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### Determination of the effect of some biological products of *Fischerella muscicola* on some pathogenic bacteria and fungi

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#### Abstract:

This study was done to indicate the exertion of secondary metabolites of cyanophyta algal excerpt for *Fischerella muscicola* which isolate from one of the running water systems in Tikrit City nearby Tikrit University campus. Study the effectiveness of secondary metabolites in *Fischerella muscicola* ethanol extract to inhibition species of different bacterial and fungi, The *Fiscerella muscicola* protein extract showed inhibitory activity, as a basic dose of 10,000 micrograms/ml was given at 21 mm for Escherichia coli, and 7,000 micrograms/ml gave a maximum dose of 16 mm for and a concentration of 5000 µg/ml E.coli, diameter 15 mm. It showed its extract effect *Fiscerella muscicola* on fungi, A concentration of 10,000 and 7000 micrograms/ml gave the extract a maximum diameter of 20 and 17 mm for Cryptococcus laurentii. A concentration of 5,000 micrograms/ml gave a maximum diameter of 9 mm for Candida albicanus. . Also used was GC- mass analysis to detect some of active secondary metabolites in ethanol extract and primary statements that used extract of blue- green alga *Fischerella muscicola*. The most important active composites had been detected that , 39 different compounds with antimicrobial ,and antifungal conditioning. Bioactive composites such as, Hexadecaonic acid ethyl ester, Heptadecanoic acid, (E) Octadec-9-enoic acid ethyl , Tetradecanoic acid , Pentadecanoic acid and Ethyl 9- hexadecenoate .

**Keywords:** Antimicrobial, Cyanobacteria, *Fischerella muscicola*, GC-MS) analysis.

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## 1. Introduction

Cyanobacteria, appertained to as blue-green algae, are believed to be one of the oldest organisms on Earth, which they've been inhabited throughout the last many billion times. Cyanobacteria are photoautotrophic, Gram-negative bacteria that can thrive in colorful surroundings ,including brackish, marine, and soil. Although cyanobacteria have analogous origins and introductory anatomical features to bacteria, their ecological, natural, and physical characteristics are relatively distinct and different from bacteria. The capability to gather solar energy and perform photosynthesis through chlorophyll- a by fixing CO<sub>2</sub> and generating O<sub>2</sub> makes the cyanobacteria the largest photosynthetic prokaryotes (Gaysina,2019).

Most Cyanobacteria are an immense source of several secondary natural products with operations in the medicinal, food, ornamental, husbandry and energy sectors(Lau et al, 2015). The increase in the rate of infection by antibiotics resistant microorganism's admonitions for exploration of natural sources of antimicrobial compounds(Pandy,2015). The hunt for cyanobacteria with antimicrobial exertion has gained significance in recently due to their uproariousness in natural metabolites with medicinal and pharmacological uses (Gheda and Ismall,2020). Antibiotics can be defined as composites produced by microorganisms and can inhibit the growth of other microbes and kill them. One of the hazardous epidemiological and remedial marvels to public health is the miracle of pathogen resistance to antibiotics(Al-Shaheryand Israa,2021) . Cyanobacteria produce natural products, which increase the capability to survive various environmental stress. Natural products have been used in complaint control for decades. Cyanobacteria have new medicines to treat incorrigible conditions These composites are necessary for the treatment of different human diseases and disorders. In this respect, numerous species of cyanobacteria are known to have an important part in treatment of colorful mortal conditions ,e.g. antibacterial , and antifungal(Ahmed at al,2018). the present work aims to study the determine the effect of the natural excerpt of the cyanobacteria species *Fischerella muscicola* against some species of insulated bacteria and fungi, Diagnoses chemical factors of cyanobacterial strains insulated from soil and submarine niche, identify the chemical ingredients for possible exploitation.

