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Bioseparation and Purification of Hepatotoxins Microcystin–LR from Cyanobacteria Species Westiellopsis Prolifica

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Abstract:

The presents study included bioseparation and purification of hepatotoxins microcystin-LR from cyanobacteria species westeillopsis prolifica the concentration of toxin was determined by preparative high performance liquid chromatography by comparing peak area and retention time of analytical standard of microcystin-LR with peak area and retention time of extraction of each species of cyanobacteria, the retention time of analytical standard of microcystinLR were 9.55 min and it's concentration was 10µg/ml, W. prolifica retention time was 9.5 min, it's concentration was 28.385 µg/ml.

Keywords: *Biological toxins, Cyanobacteria, MicrocystinL.*

1. Introduction

Cyanobacteria are prokaryotic, autotrophic microorganisms, gramnegative bacteria with thick peptidoglycan layer and lipopolysaccharide , are present in fresh and brackish waters, cyanobacteria can be produced secondary metabolites which are toxic to other organism called cyanotoxins [2] [1] . Cyanotoxins are chemical substance which is produced at stationary phase as secondary metabolite by cyanobacteria that can induce the toxic effects or a diverse groups with natural substance both from toxicological , chemical properties and cause direct intoxication to aquatic and terrestrial organism due to their ability to accumulate in tissue of organism and transfer through food chain Cyanotoxins like microcystins can produce via decrease of Phosphate, nitrate, ferric Fe+2 and zinc Zn+2 [1] [3]. Cyanobacteria can be released several toxins at the same time, while some species of cyanobacteria does not produce toxins at all. The major cyanobacteria producer of cyanotoxins are

Anabaena, Microcystis, Aphanizomenon, Nostoc ,Cylindrospermopsis, Lyngbya, and Oscillatoria (Planktothrix) [4].

2. Materials and methods

2-1-Sterilization and Preparation of BG11 medium

All glassware and BG11 medium had been sterilized in autoclave at 121C ,15J for 15 min and the BG11 medium was utilized for cyanobacterial growth [5] [6]

2-2-Culturing of Cyanobacteria

Species Unicellular algae of Cyanobacteria was taken of 10 ml of isolate of Cyanobacteria in log phase which added to a flask contained 90ml of BG11 media and incubated at 27±2Ċ with a photo period of 8 hour darks:16 hour lights for 14 days, this flask that contained 100ml growth of cyanobacteria would transport to flask contained 900 ml of media and incubated for 14 days and ultimately the growth of cyanobacteria in the flask that contained 1000ml, would be transported to pools 20 liter and harvested after 5-6 days at stationary phase (Cyanotoxin was formed in this phase) and concentrated by centrifugation at 3000rpm for 15min and lyophilized by Oven at 35Ċ for 48h, repeated culturing of each Species of cyanobacteria four time to obtain large amount of biomass[7] (Tredici,2004)

2-3-Extraction and Purification of microcystin-LR

The cyanobacterial cell are freeze-thaw ,three time before extraction to disrupt the cell wall lead to easy release of microcystin from cell and lyophilized cell of cyanobacteria (2g) from W. prolifica had been extracted three time by solvent mixture of water:methanol:1-butanol 75:20:5 for one hour then sonication by path sonicator for two hour and the extracts were centrifuged at 15000 rpm for 30 min at 20 Ċ and the supernatant combined, the combined supernatants would be airdried at 35Ċ to remove methanol and 1-butanol and to concentrate to 3ml and microcystins in each extract detected by using Ultraviolet-Spectrophotometer at 238nm [8]. The purification of toxins has been performed according to Namikoshi et al.,(9) above extract was loaded on glass column (2 × 15cm) which contained Silica gel (75-250 mesh), then the column washed by 120 ml of Deionized water followed by 20% methanol (20 ml methanol : 80 ml Deionized water) and finally, the toxins was eluted by 80% methanol with flow rate 3ml/min.

2-4-Analytical, Purification and collection of microcystin-LR

The toxins fraction has been dissolved in absolute methanol specialized for preparative high performance liquid chromatography (PHPLC) and 0.25ml was injected by microsyring to PHPLC type (Shimadzu in ministry of Science and technology in Lab. Of water and environmental analysis test) have the following characters C18- Octanoldodecyl column with

25cm×4.6mm l.D. and mobile phase (Methanol : H2O) 20:80 , flow rate (1ml / min) at wave length 238 nm and at 30C of temperature [10].The results compared with an absorbance and retention time of standard Microcystin - LR was purchased from sigma Aldrich Company , then peak of microcystinLR was collected and put in oven at 35C for two week to remove methanol.

