Detection of Bioactive Compound from Microscopic Blue-Green Algae *Gloeocapsopsis Crepidinum* under the Effect of Different Light Intensities

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ABSTRACT

Bioactive compounds are a component of secondary metabolism found in blue-green algae, green and red algae Rhodophyta and many other algae, which are used in many medical, industrial and agricultural applications. In general, the production of active compounds by blue-green algae depends on many factors of environmental conditions, especially light during the cultivation period. This study aimed to demonstrate the production of vitamins, proteins, phenols and other vital substances under the effect of different light intensities on the growth rate and maintaining optimal lighting by the blue-green algae Gloeocapsopsis crepidinum. the algae G. crepidinum was studied, under the effect of two different light intensities from a light-emitting diode, 50 and 70 micro mols, Photons/m2/s which were used in the laboratory. The study recorded the growth rate per day at a light intensity of 50 micromol. Photon/m2/s, and growth rate at a light intensity of 70 micromoles. photons/m2/s and the optimum illumination was set at 50 micromol. Photon/m2/s to detect the production of vitamins, proteins, phenols and other vital substances in the studied algae. the production of vitamins, proteins, phenols and other vital substances in the algae G. crepidinum recorded high concentrations after fixing the light at an average light intensity of 50 micromols. photons/m2/s, as vitamin (C) concentration, reached 1.77 milligrams/gram, vitamin (E) concentration reached 2.404 milligrams/gram, proteins reached 29%, phenols concentration reached 3.55 milligrams/gram, tannins recorded a value of 0.72 mg/g, and alkaloids recorded a value of 0.1002 mg/s. grams, while flavinoids reached 0.42 milligrams/gram, sugars reached 21.5%, and Fats were 24 mg/g, while the concentration of carotenoids was 0.87 µg/g. These results give a good impression that the production of vitamins, proteins, phenols and other vital substances from the algae G. crepidinum under moderate lighting conditions increases the growth rate and production of active bioactive compounds, but high light intensity prevents it. The results of the statistical analysis also showed that there were statistically significant differences between the light intensities used in the study at the $p < 0.05$ level.

Keywords: vitamins, proteins, phenols, blue-green algae, Gloeocapsopsis crepidinum.

INTRODUCTION

Algae are autotrophic organisms that carry out photosynthesis because they possess the pigment chlorophyll, so they are similar to plants in terms of photosynthesis. As for microscopic algae, they are unicellular or multicellular organisms that use light and carbon dioxide to grow and produce biomass. Some species can grow and produce biomass heterogeneously using an organic carbon source under dark conditions [1]. Microalgae can grow in a variety of environments, and they can grow in a wide range of environmental conditions and vary in size and shape [2].

Blue-green algae are affected by many physical and chemical factors that affect the production of biomass, one of these factors is the intensity of light. At gradually higher light intensities, some blue-green algae can produce a high amount of active bioactive compounds such as proteins, fats, and pigments [3][4].

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Microalgae have different applications in many fields, as photosynthetic organisms, they contain chlorophyll which can be used for food and cosmetic purposes [5].In natural applications as antibiotics and antioxidants, as well as in food applications as food supplements because they contain protein pigments such as phycocyanin, phycoerythrin, and allophycocyanin, high-value products can be produced by microalgae, such as tannins, proteins, lipids, vitamins, carotenoids, astaxanthin, antioxidants, and long-chain unsaturated fatty acids docosahexaenoic acid (DHA).) and eicosapentaenoic acid (EPA) and arachidonic acid (AA) that can Used as food supplements for human nutrition and as a feed ingredient in animal and fish feed, another application for microalgae is found in the food industry, where they are used as food dyes in candy, chewing gum or drinks. In addition, biodiesel can be produced from their fat due to their advantages over plants. Terrestrial, including a high growth rate, and the ability to grow on infertile lands all year round, as well as in salt water or coastal seawater. It should be noted that for biodiesel production, microalgae species must meet some basic requirements, such as high lipid content and biomass production [2][5].

MATERIAL & METHOD

Isolation and identification of G. crepidinum

A pure isolate of the alga G. crepidinum was obtained from the Environment and Algae Laboratory at the College of Education, Al-Qadisiyah University, and was genetically diagnosed based on Alubaidy, (2023) [2]. The Agar method was used to purify the algae and to ensure that the culture was free of bacteria and fungi, and a diagnosis of G. crepidinum algae was made. crepidinum by compound light microscopy and based on taxonomic keys in [6] [7].

Preparation and sterilization of BG-11 Medium

Preparation and sterilization of BG-11 Medium components which were described by [2]، to develop and propagate the algae *G. Crepidinum* (Figure 1).

