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Isolation and purification of Hepatotoxin (Microcystin-LR) from some Blue –green algae of sweage water in Basrah

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Abstract

The present study involved isolation, identification and purification of four species of Cyanobacteria *Microcystis aeruginosa* Kuetz , *Microcystis flos-aque* (Wittr.)Kirchner , *Hapalosiphon Welwitschii* (West and West), *Calothrix parietina* (Thnret.) from Basrah region at AL-Ashar and AL- Khandaq canals that have been recorded for the first time in Iraq in this habitat .The extracted species testing for its ability to produced toxins particularly hepatotoxins (Microcystin–LR). This toxin was diagnosed depending on chemical features by using the thin layer chromatography technique (TLC). Toxic study showed that the toxin proceed by the algae has an (Rf) value closely to the standard hepatotoxin (Microcystin – LR) (0. 58). However, the value for the purified toxin was (0.54-0.56). The Ultraviolet spectrum of the purified toxin showed two absorption peaks ; the first appears at (240-243) nm and the other at (275-280) nm these two peaks are so close to the absorption peaks of the standard toxins (Microcystin-LR), namely (239) nm for the first peak and (275) nm for the other. Whereas Infrared spectrum has shown that the purified toxin from the four species of toxic algae contains most of the active groups composing the structure of Hepatotoxins (Microcystin-LR) .

By using the HPLC technique, the purified test has shown that the toxins have a retention time ranging between (3.920-4.108) minutes which is quite close to the retention time of the standard hepatotoxins Microcystin-LR (4.037) minutes. The results revealed that the species *H. Welwitschii* contains the highest concentration of hepatotoxin Microcystin – LR (44.415) µg/ml among the other species. The Bioassay tests for two species extracts of toxic algae have shown that the LD50 was within the toxic range of algae toxicity which reached (740) mg dry cells/kg mice for *H.Welweitschii* and (1540) mg dry cells/Kg mice for *C. parietina*, while the concentrations (20 and 50) mg dry cells/ml for all toxic species killed half numbers or more of the *Artemia salina* larvae after 24 hours.

Key word : cyanobacteria toxins , Microcystin-LR , extraction and purification with bioassay

1-Introduction

Cyanotoxin have long been recognized as a water-based disease that causes animal illness and death. The biotoxin, Microcystin-LR and nodularin, have been implicated in causing irreversible hepatotoxity and tumer promoting reactions laboratory rats (Falconer 1998).

Microcystins (MCYSTS) are agroup of cyclic heptapeptide liver toxins produced by several genera of cyanbacteria (blue-green algae), including *Anabaena* , *Hapalosiphon* , *Microcystis* , *Nostoc and Oscillatoria* .but most often by this belonging to the genus *Microcystis* (Carmichael , 1997).

The general structure of microcystins is cyclo (D-Ala-X-D-MeAsp-Y-Adda-D-Glu-Mdha-) in which X and Y are variable amino acids, D-MeAsp is erythro- β-Methylaspartic acid, Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, and Mdha is N-methyldehydroalanine (figure 1) .

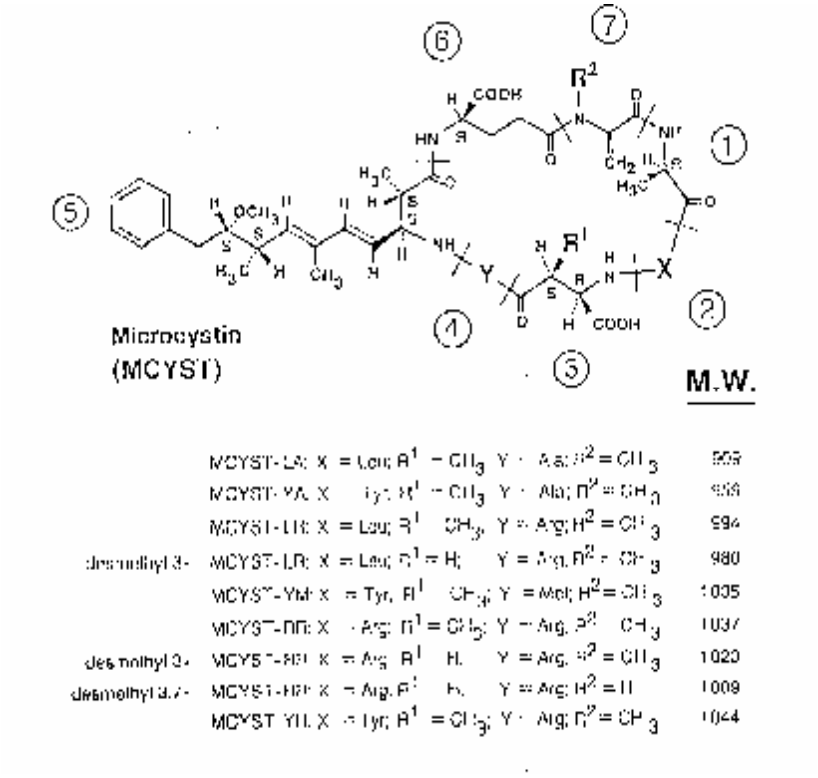


Figure-1: Chemical structure of hepatotoxins (Microcystins) that producing from cyanobactria (Carmichael , 1997)

Over 60 microcystins varients have been reported so far . The most common and toxic among them is microcystin–LR (MC-LR MW.994), in which the variable amino acids are leucine (L) and arginine (R) .

Microcystins are known to be pontent inhibitors of protein phosphatise 1 and 2A as

well as skin and liver tumer promoters in animals (Rao *et al*., 2004 ; Sivonen and Jones , 1999) .

The hepatotoxins are more famous group of toxins that producing from cyanobacteria because a highly liver toxic effects, but needing more time comparatively with neurotoxins, so that death may happen through the period between 5 and several days independently on several factores such as the size of animals, type of toxins and the dose exposure (Steffensen *et al*., 1999) .

2- Material and methods

Sample collection

The water samples are collected from two canals in Basrah region (Al-Ashar and Al-Khandaq) by using Zippline plankton net , and then stored in Plastic bottle (500 ml) until arriving to the laboratory .