Results

3-1-Culturing of Cyanobacteria

Species Cyanobacteria were obtained during the period of this study from scientific centers Iraqi Universities, Which is Westiellopsis prolifica that is growing on BG11 medium was the best media for obtaining biomass W. Prolifica was identified as producer of microcystin-LR for the first time in Iraq . Figure (1-1). The microcystin-LR of W. Prolifica was extracted at stationary phase and its entered stationary phase at nine day figures (1-2)

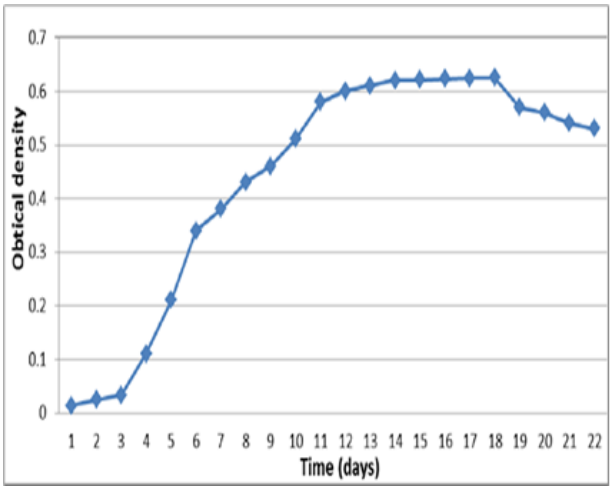


Figure (1-2) Growth curve of W. prolifica during the period of culture

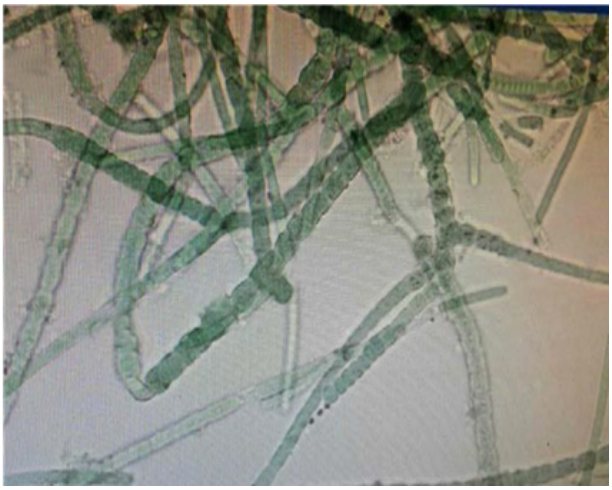
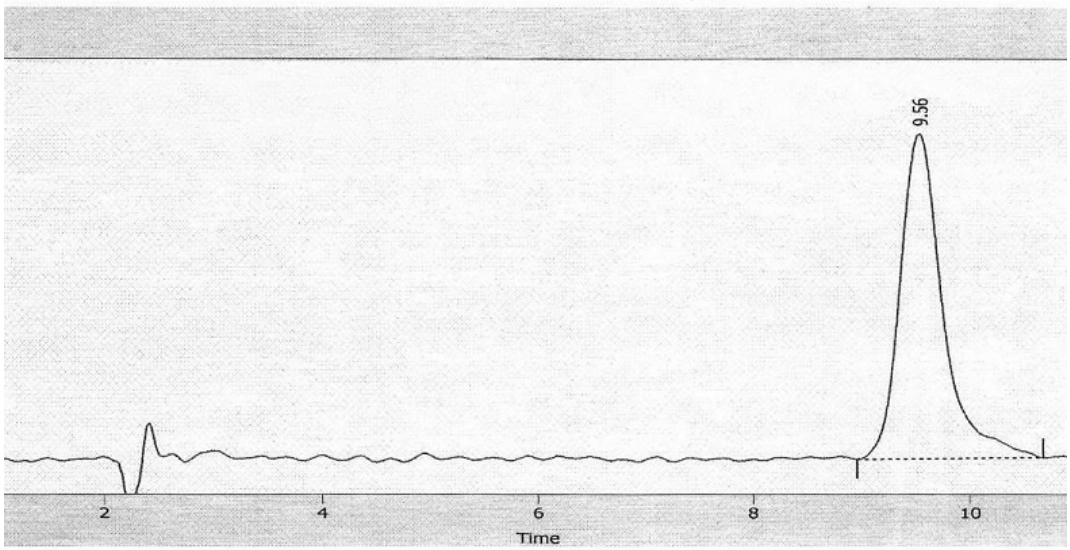