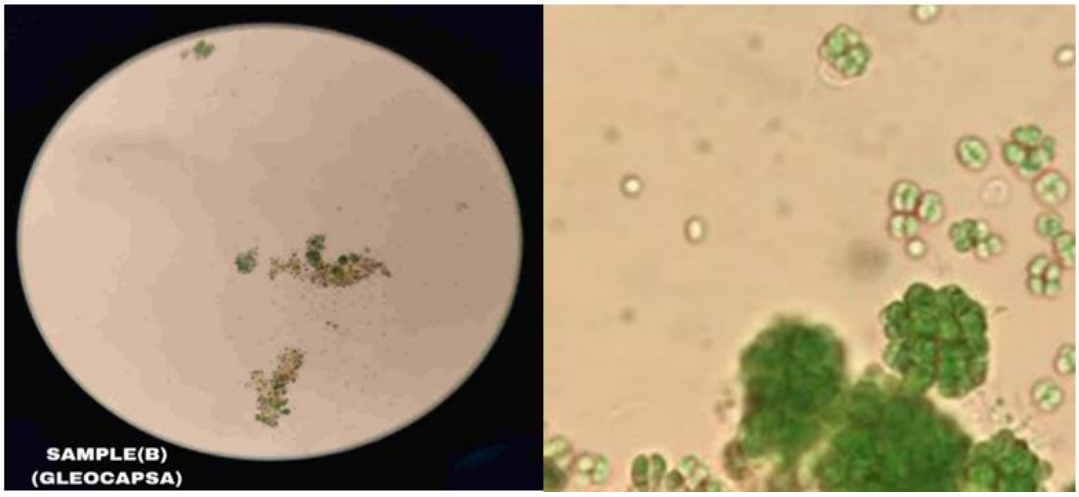

Figure 1. represented *Gloeocapsopsis crepidinum* under the microscope.

Algal Cultivation and Biomass Production:

After the process of diagnosing the algae under study and analyzing it using a spectrophotometer device to determine that it contains active biological compounds, it was cultivated to produce biomass and complete the study experiments, by growing it with a batch culture system, 10 ml was taken. The pure algal isolate was added to 400 ml of liquid culture media in 500 ml of glass flasks, then incubated in the Algae Growth Chamber incubator at 1°C, under the influence of an LED Light-Emitting Diode, and the influence of different levels of light intensity, which amounted to 50 and 70 micro mols. Photon/m2/s according to a light system of 8:16 hours light: dark. The cultures were shaken at least twice a day to prevent algae from gathering on the walls to reduce the light difference and obtain the desired growth [8][9].

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Estimation of the growth curve of algae by chlorophyll method:

To estimate the growth curve of the blue-green alga *G. crepidinum*, the Chlorophyll A. Measurement method [10][11]. where samples were collected daily by taking 5 ml from the culture and using the washing method with a spectrophotometer centrifuge at a speed of 5000 rpm for 5 minutes, after which the liquid was removed, while the algal cell sediment was taken and washed with sterile distilled water, and this was repeated The entire process consists of sedimentation and washing several times in a row. The clear solution is then removed and the sediment representing the algae is taken then 5 ml of acetone at a concentration of 90% is added to it, v/v to extract the dye, then mix it vortexed with an ultrasound device for 90 seconds, then place the sample in a shaking water bath at a temperature of 25°C for an hour and then cool. If the precipitate, i.e. the moss, does not turn white, the process is repeated and then centrifuged with a device. Centrifuge at a speed of 6000 rpm for 10 minutes and only the filtrate is taken. After that, the optical density of the filtrate (sample) is measured at the wavelength 664-720 nm using a spectrometer and the concentration of chlorophyll A is calculated according to the equation: Chl a [µg/L] = 12.9447 (A664 − A720).

The effect of different optical stresses on bioactive compounds production:

the blue-green algae *G. crepidinum* were grown on BG-11 medium under the influence of a light-emitting diode (LED) lamp and natural light at two levels of light intensity, 50 and 60 micromol. photons/m2/s, and incubated in a growth incubator. Photosynthesis under standard growth conditions with a lighting system of 8:16, lighting: darkness, and a temperature of 2 ± 25°C, with three replicates for each light intensity, while continuing to shake daily. It was left until the numerical stability phase was reached. Samples were collected and the percentage of biological compounds was measured with an atomic absorption spectrophotometer in the manner mentioned previously [12].

Detecting active biological compounds using a spectrophotometer:

Estimation of total tannin content

Tannin contents were determined by the Broadhurst method [13] [14]. using catechin as a standard compound. A volume of 400 µl was taken from the extract and added to 3 ml of a solution of vanillin (4%), in methanol and 1.5 ml of concentrated hydrochloric acid, after 15 minutes of incubation absorbance was read at 500 nm, total tannin content was determined from extrapolation of the calibration curve prepared using catechin solution. Tannin was expressed as milligrams of catechin equivalents/g of the dried sample (Figure 2).

