Growth and Biochemical composition of mucilage extracted from Basil O. basilicum and its effect on Algae Chlorella vulgaris growth

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1. Abstract

The polysaccharide mucilage derived from the seeds of sweet basil *Ocimum basilicum* L. (family *Lamiaceae*) was investigated, to examine the ability of algae *Chlorella vulgaris (V. vulgaris)* to grow on mucilage media as an possible alternative media in comparison to the commercial one (Chu-10), the extracted mucilage was tested quantitatively and qualitatively.

Algae growth on mucilage media is similar to that of on the control (commercial media) (Chu-10), where the relative constant (K) was (0.6 and 0.7) and the duplicate time was 0.5 / day for both Chu-10 and mucilage media. Chlorophyll pigments were 0.5 µg mL^{-1} for both studied media. The biochemical analysis of *C.vulgaris*, grown on both media indicated that the protein and total carbohydrates were (45, and 14%) respectively on mucilage media, while the lipid percentage was 17% (dry weight basis (dw) whereas, the percentages of protein, carbonates and total fats for chu-10 media were (48, 15, 17)% (dw) respectively. Overall, these findings indicate that the mucilage extract from the sweet basil seeds can be used as a safe, inexpensive and organic media for *C. vulgaris* growth

Keywords: carbohydrate, soluble sugar, lipids, protein, fatty acids.

Introduction:

The perceptive of knowledge in microalgae ecophysiology is essential to produce microalgae in a large scale, production of high level of bio- products such as pigments(Griffiths and Harrison, 2009), polyunsaturated fatty acids (Guschina and Harwood, 2006; Spolaore et al., 2006) animal feed (Spolaore et al., 2006), fertilizers and etc. They use solar energy (Sialve et al., 2009) to convert CO_2 and water into biomass via photosynthesis. In addition, it is known that the use of microalgae by fish larvae enhances their reproduction (Spolaore et al., 2006)

Because of the current pollution problem and the raising issue about environmental protection, in addition to the exhaustion of fossil oil reserves, impinging on a potential

for an oil crisis and having consequences on climate change, much investigation has been concerned with the production of biofuels from microalgae (Šoštarič et al., 2012), however large scale production of microalgae is expensive, and several methods has been proposed for cost reduction, such as industrial/sewage effluent (Sydney et al., 2011). However, if microalgal used for food, pharmaceutical or cosmetic industry, there is the need for keeping the microorganisms under clean conditions, free of environmental contaminants, and for such purpose, industrial material cannot be used, since the contaminants will be accumulated in the environment, therefore there is a need for culture medium selection, especially there are 19 botanical families producing mucilage (Morton, 1990).

Basil *O. basilicum* is one of the most commercially acclaimed aromatic herbal worldwide, the what we called seeds is a dry fruit that consists of pericarp (as an outer layer) and then the endocarp before the seed is coated. The mucilage is obtained after soaking the seeds in water. as the outer layer of the seeds is absorbed and the water absorbs a mucilaginous mass that surrounding the seed from the outside (Azuma and Sakamoto, 2003) .In addition, mucilage serves as a natural immobilized source for agriculturally-based polysaccharides (Melo and D'souza, 2004). The mucilaginous layer of the swollen seeds is a pectineus matrix, consisting of considerable amounts of unesterified galacturonic acid with a large capacity for hydration, the basil mucilage had 5% moisture, 95.1% dry weight, 2.1% yield, 0.8% ash and 21% protein (Fekri et al., 2008).

Using of food with high viscous fiber or soluble dietary fiber seems to be effective in reducing blood cholesterol (Anderson et al., 1994). With about 9% viscous fiber, *Ocimum canum* seeds are soaked in water causing a gelatinous coat to form, similar to psyllium seeds (Suriyasomboon et al., 2011). Chemical analyses showed that mucilage composition consisting of 2.4% fat, 1% protein, 22% crude fiber, and the gelation is composed of at least 10% of galactose and mannose were the major polysaccharides were recognized (Achi and Okolo, 2004).

