Activity Evaluation of some Plant Extracted Oils in Controling of Algal Growth

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Abstract

This study includes isolation, purification, and identification of algae from the canal around Baghdad university Al-jadriah. Four unialgal cultures were obtained. These algal cultures included 3 species of cyanophyta (*Nostoc carneum*, *Westillopesis prolifica*, *Chroococcus turgidus*), 1 species of chlorophyta (*Chlorella vulgaris*) . Different plants belonging to different families were collected and extracted for their oils which were *Ricinus communis* and *Sesamum indicum* (seeds), *Matricaria chamomilla* (flowers) .However, antialgal activity of the extracted oils were evaluated the isolated algae with 7 concentrations (0.09, 0.3, 0.5, 1, 10, 20 , 30) % using the agar wells diffusion method. Results showed that *R. communis* oil was more effective against isolated algae followed *by Sesamum indicum* oil .The volatile oil extracted from *M. chamomilla* was less effective. *C. turgidus* was the most sensitive to attack by oils followed by *N. carneum* .While *W. prolifi*ca was the less sensitive alga, followed by *Chlorella vulgaris* . Chemical composition of the most effective oil (*R. communis*) was analyzed by HPLC which showed that 5 terpens compounds found in this oil (Alpha-pinene, Thujone, Camphor,1-8Cineole, Camphene).

Keyword: *N. carneum, W. prolifica, C. vulgaris, M. chamomilla, S. indicum*, antialgal.

تقييم فعالية بعض الزيوت المستخلصة من النباتات في السيطرة على نمو الطحالب

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الخلاصة

تضمنت هذه الدراسة عزل و تنقية و تشخيص الطحالب من (القناة المائية المحيطة بجامعة بغداد– الجادرية). تم الحصول على أربعة أنواع من المزارع يحتوي كل منها على نوع واحد من الطحالب التي تضمنت ثلاثة أنواع من الطحالب الخضر المزرقة (*Nostoc carneum* , *Westillopesis prolifica*, *Chroococcus turgidus*) ونوع واحد من الطحالب الخضر (*Chlorella vulgaris*) وجمعت ثلاثة أنواع مختلفة من النباتات تنتمي إلى ثلاثة عائلات نباتية مختلفة من أجل الحصول على زيوتها وه ذه النباتات تضمنت بذور (الخروع) *Ricinus communis* وبذور (السمسم) *Sesamum indicum*، زهور (البابنك) *Matricaria chamomilla*. تم تقييم فعالية الزيوت المضادة للطحالب الأربعة والتي عزلت في هذه الدراسة و *باستعمال سبعة* تراكيز % (0.09, 0.3, 0.5, 1, 10, 20, 30) بطريقة الحفر والانتشار خلال الاكار. أوضحت النتائج إن الزيت الثابت المستخلص من بذور الخروع هو الأكثر فعالية يليه زيت السمسم ، وان الزيت الطيار المستخلص من زهور البابنك كان الأقل فعالية بين الزيوت المستعملة .

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الطحلب*C. turgidus* كان الاكثر حساسية للزيوت المستخلصة يليه الطحلب *N. carneum* أما الطحلب *W. prolifica* كان الاقل حساسية يليه الطحلب *C. vulgaris*. تم تحليل المحتوى الكيميائي للزيوت المستخلصة بواسطة جهاز HPLC ، وجدت خمسة مركبات تربينية في زيت الخروع هي: (Alpha−pinene, Thujone, Camphor, 1−8Cineole, Camphene).

Introduction

Algae are a diverse group of organisms. Previously, they were thought to fit into a single class of plants lacking leaf, stem, root, and reproductive system of higher plants such as mosses, ferns, conifers and flowering plants. However, they were realized that some of them have animals like characteristics so they were incorporated in both plants and animals kingdom [1]. Every year, increasing energy is being directed toward the control of excessive growth of algae and water weeds specific reasons for control vary, but in each case aquatic nuisance control is an attempt to protect or restore water uses that are considered valuable locally [2]. Growing awareness that harmful blooms of algae, known as harmful algal blooms (HABs), have increased globally with serious implications for human health and economy . Harmful effects of HAB species are largely mediated through the production of highly toxic compounds. The occurrence of harmful algal blooms is a problematic issue in many aquatic environments. The over growth of algae deprive fish and invertebrates from oxygen and leads to bad taste and odor and water treatment costs increased [3] A variety of methods have already been developed and applied to cope with the problems related to algal blooms, including UV-radiation, nutrient diversion, oxidation, removal, ultrasonication and biomanipulation . Even though such methods are often effective, many of them are very expensive and sometimes give rise to secondary pollution or act for a short function time. Consequently, more long-term effective and pollution free methods are still required, particularly in developing countries [4]. Medicinal plants have been prescribed and used with a strong belief in their ability to cure diseases for centuries. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents [5].

