



The effect of green algae on the growth parameters of *Linum usitatissimum* tissue and on its secondary metabolites content

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This study was undertaken to determine the effect on the growth of flax (*Linum usitatissimum* L.), of enriching the nutrient medium by an extracellular product of algae (*Cladophora glomerata*, *Chara* sp.) The effect of application of algae to Murashige and Skoog medium on plant growth and pigment content contrasted favorably with the effect of the media without algae. We recorded the highest response in seedling growth, which registered 14.8 cm and 14.4 mg, for length and dry weight of shoot, respectively, 16.4 cm for root length and chlorophyll *a* total content at 0.5%, from *Chara* extract. Phenolic compounds quercetin, syringic acid, rutin, ferulic, vanillic acid, *p*-coumaric and gallic acid were measured by high-performance liquid chromatography (HPLC) analysis, the results indicated that the highest content of phenols was found in flax seedling tissues treated by *Chara* 0.5% compared to control (without algae).

Keywords: flax; green algae; growth tissues; *Linum usitatissimum*; secondary metabolites.

Introduction

Algae can grow in a variety of habitats and are found in both fresh and marine environments. They include cyanobacteria and a broad variety of eukaryotic algae, and they have the capacity to grow quickly in photoautotrophic conditions (Cunha et al., 2023). These are the groups of photosynthetic algae that can provide the planet with a variety of food components and oxygen because they control the global climate by lowering the carbon dioxide concentration needed to synthesize sugar and release the most crucial oxygen gas as a byproduct (Wang & Komatsu, 2017). Algae are the sources of bioactive metabolites and are being considered for use in the pharmaceutical industry in drug development. A number of algae contain high-value compounds such as oils, colorants, and polysaccharides, which are used by the food additive, oil, and cosmetic industries, among others (Su et al., 2023). So algae play a vital role in the life of human beings in food, fodder and medicine and biofuels production (Diaz et al., 2023). Green algae are the most abundant chlorophyll *a* containing photosynthetic organism, they are widespread and present in large numbers across the globe. Their abundance makes them a crucial component of many ecosystems, particularly aquatic ones (Koushalya et al., 2021).

Plant tissue culture is described as plant organ or cell culture under artificial conditions for a period of incubation under sterile conditions (Hashim & Ibrahim, 2023). The effectiveness of tissue culture depends on the nature of the media used for its propagation. Specifically the best medium used is that invented by Murashige & Skoog (1962). In contrast, tissue culture companies largely manufacture using regulators and vitamins in addition to the vital elements (Yahya, 2020). Based on this standard, the application of green algae in the agricultural system might resolve some challenges impacting micro propagation. Green algae manufacture various vitamins, antibiotics and other active chemicals such as growth regulators. Among them are gibberellins, auxins, cytokinins, ethylene and abscisic acid (Ibrahim et al., 2021). Hence, extracts of these algae can be used to enhance the composition of tissue culture media. Also, the mixing of co-culturing of green algae with plant tissue would supply energy necessary for growth. It is known that protein production with green algae can change by the amino acid proportion with simple sugars. Algae can be used to produce biofertilizers; they represent food reserves. Among the algae that have been used in biofertilizers are *Acutodesmus dimorphus*, *Spirulina platensis*, *Chlorella vulgaris*, *Oscillatoria angustissi-*

ma, *Scenedesmus dimorphus*, *Anabaena azolla*, and *Nostoc* sp. (Ammar et al., 2022).

