Antibacterial activity of *Anabaena circinalis* isolated from fresh water comparison with some antibiotic

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Abstract

Cultures of the blue green algae (cyanobacteria) *Anabaena circinalis* were ~~identifications~~ identified and isolated from freshwater and their antimicrobial effect~~s were~~ was studied. The extract~~ed~~ of *the Anabaena sp.* ~~assay~~ was tested to investigate its efficiency against four bacterial strains (*Achromobacter* ~~-~~*xylosoxidans*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*~~.,~~ ~~and~~ Antimicrobial test was determined by disk diffusion method. Different concentrations of algal extracts ~~from the microalgae *Anabaena circinalis*~~ (25, 50, 75 and 100%) were ~~used~~tested. Results showed that the highest level of antimicrobial activity was recorded against ~~-~~*Shigella dysenteriae* at 100% concentration followed by 25% extract concentration ~~of~~ against the same bacteria. ~~The results also showed no effect of algae extract on at concentrations 25% against *Staphylococcus aureus* and concentrations 75% against *Achromobacter xylosoxidans*~~. In comparison with two~~-~~ antibiotics (AMP and OXA), ~~the effect by use~~ AMP ~~antibiotic~~ was the most effective on *Shigella dysenteriae* ~~was highest level and~~ followed by OXA. ~~on the same bacteria, while no effect on bacteria~~ *Staphylococcus aureus*~~,~~ and *Escherichia coli* were resistant to both antibiotics while they were sensitive to *Anabaena circinalis* extracts at low concentrations (25% and 50%). In the present study, ~~The antimicrobial activity of the~~ *Anabaena circinalis* extract is then a promising natural source, for antibiotics, worthy for more investigations

~~was higher than the antibiotics used against the test microorganisms.~~

Key words: *Anabaena circinalis*, Antimicrobial, Algal extract, Identifications, Isolation**.**

Introduction

Algae are important ~~components of~~ organisms in the aquatic ecosystems and are the primary source of food. Algae ~~is~~ are also one of the richest

Comment [u2]: *Anabaena circinalis* or *Anabaena* sp. please precise

sources of bioactive compounds, including antibacterial and antifungal compounds~~.~~ [1, 2, 3]. Secondary metabolites obtained from ~~these~~ algae have important properties. In recent years, the interest in biological activities ~~of these molecules~~ obtained from cyanobacteria molecules has increased [4, 5]. In addition, cyanobacterial secondary metabolites have been shown to have hypocholesterolemic properties (ref), enzyme inhibiting (ref), and other pharmacological effects. These natural products are not only used as raw drug material, but also as structural models in the production of synthetic molecules [6]. These organisms are ~~the~~ an excellent ~~material~~ sources for investigation by the ecologists, physiologists, biochemists, pharmacists and molecular biologists. Biologically active sub~~-~~stances were ~~proved to be~~ extracted ~~by~~ from cyanobacteria [7, 8]. Besides, t~~T~~here has been an increasing interest in cyanobacteria as a potential source of antifungal and antibacterial substances [5, 9, 10]. The antimicrobial activities are attributed to the ~~ability of~~ cyanobacteria ability to produce a large number and variety of bioactive allelochemical substances, with a diverse range of biological activities and chemical structures that can affect many biochemical processes within cells (ref). An increasing number of such metabolites are being found to be directed against oxygenic photosynthetic processes, which, in the microbial world, are unique to algae and cyanobacteria. Such chemicals are likely to be involved in regulating natural populations, and are potentially useful as biochemical tools, and as herbicidal or biocontrol agents [11]. Bacterial infections are among worldwide and important diseases that cause high mortality rates in humans. Antimicrobial agents are commonly used in the treatment of bacterial infections. However, bacteria can become resistant to available drugs. Therefore, discovering of new antibacterial compounds is required. However, increasing popularity of traditional medicine has led researchers to investigate the natural ~~Compound~~ compounds [12]. ….Our research designed to investigate the ~~Antibacterial~~ antibacterial activity of blue-green alga *Anabaena circinalis* extract against four selected pathogenic bacteria.