Isolation and purification of algal species

The isolation of algal species was made by using streaking method on the solid Chu-10 media (Stein , 1975). Axienic culture was made according to (Weidman *et al*., 1984) .

Measurement of growth

The growth curve for all species was made by measuring the concentration of chlorophyll (a) according to (Vollenwieder, 1974) ,and then growth constant (K) and generation time (G) was made .

Extraction and purification of toxin (Microcystin – LR)

The algal cultures (axienic cultures) for all isolated species are harvested after (5-6) days from stationary phase by using centrifuge (3000 rpm), the harvested algal cells was lypholized by using freezing drier type (Lab ConCo - 18) .

Extraction of toxins was made according to (Luukkainnen *et al*., 1993) by taking 0.5 g from lyophilized cell for any isolated species and then mixed with organic mixture (Methanol:n-butanol:water) in portions (4:1:15) respectively .

Purification of toxins was made by using gel filtration chromatography (Namikoshii *et al*.,1993), the column size (2×15)cm filling with silica gel (100-200) μm in size . the column was washed with three eluent repectively (Deionized water, 20 % methanol and 80 % methanol), the fraction eluted by 80 % methanol was concentrated and lypholized .

Chemical analysis of purified toxins

A- HPLC analysis

The toxin fraction was dissolved in 1ml of absolute methanol (speciallized for HPLC analysis), and then put on sonicator for 5 minutes, 20μl was injected by microsyring to the instrument (type Shimadzu) with the following conditions (Reverse phase (15×4.6 mm I.d)–C18 column 15μm particles size) .The mobile phase (65 : 35 v/v)of (Methanol : 5μm Buffer phosphate, flow rate (1ml/min) at wave length 239 nm (Mahakhart *et al*., 1998). Results were compared to a standard hepatotoxin (Microcystin-LR) (Alexis biochemical company) .

B-TLC (Thin layer chromatography)analysis

This method was made in silica gel plate in diameter (2×10)cm (type Merck silica gel GF245), the mixture (1-Butanol:acetic acid: water) as poritions (2:1:1) respectively was as eluent solvent, and the isolated spot was evaluated by Ultraviolet Lamp and ninhydrin reagent 0.2 % .

C- Ultraviolet spectrum

A(0.1)mg from both purified and standard toxin were soluted in 3ml ethanol, the spectrum ranged (200–400)nm was measured using LKB-sweeden ultraviolet UV spectrophotometer.

D- Infrared spectra

The Infrared spectra for all purified toxins were measured by using fourier transform Infrared spectrophotometer (FTIR–84005), (0.5)mg were mixed with potassium bromide (KBr) in portion 3:1 and the wave number range between (600-3600 cm^{-1}) .

E- Bioassay tests

Two types of bioassay method were made to investigating the toxicity of algal species, the first by determinating of lethal dose concentration (LD50)on mice type Albino (Balb/c) according to the (Lee *et al*., 1999), the second method by using *Artemia salina* larvae according to (Vezie *et al*., 1996 ; Lee *et al*., 1999).

3-Results

Morphological characteristic of isolated algae

In this study the isolated four species of cyanobacteria represented by *Microcystis aeruginosa, Microcystis fols-aque, Hapalosiphon Welwitschii* and *Calothrix parietina* are shown in Table-1 and picture -1 (1-4) .

The alga *M. aeruginosa* shown as colonies consist of aggregation condensed cells 2.5-7µm in diameter and always mean 5 µm . While in type *M. flos-aque* the colony cells are less condensed cell 2.5–7µm in diameter. *H. Welwitschii* is filamentous algae branched in one side that equall to or less than main filaments in diameter of its cells,the width in the main filament 7.7–10µm and 12.5–20µm in long, while cells of branch ranged between 5-6.25µm in width and 5 –15µm in long .

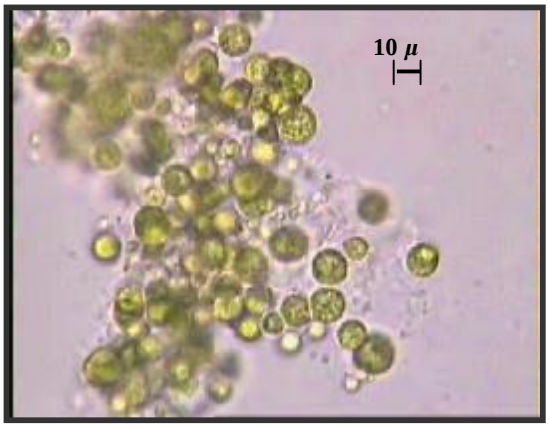
C. parietina is filamentous alga unbranched some times appear pseudobranched with basal hetrocyst, the basal cells nearly the hetrocyst 10 µm in width, the long of filaments between 87.5–250 µm .

Growth curve

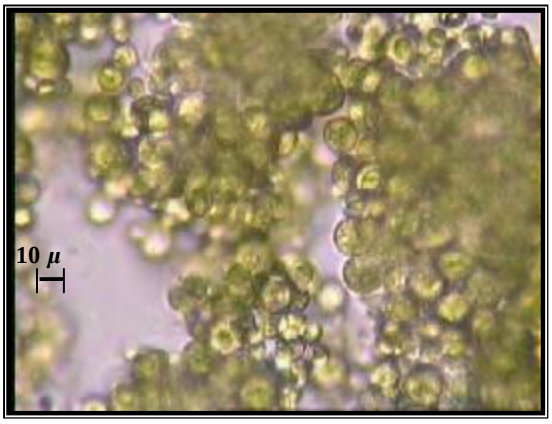
Results reveald that all isolated species were grow well in the Chu-10 medium , but the species *C. parietina* have a highly growth constant (K)1.53 and low generation time (G)0.196 following species were *M. aeruginosa* , *H. welwitschii* and *M. flos-aque* table- 2 .

Table (1): The types of the cyanobacterial culures isolates.