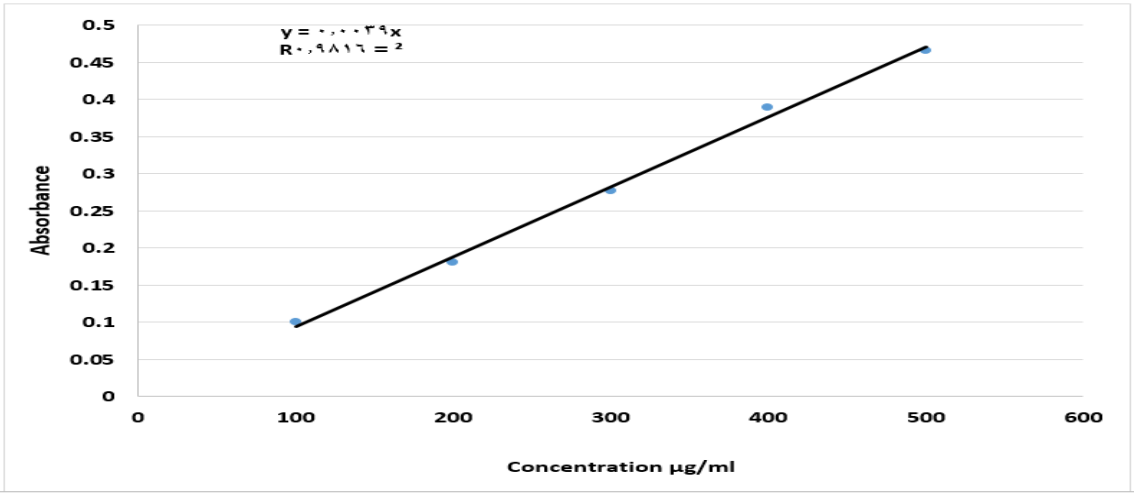

Figure 2. Calibration curve for determining tannin content

Determination of total alkaloids

To prepare the total alkaloids, three compounds must be prepared: (bromocresol green solution (1x10-4), the second is the phosphate buffer solution (pH 4.7), and the third is the standard atropine solution, with the

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following formula: Take 69.8 mg of bromocresol green with 3 ml of 2 M NaOH and 5 ml of distilled water until completely dissolved and dilute to a litre with deionized distilled water, We adjusted the pH of 2 M sodium phosphate (71.6 g Na2HPO4 in 1 litre of distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 litre of distilled water)،To prepare a standard atropine solution, we dissolve 1 mg of pure atropine (Sigma Chemical Company) in 10 ml of distilled water، The method of making these three solutions above is: We take (0.2, 0.4, 0.6, 0.8 and 1 ml) of standard atropine solution carefully into sterile glass tubes. Add 5 ml of phosphate buffer and 5 ml of BCG solution. Then we add 4 ml of chloroform gradually, with continuous stirring. We take 1 ml of the plant extract in sterile glass tubes and add 5 ml of phosphate buffer and 5 ml of BCG solution. Then we add 4 ml of chloroform gradually with continuous stirring, then the portion in the chloroform layer is read at 470nm [10] [15].

Extraction of active biological compounds

The method used by Umoren [16]،was adopted, by taking 0.2 g of blue-green algae powder *G. crepidinum* and adding 10 ml of 80% ethanol (vol/vol), placed in a 100 ml glass beaker and then placed In a shaking water bath at a temperature of 32°C at a speed of 180 rpm for an hour.

Determination of total phenolic content:

Determination of the total phenolic content of dry extracts of the blue-green alga *G. crepidinum* using the Folin-Ciocalteu reagent. 1 mL of sample (1 mg/mL) was mixed with 1 mL of phenolfolin-ciocalteu reagent. After 5 minutes, add 10 ml of 7% sodium carbonate solution to the mixture, followed by the addition of 13 ml of deionized distilled water. Mix the solution well. The mixture was kept in the dark for 90 min at 23 °C, after which the absorbance was read at 760 nm. The total phenolic content was determined by extrapolation of the calibration curve prepared using gallic acid solution. TPC was expressed as milligrams of gallic acid equivalents/g of dried sample) Figure 3 [17].

