

Antagonistic activity evaluation of *Eucalyptus camaldulensis* essential oil in control of algae and associated microorganisms

Ali M. Najem*, Abdul Latif M. Jawad* and Ayyad W. Raof

Abstract—This study includes isolation, purification, and identification of algae from different environmental places (Tigris River and canal around Al- Jadriah) during February 2008. Nine unialgal cultures were obtained which included 4 species of blue-green algae, 4 species of green algae, and 1 species of diatoms. However, contaminant bacteria and fungi were isolated from 4 of these algal cultures which were *Anabaena circinalis*, *Microcystis aeruginosa*, *Scenedesmus quadricauda* and *Chlorella vulgaris*. The contaminant bacteria included nine different species which were *Stenotrophomonas maltophilia* and *Bacillus polymyxa* which isolated from all the selected algal cultures, while the rest *Kocuria varians/rosa*, *Aerococcus viridians*, *Micrococcus* sp., *Staphylococcus auricularis*, *Staphylococcus capitis*, *Kocuria kristanae* and *Staphylococcus cohnii* varied in their presence and absence in these algal cultures. The contaminant fungi of the four algal cultures were *Aspergillus flavus* which was isolated from *M. aeruginosa* culture, *Asp.niger* isolated from *C. vulgaris* culture, *Cryptococcus albidus* that isolated from *S. quadricauda* culture, whilst *Penicillium* sp. isolated from *A. circinalis* culture. However, *Eucalyptus camaldulensis* essential oil was extracted and subsequently showed highly activity when tested against six species of the isolated algae and against the isolated contaminant bacteria and fungi species. Chemical composition of the extracted oil was analyzed by HPLC (High Performance Liquid Chromatography).

Index Terms— control algae, antagonistic activity, eucalyptus camaldulensis

1 INTRODUCTION

Algae are considered the main causes of plenty of problems in the aquatic ecosystems and they could be harmful by producing large populations in the aquatic environment. Large growths of some algae (e.g., the diatom *Chaetoceros*) can clog the gills of fish, in addition to anoxic conditions can occur at the end of blooms of other algae (e.g. green algae) as the algae die and decompose that cause release toxins that sicken and kill other organisms that prey on these algae (Hallegraeff *et al.*, 2003). Many researches were carried out to control the harmful algal bloom which included mechanical, physical and chemical methods in addition to biomanipulation (Nicholas, 1973 and Shapiro *et al.*, 1975). All these approaches were not quite sufficient to solve the problem. Therefore, in the last few decades, a variety of medicinal plants and plant extracts have been screened for their antimicrobial activity (Cowan, 1999). Essential oils, derived from aromatic and medicinal plants have been reported to be active against Gram-positive and Gram-negative bacteria as well as against yeasts, fungi, and viruses. They are mixtures of different lipophilic and volatile substances, such as monoterpenes, sesquiterpenes (Reichling, 1999). Yang *et al.* (2009) reported that essential oil from Chinese fir (*Cunninghamia lanceolata*) exhibited strong inhibitory action against one of the most harmful algal bloom causes by *Alexandria tamarensis* (dinoflagellate). However, all studies and applications which

deal with algae need to be these algal cultures free from other living organisms (axenic cultures). Thus, many studies had been done to eliminate the companion organisms especially bacteria and fungi from algal cultures included using of antibiotics, chemicals in addition to the physical methods such as use of the photo attraction which include over laying agar and unidirectional light methods

2 MATERIALS AND METHODS

2.1- Collection and identification of the studied plant

E. camaldulensis aerial parts were collected from the gardens of Baghdad University and identified. Leaves were air dried at room temperature ground to semi powdered state, the ground samples stored in and labeled in dark and clean containers.

2.2- Preparation of plant extract

Dried leaves of *E. camaldulensis* were chopped into small pieces and subjected to hydro distillation using the Clevenger apparatus for 5 hours according to British Pharmacopeia (1963).

2.3- Sampling of algae

Samples were collected from Tigris River and canal around Al-Jadriah during February 2008 by using sterile container (250 ml) and transported to the lab immediately.

2.4- Isolation and purification of algae

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 $\mu\text{E}/\text{m}^2/\text{s}$ and $26 \pm 2^\circ\text{C}$ for 7- 10 days. Each subculture was examined by using a compound microscope

A*Biology Dep., College of science, Baghdad University.

(Stein, 1973). The isolated algae identified according to the keys (Desikachary, 1959; Prescott, 1973) for Cyanophyta and Chlorophyta respectively.

