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Evaluation of some plant secondary metabolites activity to control algae

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Abstract
This study includes isolation , purification and identification of algae from different aquatic environments in Baghdad.
Two different plant species belonging to different families were selected which were *Citrilluscolocynthus* and *Cordiamyxa* for their fruits and fruits samples respectively.
Crude extracts were extracted from each plant and their antialgal activity were evaluated.Different concentrations (5,15,25) mg/ml of these extracts were prepared and their antagonistic activity was studied, and the resulted inhibition effects in (%) of concentration of chlorophyll(a) after 12 days was evaluated.
Results showed that *Chloroccumhumicola* was the most sensitive to these extracts. However, *Anabaena circinalis* was less sensitive than all other algae used in this study. Also showed that phenol was the most active against algae and terpene had less antagonistic activity comparing with all other extracts.

Keywords: Evaluation, Activity , Algae, metabolites, control

تقييم فعالية نواتج الايض الثانوي لبعض النباتات للسيطرة على الطحالب

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الخلاصة
تضمنت الدراسة عزل وتنقية وتشخيص الطحالب من بيئات مائية مختلفة في بغداد بالاضافة الى ذلك فقد تم جمع الاثمار لنبات الحنظل *Citrulluscolocynthus* واوراق البمبر *Cordiamyxa*.
استخلصت المركبات الخام من تلك الاجزاء للنباتات المذكورة واختبرت فعاليتها المضادة للطحالب المعزولة وصولاً لاستعمالها في السيطرة على نمو الطحالب والتخلص من الاضرار الناتجة منها.
استعملت تراكيز مختلفة من المركبات المستخلصة وهي (5 , 15 , 25) ملغم /مل واختبرت فعاليتها في السيطرة على الطحالب عن طريق قياس O.D للكلوروفيل A بواسطة جهاز الطيف الضوئي Spectrophotometer بعد 12 يوم .
اوضحت النتائج ان الفينول المستخلص كان الاكثر فعالية تجاه الطحال مقارنة بالمستخلصات الاخرى وكان التربين هو الاقل تاثيراً.
كذلك اوضحت النتائج ان المستخلص الناتج من ثمار نبات الحنظل *C.colocynthus* كان الاكثر تأثيراً على الطحالب المدروسة اما من حيث التراكيز المستعملة فقد كان التركيز 25 ملغم /مل هو الاكثر فعالية مقارنة بالتراكيز الاخرى. ويجدر الاشارة هنا ان المستخلصات الناتجة من نبات البمبر *C.myxa* قد حفزت وبشكل واضح نمو الطحالب المدروسة.

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Introduction

In Iraq taxonomic and morphological studies were made on different groups of algae in different aquatic systems[1].

Algae are a single class of plants which lakingleafe , stem, root and reproductive system However ,they were thought that some of them have animals like characteristics so they include in both plant and animal Kingdome. In the previous, it was thought to fit into a single class of plants lacking leaf, stem, root, and reproductive system of higher plants such as mosses, ferns, confers and flowering plants.

However, they were thought that some of them have animals like characteristics so they were included in both plant and animal kingdom. Algae are considered as oxygen producing, photosynthetic organisms that include macro algae and different groups of micro algae mainly seaweeds [2]. Algae occur in different habitats and now become very important plants , they are important providers of many bioactive compounds including plant growth hormons such as gibberellin, auxin, cytokinin,ethylene, abscisic acid and jasminc acid [3]. Many reports discussed harmful blooms of algae as the prime causes of plenty problems in aquatic environments which could be summarized in release number of the most dangerous toxins which were considered as risk to the aquatic organisms generally and human health specifically, in addition to clogging water pipelines and filters [4]. Many scienthists were worked hard to control the harmful algal bloom which included mechanical, physical and chemical methods in addition to biomanipulation[5-7].

Due to the wide uses of plant extracts to minimize and inhibit the bacterial and fungal growth [8], but additional research is wanted to know if it has a rapid breakdown in water that could cause oxygen depletion problems [9]. Therefore,The aims from this studyare to use plant extracts in control or to obtain axenic culture of algae.

Methods were suggested **are:**

1) Natural and cultivated plants were selected for this research.
2) Some algal specics were isolated , purified and identified.
3) Plant extracts efficiency to attack algae will be evaluated.
4) The efficient extracted compounds against algae will be identified.

Materials and Methods:

Different water samples from different aquatic environment were collected and transferred to the laboratory and incutated under controlled suitable conditions for algal growth 200 $\mu E/m^2/$ sec. and 26 $\pm$ 2c^o.

BG11 nutrient solidified medium was used to cultivate algal species. A small part of unialgal culture was transferred which was microscopically confirmed as unialgal culture in BG11 many times to get each specis as axenic [10].

Isolated and purified algal species were identified by using the classical phycological texts [11,12] for cyanophyta and chlorophyta respectively.

The extraction of *Cordiamyxa* leaves and *Citrilluscolocynthus* fruits were prepared by using the soxhlet apparatus for continuous extraction with an ethanol [13].

Evaluation of anti-algal activity of plant extracts by the measurement the optical density of algal Chlorophyll a:

According to The algicidal activity of plant extract were tested at 3 concentrations (5, 15,25%), was monitored throughout 12 days against algae. The plant extract was dissolved in ethanol 80% and added to a flask containing 1ml of alga and 9ml of culture media (BG-11). The culture condition (salinity, temperature, and light) during the experiments were the same. The density of alga was monitored every 2 days by estimating chlorophyll a (indirect method of biomass determination),the pigment can be completely extractable in solvents such as acetone and exhibits characteristic absorbance at 663nm, the estimation of chlorophyll a was estimated as follows: The algal culture was taken and centrifuged at 5000 rpm for 10min. the pellet was washed twice in D.W, thus the pellet was re-suspended in 4 ml of 80% acetone and shake well . Tubes were incubated in a water bath at 60°C for 1 hr. in dark with occasional shaking. The suspension was centrifuged at 5000rpm for 10 min and the supernatant was stored. Absorbance of the supernatant was read at 663nm in U.V. spectrophotometer against 80% acetone as blank. The amount of chlorophyll a in the sample was calculated using the formula .

$$\text{Chl.a} = \frac{A_{663} \times 12.63 \times \text{volume of acetone}}{\text{Volume of sample}} \ \mu g.ml^{-1}$$

A663: absorbance at 663nm.

12.63: correction factor and the amount was expressed as $\mu g.ml^{-1}$.

Plants extracts chemical composition analysis .

Chemical analysis of compounds were made by High performance chromatography (HPLC).

Mobile phase and a mixture of solvent such as water and acetonitrite were consisted in HPLC and the stationary phase comprises of a column which silica particles. 50 µl of sample was injected into the mobile phase and it – passes along the stationary phase.

The retenation time was recorded as one of the characteristic used to identify the compouned.

HPLC with separations conditions all compounds were separated and identified .C-18, 3cm particle size 50x 4.6mm internal diameter of the column.

Ultra violet detections set at 275nm, folw rate 0-7ml/min and 30^{o}c temperature. The differences were in mobile phase which was 0.1% acetic acid and a cetonitrite using linear gradient from 0-100% B in 10 min. with phenolic compounds.

Concentration of sample (µg/ml) = [[Area of the sample/Area of the standard]] × Standard Conc. × Dilution factor

Statistical Analysis

In this study the results of plant extracts of (*C.colocynthus, C. myxa*) in different concentrations and their effects on algal isolates were analysed statistically and the analysis of variance ,F-test ,t-test ,in complete randomized design .Different between means have analyzed by least significant differences (LSD) at $p \leq 0.05$ and expressed as (mean ± SEM) , The letters indicate to comparison between means in each columns , and the similar letters are not significantly different .Using spss program 2010 and excel application to find the result and draw the figures with some effects was determind[14].

Results

Isolation and identification of algae

Ten different taxa of algae were isolated, purified, and identified from Tigris River and canal around Al- Jadriah. They were included 4 species of blue-green algae,5 species of green algae, and 1 species of diatoms, as they are shown in Table-1.

Table1-The isolated algae in this study and their classification.

