Evaluation of Isolated Compounds Activity from *Convolvulus Arvensis* Against Algae

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Abstract

This study includes isolation, purification, and identification of algae from different aquatic environments in Baghdad city. Nine unialgal cultures were obtained. These algal cultures included 6 species of blue-green algae (*Microcystis aeruginosa*, *Microcystis flos-aquae*, *Oscillatoria limnetica*, *Nostoc carneum*, *Westillopesis prolifica*, *Mastigocluds lamiosus*), and 3 species of green algae (*Mougeotia sclaris*, *Scenedesmus dimorphus* and *Chlorella vulgaris*). In addition that aerial parts from *Convolvulus arvensis* were collected. Terpens, alkaloids and phenols were extracted of mentioned plant, and the antialgal activity of extracts types were evaluated in 3 concentrations (5, 10, and 20 mg/ml) by wells and diffused in the agar media. Results showed that phenols extracts from *C. arvensis* were the most effective against isolated algae than other extracts, and terpens extracts were less effective. *O. limentica* was the most sensitive to attack by extracts than other algae, while *N. carneum* was lower sensitive, also *W. prolifica*, *M. lamiosus* were the most resistance to attack by *C. arvensis* extracts.

Chemical composition of phenols and alkaloids were analyzed by High Performance Liquid Chromatography (HPLC) and showed that present 10 phenolic compounds, 8 alkaloid compounds in *C. arvensis.*

Keywords: *C. arvensis*, Extracts, Algal control, Antialgal.

تقيم فعالية المركبات المعزولة من نبات *Convolvulus arvensis* في السيطرة على نمو الطحالب

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الخلاصة

تضمنت هذه الدراسة عزل وتنقية وتشخيص الطحالب من بيئات مائية مختلفة من مدينة بغداد. وتم الحصول على تسعة انواع من الطحالب كمزارع نقية والتي تضمنت ستة انواع من الطحالب الخضر المزرقة *Microcystis aeruginosa* ,*Microcystis flos–aquae* ,*Oscillatoria limnetica* , وثلاثة انواع من *Nostoc carneum* ,*Mastigocludus lamiosus* ,*Westillopesis prolifica* الطحالب الخضر *Mougeotia sclaris* , *Scenedesmus dimorphus*, *Chlorella vulgaris* . فضلا عن ذالك تم جمع الأجزاء ألهوائية من نبات المديد *Convolvulus arvensis*.تم استخلاص كل من التربينات والقلويدات والفينولات من تلك الاجزاء الهوائية للنبات المذكور واختبرت فعاليتها المضادة للطحالب

المعزولة بثلاث تراكيز(10,5, 20) ملغم /مل بطريقة الحفر والانتشار خلال الاكار. أوضحت النتائج إن المستخلص الفينولي من نبات المديد *Convolvulus arvensis* كان الأكثر فعالية ضد الطحالب المعزولة من المستخلصات الأخرى, والمستخلص ألتربيني اقل فعالية. وكان الطحلب *O.limentica* الأكثر حساسيه من الطحالب الأخرى أما *N. carneum* كان الأقل حساسيه. بينما لم يتاثر كل من *M. , W. prolifica lamiosus* بالمركبات المستخلصة من نبات *C. arvensis*. وقد تم تحليل المحتوى الكيميائي للفينولات والقلويدات للنبات المذكور باستعمال جهاز HPLC ولوحظ وجود 10 مركبات فينوليه و 8 مركبات قلويديه في النبات.

Introduction

The development of extensive cyanobacteria and algal blooms was a worldwide problem . Cyanobacteria produce secondary metabolites, which were toxic to a variety of aquatic and terrestrial organisms, including humans. Some species also produce "0ff-flavor" compounds or create filamentous blooms that might block filters used in drinking water supply system [1].Therefore, several recent reviews provide a detailed treatment of algal blooms and their effects [2,3]. Scientists and pharmaceutical industries considered medicinal plants as a good choice, because these natural resources have ordinary fewer side effects, were costless and effective against broad spectrum of antibiotic resistant bacteria. In many parts of the world the extracts of medicinal plants have been used since ancient times [4]. However, plants which contained saponins or tannins compounds were exhibited a promising ability to attack cyanobacterial blooms [5].The aim of this research is to evaluate the antagonistic activity from plant extracts against algae.