Flax (*Linum usitatissimum* L.) or linseed is one of the oldest known crops grown for both oil seeds and fiber, it is of the family Linaceae. This plant is annual and herbaceous, and it possesses a rather shallow root system. The cultivars used dominantly for seed/oil production have comparatively less height, more branch and seed bolls or seed capsule Millam et al. (2005). Flax is one of the economic and food plants because its seeds contain large amounts of oil and protein, and the remains of these seeds are used as good animal feed and an important source of fiber. Flax seeds are rich in unsaturated fatty acids, especially omega-3 fatty acids. Flaxseed oil is the most reliable source of omega-3 acids, reaching 55% of the oil and constitutes 57% of the total fatty acids with preventive effectiveness against breast cancer. These seeds accumulate many compounds and biologically active elements such as linoleic acid, cyclic peptides, polysaccharides, phenolic compounds and cadmium. Flax is used as a remedy for cough, fatigue, aging, inflammation, diabetes, cancers and heart diseases (Arslanoglu & Aytac, 2020).

This study was conducted with the aim of evaluating the biological potential of adding *Cladophora glomerata* and *Chara* sp. extracellular products to enhance flax (*Linum usitatissimum* L.) plant growth and its phenolic compound contents.

Materials and methods

The algae samples (*Cladophora glomerata* and *Chara* sp.) were collected from the Tigris River in the forest area near the Fifth Bridge in Mosul city / Nineveh, Iraq. These types of macro algae were identified based on their morphological characteristics. They were washed with running water many times as well as distilled water to clean them from dust and dirt stuck to them and dried under darkness at room temperature to obtain the dry algal mass for the purpose of use in the current experiments (Yaqub et al., 2022).

We dissolved 4.4 g of the Murashige and Skoog (MS) nutrient medium (Himedia, India MS, powder) in an appropriate volume of distilled water and added 30 g of sugar to complete final volume to a liter of distilled water, adjusted the pH (Sartorius, Germany) to 5.8–6.0 and added 8.0 g of agar (Agar powder, Solarbio, China) to solidify it. After boiling and using a magnetic stirrer, the medium was divided into 25 mL in flasks, coated with aluminum foil and sterilized with

autoclave at 121 °C and 1.02 kg/cm² pressure for 20 minutes Yahya (2020). Green algae extracellular products were isolated by taking 0.5 and 1.0 g of each of the algae types, then added to the prepared MS medium. All samples were transferred to the growth chamber at a temperature of 25 ± 2 °C, light succession 16 h at 2000 lux intensity in the day to induce the direct shoot and root initiation to form flax seedlings.

The seeds of the flax plant (*Linum usitatissimum* L.) were washed with running water for five minutes to get rid of dust and dirt, then were sterilized superficially by immersing them in 96% ethyl alcohol solution for two minutes, then in 3.0% of sodium hypochlorite (NaOCl) solution for 10 minutes (Yahya, 2020 Khuder & Mohammed, 2025), with continuous shaking. The seeds were washed with distilled water three times / minute, then dried on filter paper. The seeds were placed in clusters of four on a surface of 25 mL of solid MS medium and samples incubated in the culture room in dark conditions at 25 ± 2 °C. After germination of the seeds, which took 15 days, all samples were transferred to photodynamic conditions in a room chamber at 16 hours of light at intensity of 1500 lux / 8 hours of darkness.

Two types of algae (*Cladophora glomerata* and *Chara* sp.) at concentrations (0.0%, 0.5% and 1.0%) were added to the medium of flax seeds cultured for studying the effect of algae on the seedlings growth, their shoot and root length and their dry weight after 15 days of growth.

Chlorophyll content of the flax seedlings was estimated according to the method described by Karthik et al. (2020). 100 mg of fresh seedling leaves were weighed and crushed with 10 mL of 80% acetone using a ceramic mortar and placed in a centrifuge for 15 minutes at a speed of 3000 rpm. The filtrate was taken and the absorbance was measured at the wavelengths of 645 and 663 nm by spectrophotometer and chlorophyll content calculated according to the equation of Arnon (1949).

$$\begin{aligned} \text{Chl } a \text{ (mg / g leaf)} &= \\ &= ((12.7) (\text{Abs663}) - (2.69) (\text{Abs645})) / (\text{leaf tissue mg}) \\ \text{Chl } b \text{ (mg / g leaf)} &= \\ &= ((22.9 \text{ Abs645}) - (4.68 \text{ Abs663})) / (\text{leaf tissue mg}) \\ \text{Total Chl} &= 20.2 (\text{Abs645}) + 8.02 (\text{Abs663}) \end{aligned}$$

