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Testing The Activities of Some of The Inside and Outside Cellular Compounds Produced by The Algae *Chlorococcum infusionum* and *Scenedesmus obliquus* Against Some Bacteria

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Abstract:

In this study *Chlorococcum infusionum* and *Scenedesmus obliquus* were isolated ،purified and identification From the Euphrates River at the city of Nasiriyah. The water of this canals originally from Tigris River. Ch-10 culture media was used for their cultivation in suitable laboratory conditions (25c°,200µE/m2/sec) for 16:8 hrs. Light: dark. Each culture was harvested at the end of exponential phase .Organic solvents used for extraction were Ethanol، Hexane and Methanol 95% to extract the crude active Intracellular and Extracellular substances, and evaporated down to dryness .Antibacterial activity of these different extracts were evaluated against 6 strains of gram positive bacteria and gram negative bacteria, Agar diffusion method was used in this evaluation.

Keywords : green algae ; extraction ; Bacterial strains.

Introduction:

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents **(Cox *et al*.,2010)**. The use of algae extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency Many algae have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant**(Dangm *et al*.,2011).** These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (20), as well as in tannin (33). The wide spread of the algae species as they represent an important and significant part of the algae is what made them .A study of many studies to identify the benefits and the possibility of practical application, especially in the medical field The pharmacists are like the rest of the other algae. If the micro-algae is emphasized as a continuous source natural products can be developed in bioreactors for large areas(Tuney *et al* .,2006). Quality can be controlled and efficient The micro algae are free from herbicide, insecticidal and poisonous by providing

them with a clean, and clean plant. They are of a wide variety compared to high-end plants(Shanmugam, and Mody, 2000; Hoppe, 1979, .Rossana, *et al.,* 2006).

The micro-algae showed their ability to release chemicals to the medium in which they live as a result of micro-organisms that are not antimicrobial. *(Rossana, et al.,* 2006). The active compounds were tested and tried to purify them and describe them to know their chemical and biological properties, and evaluate their activities and their medical value(Reichelt and Borowitzka,1984). Several studies have indicated that the species of green algae have been highly effective test their extracts in inhibiting the growth of microorganisms compared to the rest of the other algae. As she gave its extracts have positive results against positive and negative bacteria as well as fungus and large inhibition areas(Ghasemi, *et al.,* 2007; Oranday *et al.,* 2004). The aim of this study is to test the efficacy of the inside and outer extracts of the green algae.

Materials and methods :

1.Preparation of algae dryer

The two algae were isolated from the Euphrates River at the city of Nasiriyah. The two algae were diagnosed using a composite optical microscope and depending on the source of diagnosis (Prescott, 1982). The algae are grown in the middle Ch-10(Stein , 1973). Using stable farms and in stable laboratory conditions (Temperature 25°c and intensity of lighting 200 µm Einstein / m ^{2} / tha and for 6:18 hours Lighting: dark). The growth curve was determined to identify the growth stages. The plant was deposited at the end of the exponential phase by centrifugation at 3000 rpm for 15 minutes. Collect the precipitate and dry at 40 ° C for 48 hours (Taskin *et al.,*2007).

2 - Extraction of active substances inside and outside the cell

The raw and inward production of the crude produced by the extraction device was extracted Soxhelat one gram of dried algae was weighed and 250 millimeters of 95% ethanol was added and the sample was left for 2 hours The powder is saturated with the solvent. The extraction is then carried out for 4 hours and the filter is dried by using the rotary evaporator at 40 ° C and the output weight of the extraction process (Taskin *et al.,* 2007). The extraction process was also repeated for solvents methanol and hexane.

3 - Determination of the effectiveness of compounds inside and outside the cell towards bacteria

The sensitivity of 6 strains was tested *Staphylococcus aureus* , *Bacillus subtilis* , *Staphylococcus epidermidis* , *Pseudomonas aeruginos*a , *Escherichia coli* , *Salmonella typhi* Antibacterial efficacy was determined by using Agar diffusion method If bacterial isolates are developed in Nutrient broth For 18 hours and at a temperature of 37 ° C Spread the approximate number (5 10 cells / mm) on the culture Mullar Henton Agar . And configure 6 mm diameter of filter paper (Watman) and add 0.1 mL concentration of extract to each also put a tablet of solvent organic fertilizer for comparison and incubated bacterial farms at 37 ° C and for 18-24 hours after which measured diameters - inhibition if any (Taskin *et al.,* 2007).

Results and discussion

The results showed that there is a difference in the growth curve of algae, where it was found that the day 12 end of the phase of the algae *C. infusionum* (Fig. 1), while the algae record *S. obliquus* day 15 End of the exponential phase (Fig. 2) This difference is due to the type of algae, environmental conditions and consumption of nutrients (Biondi *et al* ., 2007).