Materials and Methods

Sampling: Water samples were taken from different aquatic environments of Baghdad University/Jadiria and reservoirs used for drinking water in Baghdad city. series of dilutions were made.The range of dilution were 10^{-1} to 10^{-10}. Solid and liquid modified Chu -10 and Allen's media were used to isolate algae and incubated in cooled illuminated incubator for 2 weeks. Then transferred of either single cells or filaments into new medium to obtain unialgal culture.Axenic culture was aided by either growing the parent cultures in unidirectional light so that mobile filaments spread out on the plate or by adding an extra agar layer on the top of the growing plate surface so that individual filaments would grow through[6]. Identification of algal isolates down to species level were performed by using the classical phycological texts of Desikachary [7]and Prescott [8] for Cyanophyta and Chlorophyta respectively. The plant samples included the aerial parts of *C. arvensis* Figure 1 ,were collected from the gardens of Baghdad University and different area of Wassit Government during march and april 2011, then washed by tap water and dried at room temperature then storage in clean conditions until use.

Figure 1- Convolvulus arvensis plant Preparation of plant extracts

1-Extraction of crude terpenoids: Crude terpenoids were extracted according to Harborne[8]. A quantity of 10 gm of plant powder was mixed with 200 ml of chloroform and then placed in soxhlet apparatus for 8 hours of extraction. The solution then evaporated at 45°C in rotary evaporator, and were kept in refrigerator until use .

2-Extraction of crude alkaloids: Crude Alkaloids were extracted according to Harborne [8]. 100gm of plant powder was mixed with 350 ml ethanol: D.W. mixture in a ratio of 1:4 in electrical blender for 5 minutes. The solution was filtered through muslin cloth, then through Bekhnar funnel under reduced pressure by using Whattman No.1 filter papers. The supernatant was evaporated at 45 °C in a rotary evaporator, drops of 2% sulphuric acid was added to make pH (1-2), then transferred to a separation funnel

and extracted with chloroform three times. The solution was separated into two layers, the lower layer is chloroform layer, was neglected. The upper layer is the aqueous layer. Concentrated ammonium hydroxide was added to this layer to make pH (9-10), then the solution was extracted in a separation funnel with chloroform: methanol mixture in ratio of 3:1 twice, and one time with chloroform alone. The solution was separated into two layers, the lower layer, chloroform layer or chloroform: methanol layer, was evaporated in a rotary evaporator. The upper layer, the aqueous layer, was evaporated in rotary evaporator, then the extract was kept in refrigerator.

3-Extraction of crude phenols :Crude Phenols were extracted according to Ribereau-Gayon [10] and Harborne [8]. 200gm of plant powder was divided into two equal parts, 300 ml of 1% hydrochloric acid was added to one of them, and 300 ml of D.W. was added to the other, the two quantities were transferred to electrical blender for 5 minutes, then the two mixtures were transferred to boiled water bath for 30-40 minutes, the two mixtures were cooled and filtered through muslin cloth, then transferred to centrifuge with speed of 3000 rpm for 10 minutes, the two supernatant were mixed. Equal quantity of n-propanol was added to the mixture and sodium chloride was added until the solution was separated into two layers. The lower layer extracted in separating funnel with Ethyl acetate, and the solvent layer was collected and evaporated in a rotary evaporator under reduced pressure at 40°C. The upper layer was evaporated in a rotary evaporator at 10 °C the dried material of both layers were mixed and dissolved in 5ml of 96% ethanol, then transferred to oven at 40°C then the extract was kept in refrigerator until use.