Table 1
Effect of algae types on the vegetative and root growth of flax after 15 days of growth

Medium components	Concentration of algae added to MS medium, %	Shoot length, cm	Shoot dry weight, mg	Roots length, cm	Roots dry weight, mg
MS alone (control)	0.0	7.0	6.2	6.1	2.2
MS + <i>Chara</i>	0.5	14.8	14.4	16.4	6.0
MS + <i>Chara</i>	1.0	9.8	7.8	7.4	1.0
MS + <i>C. glomerata</i>	0.5	12.8	13.0	13.0	3.6
MS + <i>C. glomerata</i>	1.0	10.2	11.6	12.2	3.4

The results showed the efficacy of *Chara* alge at 0.5% in inducing the maximum value of each growth parameter as length and dry weight of shoot (14.8 cm, 14.4 mg), length and dry weight of roots (16.4 cm and 6 mg) respectively (Fig. 1B) compared to control (Fig. 1A).

These results are consistent with those of Dar et al. (2021), who also observed that the length and number of shoots in higher plants' propagation were affected by, among other factors, the type of culture medium and cultivar. In red algae, extracellular products are suggested to include auxins, cytokines, gibberellins, and jasmonic acid, which are known to promote growth, cell division, elongation, and differentiation. These substances were synergistically combined with growth regulators incorporated into the medium, which enhanced the vegetative traits of direct shoots along with root plant traits in MS medium (Dar et al., 2021).

On the other hand, the low response to direct organic formation, poor rooting, and low numbers of shoot and roots for seedling growth in the Murashige and Skoog alone medium (control) (Fig. 1A), were due to the low content of nitrogen, phosphorus, and potassium in the medium. These results indicate that the extracellular products of cyanobacteria added to the medium were in themselves unable to provide the cultures with the materials required for the various biological processes involved. This deficiency had a negative effect on the vital processes occurring in the cultured tissue cells (Fig. 1C, 1D and 1E).

One gram of flax seedlings shoots (growing on MS medium alone (control) and the other growing on MS medium supplemented with 0.5% of *Chara* sp.) were dissolved in 30 ml of ethanol individually, agitated in ultrasonic bath for 30 minutes. The extract was filtered on Whatman filter paper (0.5 µm) to remove the fibers and undissolved textures. The extract was concentrated by rotary evaporator under vacuum to about 0.5 mL and then the volume was brought to 1.0 mL by mobile phase solvent, then 100 µL of the aqueous filtrate was injected to the HPLC column (Jeyaprakash, 2017).

Quantification of individual phenolic compounds of flax seedlings tissues was determined by reversed phase HPLC analysis, using a SYKAM HPLC chromatographic system equipped with a UV detector, the column was C18-OSD (25 cm, 4.6 mm). The column temperature was 30 °C, the mobile phase was methanol: formic acid in water (80 : 20 v/v) at flow-rate of 1 mL/min. The injected volume of samples was 100 µL and standards was 100 µL and it was done automatically using an auto sampler. The spectra were acquired at 280 nm (Radovanović et al., 2015). The concentration of separated phenolic compounds was calculated according to the following law: amount of separated substance = area of the sample / area of the standard compound x concentration of the standard compound x sample volume / weight of the sample (Harborne & Harborne, 1973).

Results and discussion

The results of the surface sterilization of the flax seed plant with 96% ethyl alcohol solution and NaOCl solutions showed its efficiency in terms of obtaining complete vital seedlings. The sterilization process is of great importance in producing healthy explants (Srivastava et al., 2010). The results also confirmed the role of sterile solutions, their concentration, the time period for sterilization and the balance between them in preserving the vitality of the plant part and getting rid of pollutants at the same time (Khuder & Mohammed, 2025) for the seeds cultured and grown on the MS medium supplemented with the two types of algae.