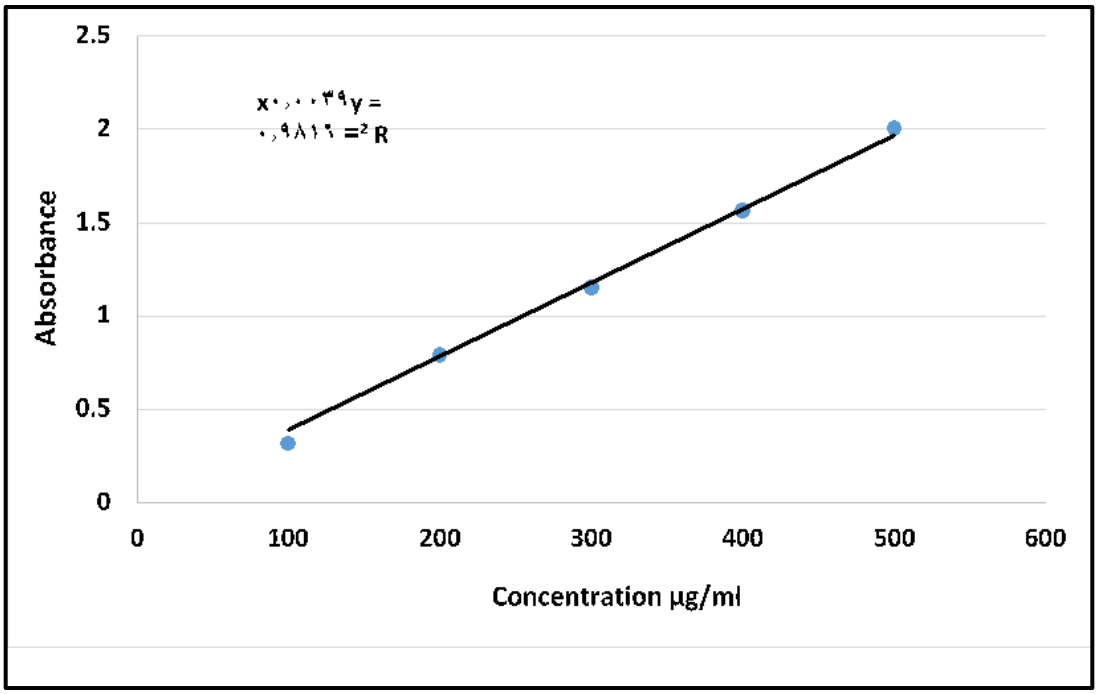

Figure 3. Estimation of total phenolic content

Protein estimation: Protein assay

The method described by [18] ،was used to determine the protein concentration in the crude and purified extract. Determination of protein concentration (Figure4).

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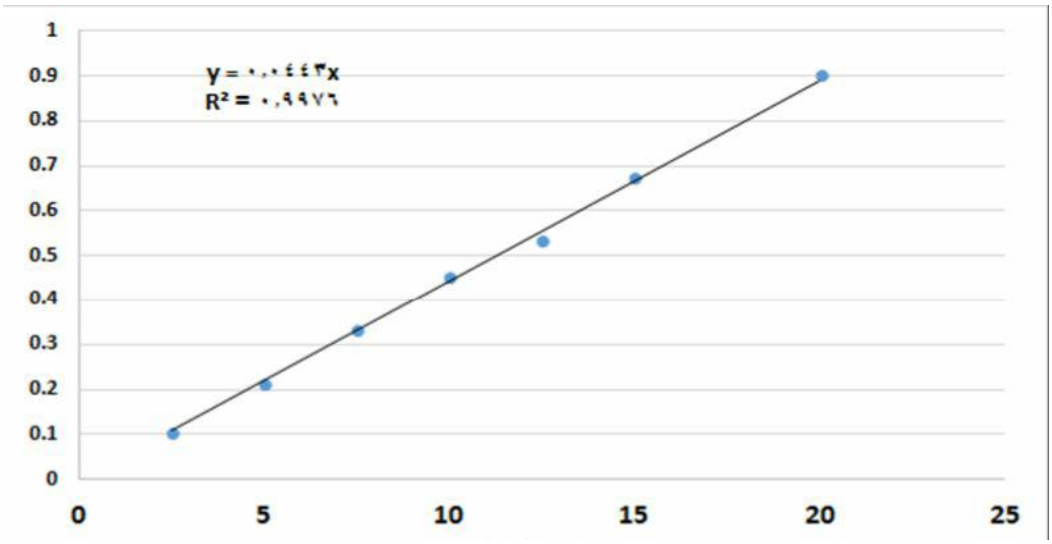

Figure 4. Standard curve for proteins

Estimating the total amount of sugars (carbohydrates):

Among the many colourimetric methods for carbohydrate analysis, the sulfuric acid-phenol method is the easiest and most reliable. The method was used to measure total sugars. The method is widely used because of its sensitivity and simplicity،The working method is by adding 50 microliters of (standard sugar or milk sample) were added to a clean and sterilized microplate, then 150 microliters of concentrated sulfuric acid was added slowly and gradually, then immediately 100 microliters of 5% phenol was added. The microplate was heated for 5 minutes at 90°C. It was then cooled to room temperature for 5 minutes, and the absorbance was measured in a microplate reader at a wavelength of A490 nm, as in the equation below: [18].

$$\text{Total sugar concentration} = \frac{\text{A.test}}{\text{A.STD}} * Conc\,of \quad STD$$

Estimating the total amount of vitamin C (ascorbic acid)

The 2,4-dinitrophenylhydrazine (DNP) method is widely used for the determination of total ascorbic acid in biological fluids. Ascorbic acid is oxidized by the cupric ion to form dehydroascorbic acid and then to 3-diketo-gulonic acid, which reacts with 2,4-dinitrophenylhydrazine to form red dihydrazone, which is measured at a wavelength of 520 nm (Figure 5) [20] [21].