Determination of Plant Extracts Antialgal Activity

Wells of each algal lawns were made by using 6 mm in diameter cork borer. Different concentrations: (5, 10, and 20 mg/ml) of different plant extracts from different plant parts were added in the peripheral wells and the medium was added in the middle well as a control. The plates were left in refrigerator until the extracts diffused in the agar, then transferred to the cooled illuminated incubator at 26±1°C and illumination intensity about 200 µE/m²/sec. for 2 weeks. Zones of inhibition were determined.

Analysis of Chemical Composition of The Plants Extracts

The analysis of the chemical composition was made by injected 20 µl from the extract of each sample in High Performance Liquid Chromatogram (HPLC Shimadzu-C-6A) for identification. Procedure used outlined by Hartley and Buchan. In the different separation conditions of The peaks were detected by UV detector. The analysis was carried out in the laboratories of Ministry of Science and Technology. Concentration of each isolated compound was determined by the following equation:

$$\text{Concentration of sample (µg/ml)} = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \text{standard con.} \times \text{Dilution factor}$$

Results

Nine different taxa of algae from different aquatic environments were isolated, purified, and identified. They were included 6 species of blue – green algae (cyanophyta), 3 species of green algae (chlorophyta). These isolates and their classification are shown in Table 1.

Table 1- The isolated algae in this study and their classification.

The isolation	Division	Class	Order	Family
1- Microcystis aeruginosa	Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae
2- Microcystis flos_aquae	Cyanophyta	Cyanophyceae	Chroococcales	Chroococcaceae
3- Oscillatoria limnetica	Cyanophyta	Cyanophyceae	Oscillatoriales	Oscillatoriaceae
4- Nostoc carneum	Cyanophyta	Cyanophyceae	Nostocales	Nostocaceae
5- Westillopesis prolifica	Cyanophyta	Cyanophyceae	Nostocales	Stigonemataceae
6- Mastigocludus lamiosus	Cyanophyta	Cyanophyceae	Stigonematales	Mastigocladaceae
7- Mougeotia sclaris	Chlorophyta	Chlorophyaceae	Zygnematales	Zygnemataceae
8-Scenedesmus dimorphus	Chlorophyta	Chlorophyaceae	Chlorococcales	Chlorococaceae
9- Chlorella vulgaris	Chlorophyta	Chlorophyaceae	Chlorococcales	Chlorococaceae

Plant Extracts Preparation

The aerial parts of *C. arvensis* were extracted for detection of terpens, alkaloids and phenols. The yield of major compounds in extracts was determined in Table 2.

Table 2- Extract compounds yielded from plant parts expresses in %

Plants Name	Type of extract	Yield (%)
Convolvulus arvensis	Terpens	3.8
	Alkaloids	8.90
	Phenols	6.29

Determination of Plant Extracts Inhibitory Activity Against Algae

Results in Table 3 showed that all extracts from *C. arvensis* were revealed significant inhibition against all tested algae except *W. prolifica* and *M. lamiosus* which were very resistant to these types of extracts in all concentrations. However, *N. carneum* was resistant to attack only by terpen compound extracted from this mentioned plant. All other algae were sensitive to attack by all type of extracts in all concentrations Figure 2. Phenols which obtained from *C. arvensis* were have highest antagonist activity against algae. The inhibition zone of *C. arvensis* were (33mm), (32mm) and (31 mm) in diameter when the concentration of 20 mg /ml was used, against *O. limnetica*, *M. sclaris* and *S. dimorphus* respectively, followed by *M. flos-aquae* (26 mm), *C. vulgaris* (25 mm), *M. aeruginosa* (24 mm)and the lowest effects (16 mm) in case *N. carneum*. Alkaloids were have varied activity against algae. The highest effects (29 mm), (25 mm) and (25 mm) against *O. limnetica*, *M. sclaris* and *S.dimorphus* respectively, followed by *M. flos-aquae* (24 mm), *C. vulgaris* (23 mm) and *M. aeruginosa* (21 mm) and the lowest effects (14 mm) in case *N. carneum*, while terpens were have less antagonist activity comparing with phenol and alkaloid compounds. The inhibition zone (28 mm) and (25 mm) against *O. limnetica* and *M. sclaris* respectively, followed by *M. flos-aquae* (22 mm), *S. dimorphus* (21 mm), *C. vulgaris* (19 mm) and *M.aeruginosa*(18mm).

