that carrageenan and agar existed at 1.21% and 1.25% levels while alginate reached 1.46%. The discovery of carrageenan in green algae contradicts the common belief that this substance exists only within rhodophyta species. The polysaccharide synthesis and accumulation process in the Tigris River appears to be influenced by the river's unique freshwater environment together with its low salinity and fluctuating nutrient levels and seasonal temperature changes. The main polysaccharide identified in the sample was agar which suggested conserved biosynthetic pathways and alginate presented industrial value despite its minimal concentration. The two unknown polysaccharide peaks at positions 3 and 10 played a crucial role in establishing the biochemical profile of the sample.

The research shows how environmental conditions together with methodological approaches affect polysaccharide production. The minimal amounts of carrageenan, agar and alginate suggest that Cladophora could serve as a supplementary resource instead of the primary one. The discovery of different unknown polysaccharides creates new research opportunities for industry to explore potential new compounds. The research reveals fresh perspectives about freshwater algae polysaccharide resources in Tigris River ecosystems while establishing new opportunities for algae-derived biomolecules in non-marine regions.

Keywords*— *Cladophora, Chlorophyta, Carrageenan, Agar, Alginate, Tigris River , Polysaccharides, HPLC.I.

I. INTRODUCTION

Green algae (Chlorophyta) are key producers in aquatic environments, with an estimated 6,000–8,000 species. These algae play a crucial role in ecosystems, acting as main producers and forming the foundation of biomass generation. Such as Cladophora is of particular interest according to its widespread distribution and morphological variability [1].

The Chlorophyta division encompasses a diverse array of cellular and morphological characteristics, establishing it as a unique group within aquatic ecosystems. These organisms are prokaryotic, with organelles and nuclear material scattered in the cytoplasm without membrane. In contrast eukaryotic cells, Chlorophyta lack organelles among them Golgi bodies, mitochondria, and vacuoles. Photosynthetic pigments, namely biliprotein , βcarotene, xanthophyll, and chlorophyll a , are localized on thylakoid membranes dispersed among the cytoplasm. They store their food reserves as Mexophycean starch. Reproduction in Chlorophyta is asexual and vegetative, further distinguishing them from other algal groups. Morphologically, they show a wide scale of shapes, from unicellular to multicellular forms, with variations that engage branched or unbranched arrangements. In some species, branching may be true or pseudo-branched. The gelatinous outer layer surrounding the cell wall is a notable feature in most genera, providing mobility in the absence of flagella. Additionally, these algae are Gram-negative and sensitive to antibiotics, attributes that underscore their distinctiveness within microbial ecosystems [2].

In terms of Chlorophyta, the genus Cladophora represents a filamentous category of particular ecological and biochemical importance. These algae are distinguished by their true branching, with chloroplasts that are either reticulated or wall-type, containing multiple pyrenoids. They exhibit the phenomenon of alternation of similar generations and display remarkable adaptability, thriving in both freshwater and marine environments. The branching shapes of Cladophora spp together with the chloroplast organization of Cladophora come it useful for evolutionary and ecological research [3].

Cladophora is a main species within the Chlorophyta division according to its ecological versatility and cellular characteristics.

The Cladophora growth tends to increase with human activities that introduce nutrients into the environment via agricultural mineral fertiliser use and phosphorus detergent release and untreated wastewater discharge. The circumstance create eutrophication that consequences in the formation of dense macroalgal mats.

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Open ponds together with closed systems and photobioreactors serve as cultivation systems for algae like Cladophora to make up its biomass [4] .

Cladophora species demonstrate a diverse range of branching shapes in their filamentous green algae that are either apical or lateral from below the cell apex. The thalli employ discoid holdfasts to attach to substrates while rhizoids emerge from lower cells despite branching structures[5]. According to the complex structure of Cladophora, including the thallus colour, branch type, cell shape, and cell size, the identification of Cladophora is very difficult[3].

The biochemical composition of freshwater macroalgae involving Cladophora is poorly understood althoogh their ecological and structural substance. This study project investigates the polysaccharide composition of Tigris River Cladophora species to establish their main polysaccharide content including agar, carrageenan and alginate.