Figure (1-1) Westiellopsis prolifica

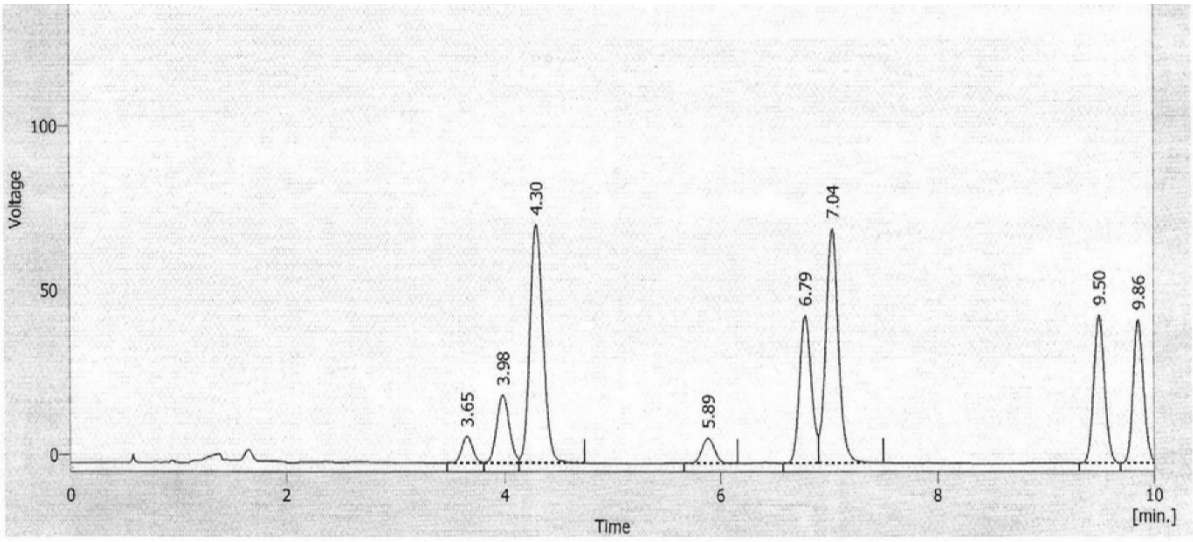
3-2-Analysis and Purification of Microcystin-LR by Preparative HPLC

Extraction Crude of each species of cyanobacteria were occurred by utilizing water: methanol: butanol and partially purified by silica gel column, then analyzed by preparative HPLC to detect the present of microcystin-LR, the concentration of toxin was determined by comparing peak area and retention time of analytical standard of microcystin-LR with peak area and retention time of extraction of each species of cyanobacteria, the retention time of analytical standard of microcystin-LR were 9.55 min figure (1-3) and it's concentration was 10µg/ml, W. prolifica retention time was 9.5 min figure (1-4) , it's concentration was 28.385 µg/ml.



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]
1	9.557	1327.449	48.527	100.0	100.0	0.39
	Total	1327.449	48.527	100.0	100.0	

Figure (1-3) HPLC analysis of standard Microcystin-LR



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	3.650	56.351	8.050	2.3	2.6	0.11
2	3.983	168.959	20.682	7.0	6.6	0.13
3	4.297	615.509	72.290	25.4	23.1	0.14
4	5.887	61.429	7.528	2.5	2.4	0.13

Figure (1-4) Preparative HPLC Analysis of W.prolifica microcystin LR toxin 4-Discussion of W.prolifica microcystin LR toxin 4-Discussion

4. Discussion

The Peptide toxins are intracellular toxins that are released to medium by breaking cells, the microcystins have been not actively secreted to the surrounding water, Studies with laboratory cultures of cyanobacterial strain demonstrated that most (<80%); of the toxin is intracellular in healthy toxin occurs during the cultures senescence, and the shift from growth to stationary phase and cell death.

