

<https://doi.org/10.48047/AFJBS.5.4.2023.45-54>



African Journal of Biological Sciences



Research Paper

Open Access

Studying the impact of various *Nostoc piscinale* biological products on some pathogenic bacteria.

Israa Salman Dalas¹, Hameed Salman Khamees², Abdulla Hussein Abdullah.³

Department of Biology , College of Education for Women, Tikrit University, Tikrit, Iraq.1,2,3

¹israasalman85@gmail.com, ²[Hameed s khamees s@tu.edu.iq](mailto:Hameed_s_khamees_s@tu.edu.iq) , ³abd54@tu.edu.iq

Article Info

Volume 5, Issue 4, October 2023

Received:19 Aug 2023

Accepted :17 Sept 2023

Published: 10 Oct 2023

doi:10.48047/AFJBS.5.4.2023.45-54

Abstract

The samples were obtained from different environments, and the growth and diagnosis of Cyanobacteria species *Nostoc piscinale* were conducted in AL-Alaam district, Salahaldeen governorate. They were collected from the edge of the river, a water sample and mud sample from the river, and a soil sample from planted land. Its bio- products were also extracted and the inhibitory efficacy of biological products of *Nostoc piscinale* was selected for species of bacteria isolated from the urinary tract and wounds, *Staphylococcus aureus* ,*Staphylococcus saprophyta* ,*Staphylococcus epidermis* ,*Klebsiella pneumonia* ,*Enterococcus faecium* ,*Burkholderia cepacia* , *Streptococcus pyogens* , *Pseudomonas putida* ,*Klebsiella oxytoca* and *Sphingomonas pauciumoblis* ,with the concentrations 10,000 , 7000 , 5000, µgm/ml concentration of 10,000 micrograms/ml gave the highest inhibitory diameter for *Enterococcus faecium*, where the diameter of inhibition reached 19 mm, a concentration of 7000 µg/ml recorded a minimum inhibitory diameter of 5 mm for Staph. epidermis, and no inhibitory activity was shown for the same bacteria at a concentration of 5000 µg/ml, used were GC-mass analysis to detect some of the active secondary metabolites in ethanol extract and primary statements that used an extract of blue- green alga *Nostoc piscinale* . The most important active composites had been detected in 48 different compounds with antimicrobial and antifungal conditioning.

Keywords: Blue green algae, Antibacterial Activity, biological products .

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1.Introduction

Cyanobacteria, or so-called blue-green algae, are among the oldest living organisms present on the surface of the Earth (Gademann and Portmann,2008) As some of them existed up to 3.5 billion million years ago (Mazard et al., 2016; Carpine *et al* 2020). Cyanobacteria are Gram-negative bacteria and are the first organisms on earth perform oxygenic photosynthesis(Talaro and Chess, ,2018) . Cyanobacteria possess unique characteristics among microscopic particles, which gives them the freedom to obtain special specificity. Several cyanobacteria produce cyan toxins, which can have detrimental impacts on human health via direct consumption of cyan-toxin-contaminated food and water (Mohamed, 2016). Some species of cyanobacteria produce antibiotics and medical drugs of therapeutic effects ,most notably the genus of *Microcystis sp.*, *Sytonema sp.*, *Nostoc sp.*, *Phormidium sp.*, *Oscillatoria sp* (Carpine *et al* ,2020) . where the compound diterpenoids has been isolated from Cyanobacteria species *Nostoc commune* which is considered an active anti-bacteria(Gaysina *et al.*,2019) . Studies and research referred to the fact that cyanobacteria are considered a potential power source for a number of chemicals and medical drugs not yet exploited. There are many isolates of cyanobacteria that secrete toxic substances that can be used as anti-cancer drugs, an aesthetic substances and others (Semary and Fouda, 2015).

Thus , the aim of this study was to know determine the effect of the biological extract of the cyanobacteria species *Nostoc piscinale* against some species of isolated bacteria from wounds and urinary tract, and evaluate its efficiency compared to some antibiotics and to detect its active compounds.Hence the present investigations are carried out to determine the GC-MS analysis of cyanobacterial extracts for detection of bioactive compounds.