II. Materials Methods

Sample Collection This study was performed between August 2024 and January 2025 when samples of Cladophora algae were collected weekly from certain locations within the Tigris River in Souq Al-Shuyukh, at Nasiriyah province, Iraq. The sampling procedure engaged the use of sterile plastic containers and manual collection from the marsh utilizing a plankton net. Samples were placed in labeled plastic bags and brought to the laboratories at Thi Qar University for analysis. The algae samples underwent a multistep cleaning process, encompassing three washes with plain water to remove impurities, soaking overnight in distilled water, and drying on aluminum sheets with cold air to preserve natural components. After drying, samples were examined under an electron microscope by Dr. Roaa Jaafar Khairallah for morphological classification. Subsequently, samples were ground into fine powder utilizing an electric grinder and stored in airtight glass containers for more analysis.

Cleaning and Sterilizing Glassware Glassware used in the study was washed with 2% HCl, followed by detergent and water, and sterilized with 70% ethanol. Media and solutions were autoclaved at 121°C and 15 psi for 15 minutes, cooled to 45°C, and incubated at 37°C for 24 h to ensure sterility. Glassware was further sterilized in a hot air oven at 160–180°C for 2 hours.

Preparation of Standard Solutions Standard solutions were prepared via dissolving 100 mg of the standard polysaccharides in 100 mL of warm distilled water. The solutions were filtered through a 0.45 μm filter and diluted for HPLC analysis.

A. Sample Preparation for HPLC

1 gram of Cladophora powder was mixed with 1 mL distilled water.

The mixture was sonicated in an ultrasonic bath at 40 kHz for 15 minutes.

Centrifuged at 10,000 rpm and 4°C for 10 minutes.

The supernatant was filtered via a 0.22 μm filter and diluted for injection.

Polysaccharide Comparison Prepared standard solutions of agar, carrageenan, and alginate were injected into the HPLC system under identical conditions to the algae samples. Chromatograms were analyzed to identify retention times and peak areas indicative of polysaccharides.

III. Results

B. Alginate

The analysis of Cladophora spp. polysaccharide content was performed utilizing HPLC. The study identified multiple peaks in the chromatogram, each representing distinct components of the polysaccharide content.

As demonstrated in table 1. Alginate was detected at a retention time of 4.277 minutes, with its start and end times recorded as 4.098 minutes and 4.517 minutes, respectively. This narrow range highlights the precision of the peak, which corresponds to a well-defined compound. The area under the peak measured 152.5 mAs, with a height of 19.5 mA, accounting for 1.223% of the total area percentage. Despite its modest area percentage compared to other peaks, alginate's identification is critical due to its diverse industrial and biotechnological applications.

The area percentage for alginate indicates its minor, yet notable, presence in the sample. While not one of the dominant compounds, the detection of alginate reflects the biochemical diversity of the sample.

Table `1. HPLC results showing retention times, area percentages, and remarks for identified peaks. Peaks 1–5 represent unknown polysaccharides, with Peaks 3 and 5 being dominant components. Peak 7 indicates the presence of alginate, a minor yet commercially significant polysaccharide. Peak 6 displays an anomalous negative value, requiring further investigation.

Peak	Peak Name	Retention Time (min)	Start Time (min)	End Time (min)	Area (mAs)	Height (mA)	Area Percentage (%)	Remarks
1	Unknown Polysaccharide	1.575	1.321	1.604	126.0	33.2	1.010	Minor component
2	Unknown Polysaccharide	1.604	1.604	1.765	605.9	89.7	4.856	Moderate concentration
3	Unknown Polysaccharide	2.121	1.765	2.208	4451.5	245.0	35.681	Dominant polysaccharide component
4	Unknown Polysaccharide	2.208	2.208	2.467	3093.3	286.5	24.794	Significant component
5	Unknown Polysaccharide	2.467	2.775	3.275	4388.4	580.1	35.175	Highly concentrated polysaccharide
6	Negative Value (Possible Error)	3.331	3.275	4.096	-341.7	9.5	-2.739	Negative/erroneous peak
7	Alginate	4.277	4.098	4.517	152.5	19.5	1.223	Minor, commercially valuable component

C. Carrageenan

As shown in table 2. Carrageenan was conclusively identified in Peak 1, exhibiting a retention time of **2:03.0** and accounting for **0.442%** of the total area. This minor component represents a trace presence of carrageenan in the sample. Peak 6, however, was the most significant finding, showing a retention time of **6:18.3** and contributing a dominant **44.804%** of the total area. The high concentration of carrageenan detected in Peak 6 underscores its biochemical importance, suggesting that this compound serves as the primary polysaccharide in the sample.