Isolated algae		Division	Class	Order	Family
1	*Microcystisaeruginosa*	Cyanophyta	Cyanophyaceae	Chroococcales	Chroococcaceae
2	*Calothrixbraunii*	Cyanophyta	Cyanophyceae	Nostocales	Rivulariaceae
3	*Anabaena circinalis*	Cyanophyta	Cyanophyceae	Nostocales	Nostocaceae
4	*Nostoc commune*	Cyanophyta	Cyanophyaceae	Nostocales	Nostocaceae
5	*Kirchinella*sp.	Chlorophyta	Chlorophyceae	Chlorococcales	Oocystaceae
6	*Pediastrumboryanum*	Chlorophyta	Chlorophyceae	Chlorococcales	Hyrodictyaceae
7	*Scenedesmusquardicauda*	Chlorophyta	Chlorophyceae	Chlorococcales	Scenedesmaceae
8	*Chlorella vulgaris*	Chlorophyta	Chlorophyceae	Chlorococcales	Oocystaceae
9	*Naviculaanglica*	Chrysophyta	Bacillariophaceae	Pennales	Naviculaceae
10	*Chlorococcumhumicola*	Chlorophyta	Chlorophyceae	Chlorococcales	Chlorococcaceae

Plant extracts preparation

For the same reason ,the leaves of, *Cordiamyxa,* and the fruits of*Citrulluscolocynthus* were extracted by Soxhlet(ethanol 80%) to obtain crude extracts. The yeild of extracts was determined (Table -2). The yeild of the highest yield of crude extracts was 14.3% (v/w) and the lowest 10% (v/w) were obtained from *C. colocynthus*.

Table 2-Yields of the extracts obtained from plants involved in this study.

Plant	Family	Type of extract	Yield of the extract % (v/w)
1.Citrullus colocynthus	Cucurbitaceae	Crude ethanol extract	14.3
2.Cordia myxa	Boraginaceae	Crude ethanol extract	13.6

Evaluation of extracts inhibitory effects against algae

The algicidal activity of *Citrullus.colocynthus,* and*Cordia.myxa* alcoholic crude extracts were tested at 3 concentrations (5, 15, 25%) was monitored throughout 12 days against these four algae. The crude extracts were dissolved in DMSO (Dimethyl sulfoxide) and add to the flask containing 1ml of alga and 9ml of culture media (BG11). The culture condition (PH, temperature, and light) during the experiments were the same. The highest concentration of DMSO used in the experiment equaled to the volume of crude extract. The density of alga was monitored every 2 days by progressed along with time of the experiment reaching to minimum amount of chlorophyll . However, growth rates of control (alga without added extracts) representing by chlorophyll-a concentration were exponensially increased along with time.The inhibitory effects of the *C.colocynthus,* on the tested algaeincreased as the time and concentrations of the crude extracts increased, while *C.myxa*crude extract showed stimulatory effect on the tested algae increased along with time and extract concentration as shown in Tables-(3,4,5and 6) .Tables-(3,4,5and 6) were showed that the concentration of chlorophyll a varied according to algal species, origin of the extracts and their concentrations.The results showed that in general, the inhibitory effect was highest effect in *C.colocynthus* on25% concentration. While *C.myxa*had enhancement effect with all tested algae. The most sensitive algae was*C.humicola,* While the most resistance algae was*A.circinalis.*

Different results were achieved toward different treatment of extracts against *Chlorella.vulgaris* as shown in Table-3,the interaction between *C.colocynthus*extract at 25% concentration was significant and showed the highest inhibition effect (1.035%) among other treatments, and *C.myxa*had enhancement effect (1.821%). While 15% of*Citrulluscolocynthus* extract also showed the highest inhibition zone among other treatments (1.049%) and *C.myxa*extract had enhancement effect (1.721%). However 5% *C. colocynthus* extract showed (1.080%) and *C.myxa*extract gave enhancement effect (1.671%).Results obtained by using the spectrophotometer to determine the optical density of chl.(a) in each algae after 2-12 days as in *C.vulgaris*were shown in Figures-(1, 2).In these Figures it was obviously cleared that 25% of *C.colocynthus*was the most effective against *C.vulgaris*. While *C.myxa*has enhancement effect on this algae and this effect increased with the increasing of concentration as shown in Figure-2.

Table 3-Variation in chlorophyll a of *Chlorella vulgaris* treated with the studied plant crude extract concentrations comparing with algae free from extract (standard) along with time.

Concentration of chlorophyll a of *Chlorella vulgaris* (mg/ml) along with time	**Concentration of *Citrulluscolocynthus* crude extract %**			
	0.0(standard)	5	15	25
0 day. (initial)	1.135	1.133	1.121	1.113
2 day	1.168	1.132	1.110	1.101
4 day	1.240	1.129	1.096	1.095
6 day	1.309	1.131	1.120	1.110
8 day	1.372	1.102	1.075	1.061
10 day	1.401	1.097	1.063	1.042
12 day	1.637	1.080	1.049	1.035
8 day	1.372	1.105	1.093	1.082
10 day	1.401	1.093	1.054	1.065
12 day	1.637	1.082	1.050	1.042
Concentration of chlorophyll a of *Chlorella vulgaris*(mg/ml) along with time	**Concentration of *Cordiamyxa* crude extract %**			
	0.0(standard)	5	15	25
0 day. (initial)	1.135	1.139	1.141	1.152
2 day	1.168	1.165	1.169	1.173
4 day	1.240	1.250	1.265	1.285
6 day	1.309	1.307	1.312	1.321
8 day	1.372	1.374	1.383	1.395
10 day	1.401	1.415	1.422	1.452
12 day	1.637	1.671	1.721	1.821

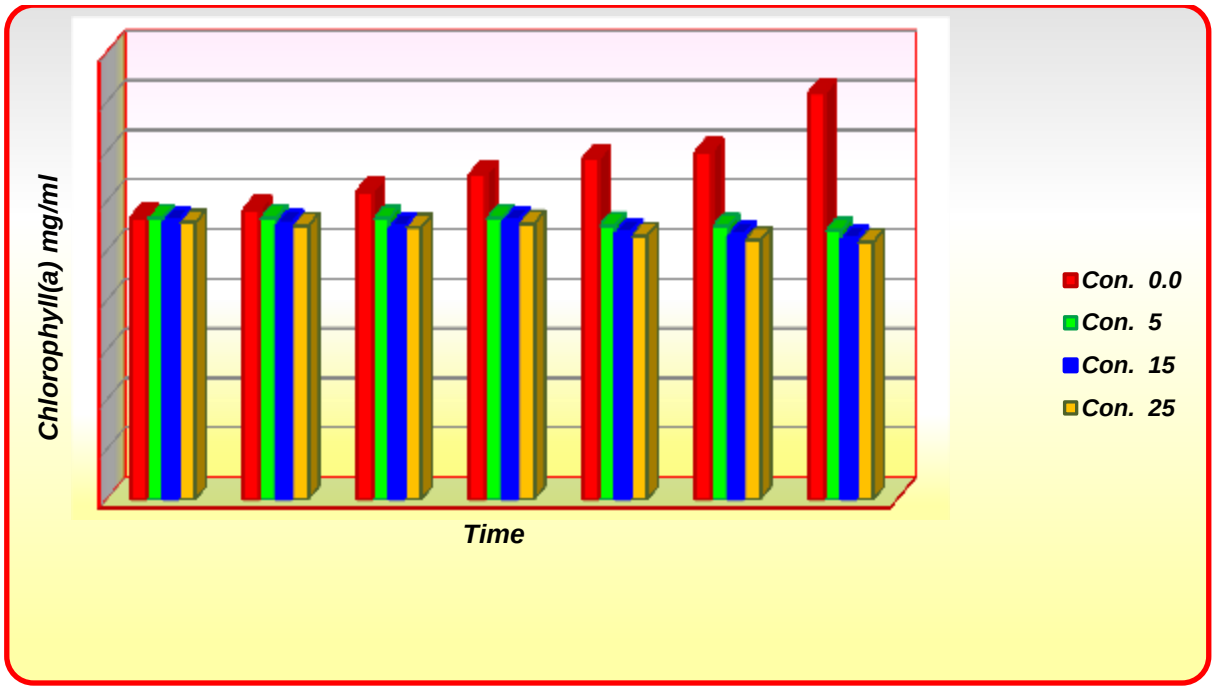

Figure 1-Statistical analysis of the results of three concentrations of *C.colocynthus*in control of *C.vulgaris*during 12 days.