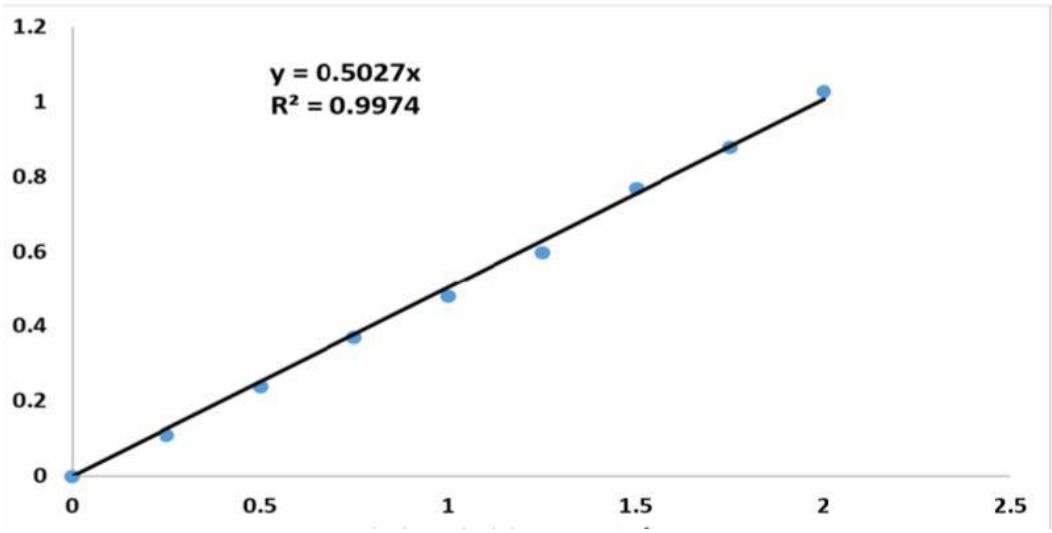

Figure 5. Standard curve for Vitamin C.

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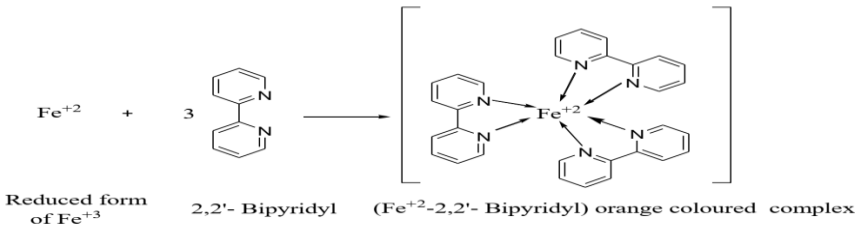
Estimation of vitamin E Vitamin E (vitamin E)

was evaluated by the method of [22]. involving an Emmerie-Engel colorimetric reaction with ferric chloride and dipridyl to give the red color, as in the equation below:



Total fat estimation

According to the method used by [23] ،using the reagents concentrated hydrochloric acid, ethyl alcohol 96%, diethyl ether, and petroleum ether reagent (light petroleum ether), at a boiling point of 40-60 degrees Celsius and using the following equation:

$$\text{Fat, \% (w/w)} = \frac{100\ (W_1 - W_2)}{W_3}$$

Estimation of carotenoids

The extract was measured at 480, 645, 663 nm, in a spectrophotometer to estimate the percentage of carotene as in the equation below: [19].

$$\text{Carotenoid (mg g}^{-1}) = \frac{(A_{480} + 0.114A_{663}) - 0.638\ A_{645}}{a \times 1000 \times w} \times V$$

Chlorophyll

Weigh 1 gram of each section and mix well with 10 ml of 80% acetone in a glass test tube and leave it in the refrigerator for twenty hours at a temperature of 4 degrees Celsius in the dark, then shake it again and also leave it for a period ranging between 1-2 hours in the same conditions, after which it is quickly centrifuged. 3000 revolutions per minute for ten minutes, then re-acidify the filtrate by adding 1-2 drops of hydrochloric acid (0.1 N). The filtrate is taken to measure the absorbance using a spectrophotometer at wavelengths of 663 and 645 nm [24]. The chlorophyll concentration is calculated from the equations below:

$$\text{Chlorophyll a (mg g}^{-1}) = \frac{12.7\ A_{663} - 2.69\ A_{645}}{a \times 1000 \times w} \times V$$

$$\text{Chlorophyll b (mg g}^{-1}) = \frac{22.9\ A_{645} - 4.68\ A_{663}}{a \times 1000 \times w} \times V$$

$$\text{Total chlorophyll (mg g}^{-1}) = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times w} \times V$$

Where

a = length of light path in the cell (1 cm)

v = volume of the extract in ml and

w = fresh weight of sample in gram

Statistical Analysis

Through the Statistical Package for the Human Sciences (SPSS), the results of the study were analyzed statistically using Two Way ANOVA, with the value of Least Significant Difference (LSD) calculated for

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comparing the averages of the coefficients included in the study. Significant differences were determined at the 5% probability level ($P < 0.05$) [25].