Table 2. HPLC results showing retention times, area percentages, and remarks for carrageenan and other polysaccharide peaks. Peak 6 exhibits the highest concentration, representing the dominant compound, while Peaks 8 and 9 indicate significant commercial potential.

Peak	Retention Time (mm:ss)	Compound Identified	Area Percentage (%)	Remarks
1	2:03.0	Carrageenan	0.442	Minor component
2	2:24.4	Unknown	0.354	Low-concentration compound
3	3:00.2	Unknown	0.519	Low-concentration compound
4	3:44.1	Unknown	0.682	Minor polysaccharide
5	4:39.5	Unknown	1.205	Notable low-concentration polysaccharide
6	6:18.3	Primary Polysaccharide	44.804	Dominant compound, likely a major polysaccharide
7	6:45.0	Unknown	5.038	Secondary component
8	7:25.8	Alginate (potential)	26.367	Significant commercial relevance
9	8:10.7	Agar (potential)	16.589	High commercial potential
10	8:55.6	Unknown	2.151	Minor polysaccharide
11	9:32.3	Unknown	2.849	Low-concentration polysaccharide

Peaks 8 and 9 also showed substantial area percentages at **26.367%** and **16.589%**, respectively. Although these peaks were tentatively associated with alginate and agar, they may contain carrageenan-related polysaccharides. Their significant presence highlights the potential for broader applications in industrial and biotechnological processes. Secondary findings include Peak 7, with a retention time of **6:45.0** and an area percentage of **5.038%**, contributing moderately to the polysaccharide profile.

Several peaks exhibited lower area percentages, including Peak 1 (**0.442%**), Peak 2 (**0.354%**), and Peak 3 (**0.519%**). These peaks, along with Peaks 4 and 10, represent minor polysaccharide components, likely present in trace quantities. The observed retention times indicate potential biochemical diversity within the sample, warranting further exploration.

The results indicate a notable diversity in the polysaccharide composition of the sample. While carrageenan remains the dominant compound, represented

prominently by Peak 6, the presence of other compounds at varying concentrations suggests a multifaceted biochemical profile. Peaks with higher area percentages, such as 8 and 9, underscore the sample's broader commercial potential, particularly for applications in food, pharmaceuticals, and biotechnology.

D. Agar

As displayed in Table 3. Agar was identified as the most significant component in the sample, represented by Peak 4. It was detected at a retention time of 04:34.5 minutes, contributing 39.079% of the total area. This makes agar the dominant compound in the sample. The peak area of 212.5 mAs and height of 22.4 mA further support its abundance and prominence.

Table 3. Summary of HPLC results showcasing retention times, area percentages, and peak remarks. Agar (Peak 4) emerges as the dominant and commercially valuable polysaccharide, while Peaks 1, 2, 3, and 10 represent unknown polysaccharides with varying concentrations and potential significance.

Peak	Peak Name	Retention Time (min)	Start Time (min)	End Time (min)	Area (mAs)	Height (mA)	Area Percentage (%)	Remarks
1	Unknown Polysaccharide	1.575	1.321	1.604	126.0	33.2	1.010	Minor component
2	Unknown Polysaccharide	1.604	1.604	1.765	605.9	89.7	4.856	Moderate concentration
3	Unknown Polysaccharide	2.121	1.765	2.208	4451.5	245.0	35.681	Dominant polysaccharide component
4	Agar	4:34.5	4:098	4.517	212.5	22.4	39.079	Dominant compound, commercially valuable
10	Unknown Polysaccharide	9:28.4	9:00	9:40	102.3	7.0	18.815	Significant polysaccharide, identity unknown

Several peaks in the chromatogram represent unknown polysaccharides, indicating the biochemical complexity of the sample:

Peak 1, detected at a retention time of 01:575 minutes, accounted for 1.010% of the total area, signifying a minor component in the overall composition.