Table 3 - Inhibition zone diameters in mm caused by extracts types (mg/ml) of *Convolvulus arvensis* against selected algae .

Selected algae / Con. mg/ml	Terpens			Alkaloids			Phenols		
	5	10	20	5	10	20	5	10	20
Microcystis aeruginosa	15	18	18	16	18	21	17	22	24
Microcystis flos_aquae	17	18	22	17	20	24	18	23	26
Oscillatoria limnetica	23	25	28	23	26	29	23	28	33
Nostoc carneum			_		12	14	10	13	16
Westillopesis prolifica	_	_	_	_	_	_	_	_	_
Mastigocludus lamiosus	_	_	_	_	_	_	_	_	_
Mougeotia sclaris	19	23	25	19	22	25	23	27	32
Scenedesmus dimorphus	18	19	21	18	21	25	23	26	31
Chlorella vulgaris	16	17	19	16	20	23	17	22	25

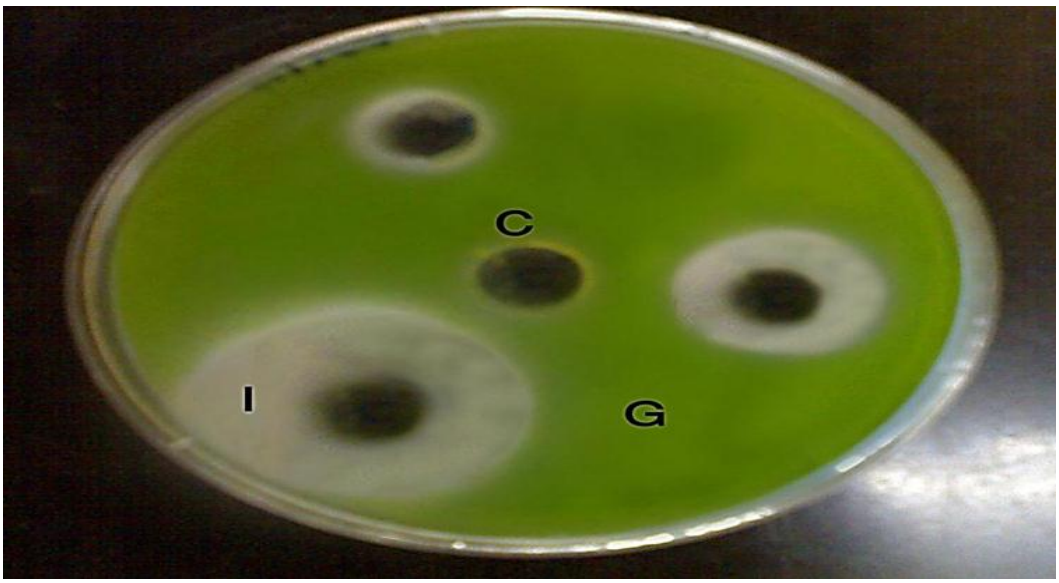

Figure 2- Inhibitory zones caused by plant extracts against algae I : Inhibition zone, C: Control, G: Normal algal growth

Chemical Constituents of The Plants Extracts

Results of HPLC analyses indicated the presence of 10 phenolic compounds in *C. arvensis*. The major phenol constituent in *C. arvensis* was β- Phellandrene (368.17 µg/ml) and Caffeic acid (53.53 µg/ml)was the minor. These compounds concentration showed in Table 4 and Figures 3, 4.