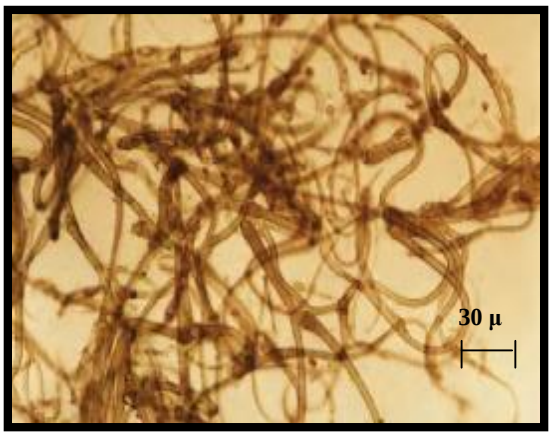
Cyanobacterial species	Unialgal culture	Axienic culture
Microcystis aeruginosa	+	+
Microcystis flos – aque	+	+
Hapalosiphon Welwitschii	+	+
Calothrix parietina	+	+



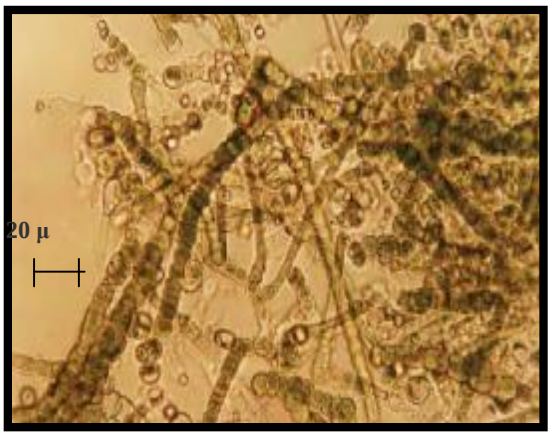
2- *Microcysts flos-aque*



1- *Microcysts aeruginosa*



4-*Calothrix parietina*



3- *Hapalosiphon Welwitschii*

Photo (1): Morphological characters of cyanobacterial species isolates .

Table(2): Growth constant (K), Generation time (G) , Harvisting time and different growth phases of cyanobacterial isolates.

Algal isolates	Lag phase (days)	Exponantial phase (days)	Stationary phase (days)	Havesting period (days)	Growth constant(K)	Generation time(G)
M. aeruginosa	4	9	13	19 - 20	1.053	0.286
M. flos - aque	4	21	25	29 – 30	0.149	2.1
H. Welwitschii	2	13	15	20 – 21	1.005	0.29
C. parietina	4	10	14	20 – 21	1.53	0.196

Chemical analysis

A- Thin layer chromatography (TLC)

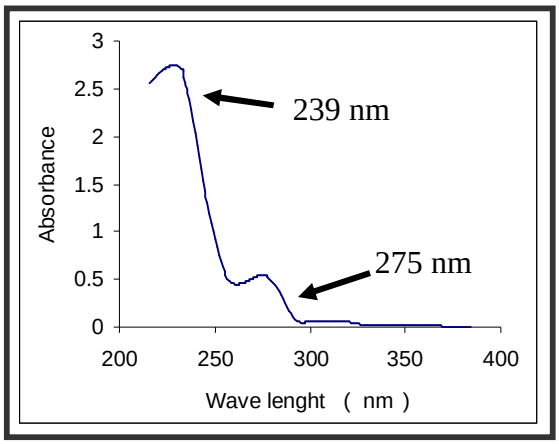
IAll purified toxin from four cyanobacterial species were found closed to the Rf value of standard hepatotoxins (Microcystin-LR) that equal to Rf = 0.58 , so that the purified toxins from *M. aeruginosa* and *M. flos-aque* reached to (Rf=0.54) and Rf=0.56 for the species *Hapalosiphon Welwitschii* and *Calothrix parietina* (Table-3) .

Tabl (3): The rate of flow value for standard toxins (MC-LR)and purified toxins .

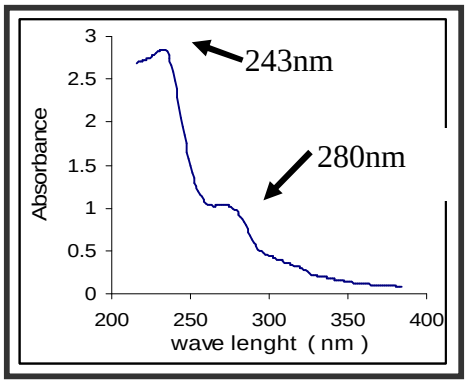
Standard hepatotoxins with purified toxins from different species algae	Rf value
Standared toxins (MC-LR)	0.58
M. aeruginosa	0.54
M. flos-aque	0.54
H. Welwitschii	0.56
C. parietina	0.56

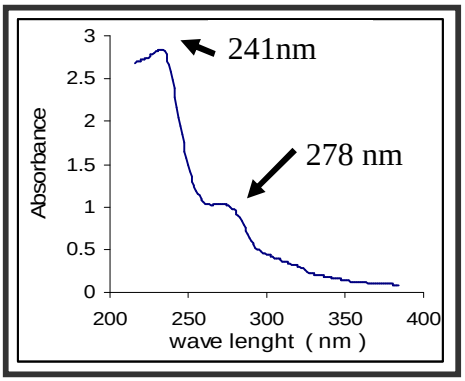
B- Ultraviolet spectra

Figure-2 showing that the standard toxin (MC-LR) UV spectrum is consisting of two peaks , the first at 239 nm and the second at 275 nm. In contrast the UV-spectra of the standard hepatotoxins are showing: (243, 280), (241, 278), (240, 275) and (240, 278)nm for *M. aeruginosa* , *M. flos-aque* , *H. Welwitschii* and *C. parietina* respectively .