Results showing the effect of all concentrations used of the two algae types on the shoot and root elongation and production are displayed in Table 1.

The results of the present work were similar to those of previous works conducted on micropropagation of other plants, which indicated the effect of extracts of different algae species on culture media that enhanced vegetative and root traits (Ibrahim et al., 2021). This is consistent with the results of other investigations conducted on several plants grown in laboratory conditions regarding the addition of extracts of extracellular products of algae of different species to nutrient media. This addition was identified by researchers as enhancing vegetative and root characteristics during tissue culture stages by promoting nutrient withdrawal, cell division, organ differentiation, growth during adventitious bud formation, and production of secondary metabolites (Ozyigit et al., 2023). The reason for the greater increase in shoot length, including the extracellular material extract from algae, is due to the reduced level of competition for nutrients in the medium, which are obtained by the few shoots that have elongated and increased their cell division.

Table 2, shows that application of algae extract enhanced chlorophyll content in leaves of flax seedlings in both treatments: 0.5%, 1.0% for *Chara* and *C. glomerata* when compared to the leaf chlorophyll content of seedlings' grown on MS alone (control). The greatest chlorophyll *a* and total chlorophyll content was found at 0.5% of *Chara* extract, 22.46 and 29.18 mg/g, while the greatest increase for chlorophyll *b* was recorded at 0.5% and 1.0% from *C. glomerata*.



Fig. 1. Effect of algae on flax seedlings after 15 days of growth on: A – MS alone (control), B – 0.5% *Chara*, C – 1.0% *Chara*, D – 0.5% *C. glomerata*, E – 1.0% *C. glomerata*

Table 2
Effect of algae types on chlorophyll content of flax seedling leaves after 15 days of growth

MS medium supplemented with	Chlorophyll <i>a</i> content, mg/g	Chlorophyll <i>b</i> content, mg/g	Total chlorophyll content, mg/g
Control	10.22	2.10	12.32
0.5% <i>Chara</i>	22.46	6.72	29.18
1.0% <i>Chara</i>	18.36	2.15	20.50
0.5% <i>C. glomerata</i>	18.90	6.81	25.71
1.0% <i>C. glomerata</i>	14.53	8.18	22.70

The concentration of pigments in the plant is one of the major quality factors that goes a long way in influencing the consumer's tendency to choose the product. The chlorophyll and carotenoid content depends on the physiological, biochemical and genetic characteristics of the plant, environmental conditions, light, temperature and fertilization (Barickman et al., 2016; Sultan et al., 2024). The use of algae improved the pigments of flax and in this regard, there was a steady increase in chlorophyll *a*, chlorophyll *b* and total content respectively. The results of this analysis are in agreement with other studies on the application of biostimulants. Dineshkumar et al. (2020) and Patel

Table 3
HPLC analysis of phenolic compounds in the extracts of flax seedlings growing on MS medium alone

Compound	Ret. time, min	Area, mAU/s	Height, mAU	Area, %	Height, %	W 05, min
Quercetin	3.08	2365.90	398.09	10.00	10.00	0.02
Syringic acid	3.85	3011.50	507.44	13.00	13.00	0.03
Rutin	4.48	6112.05	740.14	17.00	17.00	0.05
Ferulic acid	5.41	6055.91	731.65	17.00	17.00	0.05
Vanillic acid	7.97	2124.11	391.22	10.00	10.00	0.03
P-coumaric acid	9.94	3895.08	560.32	13.00	13.00	0.03
Gallic acid	10.31	7211.55	1068.70	20.00	20.00	0.08
Total		30776.49	4414.19	100.00	100.00	–