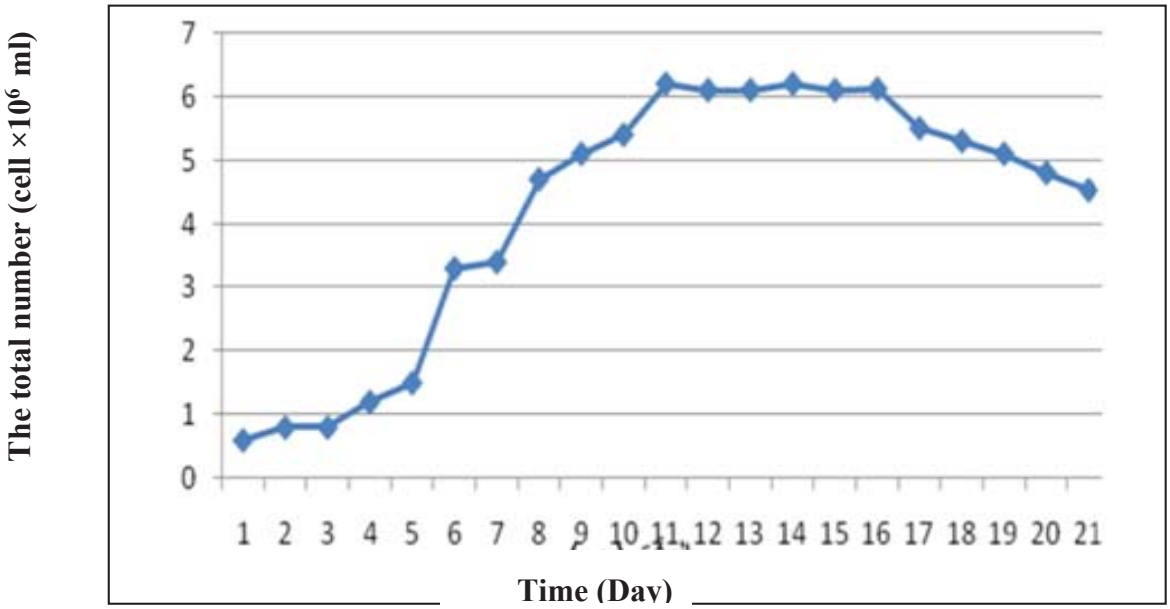

Figure 1: Growth curve for algae *C. infusionum* In terms of the total number cultivated in the plant medium Ch-10 at temperature 25±2 And intensity of lighting 200 mA\m\t 8: 16 hours Light: dark for 20 days

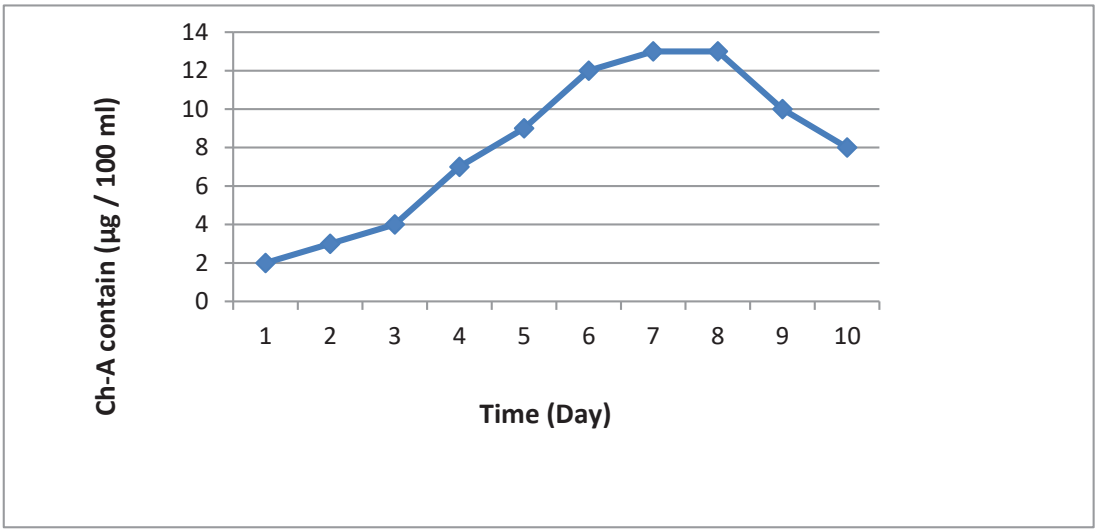

Figure 1: Growth curve for algae *S. obliquus* In terms of the total number cultivated in the plant medium Ch-10 at temperature 25±2 And intensity of lighting 200 mA\m\t 8: 16 hours Light: dark for 20 days