2.5- Algal lawns preparation

Appropriate media for algal growth were prepared in flat bottom flask which were either Chu-10 (Kassim *et al.*, 1999) or Allen's (Allen, 1968) depending on algal species, agar-agar 2% was added for solidification, then sterilized by using the autoclave. The media cooled down to 40-45 °C algal culture was added to the agar media, shaken well and poured in petri dishes immediately. Petri dishes were incubated in an illuminated incubator with 200 µE/m²/Sec and 26± 2 °C for 2-3 days until the plates turn into greenish color (Jawad, 1982).

2.6- Algal antagonistic activity evaluation

Certain numbers of wells were prepared on algal lawns with the help of sterile cork borer (8 mm in diameter), 0.2 ml of the oil concentrations (0.05, 0.1, 0.2, 0.3, 0.5, 1, 3, 5, 10, 15%) were introduced into the wells. Controls were made by using the solvent (ethylene glycol) which was used to dissolve oil. The plates incubated in an illuminated incubator for 24 hrs.. Inhibition zones were determined by measuring their diameters. Replicates were made and the mean values were recorded (Jawad, 1982).

2.7- Isolation and identification of contaminant bacteria and fungi

Enrichment culture media for bacteria (Nutrient agar and Mueller Hinton agar) and (Sabouraud dextrose agar) for fungi were prepared. These media inoculated with 1ml of selected algal culture, then plates for bacterial enhancement were incubated for 24-48 hr at 26± 2 °C and 72 hr. at 28 °C to those for fungal growth improvement. Colonies appeared after incubation were sub cultured continuously till pure isolated colony was obtained.

A- Identification of the isolated bacteria

Gram's Stain was used as primary identification of bacterial isolates (Benson, 2002). In addition, analytical profile index was used for the identification of bacterial isolates, and allowing fast, easy and accurate identification of bacteria, four types of API (API 20E, API 20NE, API 20Strep, API Staph) were used in this study according to Benson (2002).

B- Identification of the isolated fungi

Shapes, colors and textures of some isolated colonies of fungi were primarily used to identify their types. Then, a small part from a fresh fungal isolate was transferred on a slide and a drop of lacto phenol dye was added and pressed together gently with a cover slide, then examined under (10x) and (40x) of a compound microscope (Milne, 1996). While API Candida was used to find out the type of yeast isolate.

2.8- Evaluation of anti-bacterial activity of plants extracts

The anti-bacterial activity of the extracted oils from the studied plants was tested against the isolated bacteria from selected algal cultures by using agar-well diffusion method (Perez *et al.*, 1990).

2.9- Evaluation of anti-fungal activity of the extracted oils

A- The anti fungal activity for molds precisely was evaluated

by measuring the fungal growth inhibition assay method using the following formula (Wang *et al.*, 2005)

$$\text{Growth inhibition\%} = \left[\frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \right] \times 100$$

b- Agar well diffusion method was used to evaluate the anti fungal activity of plant extract against yeast isolates (Perez *et al.*, 1990).

10-Statistical analysis

Complete Randomized Design (C.R.D.) was used as an experimental design. Data were analyzed by using statistical analysis system- SAS (2004) 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 ≤ 0.05.

3. Results and discussion

3.1- Isolation and identification of algae

Nine different taxa of algae were isolated, purified, and identified from Tigris River and canal around Al-Jadriah, as they are shown in Table (1).

Table (1) The isolated algae in this study and their classification

Isolated algae	Division	Class
<i>Microcystis aeruginosa</i>	Cyanophyta	Cyanophyceae
<i>Calothrix braunii</i>	Cyanophyta	Cyanophyceae
<i>Anabaena circinalis</i>	Cyanophyta	Cyanophyceae
<i>Nostoc commune</i>	Cyanophyta	Cyanophyceae
<i>Chlorella vulgaris</i>	Chlorophyta	Chlorophyceae
<i>Kirchinella sp.</i>	Chlorophyta	Chlorophyceae
<i>Pediastrum boryanum</i>	Chlorophyta	Chlorophyceae
<i>Scenedesmus quadricauda</i>	Chlorophyta	Chlorophyceae
<i>Navicula anglica</i>	Chrysophyta	Bacillariophyceae

The algal isolates which obtained did not represent the whole algae in the collected samples in this study, this was due to the growth media used and other environmental factors adjusted for their growth. However, some algae need typical media with typical environmental factors to be grown which were different from those used in this study (Abedin and Taha, 2008). How-

ever, the density of algae during February is very low due to algal seasonal life.