Retention time for phenolic compounds (quercetin, syringic acid, rutin, ferulic, vanillic acid, p-coumaric and gallic acid) separated from flax seedlings extract treated with *Chara* algae was near to the retention time of standard compounds explained above, recording 3.00, 3.89, 4.40, 5.45, 7.99, 9.90 and 10.33 minutes, respectively, as shown in Figure 4. It was noted in Table 4 that supplementing MS medium with 0.5% of *Chara* algae raised the concentration of each phenolic compound in flax seedlings (quercetin, syringic acid, rutin, ferulic, vanillic acid, p-coumaric and gallic acid) compared to the phenolic compounds content of flax seedlings grown on MS without algae (control). The highest concentration reached 24.87 ppm for the gallic acid compound. Since green algae are able to synthesize many chemicals and by-products of cell metabolism necessary for growth through diffusion and aggregation, these substances are very useful in the process of cell division, cell growth, and organ development.

Phenolic acids are a group of secondary metabolic substances derived from the phenylpropanoid pathway, and they are among the most widespread and important compounds because of their major role in environmental processes in many ecological communities (Jasim et al., 2023; Mahdi & Yahya, 2024), through their effect on the activi-

ty of decomposers (soil microorganisms), and on the rate of decomposition of organic matter, as well as their effect on the pH of the soil, the increase in the solubility of nutrients, and the aggregation and cohesion of the soil's particles (Đurđević et al., 2009; Al-Tai & Mohamed, 2024).

HPLC results showed absorption curves for the standard compounds quercetin and syringic acid with a retention time of 3.08 and 3.88 minutes, respectively Fig. 2A and 2B. While the two standard samples of the compounds rutin and ferulic acid were separated, each of them gave one absorption curve with a retention time of 4.47 and 5.47 minutes, respectively, Fig. 2C and 2D. However the standard sample of the compound vanillic acid and p-coumaric acid gave one curve with a retention time of 7.90 and 9.40 minutes (Fig. 2E and 2F). Also, the results referring to the presence of the compound gallic acid in Figure 2G indicate the absorption peak with a retention time of 10.22 minutes.

The results of HPLC measurements of the ethanolic extract of flax seedlings' tissue (control) not treated with algae showed the absorption peaks for each of the phenolic compounds (quercetin, syringic acid, rutin, ferulic, vanillic acid, p-coumaric and gallic) with a retention time of 3.08, 3.85, 4.48, 5.41, 7.97, 9.94, and 10.31 minutes, respectively, which is near to the retention time of the standard compounds in Figure 3 and Table 3.

Table 4
Concentration of identified phenolic compounds (ppm) in ethanol extracts of flax seedlings growth on MS medium supplemented with 0.5% of *Chara* algae

Types of phenolic compounds	Phenolic compounds concentration (ppm) in seedlings on MS	
	alone (control)	with <i>Chara</i> 0.5%
Quercetin	7.24	7.78
Syringic acid	12.13	12.95
Rutin	19.24	20.54
Ferulic acid	14.90	15.29
Vanillic acid	5.65	6.41
P-coumaric	14.21	15.15
Gallic acid	22.72	24.87