Given the importance of basil seed mucilage in food and pharmaceutical industries, but to date mucilage doesn't used as a substance in microalgae media, therefor the current study is conducted to use the mucilage extract as a substance in microalgae media in comparison to the commercial chu-10 media.

2. Materials and methods:

2.1. Basil Seed and medium Collection:

Sweet basil seeds were obtained from the local markets in Baghdad/Iraq. The seeds were confirmed by plant taxonomy lab, College of Science for women, Biology department. Seeds were cleaned and kept in a dry place for further use .

Chu-10 media (Chu medium M697-100G) No.10. were obtained from HIMEDIA® the official company website.

2.2. Chu-10 media:

Chu-10 media was prepared according to method of (Stein et al., 1973). , A stock solution was prepared according to table 1 using sterilized distilled water, then1 mL of stock solution was taken and the volume was completed to 1 L with distilled water ,pH was adjusted to 7-8 by adding of (0.01 N) NaOH solution. The medium was sterilized in autoclave at 121° C and 15 lbs./ang for 15 min, after which, the phosphate salts were added and the liquid media was kept at 4 ° C until use**.**

2.3. Mucilage media preparation

Mucilage was prepared by placing 10 g of basil seeds in 1 L beaker and distilled water was added in 1:20 proportions. The pH was adjusted and maintained at 4, 6 and 8 through continuous adjustment using 0.2 M NaOH or HCl solutions, and the temperature during extraction was maintained at 4, 40 and 80 ± 1.5 ℃ using temperature controller. The mixtures were stirred with a magnetic stirrer and hydrated for 2 h. Then the aqueous suspension was spread on a drying tray and exposed to temperature of 50 °C for 10 h. The dried mucilage was separated from the seed by rubbing over a 40 mesh screen, and the weight was recorded. (Muñoz et al., 2012). Then 5 g from Mucilage powder was added to 100 mL of distilled water after 15 min, the Mucilage suspension autoclaved for 15 min, after that the new media was inoculated with *C. vulgaris* algae, and the Chu-10 media was used as a control after adjusting pH between (7-8) (Reqapapi 2003) and by 10 bis per media, the incubator conditions were 25°C and 200 micro Einstein/m^2 light intensity for 14 days with continuous shacking.

2.4. Detection of effective compounds in basil seed mucilage:

2.4.1. Qualitative disclosure:

2. 4.1. Alkaloids

Alkaloids were detected using Mayers reagent (Smolensk et al., 1972), which was prepared by weighing out 1.58 g of $HgCl_2$ and melted in 60 mL of distilled water, then it was mixed until $HgCl_2$ is completely dissolved. A 5 g of Potassium was added and mixed with 10 mL of distilled water, after no residues left the volume was completed to 100 mL with distilled water. The addition of three drops from the reagent to the plant extract will result a white precipitant, which indicates that the test is positive, and the solution contains Alkaloids (Smolensk et al., 1972).

2.4.2. Tannin

Tannin was detected by adding drops of ferric chloride solution $FeCl_3$ to 0.5 mL of extract and the appearance of bluish green color conforms tannin presence (Diaz and Abeger, 1986).

2.4.3. Saponins

In a test tube, 0.5g of the extract was shaken with water. A stable frothing was taken as an evidence for the presence of saponin. The following ranking was used: + = Present (Nyam et al., 2009).

2.4.4. Glycosides

Detection of glycosides was done by mixing 1 g of dried plant extract with 10 mL of distilled water and after filtration, Fehling's reagent was added, and the appearance of dark red color is an evidence of Glycosides presence (Diaz and Abeger, 1986).

2.4.5. Qualitative Phenolic compounds

Phenolics compounds was detected by using ferric chloride solution at a concentration of 1% , and then 2 mL to 3 mL of plant extract was added , the presence of bluish-green color is the indicator for Phenolic compounds (Harborne, 1973).