3-2 Oil yield of the extracted oil

Results were showed that *E. camaldulensis* essential oil yield was 2%, which is approximately the same that extracted by Pappas and Sheppard- Hanger (1999) which was 2.3%.

Table (2) Yields of the extracted oils from plants involved in this study.

Plant	Family	Yield of the extracted oil%(v/w)	Type of oil
<i>Eucalyptus camaldulensis</i>	Myrtaceae	2	Essential

3-3 Evaluation of inhibitory effects of the extracted oils against algae

Inhibitory effects of *E. camaldulensis* essential oil were tested against 6 taxa of the isolated algae (*N. commune*, *A. circinalis*, *M. aeruginosa*, *C. vulgaris*, *S. quardicauda* and *K. sp.*) depending on the diameters (mm) of inhibition zones according to agar-well diffusion method. The selection of those algal taxa depend on their ecological role, *A. circinalis*, *M. aeruginosa* and *N. commune* are responsible for producing a common toxin (microcystin) which considered as a threat to the aquatic organisms (Sivonen and Jones, 1999). *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 rarely form mass occurrences (Sivonen and Jones, 1999). Cooradi et al. (1999) mentioned that *Scenedesmus acutus* filtrate had toxic effects on *Daphnia magna*. However, Rise (1984) reported that *C. vulgaris* produces substances that inhibit the growth of other algal species. Results in Table (3) showed that *E. camaldulensis* oil had a wide range of concentrations which showed a significant inhibitory action against the tested algae, the highest inhibitory effect of *E. camaldulensis* oil was (35 mm) against *M. aeruginosa* at 15% as showed in Figure (1), while (2) showed the moderate effect and the lowest effect was (9.5 mm) against both of *A. circinalis* and *M. aeruginosa* at 0.1%, figure (3) pertaining to control plate, the most susceptible alga to attack by *E. camaldulensis* oil was *M. aeruginosa*, and *K. sp.* was the most resistance. The highest antialgal activity of *E. camaldulensis* oil could be explain with the help of recorded results of profile HPLC, which showed that the total concentrations of phenolic compounds, terpens and alkaloids that surveyed were high in *E. camaldulensis* oil. Nakai et al. (2001) referred that phenols are very active antialgal substances. Nakai et al. (2004) found that the active antialgal allelochemicals which they isolated from *Myriophyllum spicatum* were ellagic, gallic and pyrogallic acids and catechin which are simple and polyphenols. Greca et al. (1998) investigated the antialgal substances from *Zantedeschia aethiopica* which were phenols and polyphenols. Kobaisy et al. (2001) examined the essential oil of *Hibiscus cannabinus* leaves against *Oscillatoria perornata* which showed slightly inhibitory effect while it had no effect against one type of green algae (data not published), no one of these components (terpens) as they shown in the results is match to any of terpens which recorded in the oils in this study

which showed significant inhibitory effects against number of both green and blue- green algae.

Table (3) Variations in the algicidal effects of the extracted oil concentrations against selected algae according to the diameters of the inhibition zones (mm). Controls showed no inhibition.

Conc. %	Cyanophyta			Chlorophyta			LSD value
	<i>N. commune</i>	<i>A. circinalis</i>	<i>M. aeruginosa</i>	<i>C. vulgaris</i>	<i>S. quardicauda</i>	<i>Kirchiana sp.</i>	
15	31.0	30.0	35.0	29.0	30.0	30.5	3.520 *
10	29.0	26.0	31.0	26.0	26.0	25.0	2.733 *
5	23.0	21.5	27.0	23.0	20.0	22.5	2.640 *
3	20.0	20.5	18.0	15.5	17.0	16.0	2.648 *
1	16.5	18.0	16.0	15.0	16.0	14.0	2.044 *
0.5	11.5	16.0	14.0	13.0	15.5	11.0	2.522 *
0.3	0.0	11.0	11.5	12.0	12.0	0.0	2.164 *
0.2	0.0	10.5	11.0	10.0	11.5	0.0	1.791 *
0.1	0.0	9.5	9.5	10.0	10.5	0.0	1.379 *
0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.00 ns
-	4.526 *	5.611 *	5.872 *	4.839 *	5.155 *	4.309 *	-

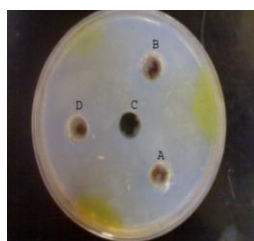


Figure (1) High inhibitory effects of *E. camaldulensis* essential oil concentrations against *M. aeruginosa*. (A) 15%, (B) 10%, (D) 5%, (C) Control.