RESULTS AND DISCUSSION

A wide range of bioactive compounds are found in blue-green algae, which is used in many countries as a dietary supplement. Algal pigments have enormous commercial value as natural colourants in the nutraceuticals, cosmetics and pharmaceutical industries, in addition to their health benefits [12]. One optimal condition is the light factor. Since photovoltaic cell factories are driven by sunlight, microalgae provide an efficient means of converting solar energy into biomass, producing a wide range of bioproducts, including sugars, lipids, pigments, proteins, vitamins, bioactive compounds and antioxidants [26]. The estimated growth curve of chlorophyll concentration (Figure 3) showed good growth of G. crepidinum under light intensity of (50, 70) µmol.photons.m2/s, and the highest growth was recorded at 50 µmol.photons.m2/s. The results of the production of active bioactive compounds by G. crepidinum are shown in Table (1) and Figure (6), if a high growth of the blue-green algae was observed, which reached the highest production of 11.94 ng/mg on day 8 at the average light intensity of 52 µmol. photons.m2/ It was in the stationary phase until growth decreased at the beginning of the death phase, which was on day 15 and continued until the death of the moss. The increase in growth is due to the presence of several types of spatial and temporal interactions between environmental factors that affect the growth rate. And behaviour, as well as the characteristics and activities of other living organisms [27].

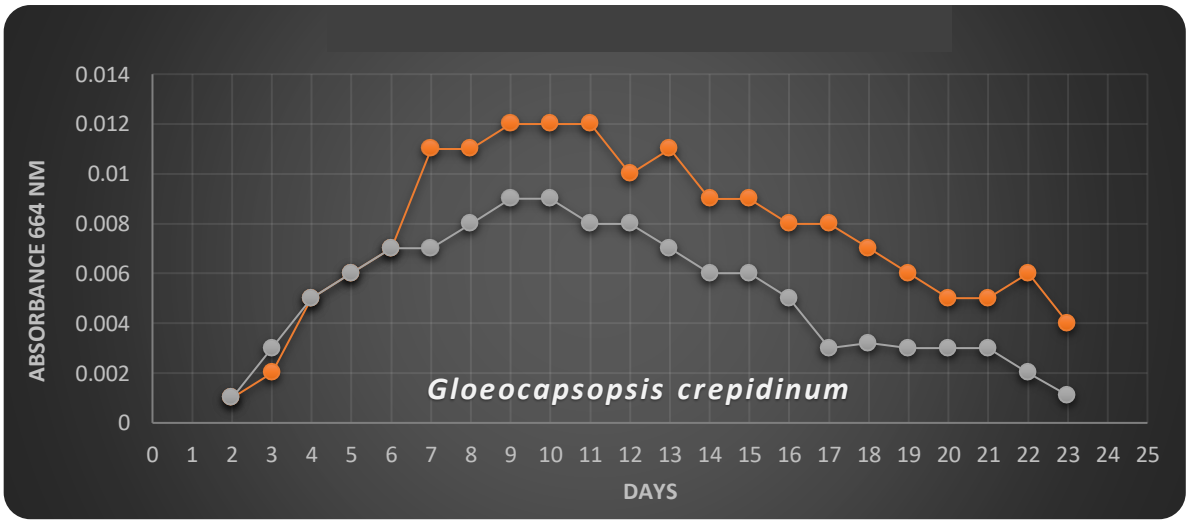

Figure 6. Orange Colour Represented illumination 50 While grey Colour illumination 70 for the Growth Curve of *G. crepidinum* as a Function of Absorbance

Through the results of the current study, as shown in Table (1) and Figure (7), and after setting the lighting intensity at 50 µmol. photon/m2/s as the best lighting for growth, an increase in the production of active biological compounds was observed, as tannins reached 0.72 mg/g, and alkaloids. 100.2 micrograms/mg, flavinoids 0.42 mg/gram, phenols 3.55 mg/gram, the percentage of proteins reached 29% and sugars 21.5%, the percentage of vitamin (C) reached 1770 micrograms/mg and vitamin (E) 2.404 micrograms/mg, as the percentage of vitamins also increased. Fats reached 24 micrograms/mg, where the percentage of fats rose to 24 mg/gram, chlorophyll 1.1 mg/gram, and carotenoids amounted to about 0.87 micrograms/mg.