2.Materials and Methods

2.1.Collecting samples

The original Al-Alam City environment sample was grown on a Petri dish using agar-based BG11 solid medium under sterile conditions. Cyanobacteria colony growth was observed after the plates were cultivated in an incubator for 4 weeks to 6 months under constant lighting conditions of 2500 Lux at a temperature of 28 °C. The sample of water was collected at the specified location and then dispersed in large quantities. Each colony is transferred to its own Petri plate filled with BG11 that has been allowed to solidify. A further step involved transferring the culture from Petri dishes to 250 ml glass beakers with 100 ml of BG11 liquid medium. The beakers were then placed in a shaker incubator at 28 degrees Celsius, 2500 lux of light, and 100 cycles per nanosecond of shaking until the relevant growth achieved sterile conditions. Willey's expert opinion and images captured with an optical microscope camera were utilized to identify the isolated *Nostoc piscinale*.

2.2Nostoc piscinale biological products extraction

On day 16 post-implant, *Nostoc piscinale* cells were harvested and centrifuged at 3000 rpm for 5 minutes to separate the culture's supernatant from its precipitate. After removing

the precipitate and dissolving it in ethanol at a g/ 10 ml (Moncheva et al., 2002), The rush was broken up using an ultrasonic device with a power of 24,000 frequency/second. The result is then centrifuged at 3000 rpm for 10 minutes. To remove the leachate and the ethanol detergents from the supernatant, a Rotary Evaporator was utilized at temperatures below 40 °C. The extract was diluted in a little amount of distilled water, and the bioproducts were made using 70 parts per thousand of ammonium sulphate. The precipitate and supernatant were separated by centrifuging the mixture at 3,000 rpm for 30 minutes in chilled centrifuge tubes. Dialysis was used to separate the protein-containing precipitation from the ammonium sulphate swab, allowing the dry proteins containing the natural products to be obtained. To extract the natural product-containing proteins, the contents of the bag were put into an airless discater at an unstable pressure. During the course of the investigation, researchers obtained samples of pathogenic bacteria from the microbiology lab at Tikrit University's College of Education for Pure Science. At the end of the 24 hour incubation period at 37°C, 0.1 ml of a bacterial suspension was injected into wells containing 0.05 ml of the aforementioned natural product attention. The perimeter of the inhibitory zone was used to record the data from the dishes (Hamoshi, 2012). The natural product samples for *Nostoc piscinale* were analyzed at the laboratory of the College of Applied Sciences -Samarra University utilizing a gas chromatography instrument of the GC-Mass type Shimadzu Ultra 2010 with Helium as the gas 99.9%.

3. Results and discussion

The inhibition results of the *Nostoc piscinale* protein extract against pathogenic bacteria appeared as shown in Table (1) After cultivating the pathogenic bacteria on Muller Hinton agar medium, the *Nostoc piscinale* protein extract at a concentration of 10,000 micrograms/ml gave the highest inhibitory diameter for *Enterococcus faecium*, where the diameter of inhibition reached 19 mm, while the smallest diameter of inhibition was for *Pse bacteria. putida*, it has reached 8 mm, due to a decrease or change in the permeability of the membrane and the bacteria's possession of efflux pumps, which leads to a significant increase in resistance (Breidenstein et al., 2011). As for the two concentrations of 7000 and 5000 µg/ml, their maximum inhibitory diameter was 17 and 15 mm, respectively, for *Enterococcus faecium*, a concentration of 7000 µg/ml recorded a minimum inhibitory diameter of 5 mm for *Staph bacteria. epidermis*, and no inhibitory activity was shown for the same bacteria at a concentration of 5000 µg/ml.