Comment [u3]: Give reference please

Comment [u4]: What do you mean by natural population, please specify

Comment [u5]: Here, Try to introduce few references that have already demonstrated the interest of cyanobacteria of the genus *Anabaena*

Material and Methods~~:~~

Isolation and ~~Cultivation~~ culture of *Anabaena circinalis* ~~:~~

Algae samples were taken from spring water in the city of Shahat in the laboratory under suitable culture conditions. The medium used throughout the ~~maintenance and~~ experimental studies was medium (MBL) [13]. MBL medium consists of stock solutions of macro and micronutrients It consists mainly of the following: Macronutrient stock solutions (each g/L distilled water) ($CaCl_2$. $2H_2O$: 36.76, $MgSO_4$: 36.97, $NaHCO_3$: 12.60, K_2HPO_4: 8.71, $NaNO_3$: 85.01, Na_2SiO_3. $9H_2O$: 28.42). Micronutrient stock solutions (all g/L distilled water) (Na-EDTA: 4.36, $FeCl_3.6H_2O$:3.15,$CuSO_4.5H_2O$: 0.01, $ZnSO_4.7H_2O$: 0.022, $CoCl_2.6H_2O$: 0.01, $MnCl_2.4H_2O$: 0.18, Na $MoO_4.2H_2O$: 0.006). The nutrient medium was prepared by using one ml of each of the stock macronutrient solutions and one ml of the micronutrient stock solution and making it up to one liter of distilled water. The final pH was adjusted to 7.2. Potassium phosphate solution was autoclaved separately and then added aseptically to the sterilized medium to avoid phosphate precipitation. The isolation of the algae ~~were~~ was carried out using the moist plate method ~~recommended by~~ according to ……[14].

Comment [u6]: What do you mean by: in the laboratory? Harvested? or cultured in the laboratory, please specify this sentence

Comment [u7]: Please write full name of the medium first, than the abbreviation in brackets (MBL)

Comment [u8]: Give author name ….. et al [14] .

Microalgae culture:

A~~nabeana~~. ~~–~~circinalis~~–~~ was cultivated in MBL medium and the experiments were carried out in 500 ml Erlenmeyer pyres-glass flasks containing 200 ml of culture under controlled conditions of ambient air ~~at an at laboratory temperature~~ and at room temperature. Light was provided by cool-white fluorescent lamps at 4000 Lux with a dark/light cycle of 16:8 h for 14 days.

~~Harvesting of cultures~~ Cultures preparation~~for analyses:~~

After ~~period the~~ culturing, ~~the~~ cells of *Anabeana circinalis* were ~~harvested by centrifugation~~ centrifugated at 5000 r-p-m for 30 min using angle rotor centrifuge. The supernatants were discarded. ~~and~~ T~~t~~he remaining pellets were then used to ~~study~~ test the effect of their extract against ~~some~~ four ~~strains of~~ bacteria strains [15].

Algal eExtraction ~~of algal biomass:~~

Dried algae biomass was mixed in a glass flask with methanol: acetone: diethyl ether ~~as~~ 5:2:1 volumes, respectively, and shaken for 3 days at about 20°C. The mixture was separated by filtration. Then, the combined solvents were evaporated to dryness and the residue re-dissolved in 2 ml distilled water to form a stock solution ~~as~~ at 50 mg/ml [16].

Microorganism strains:

For antimicrobial tests, 4 ~~microorganisms~~ bacterial strains (*Achromobacter xylosoxidans*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*) were ~~selected~~ shosen. These bacteria were ~~and~~ obtained from Department of ~~microbiology~~ Microbiology El- Bayda Hospital and were used as indicator pathogens for this study.

~~Determination of~~ Antimicrobial Aactivity ~~test:~~

~~The test organisms subject to determination of antimicrobial activity~~Indicator bacteria are swabbed on the air dried nutrient agar plates by using sterile cotton swabs [17, 18]. ~~The s~~Sterile discs are loaded with varying concentration of *Anabeana circinalis* (25%, 50%, 75%, 100%). The ~~flame~~ sterile disc loaded with 150 µl of ~~sample~~ alga extracts are placed ~~with~~ on the nutrient agar plates surface ~~of nutrient agar plates and swabbed bacterial cultures~~. The discs loaded with only the ~~proper~~ appropriate solvent~~s~~ are used as ~~prescribed and~~ controls. Plates are incubated at ~~are incorporated in order to maintain the culture at the incubation temperature of~~ 37°C ~~at~~ for 18-24 hours. ~~Trials~~ Experiments were carried out under aseptic conditions. The antimicrobial activity ~~is to be~~was evaluated by mesearing the inhibition diameter zone (in mm) around the disc ~~determined as per the normal procedure by measuring the zone of inhibition around the discs the diameter of the inhibition zone is measured exactly with the help of physical scales in millimeters~~ [19].

Determination of total phenols ~~by Folin Ciacalteu Method~~

Aliquots of the extracts were taken in a 10 ml flask and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteu reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added. The test solutions were warmed for 1 minute, cooled and absorbance was measured at 650 nm against the reagent used as a blank~~.~~ [20].

RESULTS:

Comment [u9]: Air dried or in a rotary evaporator

Comment [u10]: Whatman filter paper or filter paper? Please specify also the disc diameter

Comment [u11]: Alga extract is diluted in which medium or which solvent?

Comment [u12]: 15 µl or 150 µl?