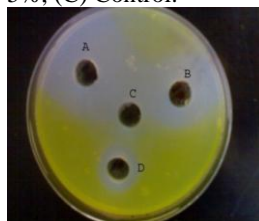


Figure (2) moderate inhibitory effects of *E. camaldulensis* es-

sential oil concentrations against *N. commune*. (A) 3%, (B) 1%, (D) 0.5%, (C) Control



Figure (3) No inhibitory effect of the extracted oil against algae.

3-4 Isolation, purification and identification of contaminant bacteria and fungi from selected algal cultures

Four algal cultures included *A. circinalis*, *M. aeruginosa*, *S. quardicauda* and *C. vulgaris* were chosen for this purpose. However, 36 pure bacterial isolates were obtained, the detected bacterial isolates were 6 genera included *Stenotrophomonas*, *Bacillus*, *Kocuria*, *Aerococcus*, *Micrococcus* and *Staphylococcus*. Those genera included 9 different species which varied in their existence with the selected algal cultures.

Table (4) Contaminat bacteria which isolated from selected algal cultures.

Alga /Bacteria	<i>Steno. maltophilia</i>	<i>K. varians/Rosasa</i>	<i>A. viridans</i>	<i>B. polymyxa</i>	<i>Micrococcus sp.</i>	<i>K. kristanae</i>	<i>Staph. cohnii</i>	<i>Staph. Auricularis</i>	<i>Staph. capitis</i>
1. <i>C. vulgaris</i>	+	-	-	+	-	+	-	-	+
2. <i>M. aeruginosa</i>	+	+	+	+	-	-	-	-	+
3. <i>A. circinalis</i>	+	-	-	+	-	-	+	-	-
4. <i>S. quardicauda</i>	+	-	-	+	+	-	-	+	-

Results revealed that the detected fungal isolates included 3 types of molds which were *Aspergillus niger*, *Aspergillus flavus* and *Penicillium sp.*, in addition to one type of yeast that was *Cryptococcus albidus*. Results showed that the *Asp. flavus* present in *M. aeruginosa* culture only, *Asp. niger* present only in *C. vulgaris* culture, *Cryptococcus albidus* present in *S. quardicauda* culture, whilst *Penicillium sp.* present in *A. circinalis*

culture.

3-5 Control of contaminant bacteria

Plant extract concentrations which did not have any inhibitory actions against the tested algae were used to control the isolated bacteria from those algae (Table 3). Results showed that *E. camaldulensis* oil concentrations had high inhibitory actions against all the bacterial isolates in this study, *E. camaldulensis* oil major concentration was 3% which caused (32 mm) inhibition zone against *Staph. capitis* (Figure 4), while the minor was 0.05% which caused (14 mm) against *K. varians*, *B. polymyxa* and *Micrococcus sp* (Figure 5). Diameters of inhibition zones in mm. Tested oil in this study showed active inhibitory effects against bacteria which isolated and identified in this study as in figure (4), except *Aerococcus viridians* which was the most resistant. All the contaminant bacteria which isolated in this study were positive to gram stain except *Stenotrophomonas maltophilia*. These bacteria isolated at 26±1 C° which is optimum temperature to algal growth and the temperature at which the isolated bacteria should be vital, but during the study it was found that isolated bacteria at 26±1 C° have the ability to grow at 37 C°. *E. camaldulensis* essential oil was the most effective against all contaminant bacteria which isolated in this study. Ghalem and Mohamad (2008) showed the antibacterial activity of *E. camaldulensis* essential oil against gram positive bacteria especially *Staphylococcus aureus* and low activity against gram negative bacteria. Akin *et al.* (2009) also reported the significant antibacterial activity of *E. camaldulensis* essential oil against gram positive bacteria. Adeniyi and Ayepola (2008) experimented *E. camaldulensis* essential oil and crude extract against 8 enteric pathogenic bacteria, the results exhibited high antibacterial activity against tested bacteria, α-pinenes, fight against pathogenic bacteria and all kinds of fungi. They are able to eliminate the microorganisms or inhibit their growth as well as intervene on their metabolism (Alma *et al.*, 2004), α-pinenes was recorded as one of the terpen components in all extracted oils in this study. Generally the essential oils mechanism of action against both bacteria and yeast represented by its ability to lyse their cell walls and also its role in metabolic activities weaken inside cell by interfering with cytoplasmic membrane functions especially the inhibition of protein synthesis which result in block the active transport of ions through the cellular membrane (Al-Chaesi, 2008).