3. Quantitative disclosures

3.1. Phenolic compound

High-performance liquid chromatography (HPLC) Shimadzu LC2010 was used to quantify phenolic compound in mucilage extracted from basil seeds. Five grams of (dry sample) were mixed with 70% methanol and vortex for 3 minutes, then centrifuged at 3000 rpm, then the supernatant was filtered using 0.22µm filter paper and 20µl was injected for phenol detection. Column type (250 x 4.6 ID) mm, 5mm particle size, the flow rate was 0.8 mL/ minute, UV visible detector was used with OD_{280}nm (Optical density) (Xu et al., 2008).

Readings were recorded on wavelength and by retention time for standard, and the concentration of the active substance for the studied samples was estimated to be quantified by comparing the standard material package area with the model package area under the same conditions, depending on the following equation:

$$Unknown\ substance\ concentration\ (g/ml\mu) = \frac{Packet\ Space\ X\ standard\ focus\ \times\ number\ of\ times\ dilution)}{Standard\ package\ Space}$$

3.2. Soluble sugars

High-performance liquid chromatography (Shimadzu HPLC LC2010) was used to quantify phenolic compound in mucilage extracted from basil seeds. Five grams of (dry sample) were mixed with 15% Acetonitrile: distil water, vortex for 3 minutes, and centrifuged at 10000 rpm, then the supernatant was filtered using 0.22µm filter paper and 20µl was injected for phenol detection. Column type (250 x 4.6 ID) mm, 5mm particle size, the flow rate was 1 mL/ minute, UV visible detector was used with OD_{190}nm O (Lv et al., 2009).

Readings were recorded on wavelength and by retention time for standard, and the concentration of the active substance for the studied samples was estimated to be quantified by comparing the standard material package area with the model package area under the same conditions, depending on the following equation

$$Unknown\ substance\ concentration\ (g/ml\mu) = \frac{Packet\ Space\ X\ standard\ focus\ \times\ number\ of\ times\ dilution)}{Standard\ package\ Space}$$

3.3. Algae Isolation and Purification:

Algae were collected form Tigris River on Al Jadriyah location in Baghdad Iraq (Figure1). The samples were collected during January 2017 using Vandron container (Stein et al., 1973), and the algae were classified using the microscope 10x and 40x WPA, (Prescott, 1982), Then the samples were purified using the technique of serial dilution from starting with 1:10. After the dilution process, 80, 90 and 100 diluted media were cultured in Chu-10 liquid media.

A sterile flask containing 70 mL Chu-10 media was prepared, and the diluted algae extract was added for each flask under sterile condition to avoid contamination. The culture was incubate at 25° C and und 200 micro-Anita Yen/m² light intensity, for 10 days, with moderate shacking (Tomaselli et al., 1981) . This process is repeated until a pure strain of algae *C. vulgaris* is obtained.

Alga *C. vulgaris* was selected among a number of algae isolated from water samples group due to its abundance in the Iraqi environment and for its simple isolation and purification. The *C.vulgaris* is a single-celled, spherical, and small-sized shape with a diameter of (2.1) in single or irregular shape, it is found in water with neutral pH and has several uses as a high protein source for animal nutrition, and as a component in dietary supplements as well

3.4. Growth Curve

The growth rate of algae was calculated according to (Andersen, 2005)by calculating the optical density at (OD_{650} nm). At exponential phase, and the beginning of the Stationary phase of the algal culture, the growth rate was expressed by the relative growth constant (K), which is calculated according to the equation:

$$K = \frac{logNt - logN0}{T1 - T2}$$ **(Fogg and Thake, 1987)**

T2: Time at the end of the experiment
T1: time zero
Nt: Optical density after the end of the experiment
N0: Optical density at the beginning of the experiment
The Doubling Time **G** is obtained from the **K** growth rate and according to the following equation:

$$G = \frac{0.301}{K}$$ **(Fogg and Thake, 1987)**

4. Determination of Protein ,Carbohydrate and lipid concentration:

4.1. Determination of total protein (%)

The total protein content in algae was determined according to (Hudson et al., 1989). 10 mL of algae cultures were centrifuged at 3000 rpm for 15 min. The supernatant was discarded, and the pellets were added to10 mL distill water and centrifuged as above. The pellets were mixed with distill water and placed in a spectrophotometer cuvette, and the absorbency is measured at A_{280}nm as maximum wavelength to A_{260}nm as a minimum for protein determination, the total protein contents was calculated by equation:

