

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water canal in Baghdad University

Maarb .S. Al-Moula

Ahmed .S. Dwaish

Department of Biology., College of Science
University of Mustansiriyah.

ABSTRACT

The present study included identification the active compounds of an Macro-algae from the Iraqi water ecosystem . The macro-algae , *Cladophora glomerata* & *Chara vulgaris* which isolated from water canal in Baghdad University during April 2016. Physic-chemical parameters were measured , such as air and water temperature, pH , Ca^{+2} , Mg^{+2} , Cl^- , No_2^- , No_3^- , Po_4^- and So_4^- . Results indicated that the active chemical compounds in the hot methanolic and ethanolic extracts for *Cladophora glomerata* & *Chara vulgaris* were Alkaloid, Tannins, Saponins , Resins , Terpenes, phenols and Flavonoids .These findings suggest the possibility of using the *Cladophora glomerata* & *Chara vulgaris* as a novel source of natural antimicrobial agents in pharmaceutical industries.

Key word : *Cladophora glomerata* & *Chara vulgaris*, Macro-algae and active compounds.

Introduction :

Macroalgae are an important source of bioactive natural compound, they are regard as a source of bioactive compounds with cystostatic, antihelminthic, antiviral , antibacterial and antifungal activities, they have also been used to cure some sicknesses such as cancer, arthritis etc. Macroalgae are naturally renewable sources which are additionally utilized as food, feed and fertilizer in many parts of the world. They have been screened broadly to confine lifesaving drugs or biologically active substances everywhere throughout the world (1).

Cladophora glomerata is a genus of filamentous branched green algae which forms free-floating mats in shallow waters or attached to the base of shallow pools for example, lakes and canals, in shaded littoral zones of lakes, and in slow streams stay connected to the substratum by a

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water channel in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

basal cell (2). while *Chara vulgaris* is a genus of submerged plant –like green algae, that has central axes hard between nodes with whorls of branches at each node, it has gray- green colour sometimes crusted with carbonates making rough to touch and it has often garlic or skunk-like odor(3).

The aquatic environment is an excellent reservoir of bioactive natural compounds, which display structural/ chemical types not found in terrestrial natural products (4). Numerous aquatic organisms make bioactive metabolites in response to ecological pressures, including competition for space, the fouling of the surface (5). In addition, they improve a chemical strategy for defence to make sure their existence, and to produce extremely active molecules, then having to act as aqueous medium very diluted (6).

In the current need to find out novel and effective drugs against resistant pathogenic strains; algal derived compounds have a wide range of biological activities for instance antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic and antimutagenic activities that could be explored more (7). Bioactive compounds: polyphenols, catechin, flavonols, glycosides, and tannins discovered from methanol extract of red, green and brown algae are been described to have exceptional activity in their molecular skeleton and structures contributing to the strong antioxidant activity (8).

The purpose of this work was to detect the active compounds of two macroalgae collected from water channel in Baghdad University, with two extraction solvents (ethanol and methanol) ,and study some physicochemical parameter of these aquatic environment which effected on quality and quantity of active compounds formation.

Materials and Methods:

Collection and Preparation of Samples:

Samplings were carried out from water channel in Baghdad University middle of Iraq, which located on longitude 33°16'09.5"N and latitude 44°20'19.41"E, during April 2016. Samples of *Cladophora glomerata* & *Chara vulgaris* were collected manually from the rock. The harvested macro algae were kept in plastic bags and transported to the laboratory. Algal specimen of species were pressed and stored in 5% formalin for identification according to (9). Biomasses of macroalgae were rinsed with fresh water to eliminate other materials such as sand, shells, etc. The macroalgae were stored in the laboratories to identified and dried at 50°C under ventilation in an oven and then grounded to powder form by the blender.

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water channel in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

Some Physio-chemical Parameters such as temperature and pH were measured in the specific location ,then in the laboratory measured some soluble cationic ions such as calcium and magnesium, as well as some of anions, such as dissolved, chloride, sulfate, nitrate, nitrite, phosphate, with three replicates for each test, according to the methods described in. (10).