The results of the test showed the effectiveness of the inorganic and inorganic methanol extract for algae *C. infusionum* (Table 2.1). All strains of positive and negative gram bacteria used in the study showed sensitivity to the extract with the exception of bacterium *S.typhi* and *S.epidermidis* Which showed no sensitivity to the inward and out-cell extractors respectively These findings are consistent with Rania and Halla (Rania, and Halla, 2008). For the sensitivity of these species to the same extracts of green algae, while the effectiveness of the extracts of hexane and ethanol were limited to bacteria

Table (1): Diameter of the growth inhibition zones (mm) shown by bacterial strains against ethanol, hexane and Intracellular extracts of algae *C. infusionum*

Isolates	control	Ethanol	Hexan	Methanol
Staphylococcus aureus	-	12	-	36
Staphylococcus epidermidis	-	-	-	27
Bacillus subtilis	-	11	13	15
Escherichia coli	-	-	-	18
Salmonella typhi	-	-	-	-
Pseudomonas aeruginosa	-	-	-	10

(-)There is no inhibition of growth of studied isolates.

Table (2): Diameter of the growth inhibition zones (mm) shown by bacterial strains against ethanol, hexane and extracellular extracts of algae *C. infusionum*

Isolates	control	Ethanol	Hexan	Methanol
Staphylococcus aureus	-	12	16	15
Staphylococcus epidermidis	-	-	11	-
Bacillus subtilis	-	15	-	20
Escherichia coli	-	10	13	25
Salmonella typhi	-	12	-	12
Pseudomonas aeruginosa	-	-	-	11

(-)There is no inhibition of growth of studied isolates.

It was also found that the crude methanol extract is cellular for algae *S. obliquus* it has high effectiveness against all positive and negative bacteria where the highest rate of 22 mm inhibition was recorded against the bacteria *S. epidermidis* ethanol extract came in second place as the inhibition diameter between 3-16 mm was limited to the different laboratory bacteria, as demonstrated by the hexane extraction that it has very weak inhibitory activity (Table 3).

Table (3): Diameter of the growth inhibition zones (mm) shown by bacterial strains against ethanol, hexane and Intracellular extracts of algae *S. obliquus*

Isolates	control	Ethanol	Hexan	Methanol
Staphylococcus aureus	-	-	15	12
Staphylococcus epidermidis	-	13	-	22
Bacillus subtilis	-	16	13	14
Escherichia coli	-	-	-	6
Salmonella typhi	-	3	-	14
Pseudomonas aeruginosa	-	10	-	10

(-)There is no inhibition of growth of studied isolates.

Table (4): Diameter of the growth inhibition zones (mm) shown by bacterial strains against ethanol, hexane and Extracellular extracts of algae *S. obliquus*

Isolates	control	Ethanol	Hexan	Methanol
Staphylococcus aureus	-	-	25	13
Staphylococcus epidermidis	-	-	-	-
Bacillus subtilis	-	12	17	6
Escherichia coli	-	-	-	-
Salmonella typhi	-	-	16	-
Pseudomonas aeruginosa	-	-	-	-

(-)There is no inhibition of growth of studied isolates.

The crude external hexane extract also showed cellular for algae *S.obliquus* high inhibitory activity against bacteria compared to ethanol and methanol (Table 4). The highest 25 mm inhibitory rate was recorded against *S.aureus* bacteria and the lowest inhibitory rate against *S.typhi* bacteria .with a 16 mm inhibition rate as shown in Fig. 3.

The difference in efficacy against some bacterial isolates of raw and inorganic extracts of *S.obliquus* and *C. infusionum* showed that there was more than one active substance and that the active substance could be distributed in more than one solvent. .(Kreitlow, *et al.*,1999). Organic compounds have a positive effect

when extracting algae, and this may reflect the chemical nature of the active agent. Organic solvents also tend to remove water-damaging compounds from the cell surface .(Kellam,. and Walker,1988).
The current study indicates that algal extracts with various solvents have a clear inhibitory effect on bacteria and fungus, whether extracted inside or outside the cell due to their containment of ring peptides, alkaloids and polycyclic fatty acids .(Kellam, *et al.*,2008*).*

The results showed that the active substance extracted from the inside and outside products of the studied algae was affected in the gram positive bacteria dye more than in the gram negative bacteria dye. The reason is that the negative bacteria are less sensitive than the active compounds positive bacteria because they contain a cell wall of several .(Ördög, *et al.* 2004).
There are many factors that affect the nature of the results obtained by the researcher in tests that relate to effectiveness algae extracts toward bacterial growth activity, there may be differences or inconsistencies in the results researchers find the same type of algae, which may be due to the time and time of collection and conservation methods samples used in the pre - extraction test, the difference in the growth population used and the prevailing environmental factors algae growth stage when harvesting the farm and the type of solvent used in extraction and extraction method.

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