Material and methods

A- Algal sampling

Different water samples were collected randomly from the canal around Baghdad University campus. AL-Jadriah .

B-Algal isolation and purification:

Chu-10 medium solidified by 2% agar-agar and sterilized with autoclave, then poured in petri-dishes which left to solidify. Then the surface of each plate was inoculated with water sample and incubated in a cooled illuminated incubator with light intensity about 200 µE/m²/s and 26± 2 C˚ for 7-10 days. Each subculture was examined by using a compound microscope. The isolated algae identified according to the keys [6,7] for Cyanophyta and Chlorophyta respectively.

C- Plant sampling:

The plants used in this study were collected from college of Agriculture\Abu-Ghreab , during April \ 2012 and have been identified by Dr. Ali AL-Mosawi in Biology department .College of science .University of Baghdad.

Oils extraction:

A-Volatile oils extraction:

Matricaria chamomilla dried flower was smashed into small pieces and subjected to hydro distillation using the Clevenger apparatus for 5 hours. The aqueous phase poured in separating funnel which contain a petroleum ether, subsequently the aqueous phase was separated into two layers, the lower layer represented by water discharged while the higher layer which contains the oils retrieved and stored in a sealed marked glass vial in a refrigerator until required.

B- Fixed oils extraction:

The oils extracted from the *Ricinus communis and sesamum indicum* were prepared by using the Soxhlet apparatus. Soxhlet apparatus used for continuous extraction of solids with hexane

Antialgal activiy determination:

Wells in algal plate were made by using 6 mm. in diameter cork borer. Different concentrations (0.09, 0.3, 0.5, 10, 20 and 30) % of different oils from each plant were added in the peripheral wells and the medium was added in the middle well as a control. Three replicates were made for each

concentration. The plates were left in refrigerator until the extracts diffused in the agar then transferred to the cooled illuminated incubator for 24 h. Zones of inhibition were determined [8].

Analysis of plant extracted oils chemical composition by HPLC

Analysis of the plant chemical composition was made by high Performance Liquid Chromatogram for identification. The procedure that used out lined by the conditions of separation. Peaks were detected by UV detector set at 285nm. 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 [9].

$$\text{Conc. of sample } (\mu g/ml) = \frac{\text{Area of the sample}}{\text{Area of the standard}} \times \text{standard conc.} \times \text{Dilution factor}$$

Statistical analysis

Complete Randomized Design (C.R.D.) was used as an experimental design, to study the effect of different factors on the diameters of inhibition zones. Least significant difference (LSD) was used to compare the significant difference between means at $P \leq 0.05$.

Results and Discussion:

Isolation and identification of algae

Four different taxa of algae were isolated, purified, and identified from the canal around the university of Baghdad , Al-Jadriah. They were include 3species of cyanophyta, 1 species of chlorophyta.

Oils yield of the studied plants

Yields of fixed oils which extracted from *R. communis* seeds *and S.indicum* seeds by soxhlet apparatus and volatile oils which extracted from *M. chamomilla* flowers *and* which are shown in figure-1. The highest yield of fixed oils from *R. communis* was 66% (v/w) and the lowest yield from *S. indicum* which was 33% (v/w) . However, *M. chamomilla* was 0.4% (v/w). Seeds of *R. communis* popularly called castor oil The result showed that the fixed oil yielded from *R. communis* was 66% which was different from the results of [10-13];which was 40-53% of fixed oil .While *S. indicum* the yield of the fixed oil was 33% which was relatively near to the result of [14] which was 22%-40% of fixed oil. Finally for *M. chamomilla* the yield of volatile oil was 0.4% this value was the same for[15] finding which was 0.24%- 2.0% .Volatile oil Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanism against predation by many micro organisms, insects and herbivores[16].