Preparation of alcoholic extract

The hot alcoholic extract was prepared by Soxhlet extraction according to (11). In this process the dried powder form of algae material extracted by using ethanol and methanol alcohol. After completion the process the concentrated active constituents from macro-algae were kept in sterilized test tubes stored in refrigerator till further use. The traces of ethanol and methanol were removed by keeping the tubes at 50°C for 1 hr.

Evaluation of some of the active compounds in the extracts :

Table (1) refers to the active compounds evaluated in hot methanol and ethanol extracts with its procedure reference.

Table (1): refers to the active compounds evaluated in hot methanol and ethanol extracts with its procedure reference.

Active compounds	Reference
Glycosides	(12)
Phenols, Alkaloids	(13)
Saponines	(14)
Terpenes	(15)
Steroids	(16)
Resins , Coumarines	(17)
Tannins	(18)
Flavones	(19)

Results and Discussions:

Algal isolates :

Cladophora is a branching, benthic, attached, filamentous green macro alga that forms a moss like structure (Figure 1). This alga is worldwide distribution in marine as well as freshwater habitats. Filaments are often longer, forming “streamers” which can be in excess of 1-2 m length (Figure 2), Coarse, dark-green to brownish-green, branching, hair-like filaments with cross walls separating segments; each segment has more than one nucleus . *Cladophora* germination and growth usually requires hard substrates for attachment, such as rocks, a further physical requirement for *Cladophora* growth is water motion, that agree with (18) .

The most common classification of this macroalgae according to (9).

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water canal in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

Division.....Chlorophyta
Class.....Chlorophyceae
Order.....Cladophorales
Family.....Cladophoraceae
Genus..... *Cladophora*



Figure (1): *Cladophora glomerata* in abstract eye

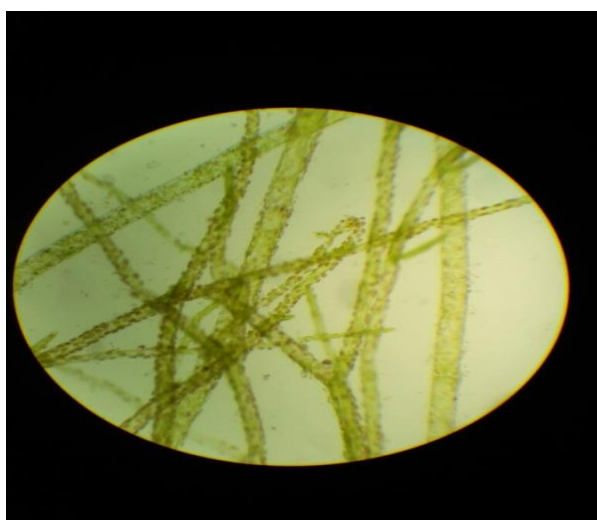


Figure (2): *Cladophora glomerata* under microscope(40x)

The alga *Chara vulgaris* distinguished to root and shoot system further comprising of well _developed vegetative and reproductive structures. Vegetative parts have been studied in detail under light microscope but it had helped very little to study the minor details particularly with regard to the reproductive organs especially the mature oospore which exhibit peculiar patterns of ornamentation in the wall as shown in figure (3,4) as well as pattern of classification (9).

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water canal in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

Division..... Charophyta
 Class..... Charophyceae
 Order..... Charales
 Family..... Characeae
 Genus..... *Chara*



Figure (3): *Chara vulgaris* in abstract eye



Figure (4): *Chara vulgaris* under microscope

Chemical and Physical Parameters:

Table (2): Refer to Chemical and Physical Parameters for water canal in baghdad university .