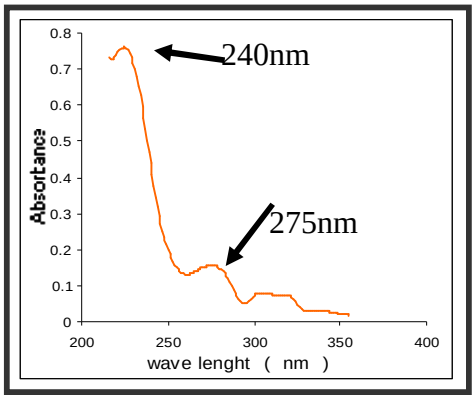


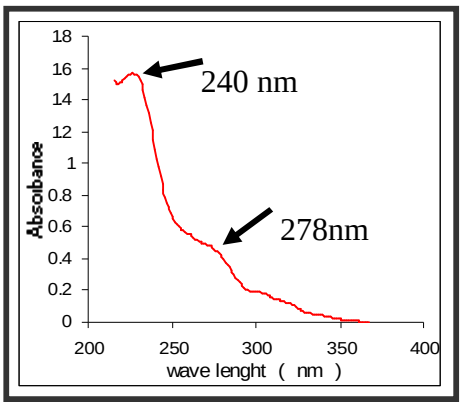
(A)





(B) **(C)**





(D) **(E)**

Fig.2:Ultraviolet spectra of purified toxins compared with standard toxins (Microcystins–LR)

A- Standard toxins (MC-LR) , B- *M. aeruginosa* ,

C- M. flos-aque* , *D- H.Welwitschii* , *E- C. parietina

C- Infrared spectra (IR)

The IR- data of the hepatotoxins (MC-LR) are listed in Table-4, which shows the involved functional groups of the purified toxins. The IR-Spectra are shown in Fig. 3.

Table (4) : Infrared data (in cm^{-1})of purified toxins from four species isolates .

Functional groups	*M. aeuginosa*	*M. flos - aque*	*H. Welwitschii*	*C. parietina*
O-H , N –H Str.	3421 S.,Br.	3409 V.S.	3423 V.S. , Br.	3409 V.S. , Br.
Alkyl group CH2 ,CH2 , CH 3 Str.	2931 - 2854 M.	2933 - 2857 V.S. , Br.	2927 - 2848 S.Br	2928 - 2862 V.S.
Carbonyl in Carboxyl group Str.	1736 M. , Br.	1729 S. Br.	1732 S. Br.	1726 V.S.
Carbonyl in amide Str.	1651 - 1465	1466 - 1424	1621 - 1465	1649 - 1465

s.br. = Strong braod u.s. = Very strong m. br = Medium braod str. : Stretching

D-High performance liquid chromatography (HPLC)

The purified toxins from all species isolates having retention times very closed to the standard toxins (Microcystin-LR) ranging between (3.950–4.108) minutes compared to the standard retention time (4.037) minutes . the results revealed that the species *H. Welwitschii* contains the highest concentration of hepato-toxins (MC-LR) reached to (44.415 ug /ml) among the other species Table -5 and figure-4 (A-E).

Bioassay

A- Mouse bioassay

The two species *H. Welwitschii* and *C. parietina* were tested of their toxicity on laboratory albino mice type Balb/c , because their have a highly concentration of toxin (Microcystin-LR), so that the statistical analysis by using propapility paper revealed that the LD50 for the species *H. Welwitschii* reached to 740mg dry biomass/kg animal weight and 1540 mg dry biomass/kg animal weight for the second species *C. parietina* figure-5 .

B- Artemia bioassay

Result revealed that all algal extracts was have ahighly significant effects on the means of larval numbers and its mortality .in addition to that the concentration 20 and 50mg/ml led to dead ahalf of larvae in all algal extracts table (6) .