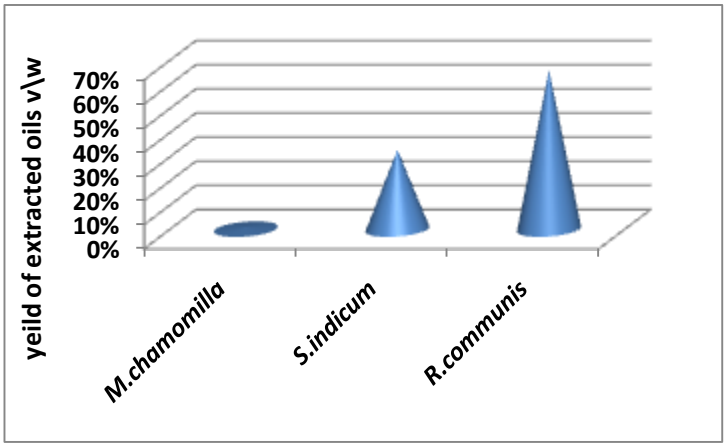

Figure 1- Yield of the extracted oils v\w%

Antagonistic activity evaluation of the extracted oil :

Inhibitory effects of the extracted oils were tested depending on the diameters (mm) of inhibition zones according to agar-well diffusion method Inhibition zones .Diameters were differed according to the algae species , *N. carneum* was the most sensitive among other algae when treated with extracted oils which was the most powerful extracted oil in this study . As shown in figure-2, and figure-3.This maybe due to the structure of their cell wall. The plants differed significantly in the antagonistic

activity against algae. These differences could be attributed to structural nature of the microorganisms [17, 18]. *C. vulgaris* was the most resistant, the reason could be due to algal type or to its cell wall structure Results showed that the inhibition zones varied according to algal species, origin of the extracted oils and their concentration. Table-1 showed that *R. communis* oil showed the highest inhibition zone (9mm) among other treatments at 30% concentration against *Nostoc carneum.* , followed by *S. indicum* (6.3mm) and finally *M. chamomilla* (4.6mm) .While 20% *R. communis* oil showed (2.6mm) the highest inhibition zone among other treatments followed by *S. indicum* (6mm) , *M. chamomilla* (3.3mm) ,10% *S. indicum*(0.3mm) and *R. communis*, *M. chamomilla* with no inhibition zones. However (1, 0.5, 0.3, 0.09)% no inhibition zones were detected for all treatments. (MIC) of *R. communis* was 20%, *S. indicum* 10%, and *M. chamomilla* was 30% . According to the statistical analysis many significant differences appeared between the different concentrations and other differences appeared between the three oils that used against *N. carneum*.

Table 1- Mean of inhibition zones diameters in mm caused by treatments with different concentrations of different oils (mg/ml) against *Nostoc carneum*.

Treatments	Concentrations							Average	L.S.D of treatment
	30%	20%	10%	1%	0.5%	0.3%	0.09%		
R.communis oil	9mm	2.6mm	-	-	-	-	-	1.65*	0.87
S.indicum oil	6.3mm	3.3mm	0.3mm	-	-	-	-	1.41*	
M.chamomilla	4.6mm	-	-	-	-	-	-	0.65 ns	**highly significance *significance Ns: no significant
Average	6.6**	1.9*	0.1 Ns	0 Ns	0 Ns	0 Ns	0 Ns		
L.S.D of concentrations:	0.65								

Different results were achieved toward different treatment of oils against *C. turgidus* . As shown in table-2, *M. chamomilla* oil showed the highest inhibition zone (11.3mm) among other treatments at 30% concentration, followed by *R. communis* (6.6mm) and *S. indicum* (5.6mm). While 20% *R. communis* oil also showed the highest inhibition zone among other treatments (4.6mm) followed by *M. chamomilla oil* (4mm) and *S. indicum* oil (2mm).However 10% *M. chamomilla* oil showed (6.3mm) followed by *S. indicum* (3mm)and *R. communis* (0.6mm), (1 ,0.5, 0.3,0.09) % of all these oils concentrations have no antagonistic activity against *C. turgidus*. (MIC) of *R. communis* was 10% while for *S. indicum* and *M. chamomilla* was 10%. According to the statistical analysis many significant differences appeared between the different concentrations and other differences appeared between the three oils that used against *C. turgidus* .