Comment [u13]: Give volume or weight

~~The extracted obtained from *Anabaena* A.~~ *circinalis* extracts showed the highest level of antimicrobial activity against *Shigella dysenteriae* (how?). ~~It was found the most sensitive microorganism against at the~~the alga extract at concentration (100%) was the most effective against indicator bacteria, ~~and~~ followed by the concentration (25%) ~~of the same bacteria and extracts~~. Whereas, *Staphylococcus aureus* was inhibited ~~at the~~by the extract at a concentration of 25% and *Achromobacter xylosoxidans* at a concentration of 50%. This indicate that these two pathogens are ~~proved to be~~ the most resistant microorganism. The results also showed no effect of algae extract on concentrations 25% against *Staphylococcus aureus* and 75% concentrations~~75%~~ against *Achromobacter xylosoxidans*~~,~~. Results were illustrated in figure ~~(~~1~~)~~ .

Comment [u14]: With which diameter?

Comment [u15]: Verify this result; it shows that there is a contradiction since in line 147 you mention that *Staphylococcus aureus* was inhibited by the extract at a concentration of 25%

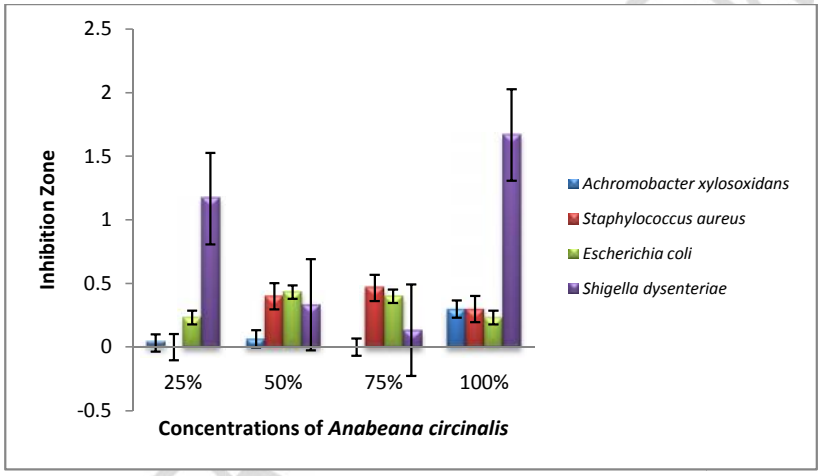

Figure 1: Inhibition zone diameter values of the *Anabaena circinalis* extracts on test microorganisms

The results also showed that the effect of ~~antibiotic (~~AMP and OXA~~)~~ on *Shigella dysenteriae* ~~the same strains of bacteria~~ was ~~the~~ highest ~~in~~ for AMP ~~on *Shigella dysenteriae*~~ followed by OXA. ~~on the same bacteria, and~~ While OXA was more effective than AMP against *Achromobacter xylosoxidans*~~lowest effect in AMP on *Achromobacter xylosoxidans* , while more effect in OXA on the same bacteria~~ . Whereas, ~~Where as no effect on~~ *Staphylococcus aureus* and *Escherichia coli* were resistant to both antibiotics tested~~.~~ (Fig. ~~(~~2).

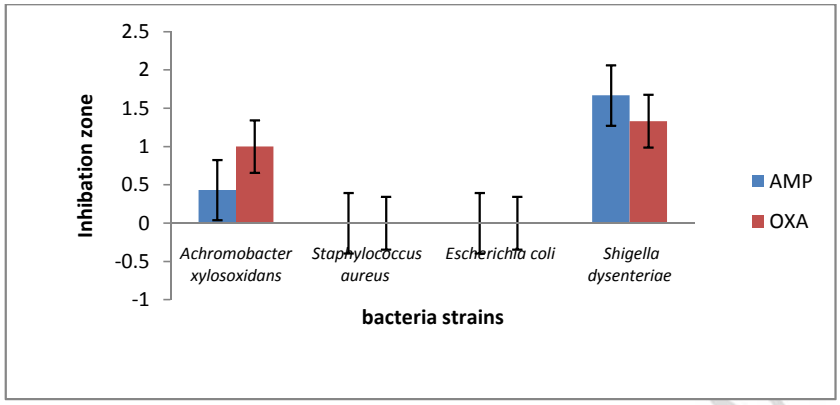

Figure (2): Inhibition zone diameter values of the AMP, OXA on test microorganisms

The content of total phenolic compounds of the ~~*Anabeana*~~ *A. circinalis* growth recorded was 28.76 ppm.

Discussion :

Antibiotic-resistant bacteria species seriously threaten animal and human health. Clinical studies on the resistance mechanism has allowed for the defining of clinical uses of all antimicrobials [21]. ~~A s~~Sensitive bacterium ~~becomes a resistant bacteria by~~ develop~~ing~~ resistance against antimicrobials through either intrinsic or extrinsic factors. Intrinsic resistance to antibiotics is an innate property given by the bacterial genome and includes the existence of resistance genes, transformation of toxic compounds, impermeability and biofilms.