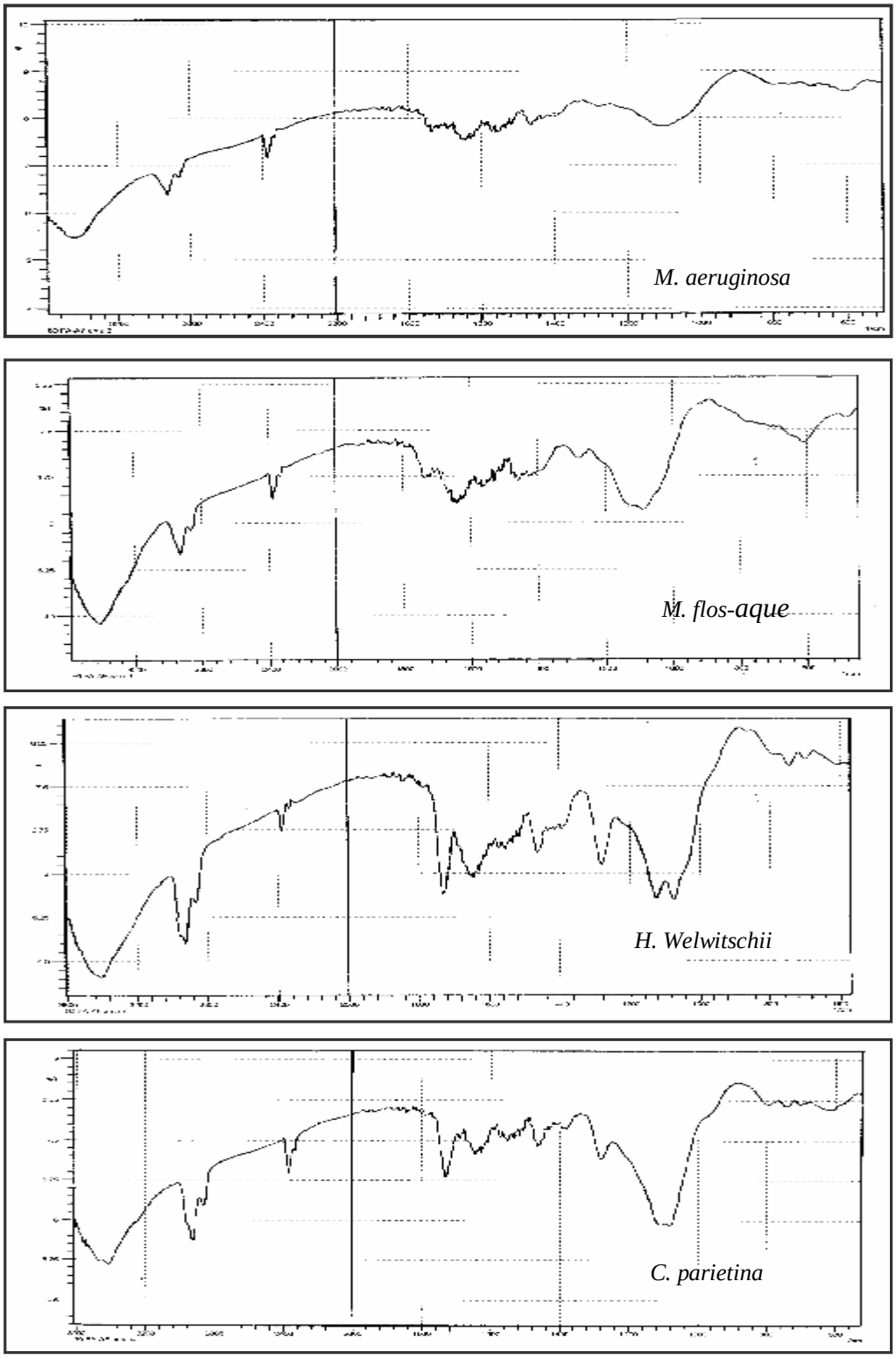


Figure 3: IR spectra of purified toxins (MC-LR) for algal isolates.

Table(5): Concentration of Microcystin in purified and standard toxins (MC-LR) and their own retention time.

Algal isolates	Aeria under curve	Retention time (min)	Conc. Of hepatotoxin (MC-LR) µg / ml
Standard Toxins (Microcystin - LR)	35190	4.037	20
M. aeruginosa	65141	3.975	37.022
M. flos-aque	58583	3.950	33.295
H.Welwitschii	78149	3.974	44.415
C. parietina	64778	4.108	36.816

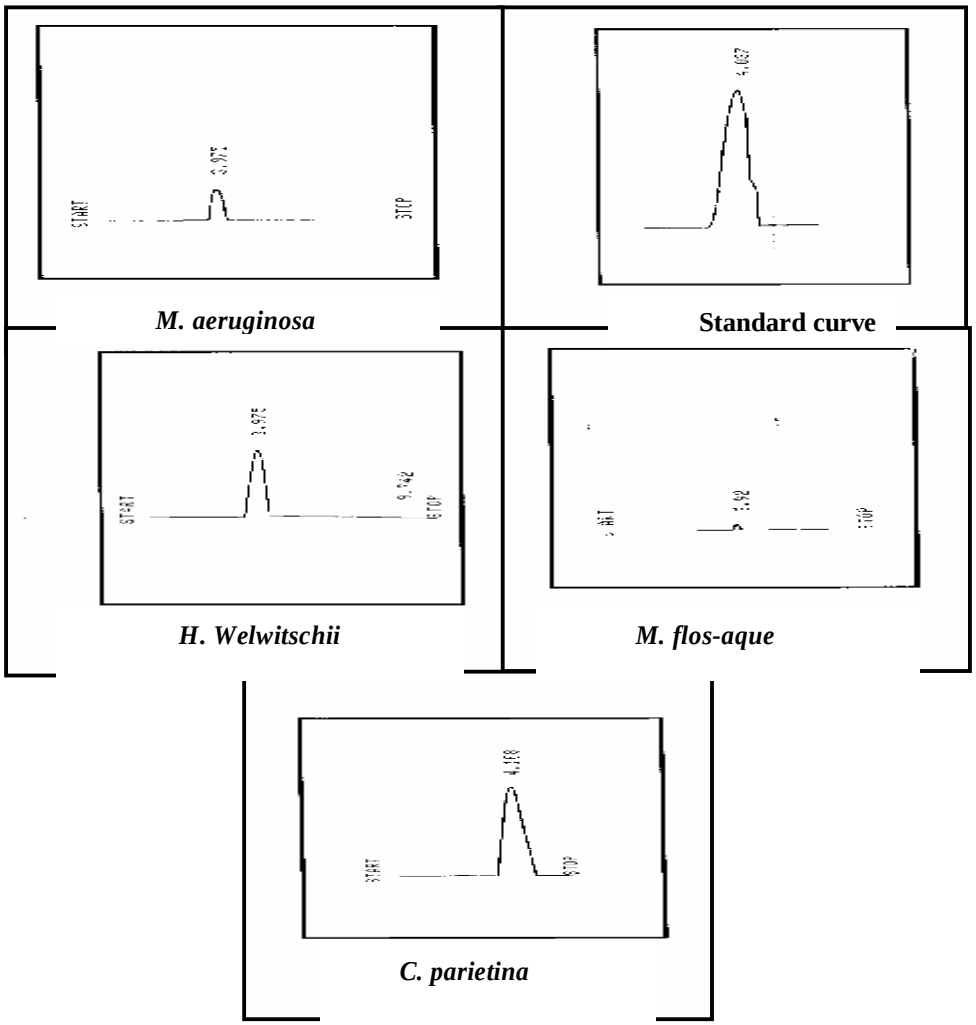


Fig. 4 : HPLC analysis of standard toxins (MC-LR) and purified toxins from algal isolates .