Table 2- Mean of inhibition zones diameters in mm caused by treatments with different concentrations of different oils (mg/ml) against *Chroococcus turgidus.*

Treatments	Concentrations							Average	L.S.D of treatment
	30%	20%	10%	1%	0.5%	0.3%	0.09%		
R.communis oil	6.6mm	4.6mm	0.6mm	-	-	-	-	1.7*	0.86
S.indicum oil	5.6	2mm	3mm	-	-	-	-	1.5*	
M.chamomilla	11.3mm	4mm	6.33mm	-	-	-	-	3.09**	**highly significance *significanc e Ns: no significant
Average	7.8**	3.5**	3.31**	0 Ns	0 Ns	0 Ns	0 Ns		
L.S.D of concentrations:	0.65								

Results in table-3, showed that *S. indicum* oil showed (4.6mm) the highest inhibition zone among the other treatments at 30% concentration against *Westilliopsis prolifica* followed by *R. communis* (2.3mm), *M. chamomilla* (0.6mm) .while 20% *S. indicum* (2.3mm) then *R. communis* (0.3mm) and no inhibition zones were detected for *M. chamomilla* , (10 ,1 ,0.5 ,*0*.3,0.09) % no inhibition zones were detected for other treatments . The (MIC) of *R. communis* was 20%, *S. indicum* 20% and *M. chamomilla* was 30% . According to the statistical analysis many significant differences appeared

between the different concentrations and other differences appeared between the three oils against *W. prolifica* .

Table 3- Mean of inhibition zones diameters in mm caused by treatments with different concentrations of different oils (mg/ml) against *Westillopesis prolific*.

Treatments	Concentrations							Average	L.S.D of treatment
	30%	20%	10%	1%	0.5%	0.3%	0.09%		
R.communis oil	2.3mm	0.3mm	-	-	-	-	-	0.3ns	0.49
S.indicum oil	4.6mm	2.3mm	-	-	-	-	-	0.9*	
M.chamomilla	0.6mm	-	-	-	-	-	-	0.08 Ns	**highly significance *significance Ns: no significant
Average	2.5**	0.8*	0 Ns	0 Ns	0 Ns	0 Ns	0 Ns		
L.S.D of concentrations:	0.37								

Results in table-4, showed that the mean of inhibition zones for *R. communis* was (8.6mm) the highest inhibition zone among other treatments at 30% concentration against *chlorella vulgaris*, followed by *S. indicum* (2.3mm) finally *M. chamomilla* (2.3mm) .While 20% *R. communis* (3.6mm) then *S. indicum* and *M. chamomilla* with no inhibition zone, All of (10 , 1 , 0.5, 0.3 ,0.09) % didn't show any inhibition zone for all treatments at those concentrations .(MIC) of *R. communis* was 20% , *S. indicum* 30% and *M. chamomilla* was 30% . According to the statistical analysis many significant differences appeared between the different concentrations and other differences appeared between the three oils against *C. vulgaris*.

Table 4- Mean of inhibition zones diameters in mm caused by treatments with different concentrations of different oils (mg/ml) against *Chlorella vulgaris*

Treatments	Concentrations							Average	L.S.D of treatment
	30%	20%	10%	1%	0.5%	0.3%	0.09%		
R.communis oil	8.6mm	3.6mm	-	-	-	-	-	1.7*	0.41
S.indicum oil	2.3mm	-	-	-	-	-	-	0.3 Ns	
M.chamomilla	2.3mm	-	-	-	-	-	-	0.3 Ns	**highly significance *significance Ns: no significant
Average	4.4**	1.2*	0 Ns	0 Ns	0 Ns	0 Ns	0 Ns		
L.S.D of concentrations:	0.31								

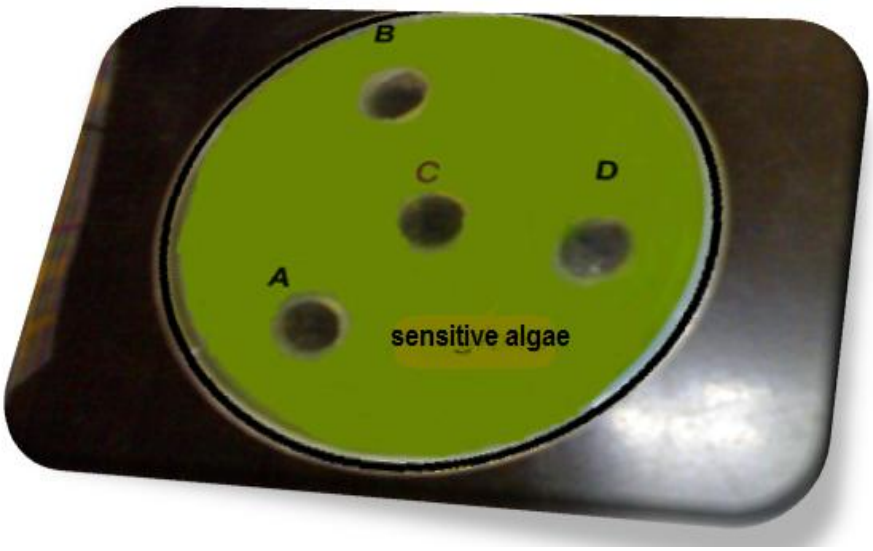

Figure 2- Not treated algae (control plate)