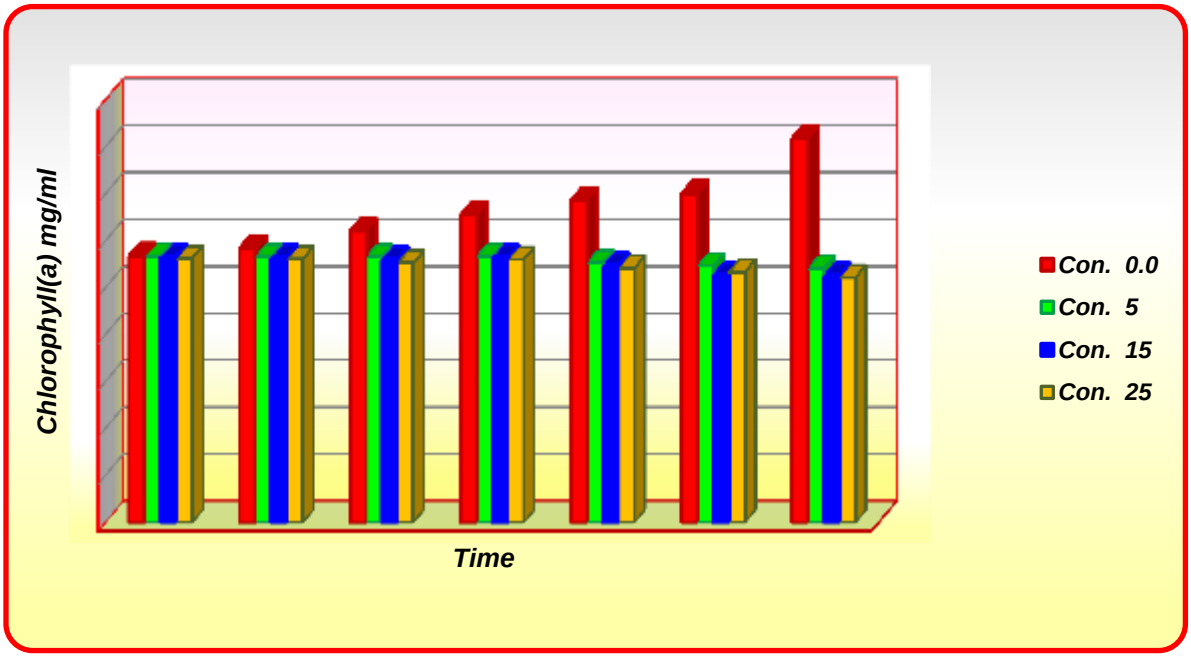

Figure 2- Statistical analysis of the results of three concentrations of*C.nilotica*in control of *C.vulgaris*during 12 days.

The results in Table-4 showed that the growth inhibition of*Chlorococcumhumicola*was very high in all plants used in this study except *C.myxa*plant extract which showed enhancement growth particularly in 25% concentration of crude extract and it was less enhancement effect in other less concentration.*C.colocynthus*extract had an antagonistic activity against *Chlorococcumhumicola*at 25% concentration and the highest inhibition effect was(0.009%) comparing with other concentration, and finally *C. myxa*also had enhancement effect on *Chlorococcumhumicola*(1.596%). However, 15%*C. Colocynyhus*extract also showed the highest inhibition effect among other treatments (0.025%) and *C. myxa*had enhancement effect (1.573%). While 5% the *C.colocynthus*extract showed (0.052%), but *C.myxa*had enhancement effect (1.554%).

Results obtained by using the spectrophotometer to determine the optical density of chl.(a) in each algae after 2-12 days as in *C.humicola*were shown in Figure-3.In these Figures it was obviously cleared that 25% of *C.colocynthus*was the most effective against *C.humicola*and the 5% of *P.australis*was the less effective compared with other extracts . While *C.myxa*has enhancement effect on this algae and this effect increased with the increasing of concentration as shown in Figure-4
According to the statistical analysis which shown in appendix (5,6,7,8), many significant differences appeared between the different concentrations and other differences appeared among the extracts that used to treat *Chlorococcumhumicola*atp≤ 0.05 **.**

Table 4-Variation in concentration of chlorophyll a of *Chlorococcumhumicola* treated with the studied plant crude extract concentrations comparing with algae free from extract (standard) along with time.

Concentration of chlorophyll a of *chlorococcumhumicola*(mg/ml) along with time	**Concentration of *Citrulluscolocynthus* crude extract %**			
	0.0(standard)	5	15	25
0.0 day (initial)	1.120	1.121	1.118	1.115
2 day	1.146	1.109	1.102	1.095
4 day	1.206	1.102	0.093	1.052
6 day	1.280	1.106	0.165	1.091
8 day	1.359	0.082	0.043	0.013
10 day	1.398	0.077	0.032	0.010
12 day	1.529	0.052	0.025	0.009
Concentration of chlorophyll a of *chlorococcumhumicola* mg/ml) along with time	**Concentration of *Cordiamyxa* crude extract %**			
	0.0(standard)	5	15	25
0.0 day (initial)	1.120	1.120	1.132	1.142
2 day	1.146	1.146	1.149	1.163
4 day	1.206	1.212	1.223	1.227
6 day	1.280	1.285	1.292	1.325
8 day	1.359	1.367	1.379	1.396
10 day	1.398	1.410	1.423	1.462
12 day	1.529	1.554	1.573	1.596

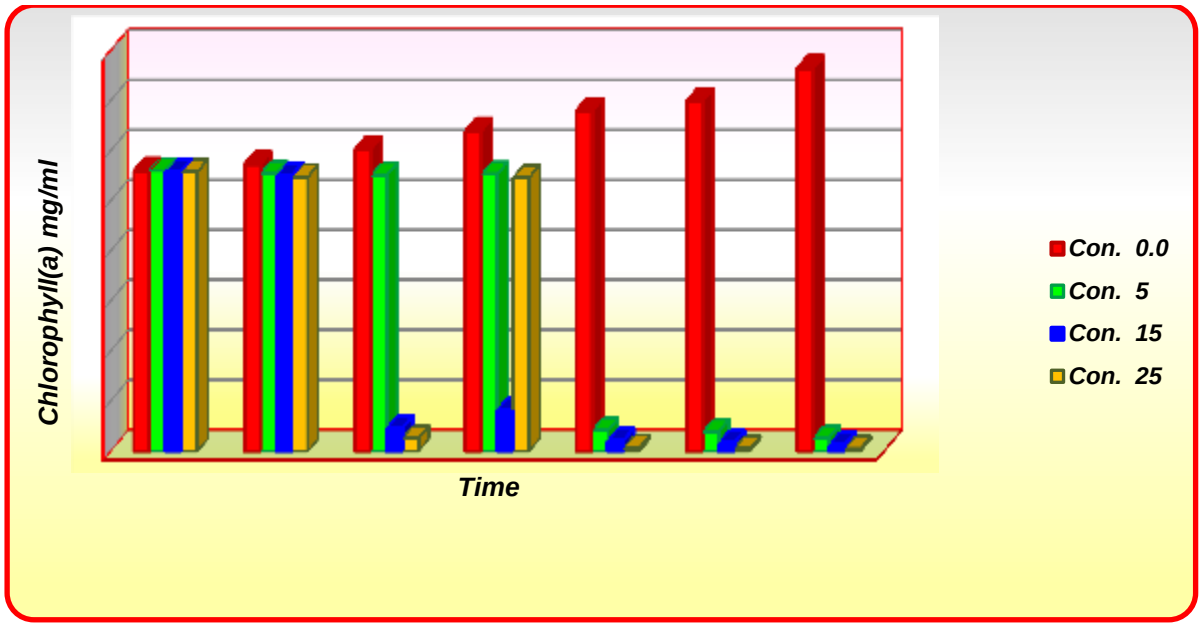

Figure3-Statistical analysis of the results of three concentrations of *C.colocynthus*in control of *C.humicola*during 12 days.