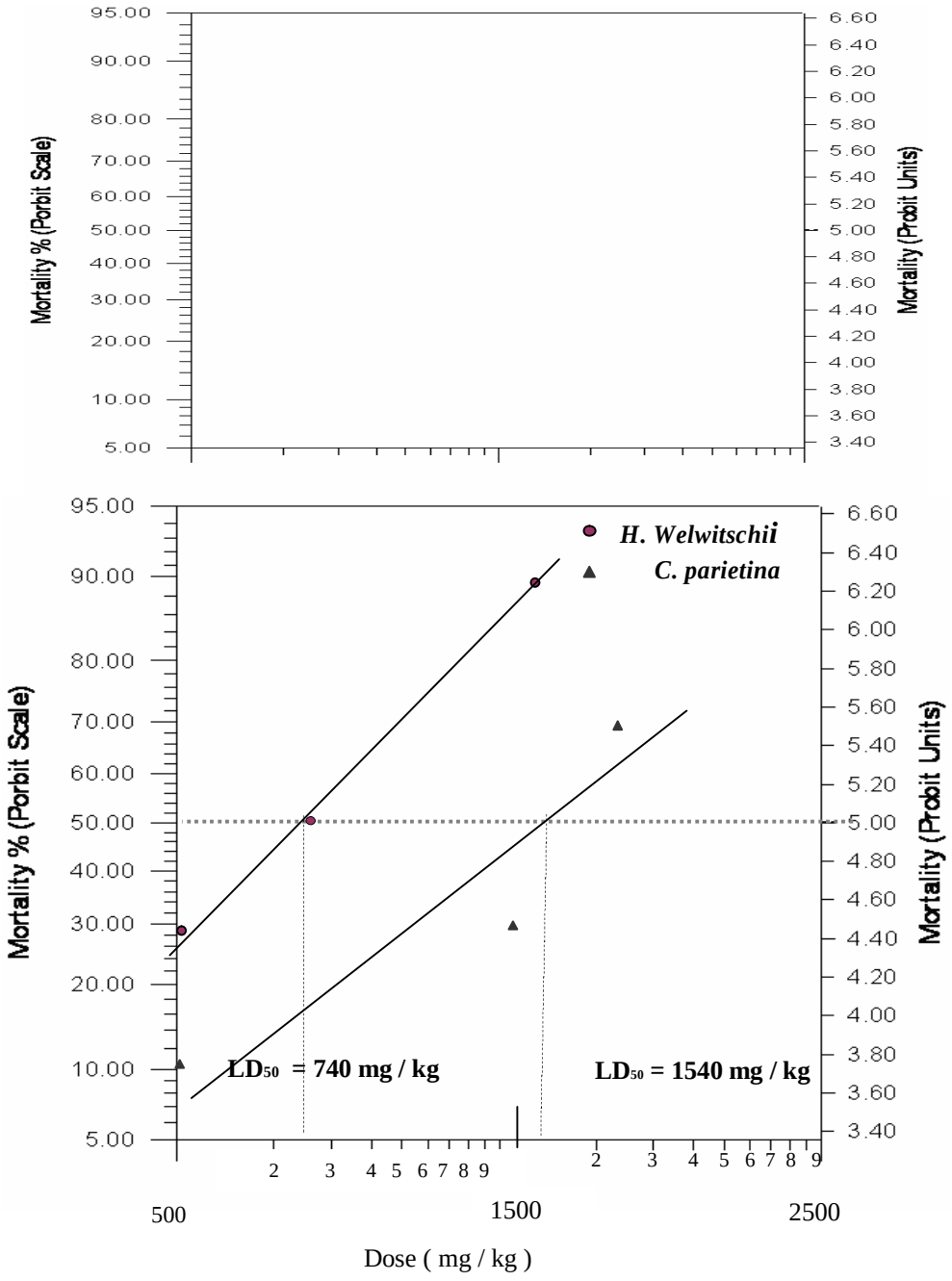


Figure 5 : Propability papers constituting determining the LD50 conc. For algal extracts *H.Welwitschii* and *C. parietina* .

Table (6): Mean of *Artemia salina* shrimps numbers after 24 h treatment with different concentration of cyanobacterals extracts .

Algal extract mg / ml	Algal isolates			
	M.aeruginosa	***M.flos-aque***	***H.Welwitschii***	***C.parietina***
Control	20 ± 0.00 [a]	14 ± 0.86 a	18.5 ± 0.86 a	14 ± 0.28 a
2	14 ±1.15 b	13 ± 0.57 a	15 ± 1.73 b	14 ± 0.00 a
10	11 ± 2.30 c	13 ± 1.15 a	14 ± 2.30 b	12 ± 0.28 b
20	10.5 ± 1.44 c	10 ± 0.00 b	12 ± 2.30 b	10 ± 0.28 c
50	10.5 ± 0.86 c	10 ± 0.00 b	11 ± 0.57 b	7 ± 0.57 d
100	6 ± 1.15 d	6 ± 0.60 c	4 ± 1.15 c	2 ± 0.57 e
R.L.S.D	2.86	1.218	3.29	0.730

4- Discussion

Recent study revealed that many genus of toxic algae exactly cyanobacteria are found in our environment (Basrah city) such as sweage water (AL-Ashar and AL-Kandak canals) such as *M. aeruginosa* is well Known awide distributed in the world and it's the first genus recorded capable to produce hepatotoxins (MC-LR) (Botes *et al*., 1982 ; Carmichael, 1988). While the species *M.flos-aque*, *H. Welwitschii* and *C . parietina* was the first recorded in Iraq by the ability to produce hepatotoxins (Microcystin-LR) .

The growth rate for all species having a highly growth constant (k) and low generation time (G) in Chu-10 medium, so this revealed that this medium is very suitable for growth of algal isolates .In addition, the growth of species *M. aeruginosa* have a highly growth compared to AL- Rekapy (2003).

The thin layer chromatography (TLC) is a classical planar chromatographic technique widely utilized by analysis until the papuarity of gas and liquid chromatographic techniques in the late nineteen seventies (Pelander , 2000) . The results of TLC technique for purified toxins and standard toxin (Microcystin-LR) were similar to (Pelander, 2000) ,Which found that the Rf value reached to 0.59 for hepatotoxins (MC-LR) and agreed with (Pelander, 1998 ; Al-Shaheen , 2000). Who found that the Rf = 0.55 and Rf = 0.54 for toxic substances purified from *Oscillatoria tenuis* .

The ultraviolet spectra of all purified toxin from cyanobacterial isolates are consisting of two peaks similar to the standard toxins (MC-LR), the first range between (240 -243) nm and the second peaks between (275-280)nm. So these results on agreement with many researchers such as (Namikoshi *et al*., 1992 ; Brittain *et al*., 2000). The observed UV-signals above 200nm may be assigned to π-π^* transition of the aromatic system of hepatotoxins (MC-LR) (Silverstein *et al*., 1991) .