Table 4- Types and concentration of phenolic compounds in *C. arvensis* extracts

Phenolic compounds	Concentration µg/ml
Caffeic acid	**53.53**
Thujone	**197.55**
Cymene	**265.24**
Ferulic acid	**103.17**
Isoferulic acid	**76.77**
Cimiracemoside	**54.31**
B-Phellandrene	**368.17**
Gentisic acid	**191.28**
Leutoline	**69.32**
Coumaric acid	**99.78**
Total concentration µg/ml	**1479.12**

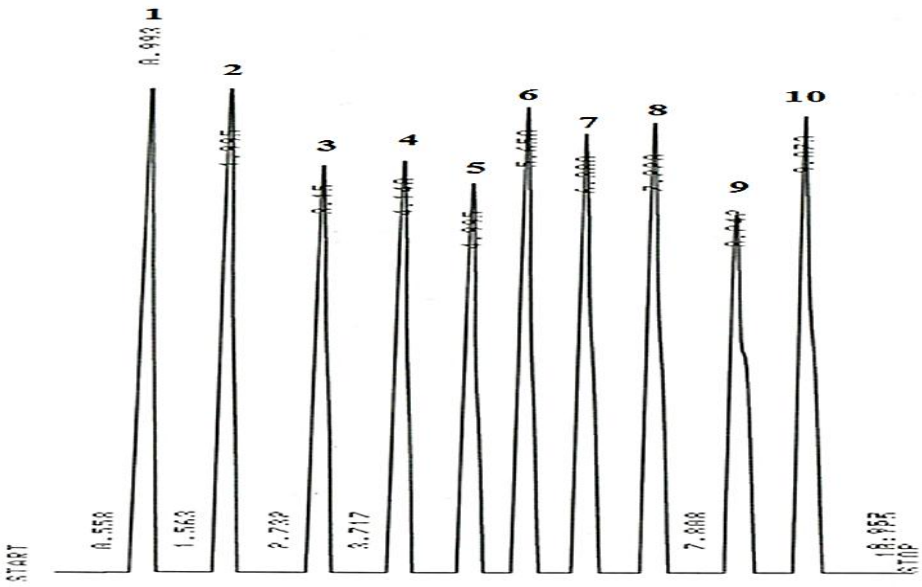

Figure 3- HPLC profile of Phenols Standards (1)Caffeic acid,(2)Thujone ,(3) Cymene, (4) Ferulic acid,(5)Isoferulic acid ,(6) Cimiracemoside, (7) β phellandrene, [8] Gentisic acid, [8] Leutoline , [10] Coumaric acid