## 2. Materials and methods:

### Collection of Samples and Culture medium used for cultivation of cyanobacteria:

The sample collected from the original environment of Tikrit City was cultured under sterilization conditions on BG11 solid medium that contains agar substance in Petri dish . A many drops of water sample brought from the designated area were dispersed, and the plates were incubated in an incubator in continuous light conditions of 2500 Lux and a temperature of (28) °C for 4- 6 weeks, after which the growth of cyanobacteria colonies was observed. Each colony is transferred collectively onto a Petri dish containing solidified BG11. The culture was also transferred from Petri dishes to BG11 liquid medium in 250 ml glass beakers containing 100 ml of medium. The beakers were also placed in a shaker

incubator under sterilization conditions and with a shaking speed of 100 cycles/ per nanosecond, illumination intensity of 2500 lux, and temperature of 28 °C until the applicable growth is get to gain a pure isolation free of any other microbial pollutants similar as fungi and bacteria . The isolated *Fischerella muscicola* was diagnosed by an optic microscope equipped with a camera, and opinion depended on (Willey *at al*,2011).

### **2.1 Extraction of biological products of cyanobacteria:**

The natural products of *Fischerella muscicola* were extracted on the 16th day of the implantation by collecting its cells using a centrifuge with a speed of 3000Prm for 5 minutes to gain the supernatant and precipitate from the cultivation. The precipitation was taken and dissolved in ethanol of g/ 10 ml( Moncheva *et al*,2002) . The rush was broken using an ultrasonic device with a power of 24,000 frequency/ second. The result is also placed in the centrifuge with a speed of 3000 rpm for 10 minutes . The leachate was taken , and the ethanol detergents were faded from the supernatant using a Rotary Evaporator device with a temperature lower than 40CO to gain on excerpt. The extract was dissolved in a small volume of distilled water , and the bio products using 70 attent of Ammonium sulphate. The precipitate was separated from the supernatant by placing the result in the cooled centrifuge tubes at a speed of 3000 for 30 minutes. The Ammonium sulphate swab was removed from the precipitation containing the protein by the process of dialysis, and the contents of the bag were poured into a discater which is empty of air under unstable pressure to gain the proteins which contain the natural products in their dry form, and also dissolved into the result Dimethyl Sulphur Oxide( DMSO) with the attention 10,000, 7000,5000 microgram/ml .

### **2.2 Bacterial and fungal Samples :**

The *Fisherella muscicola* was tested against six reference strains, one fungal strain, *Candida albicans* reference strain. The bacterial strains included 3 Gram-positive reference isolates and 3 Gram-positive reference isolates (*Staphylococcus aureus* and *Staph. saprophytic*, *Strep.pyogen*) , 3 Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *K. pneumonia*) and three fungal strain,( *Candida albicans*, *Cryptococcus laurentii*, and *Candida guilliermondii*) reference strain . The identity of the bacterial microorganisms used in this study was further verified by the Microscan Walkaway automated instrument, In contrast, *Candida* identity was verified by germ tube and by microscopy discovery of distinctive morphological features after growth on corn mealagar. Pathogenic bacteria samples were brought during the study period from the laboratories of pathological were attained from the microbiology laboratory in the College of Education for Pure lores – Tikrit University.0.05 ml of the mentioned natural product attention were placed in wells of 6 mm periphery made by a cork Borer on the trams culture medium Mueller Hinton Agar , which is invested0.1 ml of bacterial suspenseion incubated at 37°C for 24 hours. The dishes results were recorded by measuring the inhibition periphery zone (Hamoshi,2012) . Analysis of active compound biological products using the technique of gas chromatography device GC-Mass: The samples of natural products for *Fischerella musciola*

were anatomized using the gas chromatography device GC- Mass type Shimadzu Ultra 2010 using Helium as a gas 99.99 in the laboratory of the College of Applied Sciences –Samarra University .

### 2.3 Statistical Analysis:

The results were anatomized statistically using analysis of friction (ANOVA) and using the Minitab program in the analysis, and the arithmetic means of correlations were compared according to Duncan's Multiple Range test with a significance position ( $P \leq 0.05$ ).