The results revealed that the infrared spectra for all purified toxins several functional groups, are involved e.g. amine group hydroxyl group (N-H,O-H), alkyle group, methyl group

(CH,CH2,CH3)and carbonyl of amide group (C= 0), as main groups in the structure of hepatotoxins (MC-LR) (Silverstein *et al*.,1991).

By using the technique of higher performance liquid chromatography the result showed that all purified toxins from algal isolates were closed to the retention time of a standard toxin (MC-LR) that reach to 4.037 minutes. In addition the species *H.Welwitschii* producing this toxins as more as other species and then the reproducibility for the standard toxins and purfied toxins reached to 97.28 %, this result was showing a highly similarity in the structure between purified and standard toxins. Finally this teqnique is considered a widely method using for separating cyanobacterial toxins and showing its purity and concentrations (Pelander , 2000) .

The mouse and *Artemia* bioassay is widely used methods to determine the toxicity of cyanobacteria (Wanatabe and Oishi,1882 ; Falconer, 1998 ; Larsen, 1998). Mouse bioassay was showing that the LD50 for two algal isolates *H. Welwitschii* and *C. parietina* reached to (740, 1540 mg dry weight/kg animal weight) respectively so that these results agree with those obtained by many researchers e.g. (Lanaras *el al*., 1989 ; Sivonen *et al*., 1990 ; Lee *et al*., 1999) their detected toxicity of algae ranging between (1500– 2000 mg dry weight /kg animal cells) and Carmichael (1995) refered that the death may be occurred after expouring to the algal toxins (Microcystins) in rang between 4-24 hours or after several days. The second bioassay method using *Artemia salina* shrimps that which can be considered as sutable test to determine toxicity of cyanobacteria (Vezie *et al*., 1996) showing that the concentrations from 20-50 mg/l was led to death of (50%) of *Artemia salina* shrimps of table (6). These results agreed with (Veize *et al*., 1996 ; Lee *et al*., 1999) who visualizied that the algal extract less than100 mg/l in toxicity for mice to less than 1600 mg dry cells / kg animal weight .

5-References

AL-Rekabi, H.Y. (2003). Utilization of mass cultures of certain microalgae in nutrition of chicks. PH.D Thesis,Univ. of Basrah .College of science .122 pp.

AL-Shaheen, M.A. (2002). Species composition of algae and its ability to produce toxins in drinking water stations at basrah city , Iraq. Msc. Thesis ,Uni. of Basrah. College of science . 61p p.

Botes, D.P. ; Kruger, H. and Viljoen, C.C. (1982). Isolation and characterization of four toxins from the blue-green algae *Microcystis aeruginosa* . Toxicon., 20 : 945 - 954 .

Brittain, S.M. ; Wang, J.; Jackson, L.B.; Carmichael, W.W. ; Rineh -hart , K.L. ; and Culver, D.A. (2000). Isolation and characteri zation of Microcystins , cyclic heptapeptide lake Eric strain of *Microcystis aeruginosa*. J. Great lakes Res., 26 (3) : 241 – 249.

Carmichael, W.W. (1997). The cyanotoxins In: Advances Botanical research. ed

J.A Callow;Academic press Ltd.,London, 27:211- 256 .

Carmichael, W.W. (1988). Toxin of fresh water algae. In: Hand book natural toxins. A.T. Tu ed.: Vol. 3, Marine Toxin and venoms. Marcel Dekker , New york , p. 121 - 147 .

Carmichael, W.W(1995). Cyanobacterial toxins In: Manual and Harmful marine microalgae. eds.: G. Hallegraeff , . M. Anderson, D.M. and Cembella , A.D. IOC Manual and Guides no. 33 , UNESCO . Paris. P. 156 – 177.

Falconer, I.R.(1998). Algal toxin and human health. In:The handbook of environmental chemistry. eds: J. Hrubec, Vol.5. Part c, Quality and treatment of Drinking water II. Springer- verlag ,Berlin , p. 53 - 82 .

Lanaras, T. ; Tsitsamis, S. ; Chlichlia , C. and Cook, C. .Toxicon. (1989) Toxic cyanobacteria in Greek fresh water .J. Appl. Phycol., 1 : 67 - 73 .

Larsen A. (1998) Autecology , toxicity , genetic and life history of *Prymnesium parvum* and *Prymnesium patelliferium* (Haptophyte) : Is a species separation warranted. Doctor dissertation . Dep. Of Fisheries and Marine Biology . Univ. of Begen , Norway .

Lee, T.H. ; Chen, Y.M. and Chou , H.N. (1999). Toxicity assay of cyanobacterial strains using *Artemia salina* in comparision with the mouse Bioassay. Aeta Zoologica Taiwanica , 10 (1): 1- 9 .

Luukkainen, R. ; Sivonen, K. ; Namikoshi, M. ; Farding , M. ; Rinehart , K. L and Niemeta , S. (1993). Isolation and identification of eight Microcystins from thirteen *Oscillatoria agardhii* strains and structure of new Microcystin . Applied and Envir . Microbiol., P. 2204 - 2209 .

Mahakhart,A.; Sano,T. ; Ratanachot,P. ; Tongram, T. ; Srivastava, V.S.;Watanabe, M.M. and Kaya, K.(1998). Detectionof microcystins from cyanobacterial water blooms in Thailand fresh water. Phycological Res.,46 : 25-29.

Namikoshi, M. ; Rinehart , K.L. ; Sakai , R. Stotl; s , R.R.; Dahlem , A.M.; Beasly, V.R. Carmichael, W.W. and Evans, W.R (1992) . Identification of 12 hepatptoxins from homer lake bloom of the cyanobacteria *Microcystis aeruginosa* . Nine new Microcystins . J. Org . Chem., 57(3) : 866 – 872 .

Namikoshi, M. ; Choi , B.W. Sun, F. and Rinehart, K.L. (1993). Chemical characterrization and toxicity of Dihydro Derivatives of Nodularin and Microcystin -LR potent cyanobacterial cyclic peptide hepatotoxins. Chem. Res. Toxicol., 92 :151 - 158 .