$$\textbf{Protein concentration} = 1.55 \times A_{280} - 0.77 \times A_{260}$$

4.2. Determined of total Carbohydrate and lipids %:

The total carbohydrate content in algae farm is estimated according to (Herbert et al., 1971) method, where 10 mg of dry algae was crushed with 10 mL of distilled water, after which the carbohydrate was separated by centrifugation at 3000 rpm for 10 min, and the process is repeated three times to recover the deposit. A 1 mL of the resin was taken in 10 mL glass tube containing 1 mL of phenol [w/v] (5%) and 5 mL of H_2SO_4 (96%). After 10 min incubation, the sample was continuously moved, and the glass tube was placed in a water bath at 28˚C for 10 min. The optical density (OD) of samples was

measured at 488 nm. The total carbohydrate concentration was calculated for each sample with the relative reference of the standard curve for the glucose sugar, A100 mg mL^{-1} standard glucose solution was prepared, then a serial dilution (10,20,40,60,80,and 100 mg mL^{-1}) was prepared, and only 0.5 mL sample size was used for OD measurement.

4.3. Chlorophyll content determination:

In order to determine the chlorophyll content, 20 mL of broth algal culture was centrifuged at 4000 rpm for 10 min. The pellet was collected in a test tube containing 6 mL acetone (90%) with vigorous shaking for 1 min in order to disrupt the cell wall and extract chlorophyll. The samples were left at 4˚ C for 18 h in the dark. After 24 h, samples were centrifuged for 10 min, and the resulted pellet which contains chlorophyll tincture was mixed with 10 mL acetone (90%) and by using a spectrophotometer device, dye ratio was estimated by following equation:

Chlorophyll Aper mall sample=11.9(2.43 Db Da)V/L (Vollenweider, 1969)

Da: Light density of the extraction after adding acetone
Db: Light density of the extraction before adding acetone
V: Size of used Acetone
L: Optical cell length

5-Results and discussion:

5.1. Description and classification of sweet basil:

The sweet basil plant is belong to the *Lamiaceae*, an aromatic herbal plant dating back to the *Ocimum*, particularly *O. basilicum*, an annual plant durable for warm climate conditions (Simon et al., 1990).

Local sweet basil seeds were selected for its wide spread in Iraq and its seeds produce mucilage in a sufficient quantity after water absorption (Figure 2).

5.2. Qualitative and quantitative detection of Mucilage:

5.2.1 Qualitative detection:

In order to assess the probability of mucilage to be an alternative media for algal growth, qualitative detection for sugar and phenols was performed (Table 2). The results revealed that the mucilage is consists of sugar and phenols, which they considered to be beneficial for medium content, since these materials are important to improve the emulsification properties which is an important factor in the medium, in to the antioxidant effect for the phenolic compounds (Velioglu et al., 1998). In addition, binding with fatty acids also shows the presence of other effective compounds which could be a good

indicator for mucilage purity. These results are in agreement with (Al-Aubadi and Al-Ani, 2017).

5.2.2 Quantitative detection:

The results showed in (Table 3) refer to the quantitative and qualitative assessment of phenol and sugars using HPLC. The results revealed some differences in the concentration of separated compounds where sugar compounds were diagnosed as (L-Arabinose, Xylose, Mannose, and Galactose). In addition, phenols compounds were (Catechin, Rutine, Luteoline, Myrcetin , Chrysin , Kaepferol , Quercetin , Apigenin, and Gallic acid) .