Table (2): Physico-chemical Parameters of the water canal

Test	(Mean \pm standard deviation)
Air temperature °C	25.6 \pm 0.763
Water temperature °C	32.7 \pm 0.832
pH	7.9 \pm 0.0
Mg ⁺² (mg / l)	39.87 \pm 0.616
PO ₄ ³⁻ (mg / l)	0.9724 \pm 0.171
NO ₂ ⁻ (mg / l)	5.7 \pm 0.0
NO ₃ ⁻ (mg / l)	9.961 \pm 1.086
SO ₄ ⁻ (mg / l)	275.64 \pm 5.483
Cl ⁻ (mg / l)	110 \pm 0.0
Ca ⁺² (mg / l)	56.186 \pm 1.320

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water canal in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

Active compounds are, by their very nature, biologically highly active and they are consumed in high amounts, which might pose an issue in terms of quantity and quality of emissions during their life cycle. The presence of active compounds in the environment is a globally emerging issue (19)

The quality of Water means all physical characteristics , chemical and biological all must be at an effective levels of acceptability, quality described as unacceptable in case one or more of characters change ,These properties are great important in aquatic systems ,because their influence in determining the quality of good water, this is accomplished by comparing these factors with global standard specifications for quality of water, considering that water plays an important role in maintaining the safety and health of the consumer ,the variation of these factors continuously depending on the nature of geological and climatic conditions of the study area (20).

The table (2) shows the results of measurements of some physicochemical parameters of the water canal in Baghdad University at the study period, spring , air temperature was 25 °C conditioning and 32 °C of water temperature, which are optimum for the growth of most organisms including algae, as is the temperature, one of the most important factors affecting the neighborhoods that have a direct impact in the proportion of dissolved oxygen in the water as well as pH growth (21).

The availability of nutrient and phytoplankton biomass fluctuated widely on a range of time scales from days to seasons to years .Nutrient bioassays are good indicators as to which nutrient has the potential or likely to limit phytoplankton growth at a particulate time and space (22).

Concentrations of Nitrate scored high level at summer and spring this probably due to the bacterial activity and organic compounds decomposition that associated with higher temperature. On the other hand, the decreased concentrations of NO₃ in spring were mostly caused by the decrease of aquatic plants growth during these seasons (23), that agreed with our results.

Nitrite compound considered as a medium product and non-stable, effected by the oxidation and reduction of non-organic nitrogen, present in low level of waters with good ventilation. This converts by the oxidation to NO₃ while NO₂ reduced and convert to ammonia when oxygen is low (23).

In aquatic ecosystems, phosphorus occurs mainly in the phosphate (PO₄) form. Phosphate comes in two primary forms :organic and inorganic . Organic phosphate is bound in plants and animals and is not available for

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water channel in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

phytoplankton .Inorganic, or Orthophosphate, also known as reactive phosphorus is the form that available and required by phytoplankton (24). All the ions which studied in this work most abundant natural element in the earth's crust and it is a common constituent of natural water, the sources of magnesium are ferromagnesium minerals in igneous rocks and magnesium ,calcium, it is one of the main contributors to water hardness, and also considered to be an essential element for all living organisms (25).

The finding of this study was agreed with the results of other studies conducted by other researchers in the Tigris River which is responsible for provide the study area with water in the same period(20). but didn't agree with studies of (26).

The study of quality of Water means all physical characteristics considering that water plays an important role in maintaining the safety and health of the consumer ,the variation of these factors continuously depending on the nature of geological and climatic conditions of the study area and that lead to different the active compounds which found intra algal cell , and that led to understood the variation in active compounds which found in same algal genus but with different environments and collection time (27).

Detection of some active compounds from algae

Results showed the primary detected for the active compounds which is shown in Table (3) the extract of hot ethanol for alga *Chara vulgaris* contains many of which phenols ,tannins, Saponines, alkaloids, Terpenes , Steroids, Flavones, Resins. And pH of extract was 5.While the hot ethanolic extract of *Cladophora glomerata* showed many active chemical compound , like alkaloids, Terpenes , Steroids, Flavones, Resins ,Saponines, tannins and pH of extract was 6 , results showed in the table (3) dis agree with (27) who isolated the same algae from Baher AL-Najaf and that may be because variation in invironment and collection time .

Table: (3)Active compound which found in algal extracts.