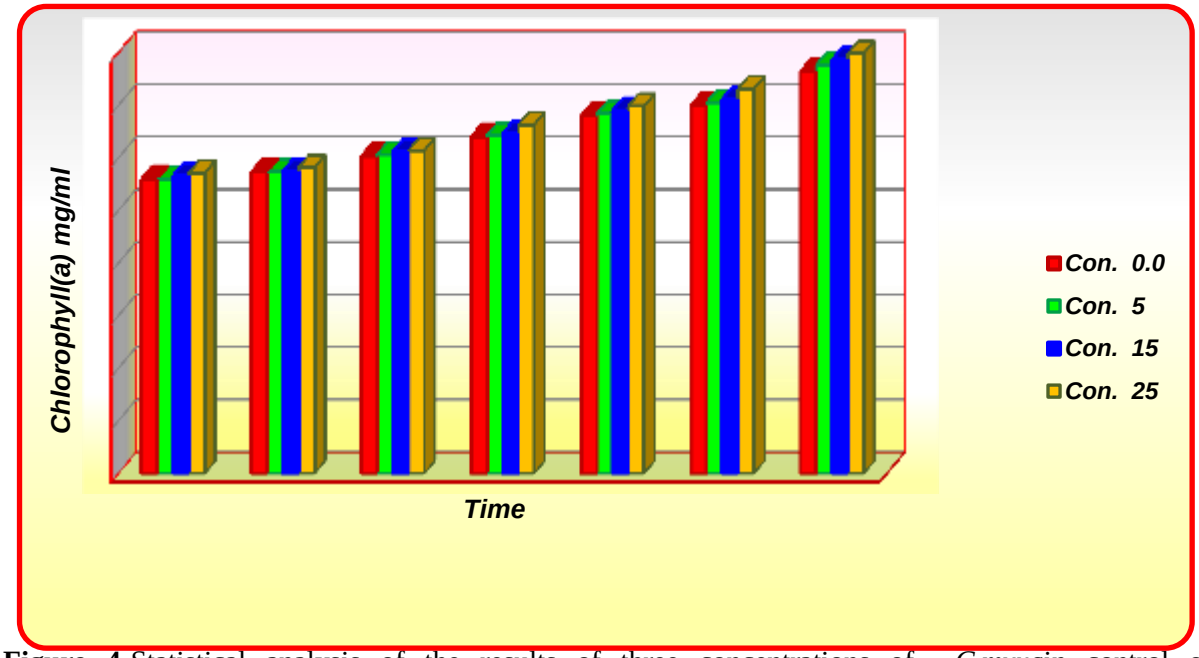

Figure 4-Statistical analysis of the results of three concentrations of *C.myxa*in control of *C.humicola*during 12 days.

Results in Table-5 showed that the interaction between *C.colocynthus* extract at 25% concentration was significant and showed the highest growth inhibition effect (0.052%) among other treatments against *Anabaena circinalis*and *C.myxa*(0.401%) hadenhancement effect .While 15% *C.colocynthus*extract showed (0.091%) the highest inhibition effect among the other treatments and*C.myxa*(0.359%) had enhancement effect .However, 5% *C.colocynthus* extract showed (0.098%) and*C.myxa*(0.321%) had enhancement effect.Results obtained by using the spectrophotometer to determine the optical density of chl.(a) in each algae after 2-12 days as in *A.circinalis*which was shown in Figures- (5, 6).In these Figures it was obviously cleared that 25% of *C.colocynthus*was the most effective against *A.circinalis*.While *C.myxa*has enhancement effect on this algae and this effect increased with the increasing of concentration as shown in Figure-6.

According to the statistical analysis which shown, many significant differences appeared between the different concentrations and other differences appeared among the two extracts that used to treat *Anabaena circinalis*atp≤ 0.05 **.**

Table 5-Variations in concentration of chlorophyll a of *Anabaena circinalis* treated with the studied plant crude extract concentrations comparing with algae free from extract (standard) along with time.

Concentration of chlorophyll a of *Anabaena circinalis* (mg/ml) along with time	**Concentration of *Citrulluscolocynthus* crude extract %**			
	0.0(standard)	5	15	25
0.0 day (initial)	0.180	0.181	0.178	0.175
2 day	0.206	0.176	0.160	0.162
4 day	0.224	0.163	0.155	0.153
6 day	0.250	0.171	0.162	0.159
8 day	0.257	0.136	0.127	0.107
10 day	0.262	0.112	0.103	0.075
12 day	0.271	0.098	0.091	0.052
Concentration of chlorophyll a of *Anabaena circinalis* (mg/ml) along with time	**Concentration of *Cordiamyxa* crude extract %**			
	0.0(standard)	5	15	25
0.0 day (initial)	0.180	0.180	0.183	0.185
2 day	0.206	0.209	0.215	0.223
4 day	0.224	0.226	0.234	0.249
6 day	0.250	0.253	0.266	0.283
8 day	0.257	0.271	0.287	0.295
10 day	0.262	0.295	0.302	0.321
12 day	0.271	0.321	0.359	0.401

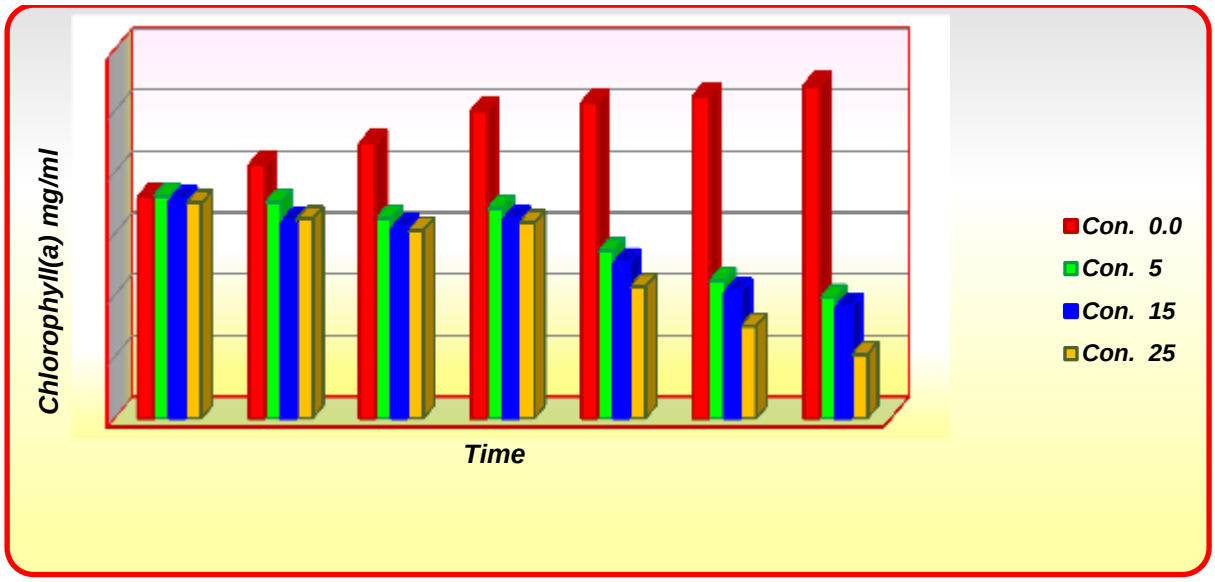

Figure 5-Statistical analysis of the results of three concentrationsof *C.colocynthus*in control of *A.circinalis*during 12 days.