Pelander, A. ; Ojanpera, I. and Vuori, E. (1998) . Analysis of cyano-Bacterial hepatotoxins by over pressured layer chromatography. J. Planer Chromato., 11 : 365 369 .

Pelander, A. (2000). Thin layer Chromatographic detection of cyanobacterial hepatotoxins. Academic dissertation . University of Helsinky , Finland .pp 287.

Rao, P.V. ; Gupta , N. and Jayaraj, R. (2004) Sccreeening of certain chemoprotectanats

against cyclic peptide toxin microcystin-LR . J.of Pharma. 36 (2) : 87-92 .

Silverstien , R.M. ; basster , G.C. and Morril , T.C. (1991). Spectrometric identification of organic compounds 5th ed. Tohn wily and Sons , Inc. USA. 419 PP.

Sivonen ,K; Carmichael , W.W. ; namikoshi, M. ; Rinehart , K . L. ; Dahlem , A.M. and Niemela, S.I. (1990). Isolation and character-ization of hepatotoxic microcystin homologs from the filamentous fresh water cyanoba-cterium Nostoc sp. strain 152. APPl. Environ. Microbiol., 56 : 2650 -2657 .

Sivonen, K. and jones, G. (1999). Cyanoba-ctyerial toxins In: Toxic cyanobacteria in water. eds.: I., Chrus, and J. ,Barton. Giude to their Public Health Consequences , p. 41 - 111 .

Stein, J.R. (1975). Handbook of phycological method. Cambridge University press. Cambri-dge . 445 pp.

Steffensen, D. ; Burch, M. ; Nicholson, B. ; Drikas, M. and Baker, P. (1999). Management of toxic Blue–green algae (Cyanobacteria) in Australia

Vezie, C. ; Benofella , F. and Sivonen , K . (1996). Detction of toxicity of cyanoba-cterial strain using *Artemia salina* and *Microtox*R assays compared with mouse bioassay results. Phycol., 35: 198 202 .

Vollenwieder, R. A. (1974). Amanual of method for measuring primary production in a quatic environment . Black well . Publ. Oxford . 225. Environ . Toxicol., 14:183-195 .

Watanabe, M.F. and Oishi, S. (1982). Toxic substances froma natural bloom of *Microcystis aeruginosa* . Appl. Envron. Microbiol., 43(4) : 819 - 822 .

Weidman, V.E. ; Walne, P.R. and Tainor, F.R. (1984). A new technique for obtaining axenic cultures of algae. Can. J. Bot., 42: 958-959 .

عزل وتنقية السم الكبدي Microcystin-LR من بعض الطحالب الخضر - المزرقة من مياه المجاري في البصرة

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الملخص

تضمنت الدراسة عزل وتشخيص وتنقية أربعة أنواع من الطحالب الخضر- المزرقة (Cyanobacteria) المنتجة للسموم في مدينة البصرة في قناتي العشار والخندق والحصول على مزارع نقية منها ، تمثلت بالأنواع *Microcystis aeruginosa* Kuetz و *Microcystis flos-aque* و *Hapalosiphon Welwitschii* West and West و *Calothrix parietina* Thnret والتي عزلت لأول مرة في القطر من تلك البيئة وأثبتت قدرتها على إنتاج السم الكبدي Microcystin – LR من خلال إستخلاصه وتنقيته .

أظهرت الدراسة السمية بأستخدام تقنية كروماتوغرافيا الطبقة الرقيقةThin layer Chromatography (TLC) إمتلاك المادة السامة المنقاة من الأنواع الأربع المنتجة للسموم قيم Rf (Refractive factor) تراوحت بين(0.54 - 0.56) وهي قريبة جدا من قيمتها للسم الكبدي القياسي Microcystin - LR والبالغة (Rf = 0.58) .

أوضح أختبار طيف الأشعة فوق البنفسجية(Ultra violet radiation UV) إمتلاك المادة السامة المنقاة قمتي إمتصاص الأولى تراوحت بين (240 - 243) نانومتر والقمة الثانية بين (275 - 280) نانومتر وهي مقاربة بدرجة كبيرة لقمتي إمتصاص المادة السامة القياسية Microcystin - LR البالغة (239) نانومتر للقمة الأولى و (275 نانومتر) للقمة الثانية ، أما إختبار طيف الأشعة تحت الحمراء Infrared spectrum (IR) فأوضح إحتواء المادة السامة المنقاة وللأنواع الأربعة من الطحالب معظم المجاميع الفعالة في تركيبة السم الكبدي القياسي Microcystin - LR .

بين إختبار النقاوة للمادة السامة بأستخدام تقنية كروماتوغرافيا الغاز السائل عالي الأداء HPLC (High performance liquid chromatography) إمتلاكها زمن أحتباس Retention time تراوح بين (3.920 - 4.108) دقيقة وهو مطابق بدرجة كبيرة لزمن إحتجاز السم الكبدي القياسي Microcystin - LR والبالغ (4.037) دقيقة ، وتميز الطحلب *H. Welwitschii* بأحتواءه على تركيز عالي من السم الكبدي MC - LR بلغ (44.415) مايكروغرام / مليلتر من بين الأنواع المدروسة .

بينت الأختبارات الحيوية Bioassay لمستخلص نوعين من الطحالب السامة على الفئران المختبرية البيضاء من سلالة Balb/c أن الجرعة نصف القاتلة الوسطى (LD50) Lethal dose concentration بلغت 740 ملغم خلايا جافة / كغم وزن حيوان للنوع *H . Welwitschii* و 1540 ملغم خلايا جافة / كغم وزن حيوان *parietina* ، أما أختبار المادة السامة ضد يرقات روبيان الممالح *Artemia salina* فأن التراكيز (20 و 50 ملغم وزن خلايا جافة / مليلتر) ولجميع الأنواع السامة أدت الى قتل أكثر من نصف العدد منها بعد مرور 24 ساعة .

كلمة المفتاح : سموم الطحالب الخضر-المزرقة ، المايكروسستين ،الأستخلاص التنقية والفعالية الحيوية