Chemicals Compound	A	B	C	D
Glycosides	-	-	-	-
Phenols	-	-	+	+
Alkaloids	+	+	+	+
Terpenes and Steroids	+	+	+	+
Resins	+	+	+	+
Saponines	+	+	+	+
Tannins	+	+	+	+
Flavones	+	+	+	+
Coumarines	-	-	-	-

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water channel in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

Extract pH	6	6	5.8	6
---------------	---	---	-----	---

A- *Cladophora glomerata* (Hot Methanol Extract)

B- *Cladophora glomerata* (Hot Ethanol Extract)

C- *Chara vulgaris* (Hot Methanol Extract)

D- *Chara vulgaris* (Hot Ethanol Extract)

As results showed the First detected which is shown in Table (3) for the active chemical compounds to extract hot methanol for alga *Chara vulgaris* it Similarity of compounds that appeared in extract hot ethanol to the same alga which is similar to the active chemical compounds of extract hot methanol for alga *Cladophora glomerata* only differ in *Cladophora glomerata* dosen't have phenols So the activity of these compounds are (Terpenes which have biological activity for viruses, bacteria, fungi and protozoa) and phenols which is one of oxidizing compounds toxic to microorganisms as well as tannins, which works to stop bleeding and secretions and inhibit enzymes and proteins tanker in the cell membrane(28) .

Alkaloids are characterized by their ability to break into the bacterial cell and intrection with the nucleic acid DNA and have important compounds in medicine intervention in the installation of several drugs as an important task has physiological effects on animals and is lethal materials .Most of them discouraging the growth of microbiology(29) . Flavones contribute in reducing the risk of heart disease and also is prese's with anti-oxidants. Flavonoids possess the effectiveness of its anti-inflammatory and anti-allergic and anti-microbial neighborhoods and anti-cancer(30).

Saponines work to reduce bacteria suger thus, a cell death and exert a wide range of pharmacological activities including expectorant, antiinflammatory, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycaemic, molluscicidal, antifungal, antiparasitic and many others (31). While Terpenes about 60% of which are anti-bacterial (28). Phytochemical analysis of the hot alcoholic extracts revealed that, Alkaloids , Terpenes , Steroids, Resins Saponins , Tannins Flavones are generally present in the macroalgae alcoholic extracts,alkaloids and phenols were found in *Chara vulgaris* (Hot Methanol Extract) *Chara vulgaris* (Hot Ethanol Extract) only .While Glycosides and Coumarines were absent in both the extracts Table (3).

This results supports the findings of many authors(32,33). Many papers have been published which discuss the methods of manufacture and the composition of algal extract the general that the composition of extracts

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water channel in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

strongly depends on the raw material (geographical location of harvested algae and algae species)as well as on the extraction method. The biologically active compounds which are transferred from the biomass of algae to the liquid phase ,they have well documented beneficial effect on humans, animals and plants, mainly by protection of an organism from biotic and a biotic stress (antimicrobial activity, scavenging of free radicals and host defence activity etc...(30,31 and 34).

The results showed that extracts of *Cladophora glomerata* and *Chara vulgaris* had Alkaloids, Tannins, phenols , Flavonoids and saponins, while Glycosides ,were absent. This results agreed with many studies such as (35,36) they screened the most active compounds in macroalgae. Biochemical analysis were being undertaken to determine the structure and nature of compounds responsible of the bio-activity of the extract.

Refrance :