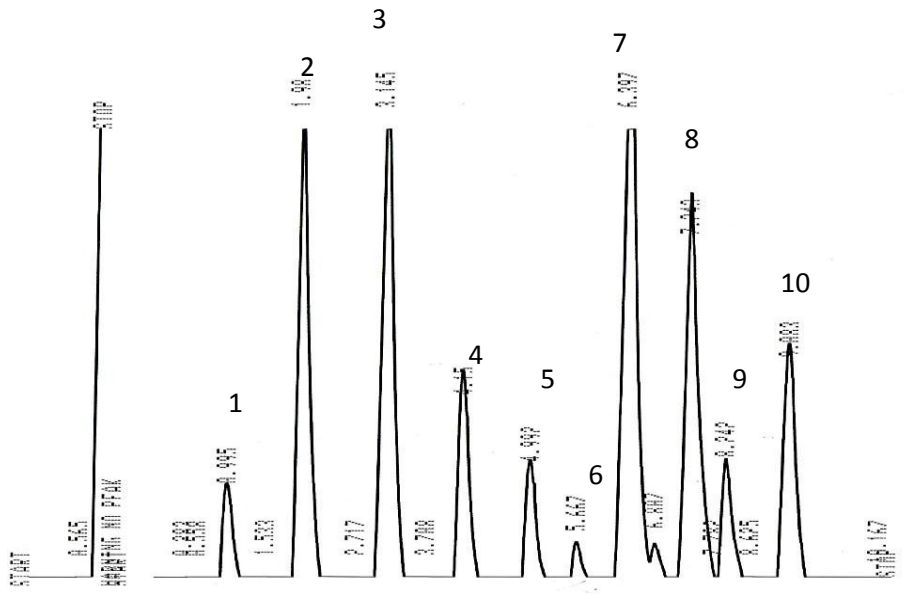

Figure 4- HPLC profile of *Convolvulus arvensis* Phenols (1) Caffeic acid, (2)Thujone, (3) Cymene, (4) Ferulic acid, (5) Isoferulic acid, (6)Cimiracemoside, (7) β – pellandrene, (8) Gentisic acid, (9) Leutoline,(10) Coumaric acid .

The *C. arvensis* having 8 alkaloids and major alkaloid compounds was Swainsonine (438.79 µg/ml),while Austaline (20.10 µg/ ml) was the minor. Table 5 and Figures 5,6.س5,6.

Table 5- Types and concentration alkaloid compounds in *C. arvensis* extracts

Alkaloid compounds	Concentrationµg/ml
Plantyneciene	**211.07**
Fagomine	**111.82**
Swainsonine	**438.79**
1-deoxynojirimycin	**50.61**
Austaline	**20.10**
1-epiaustaline	**59.37**
6-epicastanospermine	**76.87**
Castanospermine-N- oxide	**87.68**
Total concentrationµg/ml	**1056.31**

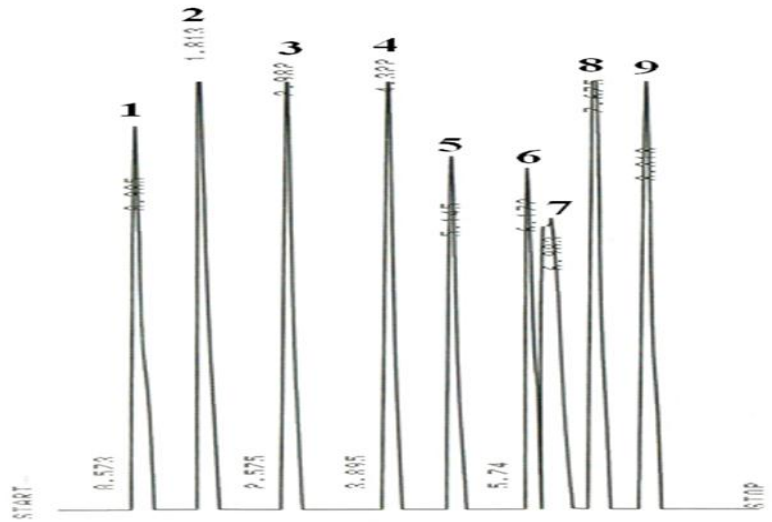

Figure 5- HPLC profile of Alkaloids Standard of *Convolvulus arvensis* (1)Aspastic acid, (2) Plantyneciene , (3) Fagomine , (4) Swainsonine , (5)1-deoxyhojirimycin , (6)Austaline ,(7) 1-epiaustaline ,(8) 6-epicastanospermine , (9) Castanospermine – N – oxide .