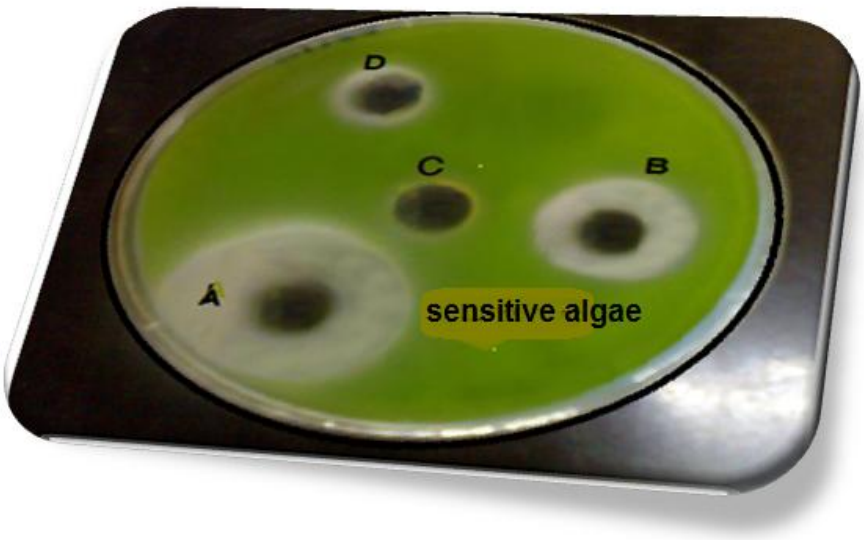

Figure 3- Representative inhibition zones observed in sensitive algae to attack by different oils concentrations A- 30% B-20% D- 10% C-control

Chemical constituents of the extracted oil:

Results of *R.communis* analysis by HPLC, showed the different concentration of each secondary metabolic compounds of the extracted oil, they were terpen , phenol or alkaloid..The chemical analysis of *R.communis* which shown showed that the majority chemical compounds was terpens (Alpha-pinene34.18 µg/ml – Thujone 65.71 µg/ml –Camphor 23.1 µg/ml -(1-8 Cineole)41.5 µg/ml - Camphene 30.1 µg/ml) .). results of HPLC showed that the chemical constituents of *R.communis* fixed oil majority chemicals were (Alpha- pinene34.18 µg/ml – Thujone 65.71 µg/ml –Camphor 23.1 µg/ml -(1-8 Cineole)41.5 µg/ml - Camphene 30.1 µg/ml) . As shown in figure-4, but beside those chemicals (triglyceride, ricinoleic acid) presence were indicated by [19].

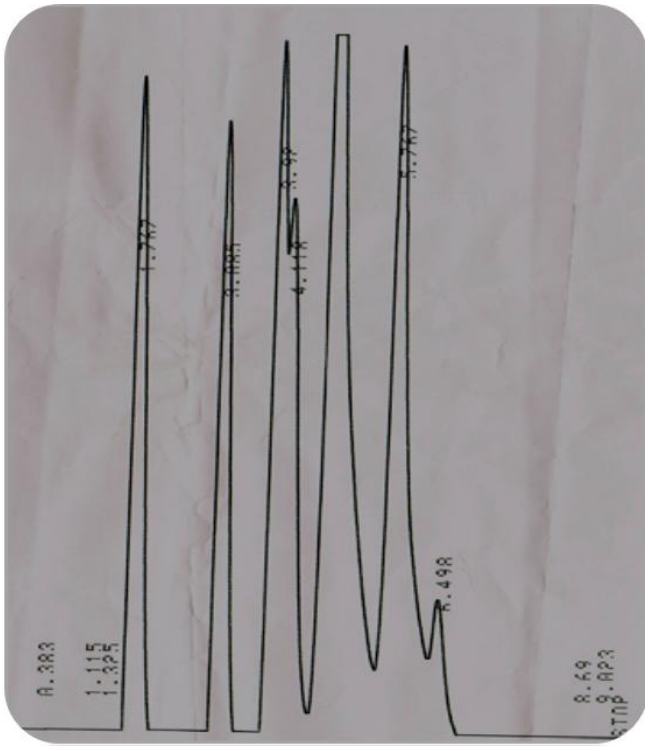

Figure 4- *R.communis* fixed oil chemical constituents

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