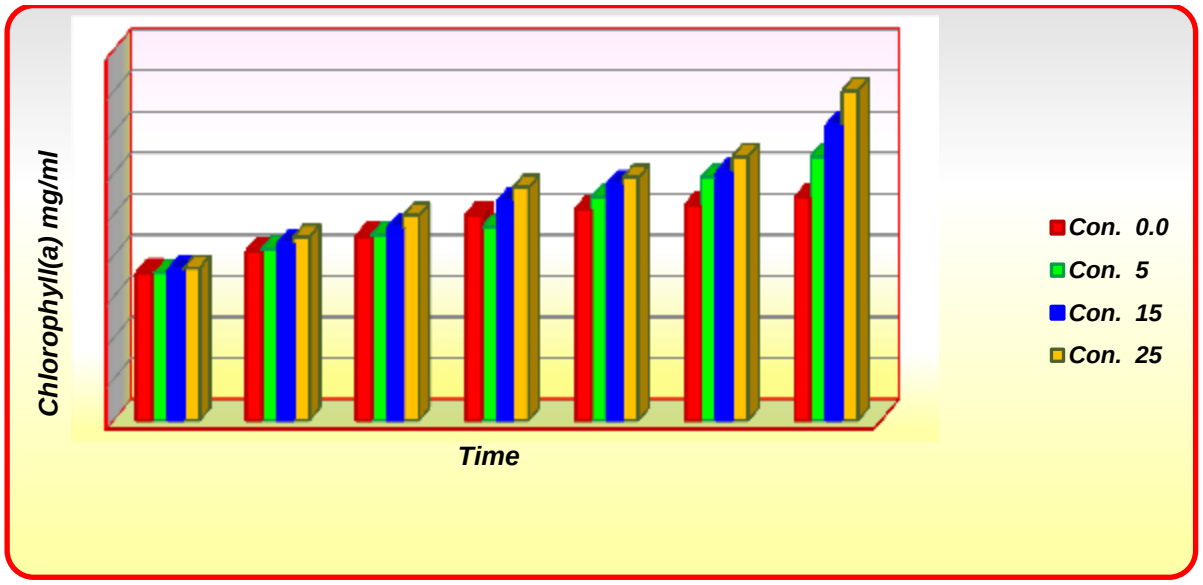

Figure 6-Statistical analysis of the results of three concentrations of *C.myxa*in control of *A.circinalis*during 12 days.

Result in Table-6 showed that *C.colocynthus*extract showed the highest inhibition effect (0.075%) among other treatments at 25% concentration against *Microcystisaeruginosa*,and *C.myxa*(0.602%) had enhancement .

WhileC.colocynthus(0.121%) *and C.myxa*(0.495%) had enhancement. 5%by *C.colocynthus*(0.151%) *Cmyxa*no inhibition zone but had enhancement effect (0.434%) . Results obtained by using the spectrophotometer to determine the optical density of chl.(a) in each algae after 2-12 days as in *M.aeroginosa*were shown in Figures- (7, 8).

In these Figures it was obviously cleared that 25% of *C.colocynthus*was the most effective against *M.aeroginosa*. While *C.myxa*has enhancement effect on this algae and this effect increased with the increasing of concentration as shown in Figure-8.

According to the statistical analysis, many significant differences appeared between the different concentrations and other differences appeared among the four extracts that used to treat *Microcystisaeruginosa*atp≤ 0.05

Table 6-Variation in concentration of chlorophyll a of *Microcystisaeruginosa* treated with the studied plant crude extract concentrations comparing with algae free from extract (standard) along with time.

Concentration of chlorophyll a of *Microcystisaeruginosa*(mg/ml) along with time	**Concentration of *Citrulluscolocynthus* crude extract %**			
	0.0(standard)	5	15	25
0.0 day (initial)	0.241	0.230	0.203	0.201
2 day	0.290	0.223	0.201	0.182
4 day	0.342	0.202	0.192	0.169
6 day	0.391	0.215	0.197	0.171
8 day	0.421	0.181	0.160	0.122
10 day	0.457	0.163	0.143	0.112
12 day	0.482	0.151	0.121	0.075
Concentration of chlorophyll a of *Microcystisaeruginosa*(mg/ml) along with time	**Concentration of *Cordiamyxa* crude extract %**			
	0.0(standard)	5	15	25
0.0 day (initial)	0.241	0.250	0.253	0.282
2 day	0.290	0.272	0.292	0.302
4 day	0.342	0.295	0.312	0.365
6 day	0.391	0.321	0.351	0.402
8 day	0.421	0.370	0.392	0.471
10 day	0.457	0.395	0.421	0.523
12 day	0.482	0.434	0.495	0.602

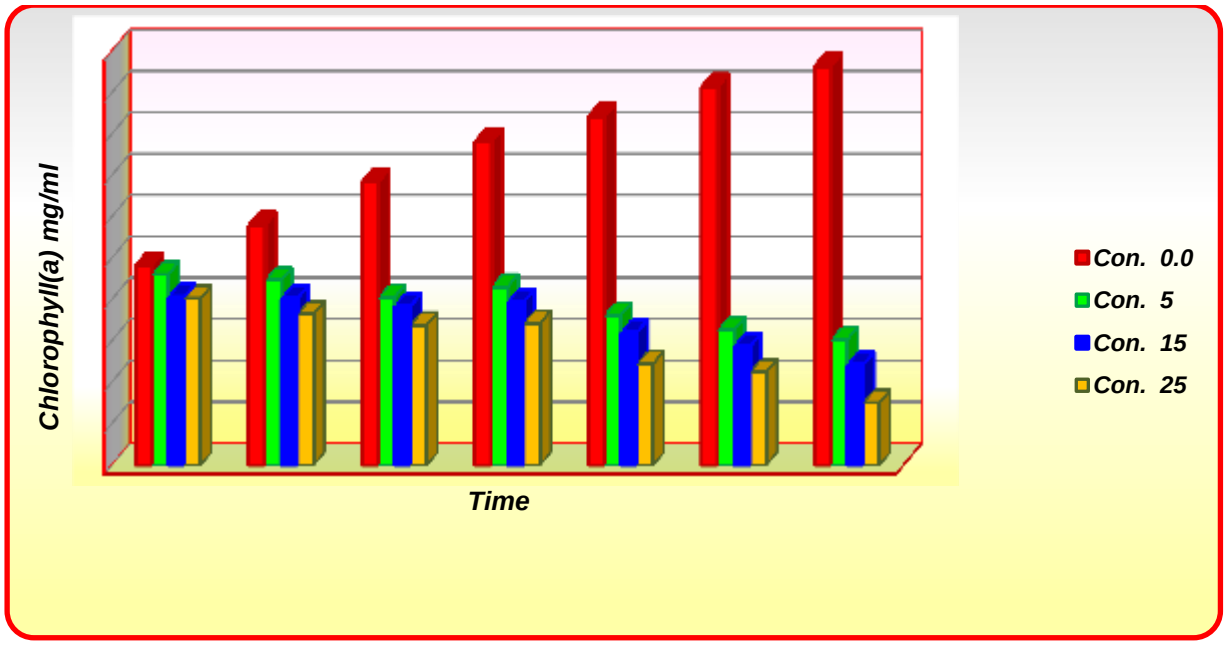

Figure 7-Statistical analysis of the results of three concentrations of *C.colocynthus*in control of *M.aeruginosa*during 12 days.

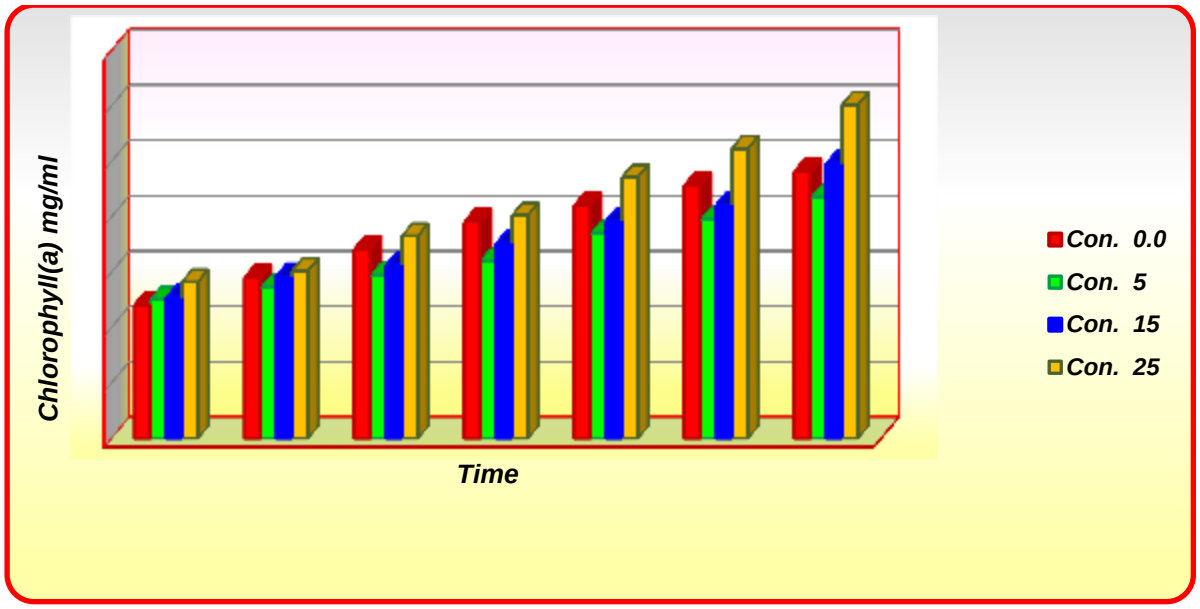

Figure 8-Statistical analysis of the results of three concentrations of *C.myxa*in control of *M.aeruginosa*during 12 days.