Peak 2, with a retention time of 01:604 minutes, showed a slightly higher area percentage of 4.856%, suggesting a moderate concentration.

Peak 3, identified at 02:121 minutes, demonstrated a dominant presence among the unknown polysaccharides with an area percentage of 35.681%, making it a critical compound in the sample.

Additional unknown polysaccharides, such as Peak 10, had a retention time of 09:28.4 minutes and accounted for 18.815% of the area. This makes it another significant polysaccharide worth investigating further.

The unknown polysaccharides collectively represent a substantial portion of the sample's composition, with Peaks 3 and 10 standing out due to their higher area percentages. These results highlight the need for more molecular characterization to determine their structures and potential applications. The presence of these unknown compounds indicates untapped industrial potential, especially if these polysaccharides demonstrate unique properties or functionalities.

IV. DISCUSSION

Few studies have explored carrageenan synthesis across different species and the levels of carrageenan in Cladophora and related algae. Most of the previous studies conducted on carrageenan levels have been carried out on rhodophyta species including Eucheuma and Kappaphycus which are known to produce high levels of carrageenan[6, 7]. Red algae have been established as the primary and most efficient source of this polysaccharide by these studies. Our results contradict the well-established view that Cladophora is a green alga that does not produce significant amounts of carrageenan, unlike the current study findings of 1.21% carrageenan in Cladophora [8]. Cladophora, as a green algae species, has not in the past been linked with carrageenan production, which is why our findings go against previous studies. This discrepancy could be due to differences in algal taxonomy, or environmental conditions. Factors including water temperature, salinity, nutrient availability, and light intensity can impact the biochemical profile of algae [6]. Elevated water temperatures in the summer may impact the metabolic processes pathway of algae, which in turn may affect the synthesis and accumulation of polysaccharides as the see in the carrageenan [9-10]. The nutrient status of the Tigris River, which may change depending on the seasonal flow or human activities, may have affected the biochemical makeup of Cladophora [11- 12].

The ecological conditions of the Tigris River are also an important factor to consider. Unlike the marine habitats

where red algae are abundant and produce high amounts of carrageenan, freshwater habitats such as the Tigris have different salinity and water quality parameters [13-14]. This may be inherently limiting for Cladophora to produce upstream levels of carrageenan.

The conditions of the summer may have affected algal growth rate and resource allocation within the cells and therefore the production of secondary metabolites such as carrageenan through increased light intensity [15].

Differences in the methods that utilized in the studies could be another reason for the differences observed in carrageenan levels. One of them is the solvents utilized in the extraction methods. Several studies may employ different solvents, temperatures, or extraction time which can directly effect the yield of carrageenan extraction.AS illustration, those optimized for red algae may not be as efficient for green algae like Cladophora, which could result in underestimation of the carrageenan content [16].

The analytical methods used to determine carrageenan can also influence the results. Some studies may implement advanced methods such as HPLC or spectrophotometry that are more susceptible and specific. Others may use less specific or indirect procedures that may result in differences in reported concentrations [17].

Sample preparation is another important factor to consider. Before treatment methods for instance drying, grinding may vary among studies, and this can affect the amount of carrageenan extracted [17].

This is because this field is not well coordinated and this can lead to lack of uniformity in the protocols utilized in this field. Variability in experimental controls, such as pH, salinity, or temperature during extraction, may result in different outcomes . Some studies may engage a wide range of polysaccharides in their analysis, while others may focus on carrageenan, which may result in different levels of the latter. [18]

Hence, the results of this study indicates that carrageenan synthesis may not be limited to the red algae species that are commonly considered. Carrageenan in algae is an area of attention for research in the biochemical production of carrageenan. Carrageenan is a sulfated polysaccharide detected in red algae especially in their cell walls [19]. The pathways of synthesis of carrageenan are a sequence of enzymatic reactions that introduce sulfate groups to galactose units to form a complex carbohydrate that gives the algal cell wall its flexibility and rigidity [20- 21]. At the molecular aspect, the synthesis of carrageenan encompass the activation of specific genes that encode for the enzymes that are engaged in the sulfation mechanism[22] . These enzymes, known as sulfotransferases, bring sulfate groups to the galactose residues that are then polymerized to form the carrageenan chains [23]. The extent of sulfation and the composition of the carrageenan can be different depending on the species of algae and the ecological factors for instance nutrient availability and water temperature [24].