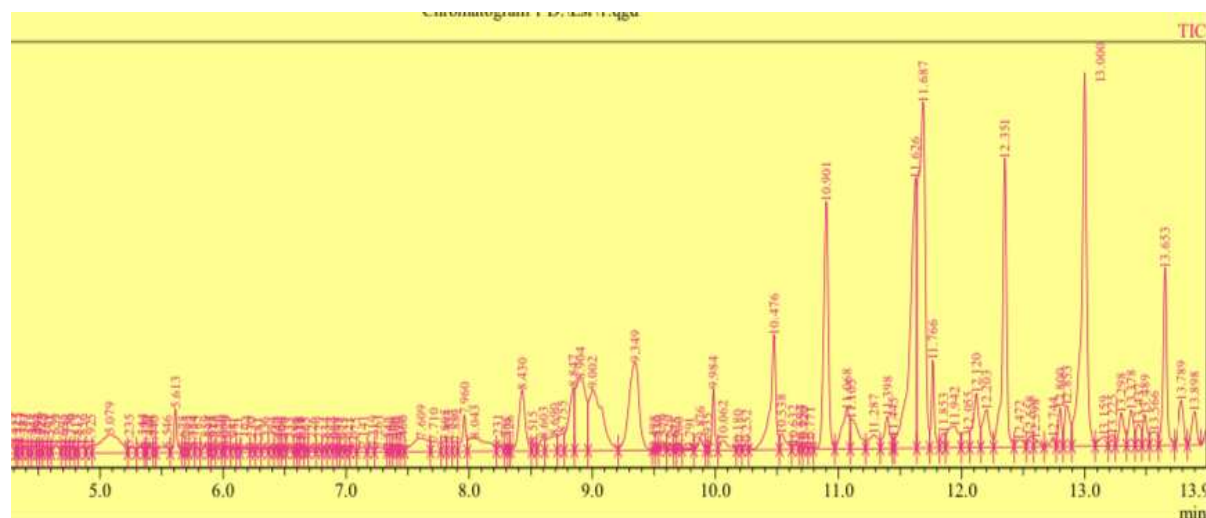
Table (1) shows the inhibition diameters biological of *Nostoc piscinale* against some species of bacteria.

<i>Nostoc piscinale</i> Extracts µg/ml			أنواع البكتريا
50000	7000	10000	
0 I c	5 F b	11 D a	<i>Staph. epidermis</i>
11 BC b	11 BC b	13 C a	<i>Staph.saprophyta</i>
10 CD c	12 B b	14 C a	<i>Staph. aureus</i>
15A c	17 A b	19 A a	<i>Entero. faecium</i>
12 B b	16 A a	17 B a	<i>Strep.pyogen</i>
9 DE c	11 B b	17 B a	<i>E. coli</i>
6 GH c	8 E b	10 D a	<i>Pse. aeruginosa</i>
5 H b	8 E a	8 E a	<i>Pse. putida</i>
8 EF b	10 CD a	11 D a	<i>K. pneumonia</i>
9 DE b	12 B a	10 D b	<i>Sph. paucimoblis</i>
6 GH c	8 E b	13 C a	<i>Burk. cepacia</i>
7 FG c	9 DE b	14 C a	<i>k. oxytoca</i>

* Small letters that are similar horizontally mean no significant difference ,only Vertically similar capital letters mean that there is no significant level (P<0.05).

By using GC-MS analysis, the bioactive substances found in the methanolic extract of *Nostoc piscinale* were discovered. The 48 distinct substances were discovered and classified as amino acids, phenols, fatty acids, alkaloides, and fatty acids. The five compounds are reported in Table (2) along with their retention time (RT), molecular formula, and peak area (%) in relation to their biological activity. Tetradecanoic acid has a total peak percentage of 10.64% and a molecular weight of 256 daltons and its chemical formula is C₁₆H₃₂O₂. and 9-octadenoic acid 8.15%, MolWeight 310 daltons and its chemical formula is C₂₀H₃₈O₂ The compounds Hexadecanoic acid, 3.63%,Molweight 284 and chemical formula C₁₈H₃₆O₂ butanoic acid,3.29% , MolWeight 116 ,chemical formula C₆H₁₂O₂ , S, and dodecanoic acid, ethyl ester 3.04% , MolWeight 288, chemical formula C₁₄H₂₈O₂. The findings revealed that these compounds had a range of effects, including anti-tumor, anti-cancer, anti-inflammatory, anti-oxidant, and anti-microbial qualities[7]. Hexadecanoic acid , Nematicide, Pesticide , Antiandrogenic, Hemolytic 5α reductase inhibitor,and antioxidant. It also lowers blood cholesterol. Tetradecanoic acid Larvicidal and repellent activity (Ganesh, and Mohankumar,2017; Mujeeb,et al.,2014). Further compounds with antibacterial activity, such as 9-Octadecenoic Acid(Sivakumar,2011).Butanoic acid,antibacterial (Kennedy,2019).Cyanobacteria are abundant sources of metabolites, including alkaloids, carbohydrates, flavonoids, pigments, phenols, steroids, and vitamins, which can be used in biotechnology and industrial fields as