The growth curve of *C. vulgaris* was estimated as the algal growth on Chu-10 and mucilage media (Figure 3) and (Figure 4). When *C. vulgaris* was grown on mucilage media, the exponential phase was initiated after the second day and reached the stationary growth phase after 12 days, afterward, it start to decline after 14 days with a constant relative growth (K = 0.65), and the rate of doubling time 0.46. In contrast, , the exponential phase on Chu-10 media is started after the second day and reached the stationary growth phase after 10 days, then they tend to decline after 12 days with relative growth constant (K = 0.6) and the doubling time was 0.5. These results are in agreement with (Al-Husseinawy, 2015) when the culture of *C.vulgaris* was cultivated on Chu-10 media under similar laboratory conditions.

According to the results above, the algae cells are able to survive longer on the alternative media before they die, where the growth is decreased after 14 days compared to Chu-10 media, however the phase was started after 12 days of the assay.

Interestingly, taking the two averages in terms of growth rate (K), the results showed that there is a convergence in values and with less doubled time as showed in (Figure 4. A & B), offset by the highest value of the Chlorophyll pigment in the two media as shown in (Figure. 4C). The successful growth of algae on the alternative media (mucilage basil Seed) may be due to the fact that the extract contains high level of sugars, a source of carbon, which is the main key in the process of respiration and photosynthesis (Chen and Johns, 1991), and these results generally indicate that the plant extract (mucilage) may be a good candidate to grow *C.vulgaris*, in comparison to the growth rate on Chu-10 media. This gives an indication of the possibility of using mucilage extracted from basil seeds as an alternative planting media for the base and is a mean of reducing the cost of planting and producing live blocks of algae, because the cost of the constituent nutrients of the base is a large proportion of the cost of production, in addition to the availability of the seeds in local market in Iraq.

5.2.3 Chemical content of *C. vulgaris* Algae:

The results of the biochemical analysis of *C. vulgaris* culture on Chu-10 media and alternative media are in table (4) ,The percentage of total proteins was (48 ± 1.7)% ,total carbohydrates (17 ± 1.5)% and total lipids (16 ± 1.4%), all values were calculated in a dry weight basis, these results are in agreement with (Becker, 2007). The results of the biochemical analysis of the studied algae developed on the mucilage media, showed that the ratio of proteins and fats and the total carbohydrates of the studied algae were close to the proportions in Chu-10 media, and may be due to the availability of appropriate nutrients in the extract consumed by the algae results in an increment in the living mass. Venkataraman (1985) indicated that the chemical content of algae depends on the ingredients of the medium and the availability of nutrients in the growth environment, therefor, these components suggested mucilage is a possible alternative to develop *C. vulgaris* with low coast and no additives. The results also showed that total protein content makes *C. vulgaris* a good source of protein, which can be used as a supplement in animal and human diet. This is what (Al-Asadi, 1977), pointed out that the protein content of *C. vulgaris* was 50 % on dry weight basis.