Carrageenan production in Cladophora and other green algae may be consistent to that in red algae, though the exact biochemical mechanisms may differ slightly according to evolutionary and environmental factors. The expression of sulfotransferase genes in Cladophora that are similar to those found in red algae could enhance the presence of carrageenan in Cladophoran[25] . Moreover, factors namely nutrient availability and water quality may be important for carrageenan synthesis [6]. Case in point, higher nutrient levels in the water can elevate the growth and metabolic rate of algae, and thus the synthesis of carrageenan [6].

The current results also demonstrated that the agar and alginate levels in Cladophora were 1.25% and 1.46% respectively. These outcomes, collectively with the carrageenan content of 1.21%, suggest the possibility of using Cladophora as a source of different polysaccharides, though in lower amounts than the traditional sources. Agar is typically derived from red algae for instance, Gelidium and Gracilaria, while alginate is collected from brown algae as evidence of Macrocystis and Laminaria [26-28] . The results of these compounds in Cladophora, green algae, is a surprising departure from the usual expectation.

The occurrence of agar and alginate in Cladophora indicates that this green algae may have some biochemical diversity, despite the yields being relatively low compared to the conventional sources. Agar, essentially derived from the red algae namely Gelidium and Gracilaria, is an integration of agarose and agaropectin[26]. Agarose is a linear polymer composed of D galactose and 3, 6-anhydro-L-galactose repeating polymers [29]. In Cladophora, the generation of agar could be facilitated through conserved biosynthetic mechanisms that are present in other algal groups, however the low content (1.50%) may be according to lower enzymatic efficiency or a decrease in the metabolic flux towards this polysaccharide [30].

On the other hand, alginate is mainly produced in brown algae such as Macrocystis and Laminaria[31]. It is made of mannuronic acid and guluronic acid residues, and its biosynthesis is highly dependent on the activity of mannuronan C5-epimerase enzymes that convert mannuronic acid into guluronic acid [32]. In Cladophora, the measured alginate content of 1.25% might be due to variations in the enzymatic apparatus involved in this conversion. If these epimerases are absent or not very active, then alginate production may be lower than in brown algae. In context, marine algae are known to have higher concentrations of polysaccharides for instance agar, alginate, and carrageenan unlike freshwater algae [32, 33] [28, 29]. This difference can be associated to a combination of ecological adaptations, biochemical

composition, and environmental factors. Marine algae thrive in saline circumstance where they are subjected to desiccation , osmotic stress, and wave action [34]. To counter these challenges, they produce sulfated polysaccharides like carrageenan and alginate, which help retain water, maintain cell integrity, and offer structural support [35] . On the other hand, freshwater algae, such as those in rivers, encounter less osmotic stress and therefore have a reduced need for such compounds [36] .

According to our knowledge, there is a notable lack of data regarding the concentrations of agar, alginate, and carrageenan in algae within Iraq and the surrounding regions, including countries that share the Tigris River ecosystem. While some studies in nearby nations, namely Turkey and Iran, have focused on marine algae, research on freshwater algae, particularly in the Tigris River, remains limited. This scarcity of information highlights the importance of our study, as its findings may represent one of the first comprehensive efforts in this field. By assessing the polysaccharide content of Cladophora in the Tigris River, our work contributes valuable insights and opens new avenues for understanding the potential of green algae as a source of these commercially important compounds.

V. CONCLUSION

In conclusion, this study contributes to the understanding of *Cladophora's* biochemical potential, particularly within the underexplored Tigris River ecosystem. While the concentrations of carrageenan, agar, and alginate are modest, their detection highlights the versatility and adaptability of this green algae. Furthermore, the discovery of unknown polysaccharides opens promising avenues for future research, aimed at uncovering novel compounds with industrial relevance. These findings underscore the importance of expanding the scope of research on freshwater algae, not only within Iraq but also across comparable ecosystems globally.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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