well as industrial applications, such as the production of many bioindependent agents with antibacterial activity(Guiheneuf et al., 2016).



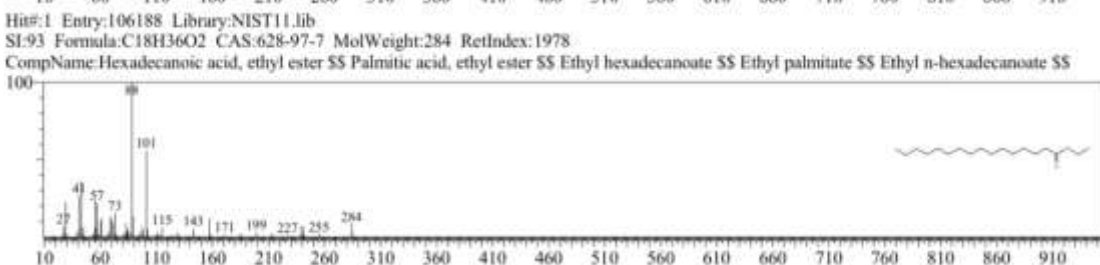
Figur 1:GC-Mass of the biological product for *Nostoc piscinale*.

Table (2) Different bioactive compounds and their biological activities of *Nostoc piscinale*. using GC-Mss chromatogram.

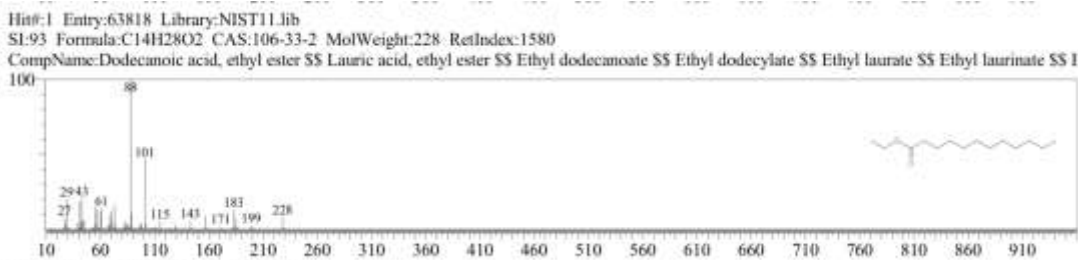
R.time	Area %	Name of Compound	No
3.021	0.23	Acetic acid, cyano-	.1
3.021	3.29	Butanoic acid,	.2
3.305	0.26	2,5-Furandione, dihydro-3-methylene-	.3
3.875	0.12	cis -Aconitic acid anhydride	.4
3.909	0.18	1,2,3-triazine,2,4-tris(cyanomethoxy)-	.5
4.069	0.12	[1,2,4]Thiadiazole, 5-amino-3-(adamantan-1	.6
4.695	0.11	1,2-Dithiolane	.7
4.856	0.17	1,2,4-Trioxolane, 3,5-dipropyl	.8
5.079	1.71	Decanoic acid, ethyl ester	.9
5.235	0.19	N-(2-Methylacryloyl)imidazole	.10
5.613	0.61	Hexanoic acid, ethyl ester	.11
7.141	0.14	Butanoic acid, 2,3-dihydroxypropy	.12