- 1- Hellio, G.B.; Brrener, A.M.; Pons ,G.; Cotlenceau ,Y. and Borgongman, L.G. (2002). Antibacterial and Antifungal activities of extracts of marine algae from Brittany. France. use as antifouling agents. Appl. Microbiol. Biotechnol, 54: 543 – 549.
- 2- Sahoo , D and Seckbach ,J. (2015). The Algae World, Springer Netherlands,1st ed , v (26): (XII, 598).
- 3- Ditomaso, J.M. and kyser, G.B.*et al.*(2013). weed control in natural areas in the western united states. weed research and information center, University of California: pp 544.
- 4- Carter, B .K.(1996) Biomedical potential of marine natural products. Bioscience; 46: 271-286.
- 5- Ireland ,C.M; Copp, Foster, M.P., MC Donald, L.A.; Raisky, D.C. and Swersey ,J.C.(2000) . Bioactive compounds from the sea. in: martin re, carter ep, davis lm, eds. marine and freshwater products handbook. lanceaster.pa/ technomic: 641-661.
- 6- König, G.M.;Wright, A.D.; Stiche, o.; Angerhofer, C.K .and Pezzuto J.M. (1994). Biological activities of selected marine natural products. Planta med, V. 60: 532-537.
- 7- Salvador N., Garreta A.G., Lavelli L. and Ribera M. (2007). Antimicrobial activity of Iberian macro algae. Sci. Mar. 71: 101-113.
- 8- Khoddami A., Wilkes M.A. and Roberts T.H. (2013). Techniques for Analysis of Plant Phenolic Compounds. Molecules.18(2): 2328- 2375.
- 9- Prescott, G.W. (1962). algae of the western great lakes area. 2nd ed w. c brown. co. dubuque, Iowa pp 997.
- 10- Apha (American Public health association) (2004). standard methods for examination of water and wastewater.16thed.New York

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water channel in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

- 11- Harbone, J.B.(1984) phytochemical methods chapman and hall. 2nd ed. New York, 288.
- 12- Tiwari ,P.; kumar ,B.; kaur, M.; kaur ,G. and kaur ,H. (2011). phytochemical screening and extraction. Internationale Pharmaceutica Scientia,V. 1: 98-106.
- 13- Harbone, j. (1973). phytochemical methods. Chapman and Hall. London.
- 14- Al-abid, M.R.,(1985). Zurrzusamme mse turungder abschla bmembrane in phoenix dactylifera. Wurzburg University. Wuzzburg, f.r. of Germany, 153-140.
- 15- Shihata, I.M., (1951). A pharmalogical study of anagllis arvensis. m.d. vet. thesis, Cairo University.
- 16- Jaffer, H.J.; Mahmood, M. J.; Jawad, A.M.; Naji, a. and al-naib, a. (1983). phytochemical and biological screening of some Iraqi plants. Fitoterpia, lix.pp:229
- 17- Geisman, T. A. (1962). Chemistry of havonoids compounds. Macmillan co. New York. pp. 90-101
- 18- Gibson, R.; Hextall, B. and Rogers, A. (2001).photographic guide to the sea and shore life of Britain and north-west Europe. Oxford University press, Oxford.
- 19- Boxall, A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T. and Ankley, G.T., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions?. Environmental health perspectives.
- 20- Al-Sarraf, M.A.(2006). Ecological and Taxanomial Study for Phytoplankton in Al-Adaim and Diyala Tributaries and their Effects on Tigris River. PhD Thesis Baghdad University. College of Science for women. (In Arabic)
- 21- Ames, M. and Smith. W. (1964) .the quality control of drinking water and waste water, J. Bach., 47: 445.
- 22- Dwaish, A. S. (2012). Ecological Study of Phytoplankton in Tigris River through Baghdad City. PhD Thesis ,College of Science, Biology Department, University of Baghdad, Iraq.
- 23- Goldman, C. P. and Horne, A. L. (1983). Limnology. Mc Groaw-Hill international book company. PP 464.
- 24- Behar, S. and Cheo, M. (2004). Hudson Basin River Watch Guidance Document: helping to coordinate monitoring of freshwater wade able Rivers throughout the watershed, River network - River watch program, Revised Draft. retrieved june, 2000, from River Network (www.hudsonbasin.org).
- 25- CCME.(1999). Canadian Water Quality Guidelines. task force on water quality guidelines of the Canadian Council of Ministers of the Environment.