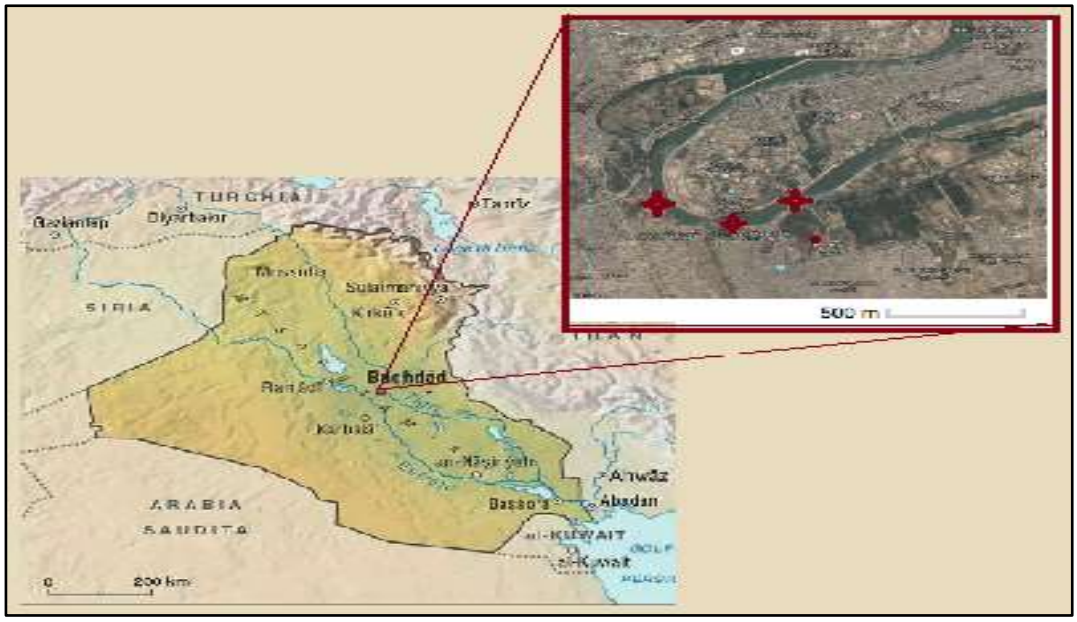

Figure (1) Location of samples collection from Tigris River

Table (1) Chemical composition of Chu-10 media (Al-Aarajy, 1996)

Compound	g L^{-1}	Compound	g L^{-1}
$COCl_2.6H_2O$	0.01	Na_2EDTA	31.8
H_3BO_3	0.72	$Na_2SiO_3.9H_2O$	6.20
$CuSO_4.6H_2O$	0.02	$FeCl_3.6H_2O$	1.45

$NaHCO_3$	25.0	$NaNO_3$	53.3
$MnCl_2.4H_2O$	0.05	$MgSO_4.7H_2O$	25.0
$(NH4)6MO7O_{24}.4H_2O$	0.01	K_2HPO_4	10.0
$ZnSO_4.7H_2O$	0.06	$CaCl_2.2H_2O$	40.0

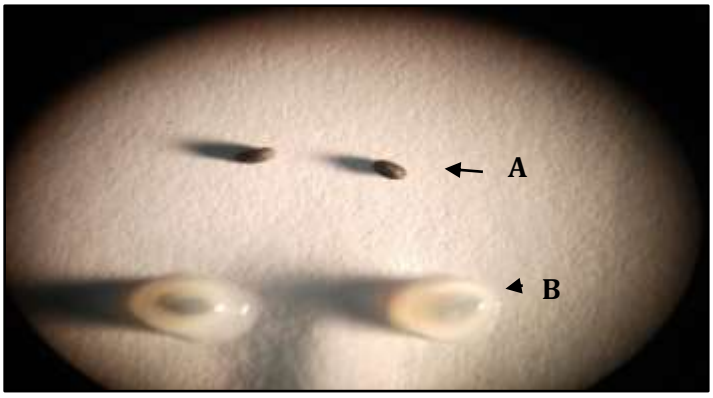

Figure (2) Basil seeds before (A) and after (B) Hydration.

Table (2) Qualitative detection of effective compounds in basil seed mucilage

Effective compound	Test Result
Alkaloids	-
Flavonoids	-
Phenolics	+
Saponins	-
Sugar	+

Table (3): Phenol compounds and concentration of sugars in the Mucilage extracted from sweet basil seeds µg mL^{-1}

Phenols compounds		Sugars compounds	
compound	**µg mL^{-1}**	**compound**	**µg mL^{-1}**
Rutine	99	L-Arabinose	3.1
Luteoline	506	Xylose	3.5
Myrcetin	789	Mannose	6.1
Chrysin	103	Galactose	7.0
Kaepferol	69		
Quercetin	594		
Apigenin	806		

Gallic acid	350

Table (4) Biochemical content of dry weight of Algae *c. vulgaris*

Media culture	Proteins%	Carbohydrates%	Lipids %
Chu-10	48±1.7	15±1.5	17 ±1.4
Mucilage	45±2.3	14±1.8	17±2.1