7.261	0.17	alpha.-D-Mannofuranoside,1-O-octyl-	.13
7.609	0.97	Propyl2-methyl valerate	.14
8.043	1.12	Butyric acid, undec-10-enyl ester	.15
8.430	1.79	Dodecanoic acid, ethyl ester	.16
8.515	0.15	Oxamide, N-(2-morpholinoethyl)	.17
8.690	0.65	1 Ethyl 13-methyl-tetradecanoate	.18
8.847	1.65	Hexadecanoic acid, ethyl ester	.19
8.904	3.36	Ethyl hexadecanoic acid,	.20
9.002	3.63	Hexadecanoic acid, ethyl ester	.21
9.349	4.25	Tetradecanoic acid, ethyl ester	.22
10.062	0.39	Butanoic acid, 2,3-dihydroxypropyl ester	.23
10.476	3.04	Dodecanoic acid, ethyl ester	.24
10.538	0.45	Pentanoic acid, 4-oxo-, butyl ester	.25
10.901	5.21	Tetradecanoic acid	.26
11.068	1.41	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy	.27
11.105	1.08	11,14-Eicosadienoic acid, methyl ester	.28
11.287	0.61	9,12.15-Octadectrienoic acid ,methyl ester	.29
11.626	8.15	(9-Octadecenoic acid	.30
11.687	10.64	Tetradecanoic acid, ethyl ester	.31
11.766	1.17	Dodecanoic acid	.32
11.942	1.07	(E)-9-Octadecenoic acid	.33
12.055	0.51	Nonanoic acid,6-oxo-,ethyl ester	.34
12.120	1.35	cis-10-Heptadecenoic acid	.35
12.203	1.17	Pentadecanoic acid	.36
12.351	5.42	Tetradecanoic acid,	.37
12.472	0.30	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	.38
		Linoleic acid etyl ester	.39
13.225	0.26	Pyrrolidine-2,4-dione	.40
13.00	8.93	Tetradecanoic acid	.41
13.298	0.78	Hexanoic acid, 2,2-dimethylpropyl	.42
13.378	0.79	Pentadecanoic acid,	.43
13.489	0.58	Ethyl 9-tetradecenoate	.44
13.566	0.43	Docosanoic acid,	.45

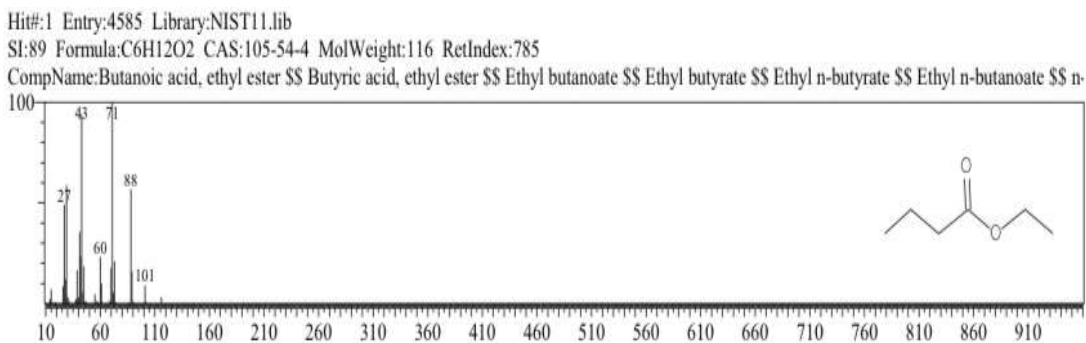
13.653	2.95	Tetradecanoic acid,	.46
13.789	1.05	Ethyl9-hexadecenote	.47
13.898	0.77	Pentadecoanoic acid	.48



Hexadecanoic acid



Dodecanoic acid



Butanoic acid,ethyl

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Cite this article as: Israa Salman Dalas (2023).

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African Journal of Biological Sciences. 5(4), 45-54. doi: 10.33472/AFJBS.5.4.2023.45-54