Table 1. informs the amounts of bioactive compounds

Active compounds	amount	measurement unit
Tannins	0.72	mg. g^{-1}
Alkaloids	100.2	microg. g^{-1}

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Flavinoids	0.42	mg. g^{-1}
Phenols	3.55	mg. g^{-1}
Proteins	29	%
Polysaccharid	21.5	%
Vitamin C	1770	microg. g^{-1}
Vitamin E	2.404	mg. g^{-1}
Fats	24	mg. g^{-1}
chlorophyll	1.1	mg. g^{-1}
Carotenoid	0.87	mg. g^{-1}

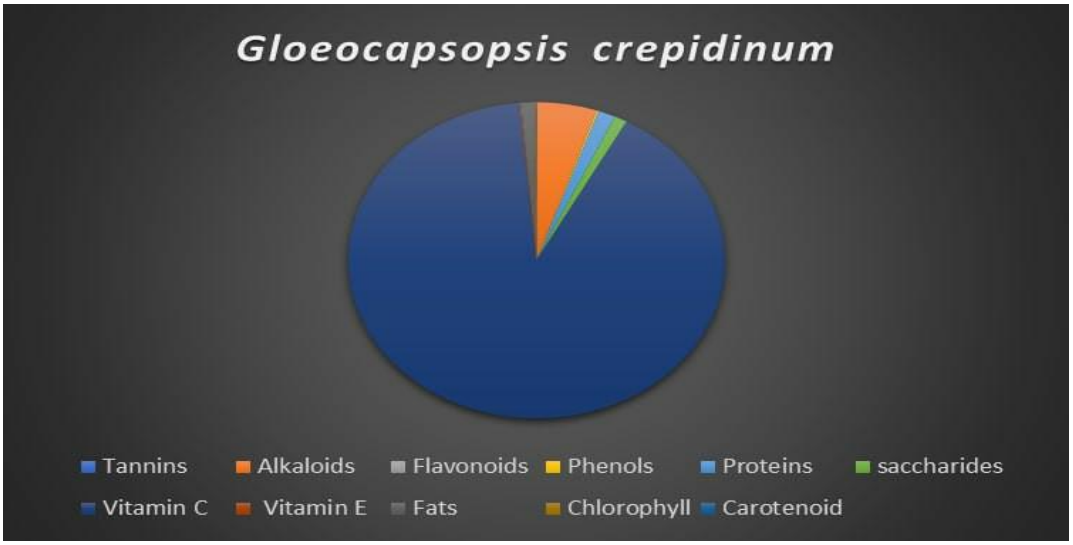

Figure 7. Indicates the distribution of values of bioactive compounds produced from *G. crepidinum*

Many recent studies have noted that the reasons for the variation in the rise and fall in the concentrations or values of active biological compounds may be due to many reasons related to the physiological state of the algae and the nature of the nutrients (nitrates and phosphates), including the ability of the algae to absorb light from building pigments. Photosynthesis and the accumulation of compounds, and the reason may be due to the nature of the growth phase and the active state of the alga [2].

Referring to the results of Table (3), in the case study, an increase in the percentage of proteins and a decrease in tannins was observed, other research [11]، noted that the increase in the production of soluble proteins is one of the main mechanisms that reduce the percentages of tannins and reduce growth in the alga M. aeruginosa.

An increase in flavonoids was observed in algae, and the concentration of flavonoids can be increased through various manipulation or enhancement techniques. For example, increasing the concentration of nutrients as salt has a significant impact on the accumulation of phenols and flavonoids, as a decrease in the growth of the cyanobacteria species *Plectonema boryanum, Anabaena doliolum, and Oscillatoria acuta* was observed when Treated with a high concentration of NaCl but increased accumulation of rutin, mineral stress can also aid in the accumulation of flavonoids. Accumulation of flavonoids has also been reported, e.g. Quercetin and catechin, in *Chlorella vulgaris* under nitrate stress conditions with L-phenylalanine supplementation. High flavonoid concentration was also observed through nitrate depletion in the medium, which led to the upregulation of flavonoid synthesis in *Leptolyngbya sp.* Such as *catechin) apigenin, naringenin, luteolin* and luteolin-7-

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glucoside were the most common flavonoids among them, *Naringenin* was the highest [28] [29] and this was confirmed by the results of the current study. Phenolic compounds consist of a structural core based on a hydroxyl group directly attached to the phenol, which gives them the ability to capture free radicals, reactive oxygen species, and chelated metal ions [30]. They are synthesized in nature in response to stimuli Harmful environmental effects such as ultraviolet radiation, attacks by pathogens, and due to the lack of factors that lead to an increase in phenols, their percentage is low [31].