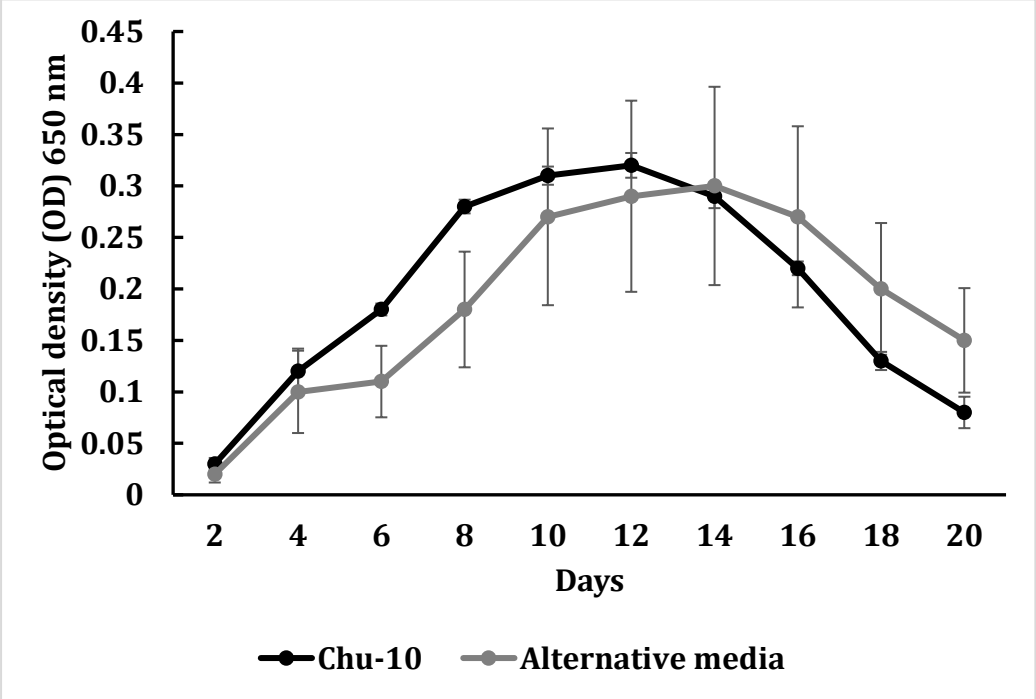

Figure (3) Growth curve of *C.vulgarisin* Chu-10 media and in alternative media

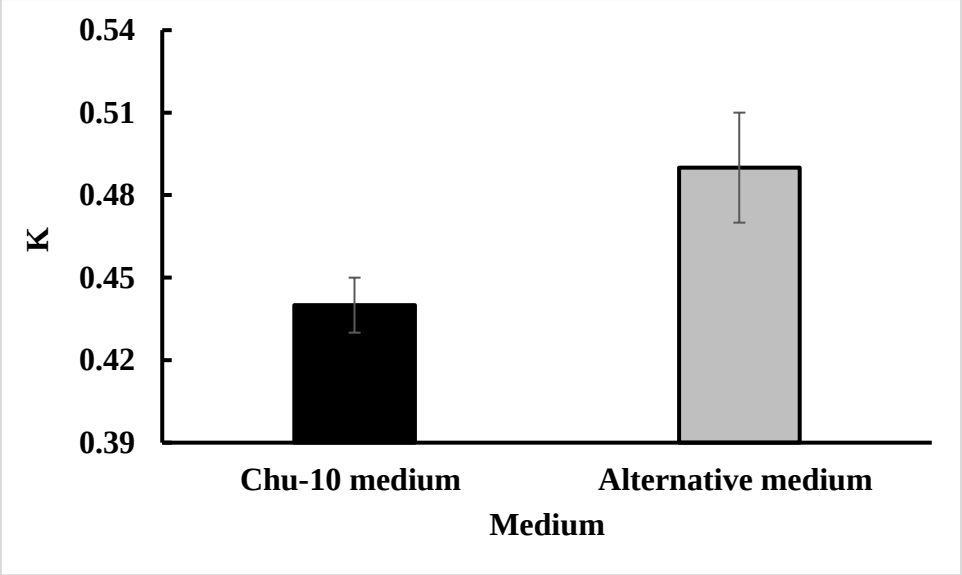

Figure (4): A growth rate of *C. svulgarisin* in Chu-10 media and in Alternative medium.