Chemical constituents of the plant extracts

Results are shown important and significant differences between different plantextracts used in this study and even their different compounds terpen, phenol and alkaloid or even between their total concentrations .However, the total concentration in the extract of *C. colocythus*, and *C. myxa* were 240.25, 155.42 µg / ml respectively. The major terpenin the extract of *C.colocynthus* wasTerpinene(1422.74 µg /ml) and B-Cymmene (16.43 µg /ml) was the minor.~ - bisabollene (1659.35 and 697.47 µg /ml) were the major terpen , while Mesembrine (76.40 µg/ml) was the minor *C. myxa* extracts .Figures-(6,7,8 and9)which shown the stardandterpens and those present in each extract. Table-7 shows the highest in the number of terpen compounds which contained and the highest in their total concentration in comparision with *C.myxa extract.*

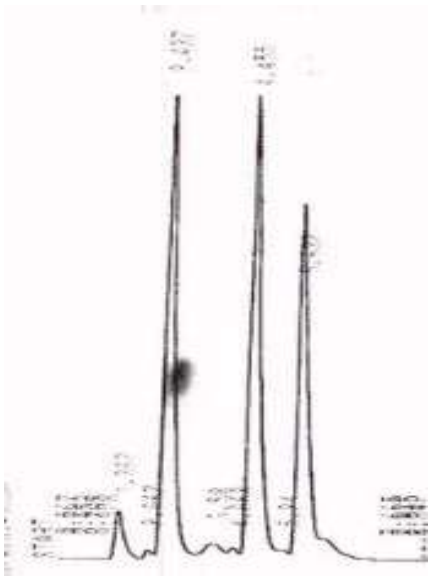

Figure 6-HPLC profile of terpenestandards for*C.colocynthusterpe*

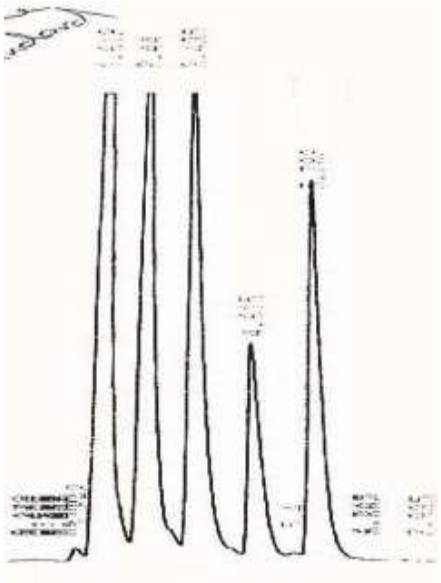

Figure 7- HPLC profile of *C.colocynthus*

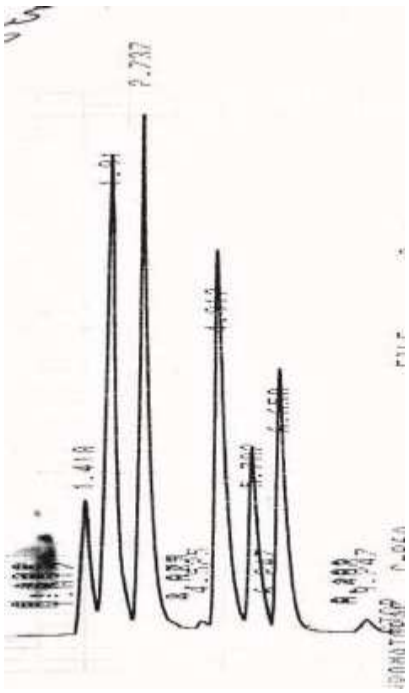

Figure 8- HPLC profile of ofterpene standaeds for C.myxa

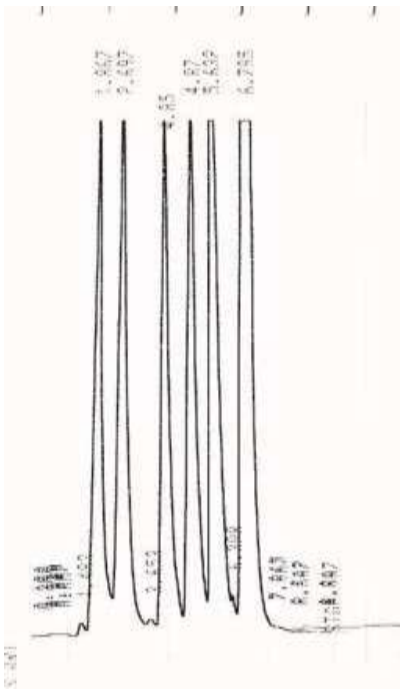

Figure 9-HPLC profile of C.myxaterpene

Table 7-Terpene compounds found in studied plant extracts:

Plants / Terpene	*C.colocynthus*	*C.myxa*
Camphene	25.19	266.75
B-pinene	270.20	____
B-cymmene	16.43	____
Terpinene	1422.74	____
~-phellandrene	507.64	____
~-pinene	____	265.50
Farnasal	___	
B-caryophyllene	-----	185.97
~-bisabollene	_____	697.47
Mesembrine	_____	76.40
Carvone	____	

Alkaloids present in the extracts from the selected plants were also identiHPLC, as elaborated in Table-8 and the peaks in Figures- (9,10,11and 12). The total concentration of alkaloids in the extracts of *C.colocynthus,* and *C.myxa*were 431.47,383.61 µg/ml respectively.
Colocynthin(829.59 fig/ml) were the major alkaloid, while Cucurbitaciri C (68.06 µg/ml) was the minor in the *C.colocynthus*extract, it was found thatMiColocynthin present only in these extract and it was not existed in the other extracts . However, Ephedralone(636.40 ,ug/ml) was the major alkaloid,Ergotamme(707.68).However, Echimidine (271.96) was the major alkaloidwhileMonocrotamine the minor alkaloid in *C.myxa*extract,