Toxin levels were expressed in volumetric units that are more suitable for the risk estimation for aquatic organisms, wildlife, and humans, The microcystinLR guideline value in drinking water was (1 µg/ml) and tolerance daily intake of microcystin in human was 0.04µg/kg bodyweight per day .

The results of present study were shown the toxin concentration were (28.385) µg/ml in W. prolifica, Zhang et al., (13) observed highest concentration of microcystin-LR was 7300 µg/g in microcystis. sp. and Lindholm and Meriluoto,(2004) shown the highest concentration 40 µg/l of demethylmicrocystinRR in O. agardhii and in microcystis bloom , the microcystin concentration were 2500 µg/l in lake water of Ostera in Aland, Al-Aarajg and Al-Sultan (14) shown that species Hapalosiphon Welwitschii contain the highest concentration of microcystin-LR(44.415) µg/ml While Lalita et al .,(15) recorded the highest concentration of microcystin-RR and was 732 µg/ml in strain of microcystis, the differences in variant of microcystins concentration between different or the same species of cyanobacteria can be caused by intra- and interspecific variability, as well as by regulation of microcystin synthesis under different condition, differences in toxin gene expression , growth phase and environmental factor such as temperature, pH and nutrients .

References

1. Almamoori A et al 2017 The effects of purified hepatotoxins , microcystin-RL from Anabaena cicinalis on some biochemical markers of common (cyprinuscarpio).j.Glo.pharm.Techn.10(9):40-46.
2. Campos A and Vasconcelos V 2010 Molecular Mechanisms of Microcystin Toxicity in Animal Cells , Int. J. Mol. Sci. 11, 268-287;
3. Khadairi M et al 2017 The biochemical alteration and DNA damage in rats rattus rattus after chronic intraperitoneally injection to purified microcystin-LR from Anabaena cicinalis . As. J. Pharma. and Clin. Rese.10(11):277
4. Adamovsky O 2010 Bioaccumulation and effects of cyanotoxins in the aquatic environment. J.En Ch. 4: .223-227.
5. Aderson R A 2005 Algal culturing techniques.physiological Society of America, El. A. p,.589P.
6. Alghanmi H and AbdulWahid M 2014.Investigation of geosmin and 2Methylisoborneol produce by some species of Cyanophyta under different condition.University of AlQadisiya, Doctor of philosophy in Biology.
7. Tredici M R 2004 Mass production of microalgae: photobioreactors. In: Richmond A, ede. Handbook of microalgae culture: Biotech. and Appl. phyc.. Oxford. Bla. Sci.2004.
8. Fastner, J et al 2006 Optimized extraction of microcystins from field samples - a comparison of different solvents and procedures. Water Res. 32, 3177-3181.
9. Namikoshi M M et al 1995 Phytochemistry 31 Rinehart, L. Rouhiainen, F. Sun, S. Brittain, A. Otsuki, 1247. Chem. Res. Toxicol. 11 143.
10. Purdie E L et al 2009 Toxicity of the cyanobacterial neurotoxin βNmethylamino-L-alanine to three aquatic animals species. Amyo. Lat. Scler. 10 : 67-70
11. Meriluoto J et al 2005 Isolation and detection of microcystins and nodularins, cyanobacterial peptide hepatotoxins. In: Holst, O. (Ed.), Methods in Molecular Biology. Humana Press Inc., New Jersey, pp. 65– 87.
12. WHO 2003 Guidelines for Drinking-water Quality. Second edition, Addendum to Volume 2, Health Criteria and Other Supporting Information, World Health Organization, Geneva.
13. Zhang Q.-X et al 1991 Cyclic peptide hepatotoxins from freshwater cyanobacterial (blue-green algae) waterblooms collected in central China. Environ. Toxicol. Chem. 10, 313-321
14. AL-Aarajya M J and Al-Sultan E Y A 2008 Isolation and purification of Hepatotoxin (Microcystin-LR) from some Blue –green algae of sweage water in Basrah. Mar. Bull. 3(1) 1-16
15. Lalita N S et al 2009 Isolation and characterization of microcystin producing Microcystis from a Central Indian water bloom. Har.l Alg. (8) :674–684
16. Marten J C and Vasconcelos V 2011 Differential protein expression in Corbicula fluminea upon exposure to a Microcystis aeruginosa toxic strain. Toxicon., 53: 409–416.