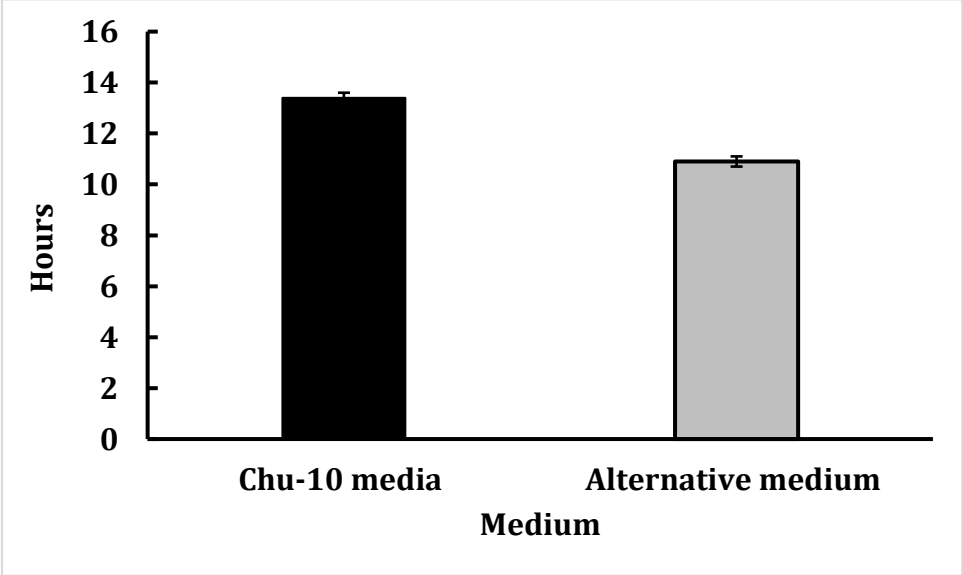

Figure (4): B. Doubling growth time of *C.vulgaris in* Chu-10 media and in alternative medium.

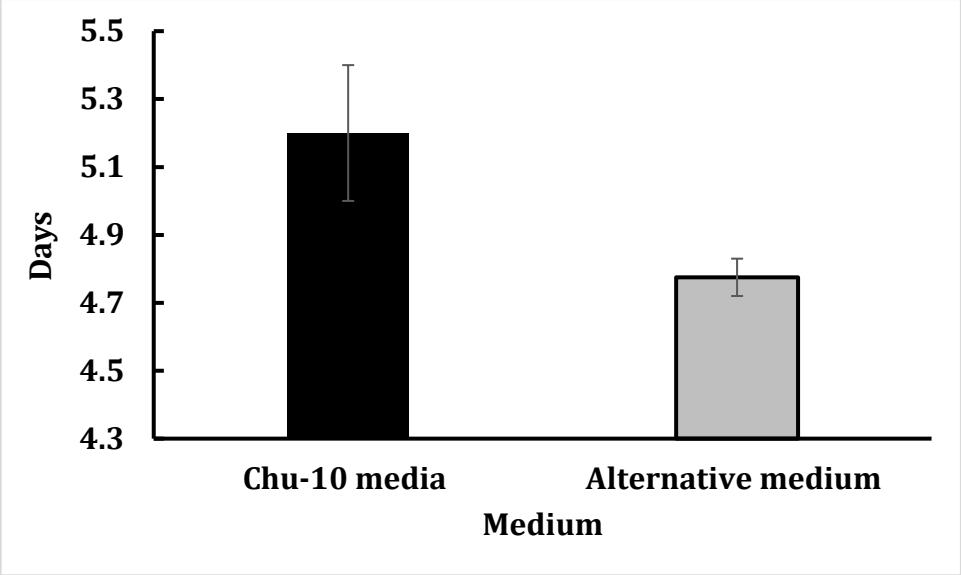

Figure (4): C. Chlorophyll content µg L^{-1} of *C.vulgaris* in Chu-10 media and alternative medium.

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