### 3. Results and Discussion:

Colonies with a bluish-green colour, were insulated and examined microscopically by a compound microscope , and opinion was made. A pure isolate of Cyanobacteria *Fischerella musciola* was attained. It is characterized by its vegetative structure, which is in the form of branched filaments, and lateral branches are relatively short . The vegetative cells are permeated with heterogeneous vesicles depending on the references (Bold,1985). The results showed, in Table (1), the cyanobacteria showed an apparent exertion against all types of the studied pathogenic bacteria. The maximum inhibition periphery was 21 mm when using a attention of 10000  $\mu\text{g}/\text{ml}$  of the excerpt against *E. coli* bacteria . The maximum inhibition periphery was 16 mm when using a attention of 7000  $\mu\text{g}/\text{ml}$  of the extract against *E. coli*, The maximum inhibition periphery was 9 mm when using a attention of 5000  $\mu\text{g}/\text{ml}$  of the excerpt *Pse. aeruginosa*. while the other bacterial general was less affected. The reason is due to the fact that the excerpt includes colorful raw chemical composites that have high inhibitory efficacy ,similar as alkaloids, steroids, and colors, as well as the poisons contained in cyanobacteria in general (Al-Khafaji,2021) . The practical impact of this excerpt against the pathogenic bacteria studied is due to the effect of the active composites against the mechanisms of natural resistance of pathogenic bacteria in general, anyhow of the acidity factors of each type . The inhibitory effect of the excerpt may be due to the presence of cyanobacteria poisons ,which may inhibit the conflation of proteins necessary for the biosynthesis of acidity factors for the studied pathogenic bacteria (Bajpai,2016) and the results of the current study are in agreement with the study of (Maadaane et al ,2017).

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

| Bacteria                | <i>Fisherella musciola</i> Extracts<br>$\mu\text{g}/\text{ml}$<br>Diameter(mm) |        |        |
|-------------------------|--|--------|--------|
|                         | 5000   | 7000   | 10000  |
| <i>Staph.saprophyta</i> | 11 EFc   | 13 Deb | 16 EFa |
| <i>Staph. Aureus</i>    | 12DEb  | 13Deb  | 15FGa  |
| <i>Strep.pyogen</i>     | 12 Deb   | 15 ABb | 17DEa  |

|                        |       |        |        |
|------------------------|-------|--------|--------|
| <i>E. coli</i>         | 15 Ab | 16 ABb | 21A a  |
| <i>K. pneumonia</i>    | 13CDb | 13 Deb | 20Aba` |
| <i>Pse. Aeruginosa</i> | 9 Gc  | 12 Eb  | 14 Ga  |

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

Table (2) shows the attention of protein extract. 10000,7000  $\mu\text{g/ml}$  had the loftiest inhibition periphery of 20,17 for *Cryptococcus laurentii*, and an attention of 5000  $\mu\text{g/ml}$  didn't record any inhibitory exertion of the excerpt for *Candida guilliermondii*. *Fisherella muscicola* extract may act in three mechanisms on these two types of fungi; the, The first one these extract affect on ergosterol(azoles) (Sheehan et al,1999). The alternate medium was the effect of inhibition of polyenes that contract with enzymes inside a cell and the third medium was substantially effect on enzymes that made fungal cell walls and inhibition of cell membrane adipose acids conflation also all of these led to inhibition these fungi (Salman and Weber,2016).

**Table (2) shows the inhibition diameters biological of *F. muscicola* against some species of fungi.**

| Fungi                    | <i>Fisherella muscicola</i> Extracts |       |       |
|--------------------------|--------------------------------------|-------|-------|
|                          | $\mu\text{g/ml}$                     |       |       |
|                          | Diameter(mm)                         |       |       |
|                          | 5000                                 | 7000  | 10000 |
| <i>C. guilliermondii</i> | 0 Dc                                 | 5 Db  | 13 Ba |
| <i>Cry. Laurentii</i>    | 2 Dc                                 | 17 Aa | 20 Aa |
| <i>C. albianus</i>       | 9 A                                  | 9 BC  | 10 B  |