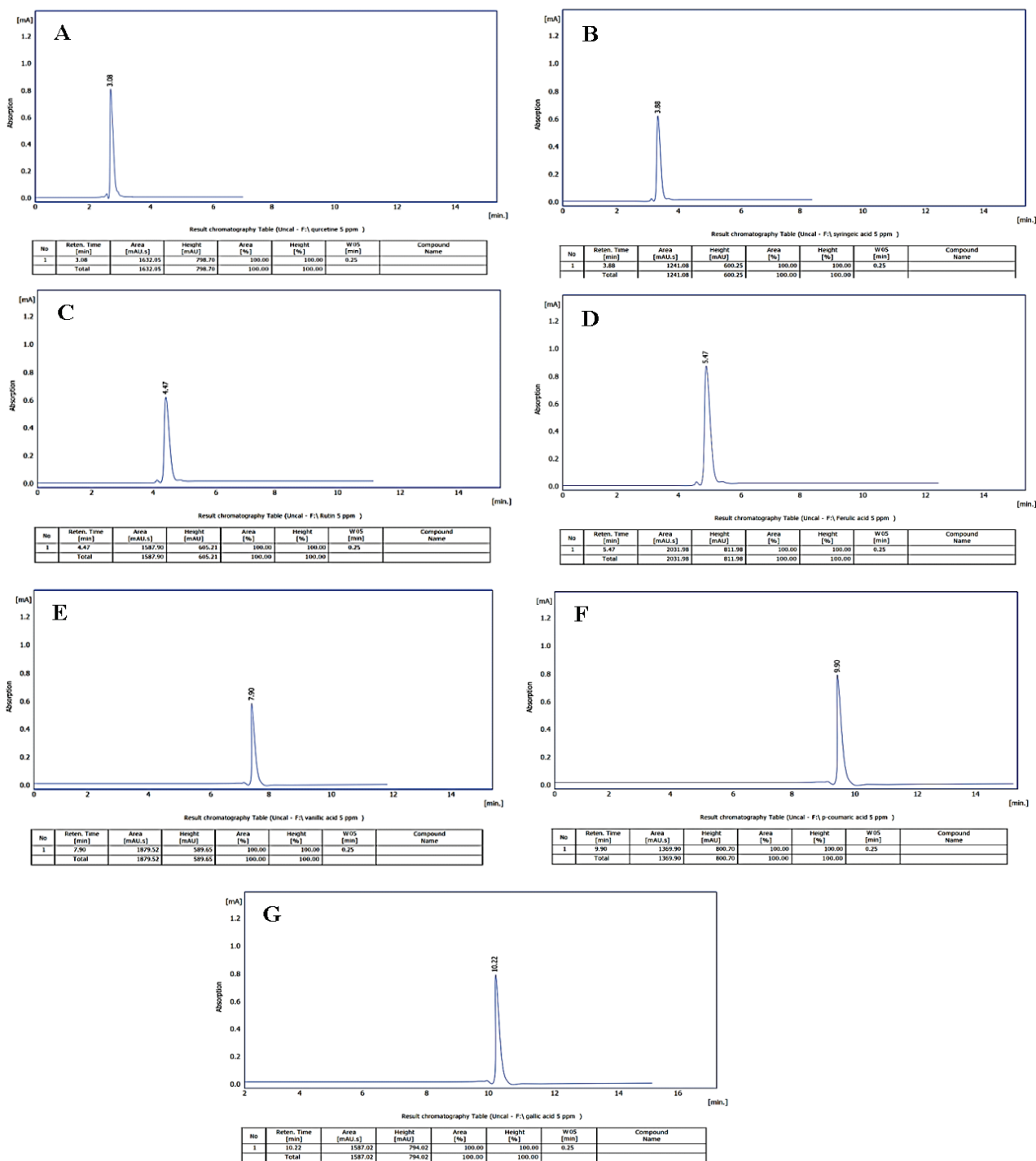


Fig. 2. HPLC curves for the standard phenolic compounds:
A – quercetin, *B* – syringic acid, *C* – rutin, *D* – ferulic acid, *E* – vanillic acid, *F* – P-coumaric acid, *G* – gallic acid