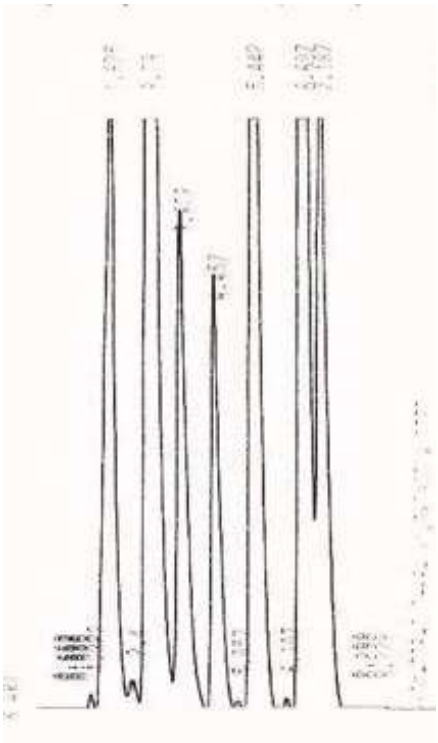

Figure 9- a- HPLC profile of alkaloid standards for*C.colocynthus*alkaloid

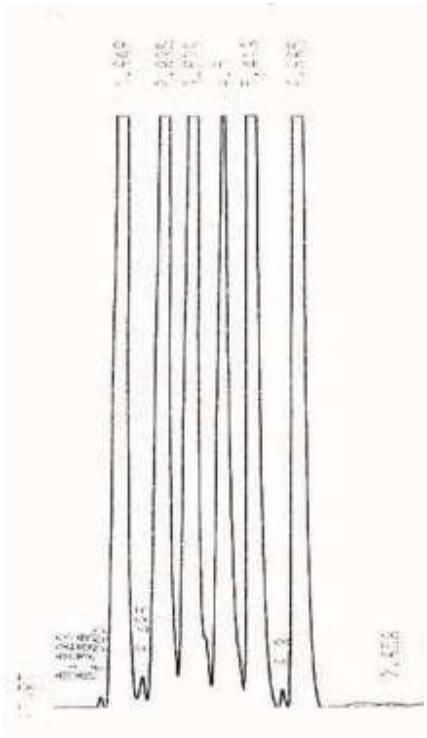

Figure10- HPLC profile of *C.colocynthus*

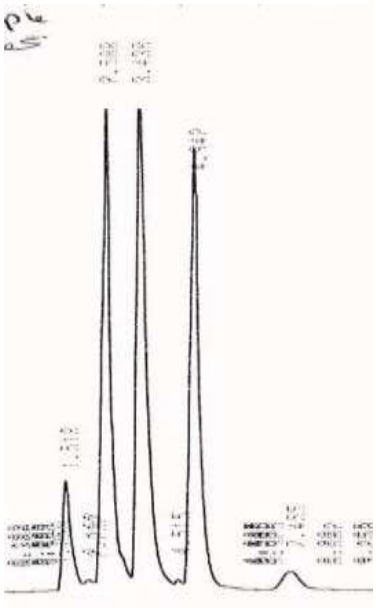

Figure 11- HPLC profile of alkaloid standards for *C.myxa*alkaloid

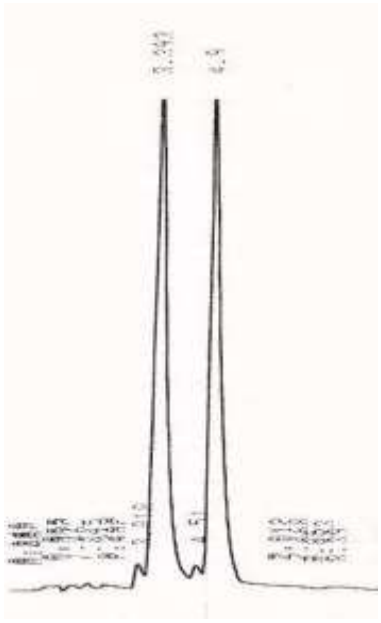

Figure12- HPLC profile of *C.myxa*

Table 8-alkaloid compounds found in studied plant extracts:

Plants	***C.colocynthus***	***C.myxa***
Citrullol	221.09	____
CucurbitacinA	628.63	____
CucurbitacinB	187.41	____
CucurbitacinC	68.06	____
Colocynthin	829.59	____
Colocynthitin	581.27	____
Ephedralone	____	____
Niloctin	____	____
Ergocristine	____	____
Ergotamine	____	____
Echimidine	____	271.96
Monocrotamine	____	217.16

Phenolic compounds were the third secondary metabolic compounds in the studied extracts which identified. The total concentration of phenolic compounds in the extracts of *C.colocynthus*, and *C.myxa* were 1081.34,and 583.49 (µg/ml) respectively. Isovitexin (531.05 µg/ml) was the major phenolic compound, while OH-Benzylisovitexin (332.27 µg/ml) was the minor in the *C.colocynthus* extract. Ferulic acid (776.85 µg/ml) was the major phenolic compound.Gallic acid (179.92 µg/ml) and Allantoin (1522.83 µg/ml) were the major phenolic compound, while Coumarins 6.55 (µg/ml) and 115.42 (µg/ml) were the minors in *C.myxa* extracts. as showed in Table-9 in addition, HPLC peaks of the phenols of each extract as showed in the Figures-(13,14,15and16).

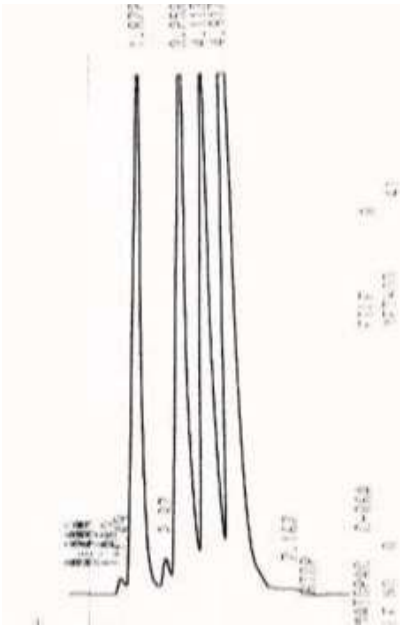

Figure 13-HPLC profile of phenold standards for*C.myxa* phenol

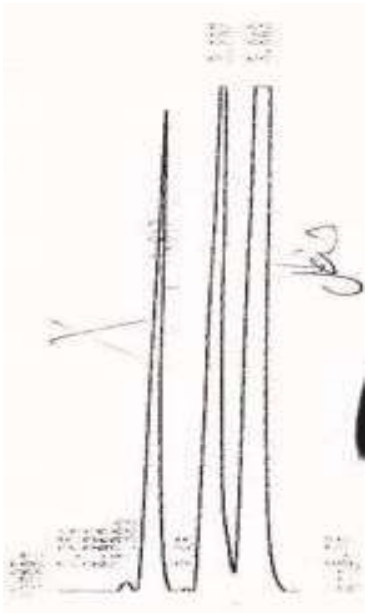

Figure 14- HPLC profile of *C.colocynthus*

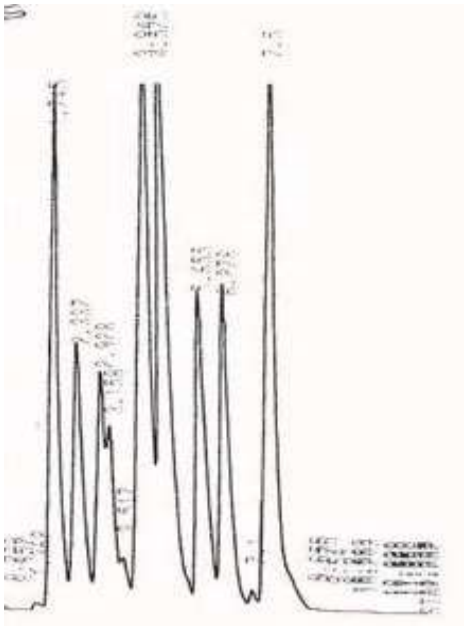

Figure 15-HPLC profile of phenold standards for *C.myxa*.

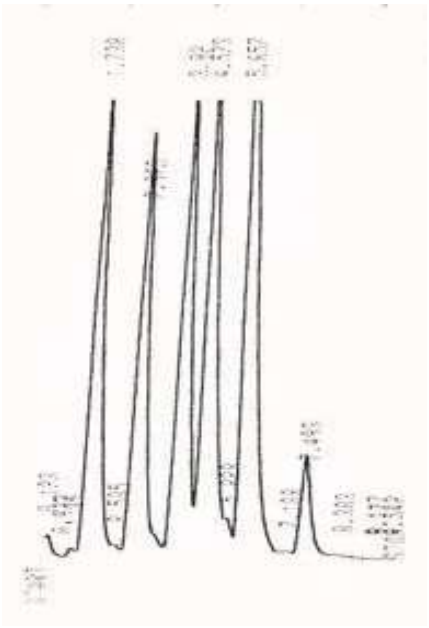

Figure16- HPLC profile of *C.myxa*

Table 9-phenol compounds found in studied plant.