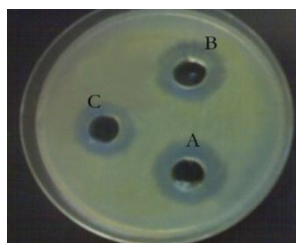


Figure (4) Variation in the inhibition zones of *Staph. capitis* due to the inhibitory effect of *E. camaledulensis* concentrations (A) 3%, (B) 1%, (C) 0.5%

3-6 Control of contaminant fungi

Antifungal activity of the extracted oils was tested against the isolated fungi from the selected algal cultures in order to ride off those fungi from algal cultures and get axenic cultures of algae. However, this achieved by fungal growth inhibition assay (for molds) and agar-well diffusion method (for yeast isolate). *E. camaldulensis* oil showed strong inhibitory effect against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* sp. and *Cryptococcus albidus* as they are clearly appeared in table (6) and figures (5-8). It was found that the isolated fungi which actually are contaminant of four algal cultures were not belong to aquatic fungi which complete their life cycle in water only and that may be due to the growth media which used were not specific to these fungi. Begum *et al.* (2005) examined the antifungal activity of *E. camaldulensis* essential oil against *Aspergillus* sp., *Penicillium* sp., *Fusarium solane* and *Verticillus* sp. which cause inhibition to the mycellium of these fungi. monoterpenes increase cytoplasmic membrane fluidity and permeability, disturb the order of membrane embedded proteins, inhibit cell respiration, and alter ion transport processes (Reichling *et al.* 2006).

Table (6) The antagonistic activity of the extracted oil against three strains of molds measuring by growth inhibition (%), and one strain of yeast measuring by diameters of inhibition zone in mm.

Plant's oil	Conc. (%)	Asp. Niger G.I %	Asp. Flavus G.I%	Penicillium Sp. G.I%	Cryptococcus albidus mm	LSD
1.Eucalyptus	3	73	74	72	23	5.39
	1	60	64	69	19	4.73
camaldulensis	0.5	49	52	55	14	4.82
	0.05	32	33	35	12	3.66



Figure (5) Highly inhibition of *Asp. flavus* due to the inhibitory effect of *E. camaldulensis* 3%



Figure (6) Highly growth inhibition of *Penicillium* sp. due to the inhibitory effect of *E. camaldulensis* 3% essential oil.

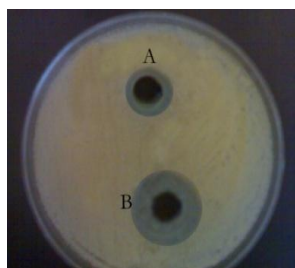


Figure (8) The inhibitory effects of (A) 3% (B) 1% *E. camaldulensis* oil against *Cryptococcus albidus*.

3-7 Chemical constituents of the extracted oils

The analysis of the secondary metabolic compounds precisely terpenes, alkaloids and phenols revealed that major terpen constituent in the oil of *E. camaldulensis* was β - phellandrene 160.2 ($\mu\text{g/ml}$) and Thujone 86.9 ($\mu\text{g/ml}$) was the minor and both are part of another detected terpenes which were α - pinene 130.8 ($\mu\text{g/ml}$), camphene 95.3 ($\mu\text{g/ml}$), myrcene 158.1($\mu\text{g/ml}$),linalool 90.6 ($\mu\text{g/ml}$), cymene 94.4 ($\mu\text{g/ml}$) while their total concentration was 816.3 ($\mu\text{g/ml}$). the peaks showed in figure (9) and (10) which are shown the standard terpenes and those present in this target oil. Alkaloids present in the extracted oil were also identified by HPLC, and they were Atropine 84.2 ($\mu\text{g/ml}$), Hyocyanine 66.8 ($\mu\text{g/ml}$), 3-OH- tropane 65.2 ($\mu\text{g/ml}$), tropine 59.8 ($\mu\text{g/ml}$), scopolamine 101.6 ($\mu\text{g/ml}$) and 7-OH Hyocyanine 72.2 ($\mu\text{g/ml}$) and the total concentration of alkaloids was 449.8 ($\mu\text{g/ml}$) as peaks are shown in figures (11) and (12). Scopolamine 101.6 ($\mu\text{g/ml}$) was the major alkaloid, while Tropine 59.8 ($\mu\text{g/ml}$) was the minor in the *E. camaldulensis* oil. Phenolic compounds were the third secondary metabolic compounds in the studied oil which identified. Gallic acid 793.4 ($\mu\text{g/ml}$) was the major phenolic compound, while Syringic acid 12.6 ($\mu\text{g/ml}$) was the minor in the *E. camaldulensis* oil, in addition to other identified phenolic compounds in this oil which they were chlorogenic acid, caffeic acid, gallic acid, protocatechic acid, syringic acid, OH -benzoic acid, D-coumaric acid, vanillic acid and catechol which their concentrations were 119, 28.5, 793.4, 212.3, 12.6, 24.9, 52.1, 178.26, 191.8 ($\mu\text{g/ml}$) while their total concentration was 1612.86 ($\mu\text{g/ml}$). As their peaks are shown in figures (13) and (14).