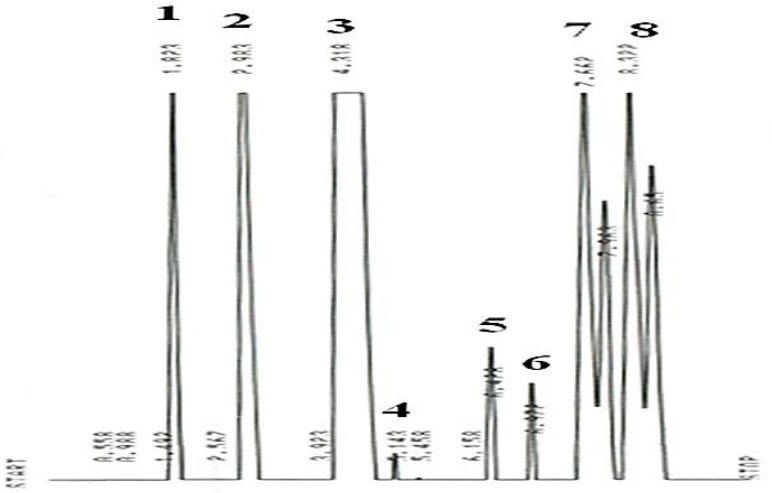

Figure 6- HPLC profile of of *Convolvulus arvensis* Alkaloids (1)Plantyneciene ,(2) Fagomine , (3) Swainsonine , (4) 1-deoxyhojirimycin , (5) Austaline , (6) 1-epiaustaline, (7)6-epicastanospermine, (8) Castanospermine – N – oxide.

Discussion

Previously antibiotics were used to control of the algal growth and this method was not recommended due to the following reasons: destroyed the whole ecosystem. Organisms which live became resistance with long exposure to these antibiotics and this treatment was very costly [6]. However, different methods were attempted to control of the algal growth such as chemical, physical, and biological methods.

Chemical methods included inorganic and organic compounds which have many disadvantages such as copper sulphate, chlorine, Alum, lime [11,12]. Due to copper sulfat caused sudden, algal die off, and oxygen depletion could result as algal dead and the fish will suffocate. However, these chemicals were lysed the algal cells, followed by the release of toxins in to surrounding water which threaten water supplies, and persist in the environment . However, these algicides were toxic to other aquatic micro–organisms and might accumulated in the sediments at harmfull concentration and cause long – term damage to the water environment ecology [11]. The physical methods were very costly [12] .Thus the development of control measures methods such as biological control using (bacteria ,fungi, viruses and protozoa) were established [13,14,15] .

Antialgal substances obtained from these organisms might be less toxic to the environment when compared with chemical methods. In recent efforts to control algae, algicides from natural biomaterials have received an attention as alternative to chemical agents and might therefore offer an environmentally friendly method for control of algal blooms when prevention was not an option [16,17,18].Natural products from terrestrial and aquatic plants reported to have inhibitory effects on growth of phytoplankton species[16,17,19]. Researchers found that different plant extracts or even algal extracts active against algae ,such as using of barley straw in control of algal growth [20].

Most of the previous works were done on these plants concentrated on the crude extracts and their effected against different organisms. However, non of these studies used these plants in the growth control of algae. Many researcher referred that *C. arvensis* have antagonistic activity against yellow fever mosquitoes, antimicrobial, antioxidant and anti-cancer [21,22,23]. Results showed that the alkaloid extract yielded form *C.arvensis* was 8.9% higher than those obtained by Al- Rubaye [24] and Todd et al. [25] which were 0.06% and 0.015% respectively .

This was due to the plant parts used and the place and time of sampling in both studies , while in this study the whole aerial parts were collected during the flowering season from different environment. The variation in quality or composition of the same plant species which could be due to differences in the environmental conditions and genetic variations.

In this study *O. limnetica* was more sensitive to attack than other selected algae such as *N. carneum.* The phenolic extract obtained from *C. arvensis* was 6.29%. This was less than that obtained from the same plant by Elzaawely and Tawata [26] which was 24.17 % or 24.75 %respectively. This was due to the different plant parts used which was only the leave, while in this study the whole aerial parts were used. Results showed that phenols have the highest antagonistic activity followed by alkaloids, while terpens have lower antagonistic activity.The main reason for this antagonistic activity presence of active compounds in phenols such as caffaic acid, cymene, ferulic acid, isoferulic acid, cimiracemoside, gentisic acid and coumaric acid. Nakai et al.,[27] referred that phenols were have active antialgal substances. Kaur and Kalia [23] demonstrated TLC and HPLC of *C. arvensis* which have polyphenolic compounds such as coumarins and phenolic acid. In the fraction of phenolic acid the occurrence of protocatechuic, caffeic, chlorogenic, gentisic, p-coumaric, p-hydroxybenzoic, p-hydroxyphenylacetic, ferulic, vannilic and salicylic acids .

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