Plants / phenols	***C.colocvnthus***	***C.mvxa***
OH-Benzyl isovitexin	332.27	_____
Isovitexin	531.05	_____
Tannin	__ ___	_ ____
Tamarixen	____	____
Nilotinin	_____	____
Hirtellins	____	____
Ferulic acid	____	____
Tetapathine	____	____
Kaempferol	____	____
Methoxymethy l gallate	____	____
Gallic acid	____	____
Furfurol	____	____
Genestic acid	____	____
Coumarins	____	115.42
Nervonic acid	____	374.07
Rosmaric acid	____	310.67
Allantoin	____	1522.83
Chlorogenic acid	____	324.65
4-hydroxy cinamic Acid	_____	545.40

Discussion

Algal isolates obtained in the present research are represent the whole algae present in the sampling areas selected and this was possibly due to the culture media used or to other environmental circumstanes adjusted in this stydy . Many algal specis to be isolated need typical environmental availalble in the culture media [15].Al-Haidari[16] isolate 20 algal species as unialgal cultures[17] isolated 9 algal species as unialgal cultures, and [18] also succeeded in isolating 9 algal species as unialgal cultures.All samples of the previous studies were collected from the same environmental places which used in this study.

Harmful algal blooms such as *Anabaena circinalis*, *Microcystisaeruginosa,*and *Nostoc strains* which have components produced to the water environment and cause toxicity to all users of contaminant water with these toxins.

The most successful one was the addition 0.1 g\l of Nystatine to control fungal contamination and 0.2 g\l actidione to control bacterial growth [10]. But the most difficult and nuisance was to get algal cultures free from other living organisms, in other hand getting axenic cultures of the isolated algae was the most complicated due to the complex interactions and relationships between algae and other organisms in the culture.

Plant extract yield and their chemical constituentes

Results were showed that *C.colocynthus* crude extract yield was 14.3%, which is approximately the same as that extracted which was 13.5%. The extract yield of *C.myxa* in this study was 13.6% which different from *M.spicata* extracted by [19] and was 2% of spearmint essential oil.

Results of HPLC of the *C.colocynthus*and*C.myxa* crude extracts referred to present of terpens, alkaloids and phenols, prescence of alkaloids, terpens and phenols in those three crude extracts due to purity of these extracts was not reach to the obsolute, inspite of repeating the extraction more than one time so it needs high performance equipments with repeating the extraction several times.

Antimicrobil activity of plants extracts depends upon their active components.

However, the biological effect is often due to a synergy between the compounds [20]. Thus, the extracts in this study were analyzed in a screening for the most active compounds (terpens, phenols and alkaloids) that most topics and studies referred that antimicrobial activity due to them.

In the case of terpens it was reported that β-pinenes has antimicrobial activity and β-pinenes was found in all extracts involved in this study. The mechanism through which the terpens β-pinenes which present in the extracts in this study was active against yeast or bacteria lies mainly in their capacity to induce toxic effects on the membrane structure and functions [21]. [22] showed that due to their lipophilic character, cyclic monoterpenes will preferentially partition from an aqueous phase into membrane structures. This results in membrane expansion, increased membrane fluidity and inhibition of a membrane-embedded enzyme.

In yeast cells and isolated mitochondria, α-pinenes and β-pinenes destroy cellular integrity, inhibit respiration and ion transport processes and increase membrane permeability [21].

Some of the phenolic compounds which recorded in this study were found that they have allelopathic effects. [23]mentioned that vanillic acid is the most identified benzoic acid derivatives involved in allelopathy, also synergic acid, protocatcheic acid and gallic acid play the same role, and also reported that gallic acid is one of the inhibitors produced byplants.

Control of algae

Different methods were used to control the growth of algae such as physical, chemical, and biological methods.Chemical methods such as classical antibiotics have been used previously to control algae and they found that some of these antibiotics such as ampicillin, erythromycin could kill 90% or more of algae. However, these antibiotics are considered not recommended to controls algae due to the followings :

These are very expensive and could make resistance to whole organisms in the environment including algae themselves. Moreover, these antibiotics found able to lyse the algal cell wall and make the release of toxins to the water environment more possible[24].

Four algal taxa included two blue greens (*A. circinalis*and *M. aeruginosa*) and two green algae (*C. vulgaris* and *C. humicola*) were selected from those obtained in this study to be controlled, because those taxa varied from unicellular to filamentous in their bodies structures, in addition to their negative impacts in some cases. *A. circinalis, M. aeruginosa*are responsible for producing a common toxin (microcystin) which considered as a threat to the aquatic organisms [25].

A.circinalis and M.aeruginosa were known as harmful algal bloom formers, but Nostoc strains can also occur as a minor component of cyanobacterial blooms but raely form mass occurrences [26].

[27]mentioned that Scenedesmusacutusfilterate had toxic effects on *Daphnia magna*.

However,[23] reported that *C.vulgaris* produces substances that inhibit the growth of other algal species. Thus, those algal strains were picked and controlled successfully. All the plant extracts in this study except *C.mayxa* showed high ingibitory effects against the selected algae especially at high concentrations which cause complete lysing to the algal cell walls which confirmed microscopally,thus, intracellular toxins could be release and become a threat to the environment, so this study is applicable for water treatment which could be use for indusrial uses.

Since phytochemical analysis of these extracts had been done by using HPLC to figure out the most active constituents in these extracts and to make clear the reasons which cause these distinctions between inhibitory effects of these extracts against selected algae isolated .

However, *C.colocynthus* extract showed the maximum inhibitory effects against the selected algae than the others .

The highest antialgal activity of *C.colocynthus* extract could be explained with the help of recorded results of profile HPLC, which showed that the total concentrations of phenolic compounds, terpensand alkaloids that surveyed were the highest in *C.colocynthus* extract than of those in other tested extracts.

When phenolic compounds represent one of the most important antialgalallelochemicals[27]referred that phenolic care very active antialgal substances Hc found that the active antialgalallelochemicals which they isolated from *Myriophyllumspicatum* were ellagic, gallic and pyrogallic acids and catechin which are simple and polyphenol.[28]Investigated the antiakgal substances from *Zantedeschiaaethiopica* which were phenols and polyphenols.[29] manifested the important role of phenols toxicity against *Chlorella vulgaris* and *Scenedesmusbijugatus*.

[30] reported that plant extracts from *Cyperusalternifolius* and *Cana generalis*, had active inhibitory effects against cyanobacteria due to releaseallelochemicals and they screened for these allelochemicals and found that they were 9 phenolic compounds (resorcinol, 3-hydroxy benzoic acid, 4-hydroxy benzoic acid, Anti- cyanobocterial antagonistic activity was due to the release of some alldochemicals such as bengoic acid, (4-hydroxyphenyl) acetic acid, protocatechic acid, P- coumaric acid, gallic acid and ferulic acid. However, gallic acid, vanillic acid, proto catechuic acid and p-coumaric acid were present in present study in most of the plant extracts obtained.

Reported eight phenolic compounds produced by plants inhibited the growth of *Anabaene* sp. and *Lyngbya*sp. which were chlorogenic acid, coumaric acid, gallic acid, isochlorogenic acid, scopoletin, α-naphthol, tannic acid and hydroxybezaldehyde. Thus, coumaric acid, gallic acid were recorded in most extracts in this study and they may played an inhibitory role of these extracts against selected algae in this study.Another secondary metabolites which recorded as it have antialgal action are terpens, [31] reported that essential oil from Chineasefir(*Cunringhamislanceolate*) exhibited strong inhibitory action against one of the most harmful algal bloom causes by *Alexandariatamarense*(dinoflagellate).

[32]examinedthe essential oil of *Hibiscus cannabinus* leaves against *Oscillatoriaperornata*which showed slightly inhibitory effect while it had no effect major components when tested under the same conditions as the essential oil, None of these components (terpens) as they shown in the results is match to any of terpens which recorded in the extracts in this study which showed significant inhibitory effects against number of both green and blue- green algae. [16] extracted*Euphorbia peplus*, *Albizialehbeck* and *Callisfrnonviminalis* to obtain terpens, alkaloids, and phenols, and found that terpens were the most effective against algae followed by phenolic compounds. Alkaloids extract had less antagonistic activity 'comparing with all other extracts which another assertion about the active role of terpens and phenols as antialgal and the opposite for alkaloids, but the antagonisty of the extract of *C.myxa* used in this study against selected algae caused stimulatiem or enhancement to the tested algae as that which occurred [16] and that could explain on the ground of that the crude extract of *C.myxa* actually have the nutrients which present in the crude extracts which they in tuifn may be responsible for the algal growth enhancement.

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