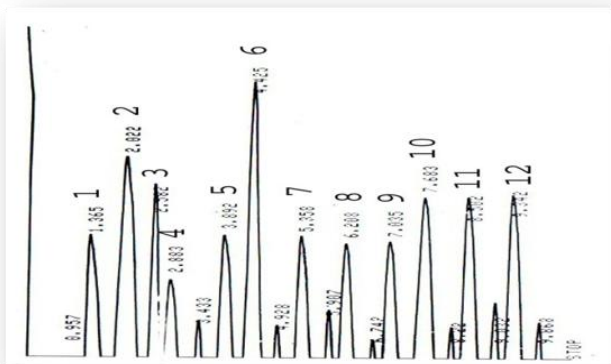


Figure (9) HPLC profile of Terpens standards (1) α - Pinene, (2) Camphene, (3) β - Pinene, (4) 3- Carene, (5) Myrecene(6) Thujone, (7) Linalool, (8) Cymene, (9) Terpinoline,(10) Terpene-4-ol, (11) Geranyl acetate, (12) β -Phellandrene.

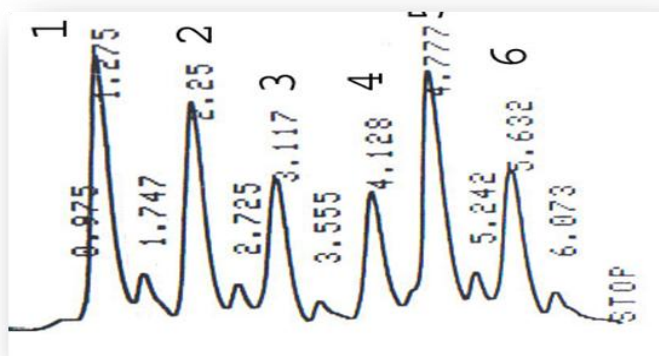


Figure (12) HPLC profile of *Eucalyptus camaldulensis* essential oil alkaloids (1) Atropine, (2) Hyocyanine, (3) 3-OH tropane, (4) Tropine, (5) Scopalamine, (6) 7-OH Hyocyanine.

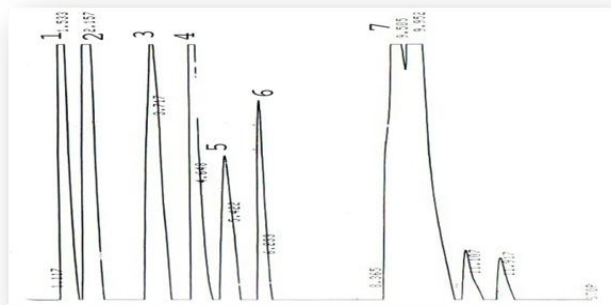


Figure (10) HPLC profile of *E. camaldulensis* essential oil Terpens (1) α - Pinene, (2) Camphene, (3) Myrecene(4) Thujone, (5) Linalool, (6) Cymene, (7) β -Phellandrene.

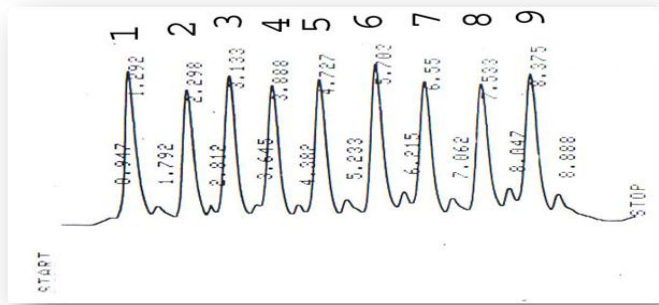


Figure (13) HPLC profile of phenols standards (1) Chlorogenic acid, (2) Caffeic acid, (3) Gallic acid, (4) Protocatechic acid, (5) Synergic acid, (6) OH-benzoic acid,(7) D-coumaric acid,(8) Vanillic acid, (9) Catechol.