Blue-green algae showed a response to moderate light intensity, which led to an increase in tannins, alkaloids, flavonoids, phenols, and carotenoids, a decrease in chlorophyll, and an increase in other biologically active compounds, as it showed this in the stage of its active growth and production of some compounds such as carbohydrates, proteins, and fats, where productivity increases in quantity and composition permanently in stages Growth in the presence of appropriate environmental factors, as microalgae can store a large amount of protein, vitamins and fats in the exponential growth phase and the phase with the availability of light. active [32]. Many studies have observed that fat production increases by 75% of neutral fats among the total fats even under conditions of nitrogen deficiency (20, 23,). Nitrogen-deficient conditions could improve microalgal lipid biosynthesis by influencing other biochemical pathways [31]. Carotenoids are associated with the photosynthetic apparatus of microalgae and provide absorption of light energy by carotenoids. Cells often produce secondary carotenoids in response to certain environmental conditions, such as strong light. They accumulate in the peripheral cytoplasm or plastid stroma [the carotenoid property of the photosynthetic apparatus of algae can accumulate, for example, Carotene, and lutein, as secondary carotenoids. The most widely known secondary carotenoid is astaxanthin, the end product of secondary carotenoid biosynthesis in several microalgae. The production of carotenoids, including β-carotene, astaxanthin, and lutein, is also enhanced by medium illumination in Other studies include nutrients (nitrogen limitation) such as the algae (*Chlorella zofingiensis*, *Dunaliella salina*, and *Neochloris oleoabundans*) [33]. On the other hand, when low levels of nitrogen reduce competition for carbon, the synthesis of both carotenoids and proteins increases, because only the latter requires nitrogen. Another possible reason may be the degradation of nitrogenous compounds such as chlorophylls and proteins [34].

The results of the current study showed that the polysaccharide compound recorded a high concentration, as the composition of the polysaccharides depends on the microalgae strain and cultivation conditions, such as moderate light intensity, abundance of nutrients (N-P), salinity, radiation, and temperature. Nutritional improvement was used to increase the production of exogenous sugars by algae. Microflora and cyanobacteria by (Botryococcus braunii UC58) are grown under different sources of nitrogen (nitrate and ammonium) and a moderate light intensity of 50 µmol.Nm2/s, as many researchers found that high concentrations of these compounds created the appropriate conditions that led to improvement in the production of sugars [33]. Many environmental factors affect the high and low content of vitamins in algae. An AP study noted an increase in vitamin A during...

Summer and decreases during winter. Despite the lack of seasonal variation in the content of vitamins in microalgae, their modification by environmental changes has been verified in various studies, and the increase in (C) is also attributed to the active growth phase in the algae [35]. From the results of Table (3), the results of the chlorophyll pigment showed a relative variation, as measuring the concentration of chlorophyll A in the water provides an estimate of the algal biomass, as the production of algae depends on the amount of chlorophyll present in a body of water on the water temperature, nutrient content, and sunlight, and the result of suitable conditions is There is an increase in the production of chlorophyll, which is in the environment where the ratio is best [9].

CONCLUSIONS

Bioactive compounds are essential secondary metabolites due to their widespread use in pharmaceutical, medical, and food applications. These compounds are sensitive to light, and to achieve good production, it is necessary to optimize light intensity. This study observed significant accumulation of these compounds in the compounds at an average light intensity of 50 µmol/photon/m²/s. These compounds could potentially be natural alternatives to synthetic pharmaceutical compounds and potential targets for treating many diseases, as nutritional supplements, cosmetics, and in various industries if algae are cultivated and propagated under

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appropriate lighting conditions for the purpose of producing and extracting compounds in environmentally friendly ways.

Suggestions

1. Using different light intensities to systematically study the effect of lighting on increasing microalgal growth and productivity.

2. Understanding the ideal lighting conditions for cultivating species and strains of microalgae of biotechnological value.

3. Identifying the ideal genera that prefer a specific culture medium and good lighting.

4. Determining the correlations between changes in lighting conditions, such as light intensity, spectral composition, and length of light duration, and increased microalgal production of biochemically useful compounds.

5. Studying algal physiology by evaluating the synergistic effects of moderate light stress, along with additional stressors, on microalgal growth and productivity, with consideration given to the biosynthesis of important bioproducts (pigments, lipids, fatty acids, and carotenoids).

6. Cultivating meals allows for significant algal growth. Algae tanks can be used for this purpose, as well as permanent cultures for the purpose of continuing the production of the desired compounds with various culture media such as CH-10, ZAROUK, BG-11, and others.

RECOMMENDATIONS

1_Unifying methods for studying the effect of lighting on the growth and productivity of microalgae

2_Knowing the optimal lighting conditions for cultivating species and strains of microalgae of biotechnological value

3_Establishing relationships between changes in lighting conditions, including light intensity, spectral composition of light, duration of illumination, etc., and increasing the synthesis of compounds valuable from a biochemical point of view by microalgae

4_Determination of the stimulatory effects of a combination of mild stress with other types of stress on the growth and productivity of microalgae, taking into account the synthesis of valuable bioproducts (pigment, lipids, fatty acids, carotenoids).

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