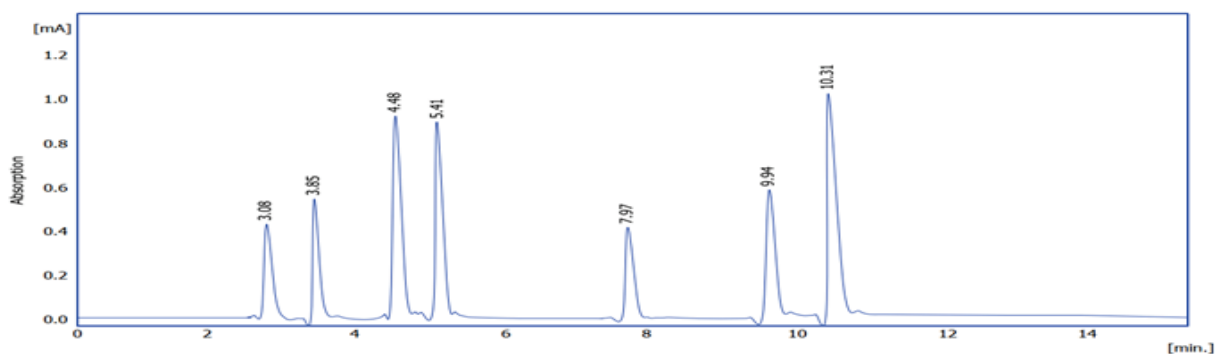


Fig. 3. HPLC curves of phenolic compounds in flax seedlings extract (control)

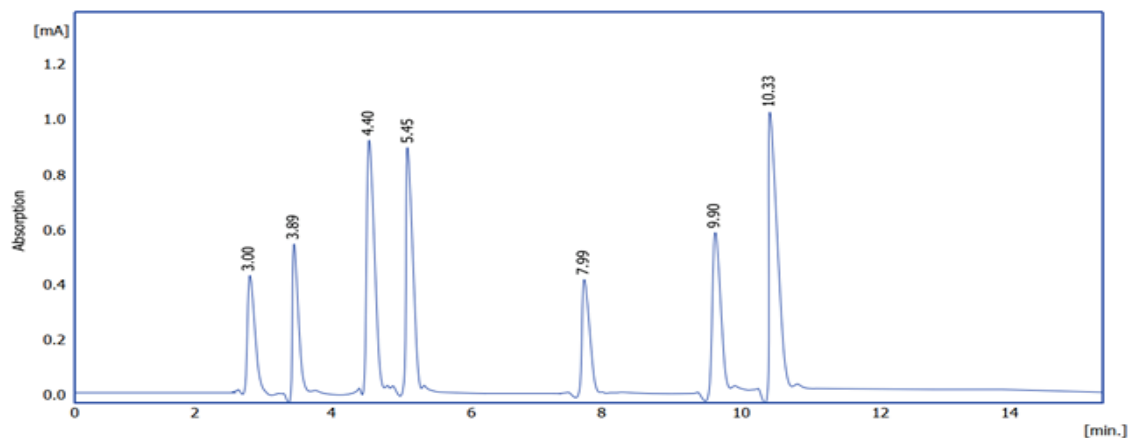


Fig. 4. HPLC analysis of phenolic compounds in the extracts of flax seedlings grown on MS medium supplemented with *Chara* 0.5%

Flavonoids act as signaling molecules for root endosymbiosis, their mechanism of phenolic compound action is through their effect on membrane permeability, protein production, photosynthesis, respiration, enzyme activity, and the mechanism of hormone regulation (Babenko et al., 2019, Abdel-Latif et al., 2018). This is reflected in the improvement of plant growth, as indicated in the presentation of the current results of the growth traits of flax plants under the influence of the algae *C. glomerata* and *Chara* sp.

Conclusion

In this current study, we consider that being environmentally sensitive is an approach that needs to be followed to meet the quantity and quality needs of foods. It further makes it possible to meet our dietary needs and it is environmentally friendly, with no pollution. The utilization of algae comprises of the utilization of a biofertilizer. The augmentation of the nutrient content of the media, the addition of an extract of green algae extracellular products (bioactive compounds, growth regulators, and nutrients) to the MS medium was important in enriching the media. This was reflected positively in stimulating the growth of flax plants and their phenolic compound content. From our perspective, we consider that there is need to conduct more research including characterization of growth hormones and other metabolites. We can also establish the influence of algae extract on the nitrogen metabolism in the roots and leaves of flax plants cultivated in medium culture richened by mineral elements.

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The authors declare that they have no financial interest.

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