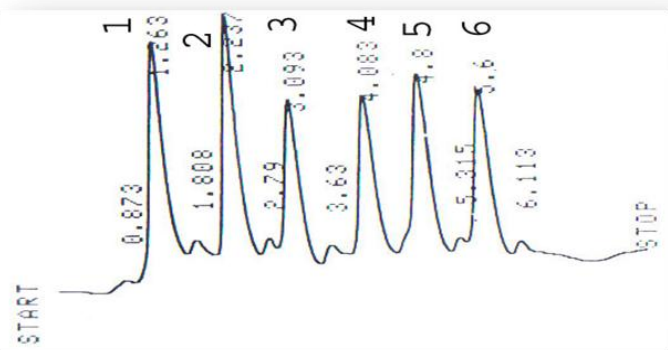


Figure (11) HPLC profile of alkaloids standards (1) Atropine, (2) Hyocyanine, (3) 3-OH tropane, (4) Tropine, (5) Scopalamine, (6) 7-OH Hyocyanine.

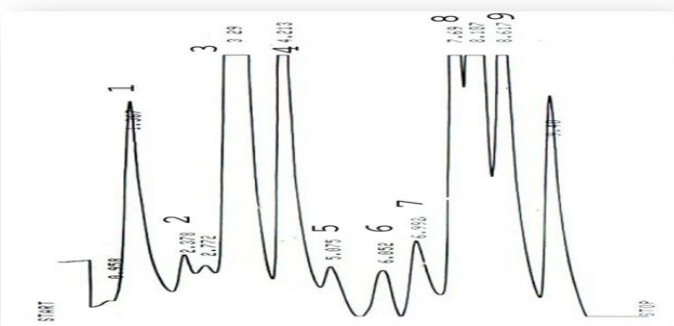


Figure (14) HPLC profile of *E. camaldulensis* essential oil phenols (1) Chlorogenic acid, (2) Caffeic acid, (3) Gallic acid, (4) Protocatechic acid, (5) Synergic acid, (6) OH-benzoic acid,(7) D-coumaric acid,(8) Vanillic acid, (9) Catechol.

REFERENCES

- 1- Abedin, R.M.A. and H.M.T. Taha. 2008. Antibacterial and antifungal activity of Cyanobacteria and green microalgae. Evaluation of medium components by Plackett- Burman design for antimicrobial activity of *Spirulina platensis*. Gobal J. Biotech. and Biochem. 3(1): 22- 31.
- 2- Adeniyi, B.A. and O.O. Ayepola. 2008. The Phytochemical Screening and Antimicrobial Activity of Leaf Extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana* (Myrtaceae). Research Journal of Medicinal Plant. 2, pp: 34-38.
- 3- Akin, M.; A. Aktumsek and A. Nostro. 2009. Antibacterial activity and composition of the essential oils of *Eucalyptus camaldulensis* Dehn. and *Myrtus communis* L. growing in Northern Cyprus. African Journal of Biotechnology Vol. 9 (4), Pp. 531-535.
- 4- Al-Chaesi, S.A.S. 2008. Effect of *Myrtus communis* essential oil on growth and activity of several pathogenic bacterial types and *Candida albicans* yeast. Om Salma Journal for Siences, 5: 8-12. (In Arabic)
- 5- Allen, M. 1968. Simple conditions for the growth of unicellular blue-green algae on plates. Journal of Phycology, 4:1-4.
- 6- Alma, M.H.; S. Nitz; H. Kollmannsberger ; M. Digrak ; F.T. Efe and N. Yilmaz . 2004. Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (*Pistacia vera* L.). J .Agric. Food Chem. 52(12):3911-4.
- 7- Begum, S.H.; S.M.M.H. Majumder; M.A. Majid and M. M. Hossain. 2005. Isolation, Characterization and Study of the Physical, Chemical and Anti Microbial Properties of Fat Obtained from *Eucalyptus* (*Eucalyptus camaldulensis* Dehnh) Leaf. European Journal of Scientific Research, Vol 10, 3.
- 8- Benson, H.J. 2002. Microbiological Applications Laboratory Manual in General Microbiology. New York. McGraw-Hill companies.
- 9- British Pharmacopoeia, 1963. Determination of Volatile Oil in Drugs. The Pharmaceutical Press, London.
- 10- Cooradi, M.G.; G. Orbi; H.M. Abdel Moneam; A. Torelli and M. Bassi. 1999. Exudates from the wild type and a CR-tolerant strain of *Scenedesmus acutus* influence differently CR (VI) toxicity to algae. Science Direct, vol 37, Pp: 3019-3025.
- 11- Cowan, M.M. 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-582.
- 12- Desikachary, T.V. 1959. Cyanophyta. Academic press. New York.
- 13- Ghalem, B.R and B. Mohamed. 2008. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. African Journal of Pharmacy and Pharmacology Vol. 2(10). Pp. 211-215.
- 14- Greca, M. D.; M. Ferrara; A. Fiorentino; P. Monaca, and L. Previtiera.1998. Antialgal compounds from *Zantedeschia aethiopica*. Phytochemistry, 49: 1299-1304.
- 15- Hallegraeff, G. M.; D. M. Andersen and A.D. Cembella. 2003. Manual on Harmful Marine Microalgae.UNESCO Publishing, Paris.
- 16- Jawad, A.L.M.1982. Interaction between cyanobacteria and other micro-organisms. Ph.D.Thesis.Liverpool University. England.
- 17- Kassim, T.I.; H. AL-Saadi and N.A. Salman. 1999. Production of some phytoplankton and zooplankton and their use as live food for fish larva. Iraqi J. Agric. Proc. of 2nd Sci. Confer., 4(5): 188-201.
- 18- Kobaisy, M.; R. Mario; R. Tellez; C. L. Webber; F. E. Dayan; K. K. Schrader and D. E. Wedge. Phytotoxic and Fungitoxic Activities of the Essen-