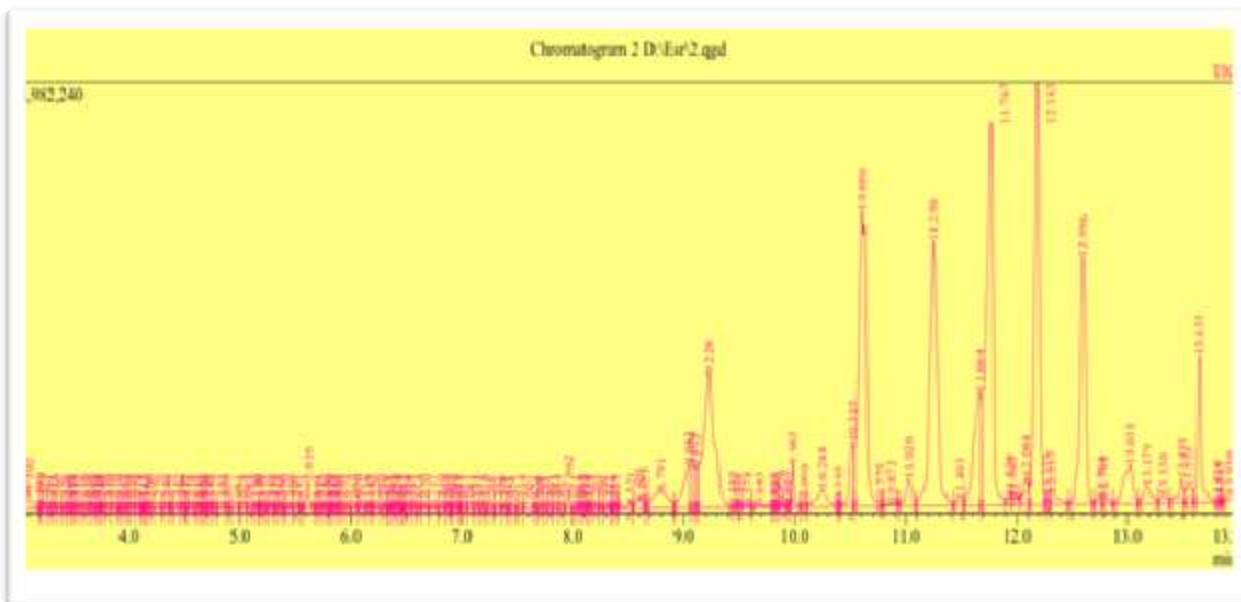
The methanol extract of cyanobacterial specie were characterized by gas chromatography- mass spectrometry (GC- MS) fashion. Table 3 shows the major ingredients present in methanol extract Fischerella muscicola. Retention time and peak area chance and Fig. 1 represents the GC- MS chromatogram of methanol excerpt of *F. muscicola*. The chemical composition of excerpt was determined using GC- mass spectrometer . Also, results showed that 39 bioactive emulsions was , Hexadecaonic acid ethyl ester , which represented 16.22 , followed by Heptadecanoic acid, ethyl ester ,which represented 13.44 ,(E) Octadec-9-enoic acid ethyl ester ,which represent 4.85, Tetradecanoic acid which represent 3.24, Pentadecanoic acid which represents 2.19 ,and Pentadecanoic acid, ethyl ester which represent 1.51, Ethyl 9- hexadecenoate which represent 1.02. Data indicated that similar composites have variable goods ,including anti-tumor, anticancer, anti-inflammatory, anti-oxidant and anti-microbial conditioning (Anini and Susilo,2023; Shaaban,2021;Kumar et al,2018). Cyanobacteria and algae are immense sources of several metabolites similar as alkaloids, carbohydrates, colors, phenols,

flavonoids, vitamins steroids, which can be employ Byed in artificial fields and biotechnology(Ahmed et al,2018; Varsha and Meeta,2019) as well as pharmacological areas including product of several bioactive metabolites that showed antibacterial and antifungal (Malathi *et al*,2014) .