Detection of some active compounds from algae *Cladophora glomerata* & *Chara vulgaris* which isolated from water channel in Baghdad University Ahmed .S. Dwaish & Maarb .S. Al-Moul

- 26- Nashaat, M. R. (2010). Impact of AL-Durah Power Plant Effluents on Physical Chemical and Invertebrates Biodiversity in tigris River Southern Baghdad. PH.D Thesis, Department of Biology, College of Science, University of Baghdad, PP 183.
- 27- Siham. N and Ahmed. S (2014). Use of *Cladophora glomerata* Extract Against Multidrug Resistant Bacterial Pathogens. World. J. Pharm. Res. Vol 3, Issue10.
- 28- Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical microbiology reviews, 12(4), 564–582.
- 29- Choudhary,C.;Kumar,C.; Gnad,F.; Nielsen,M.L.; Rehman,M.; Walther,T.C.; Olsen,J.V. and Mann,M.(2009). lysine acetylation targets protein complexes and co-regulates major cellular functions, Journals of science ,V. 325, PP. 834-840.
- 30- Aiyelaagbe, O.O. and Osamudiamen, P.M.(2009). Phytochemical screening for active compounds in mangifera indica leaves from ibadan, Oyo state. PL. Sci. RES., 2: 11-13.
- 31- Sahu ,N.P.; Banerjee, S.; Mondal, N.B. and Mandal, D. (2008). steroidal saponins. in: fortschritte der chemie organischer naturstoffe/progress in the chemistry of organic natural products, fortschritte der chemie organischer naturstoffe/ progress in the Chemistry of organic natural products, V.(89): 45–141.
- 32- Mansuya, P.; Aruna, P. ;Sridhar , S.; Kumar, J.S. and Babu, S. (2010). Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from gulf of mannar region. J. Exp. Sci. 1(8): 23-26.
- 33- Turney, I.; Cadirci, B.H.; Nal, D. and Sukatar, A. (2006). Antimicrobial activities of the extracts of marine algae from the coast of urla (Izmir, Turkey). Turk J. boil, V. 30: 171-175.
- 34- Oumaskour, K.; Boujaber, N.; Etahiri, S. and Assobhei, O.(2013). Antiinflammatory and antimicrobial activities of twenty-three marine red algae from the coast of sidi bouzid (El jadida-Morocco). Int. J. Pharm. Pharm. Sci., V. 5: 145-149.
- 35- Pelaez, F.(2002). screening of antimicrobial activities in red, green and brown macroalgae from gran canaria (Canary islands, Spain),Int. Microbiol., 435-40.
- 36- Mayer, A.M.; Rodriguez, A.D. ; Berlinck, R.G. and Hamann, M.T.(2007). Marine pharmacology in 2003-4: Marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action,Comparative Biochemistry and Physiology ,145 553-581.

الكشف عن بعض المركبات الفعالة من طحلي
Cladophora glomerata & *Chara vulgaris*
المعزولة من مياه القناة في جامعة بغداد

مأرب صالح المولى و أحمد ساهي دويش

قسم علوم الحياة /كلية العلوم /الجامعة المستنصرية/ بغداد-العراق

الخلاصة:

تضمنت الدراسة التعرف على المركبات الفعالة للطحالب الكبيرة المعزولة من البيئة المائية للعراق. عزلت الطحالب (*Cladophora glomerata* & *Chara vulgaris*) من مياه القناة المحيطة بجامعة بغداد خلال الفترة نيسان / 2016. تضمنت الدراسة قياس بعض الصفات الفيزيوكيميائية لمياه القناة المعزول منها الطحلبان, اذ شملت كل من درجة حرارة الهواء والماء , الدالة الحامضية, وتركيز بعض الايونات السالبة والموجبة للاملاح الموجودة في ماء القناة و هي كالاتي : الكالسيوم ,المغنسيوم , الكلوريدات ,نترات , نترت و فوسفات. أظهرت النتائج ان المستخلص الميثانولي الحار والأيثانولي الحار للطحلبان (*Cladophora glomerata* & *Chara vulgaris*) كانت حاوية على مركبات كيميائية فعالة هي: قلويدات , تانينات , صابونيات , راتنجات , تربينات , فينولات و فلافونيدات. وتشير هذه النتائج إلى إمكانية استخدام (*Cladophora glomerata* & *Chara vulgaris*) كمصدر جديد من العوامل المضادة للجراثيم الطبيعية في الصناعات الدوائية.

الكلمات المفتاحية : *Cladophora glomerata* & *Chara vulgaris* , الطحالب الكبيرة والمركبات الفعالة .