- tial Oil of Kenaf (*Hibiscus cannabinus* L.) Leaves and Its Composition. Journal of Agriculture and Food Chemistry, 49(8): 3768-3771.
- 19- Miline, L.J.R. 1996. Fungi In: Practical Medical Microbiology edited by Collee, J.F. Churchill, Livingstone.
- 20- Nakai, S.; Y. Inoue and M. Hosomi. 2001. Algal growth inhibition effects and inducement modes by plant-producing phenols. Water Res., 35 (7) 1855-1859.
- 21- Nakai, S.; Y. Shingo and H. Massaki. 2004. Anti-cyanobacterial fatty acids released from *Myriophyllum spicatum*. Hydrbiologia, 543:71-78.
- 22- Nicholas, S.A. 1973. The effects of harvesting aquatic macrophytes on algae. Transactions of the Wisconsin Academy of Sciences, Arts and Letters, 61: 165-172.
- 23- Perez, L.; M. Pauli and P. Bazeque. 1990. Antibiotic assay by the agar well diffusion method. Journal of Actobiology, 15: 113-115.
- 24- Prescott, G.W. 1973. Algae of the Western Great Lakes Area. OHO Koeltz. Science publishers. Germany.
- 25- Reichling, J. 1999. Plant-microbe interaction and secondary metabolites with antiviral, antibacterial and antifungal properties; in Wink M (ed): Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology. Sheffield, Sheffield Academic Press, 1999, pp 187-273.
- 26- Reichling, J.; U. Suschke ; J. Schneele and H.K. Geiss. 2006. Antibacterial activity and irritation potential of selected essential oil components – structure-activity relationship. Nat. Prod. Commun., 1:1003-1012.
- 27- Rice, E. L. 1984. Allelopathy, 2nd ed. Academic Press, Ltd., London, United Kingdom.
- 28- SAS. 2004. SAS / STAT Users Guide for Personal Computers. Release 6.12. SAS Institute Inc., Cary, NC., USA. (SAS = Statistical Analysis System).
- 29- Shapiro, J.; V. Lamarra and M. Lynch. 1975. Biomanipulation: An ecosystem approach to lake restoration. In: Brezonick, P.I. and J.L. Fox (eds.), Water quality management through biological control. Department of Environmental Engineering Sciences. University of Florida, Gainesville, Pp: 85-96.
- 30- Sivonen, K. and G. Jones. 1999. Cyanobacterial toxins, pp: 41-111. In : Chorus and J. Bartram (ed.), Toxic cyanobacteria in water. E&FN Spon, London, Great Britain.
- 31- Stein, J.R. 1973. Handbook of phycological methods: Culture methods and growth measurements. Cambridge University Press. Cambridge.
- 32- Wang, S.Y.; C. Wu; F. Chu; S. Chien ; Y. Kuo ; L. Shyur; and S. Chang. 2005. Chemical composition and antifungal activity of essential oil isolated from *Chamaecyparis formosensis* Matsum. Wood. Holzforschung, 59, Pp. 295-299.
- 33- Yang, W.; J.S. Lu; H.Y. Li; X.L. Zang and Y.Z. Oi. 2009. Inhibition of the Growth *Alexandrium tamarense* by Algicidal Substances in Chinese Fir (*Cunninghamia lanceolata*). Bull. Environ. Contam. Toxicol. 83: 537-541.