**Table (3) Different bioactive compounds and their biological activities of F. muscicola using GC-MS chromatogram.**

| P   | RT     | Area% | Name of Compound                              | Formula    |
|-----|--------|-------|---|------------|
| 1.  | 3.019  | 0.03  | Acetic acid, oxo                              | C2H2O3     |
| 2.  | 3.102  | 0.62  | Butanoic acid,                                | C6H12O2    |
| 3.  | 3.488  | 0.01  | Carboxymethanephosphonic acid                 | C2H5O5P    |
| 4.  | 3.810  | 0.04  | Propanedioic acid, dihydroxy                  | C3H4O6     |
| 5.  | 4.844  | 0.05  | 1,2,3-Butanetriol                             | C4H10O3    |
| 6.  | 5.295  | 0.03  | Thiocyanic acid, 5-amino-3-methyl-4-isoxazole | C5H5N3OS   |
| 7.  | 5.757  | 0.02  | 12-Methylaminolauric acid                     | C13H27NO2  |
| 8.  | 5.823  | 0.02  | N-[3,5-Dinitropyridin-2-yl]proline            | C10H10N4O6 |
| 9.  | 6.810  | 0.03  | Benzeneethanamine,2,5-difluoro.beta.,3,4-     | C9H11F2NO3 |
| 10. | 7.843  | 0.03  | 1,1-Cyclopropanedicarboxamide                 | C5H8N2O2   |
| 11. | 7.962  | 0.26  | Octanoic acid, ethyl ester                    | C10H20O2   |
| 12. | 8.521  | 0.15  | Morpholin-4-yl-acetic acid, methyl ester      | C7H13NO3   |
| 13. | 8.605  | 0.37  | Butanoic acid, 2-methyl butyl ester           | C9H18O2    |
| 14. | 8.791  | 1.51  | Tridecanoic acid, 13-formyl-, ethyl ester     | C16H30O3   |
| 15. | 9.043  | 1.71  | Pentadecanoic acid, ethyl ester               | C17H34O2   |
| 16. | 9.078  | 0.93  | Eicosanoic acid                               | C22H44O2   |
| 17. | 9.112  | 0.66  | Ethyl tridecanoate                            | C15H30O2   |
| 18. | 9.228  | 8.74  | Hexadecanoic acid ethyl ester                 | C18H36O2   |
| 19. | 9.440  | 0.00  | Cyclohexanone, 3-hydroxy-                     | C6H10O2    |
| 20. | 9.51   | 0.05  | 4,4-Dimethyl-2-pentyl methylphosphonofluo     | C8H18FO2P  |
| 21. | 9.876  | 0.05  | Ethyl 9-decanoate                             | C12H22O2   |
| 22. | 9.940  | 0.01  | 3-Decyn-2-ol                                  | C10H18O    |
| 23. | 9.985  | 0.57  | Decanoic acid, ethyl ester                    | C12H24O2   |
| 24. | 10.090 | 1.02  | Ethyl 9-hexadecenoate                         | C18H34O2   |
| 25. | 10.51  | 1.71  | Hexadecenoic acid, ethyl ester                | C18H36O2   |

|     |            |       |                                     |                |
|-----|------------|-------|-------------------------------------|----------------|
|     | 9          |       |                                     |                |
| 26. | 10.60<br>6 | 13.44 | Heptadecenoic acid, ethyl ester     | C18H36O2       |
| 27. | 11.66<br>4 | 4.85  | (E)Octadec-9-enoic acid ethyl ester | C20H38O<br>2   |
| 28. | 11.79<br>7 | 16.22 | Hexadecanoic acid ethyl ester       | C18H36O<br>2   |
| 29. | 12.11<br>0 | 12.60 | Hexadecanoic acid ethyl ester       | C18H36O<br>2   |
| 30. | 12.27<br>5 | 0.23  | 2-Chloroethyl oleate                | C20H37Cl<br>O2 |
| 31. | 12.31<br>7 | 0.59  | Docosanoic acid,                    | C24H48O<br>2   |
| 32. | 12.76<br>4 | 0.24  | Z-9-Pentadecenol                    | C15H30O        |
| 33. | 12.78<br>0 | 0.20  | Heptadecanoic acid                  | C19H38O2       |
| 34. | 13.03<br>1 | 2.19  | Pentadecanoic acid ethyl ester      | C17H34O2       |
| 35. | 13.17<br>9 | 0.83  | Nonadecanoic acid                   | C21H42O2       |
| 36. | 13.49<br>1 | 0.77  | z-7-Tetradecanoic acid              | C14H26O2       |
| 37. | 13.55      | 0.66  | Ethyl 13-docosenoate(ethyl erucate) | C24H46O2       |
| 38  | 13.65<br>3 | 3.24  | Tetradecanoic acid, ethyl ester     | C16H32O2       |
| 39  | 13.84<br>5 | 0.17  | 9-Octadecenoic acid, ethyl ester    | C20H38O2       |



**Figur 1:GC-Mass of the biological product for *Fischerella musciola*.**

#### 4. Conclusions:

Cyanobacteria are photosynthetic microorganisms that produce a wide range of bioactive compounds. A cyanobacterial species extract has shown significant antibacterial activity. *Fischerella musciola* methanolic extract has shown a significant antibacterial and antifungal effect, particularly against *Cryptococcus laurentii* and *E. coli*. The *Fischerella musciola* 39 active chemicals were effectively identified by the GC-MS. However, further research is needed to envision cyanobacterial crude extracts as cheap, natural, and secure for medicinal uses and drug industry.

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