~~**Intrinsic resistant development is related to the existence of resistance genes within the bacteria in question**~~. Bacterial strain can also acquire resistance (Extrinsic factors) either by the uptake of exogenous genes or by mutation.

~~Extrinsic resistance development may stem from any number of reasons, such as the use of antimicrobials without therapeutic properties, or changes in genomes due to sudden mutations~~. The use of antimicrobial substances beyond therapeutic dosage ~~in particular,~~ is one of the most common reasons ~~behind~~ for bacteria resistance [22]. Because of the growing bacterial resistance against the commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity ~~against pathogenic bacteria~~ is of increasing importance [23, 24]. Most ~~of these~~previous studies on antimicrobials from natural sources have been focused on various Cyanobacteria ~~and Chlorophyta~~ species such as

Comment [u16]: It is better to focus only on cyanobacteria in the discussion, no need to talk about chlorophyta.

Spirulina platensis, *Chrococcus* sp, *Oscillatoria* sp, *Synechocystis aquatilis, Anabaena* sp, *Oscillatoria limosa*, *O. limnetica* (Synonymous *Pseudoanabaena limnetica*), *Phormidium tenue*, ~~*Chlorella vulgaris,*~~ and *Spirulina major* [25, 26]. ~~In the antimicrobial activity the~~In our study, *A. circinalis* extract at 100% concentration, ~~(100%)~~ was ~~showed~~ the ~~highest degree of activity~~most active against *Shigella dysenteriae* compared by other concentrations. W~~w~~hereas the same extract at 25% concentration ~~25%~~ showed the lowest ~~level of~~ activity ~~amongst the extracts used~~ against the same bacteria. Besides,~~The data~~our results showed that the high content~~s~~ of total phenolic compounds in ~~the blue- green alga *Anabaena*~~ *A. circinalis* were 28.76 ppm. ~~Another~~ Previous study ~~results are~~ is agreeing with our results; *Anabaena circinalis* and *Nostoc entophytum* isolated from…. were found to be the most effective cyanobacteria species in terms of antimicrobial activity (reference?). Sánchez- Saavedra *et al* [27]~~also found~~ demonstrated that all extracts (…..) inhibited *Bacillus subtilis* growth ~~[27]~~. In the present study, ~~These~~ antibacterial activity may ~~due~~ vary with ~~to the type of~~ bacteria strain and algal extract concentration ~~of algal extract which use in each experiment~~. In line with these finding ~~These results are similar to another result that smmliring with our results~~ …. et al. ~~describe~~ reported that the mechanism of action of antimicrobial agents was based on ~~structure of~~ bacteria structure (ref). ~~The Same~~Similar results were reported by …. et al. [28] ~~was found on~~ for *Chlorella vulgaris* ~~recorded by [28]~~these authors demonstarated that…. The antimicrobial activity (of what) ~~was carried out to determine inhibition~~ against ~~some of the~~several common pathogens ~~like~~ such as *E. ~~Coli~~coli*, *Klebsilla* sp., *Bacillus* sp. and *Pseudomonas* sp. [29]. Microalgae possess the extra advantage of a substantial metabolic plasticity, dependent on their physiological state (i.e. stressed vs. non stressed); likewise, their secondary metabolism can easily be triggered by most forms of externally applied stress [30]. ~~[31]~~ regard that algae is a very interested natural source of new compounds and many of them are antioxidant, antimicrobial and antiviral activities~~. They~~ Algae are very interested natural source of new compounds [31]. They are able to produce a wide range of biologically active substances with antibacterial, antiviral, antifungal, enzyme inhibiting immunostimulant cytotoxic and antiplasmodial activities [31, 32]. ~~On another hand~~In this study, the effect of OXA and AMP antibiotics, (~~OXA and AMP~~) was ~~low~~ less interesting ~~Than~~ than the ~~effect~~ at of algal extracts ~~in this study,~~ . in addition ~~the problem that association with~~ using these antibiotics may ~~like the~~ develop resistant by ~~some~~ several pathogenic microbes. The use of algae extracts as a natural source of antibacterial compounds is an interesting alternative because of their low side effect. In a study by … et

Comment [u17]: Please put the discussion about the total phenols at the end of the discussion. Discuss antibacterial activity first than you discuss total phenol content and eventual antioxidant properties of the alga.

Comment [u18]: Which one? Results relative to total phenol content or those relative to antibacterial activity? Please clarify

Comment [u19]: Precise origin

Comment [u20]: Extract of what, please precise

Comment [u21]: Give author name please

Comment [u22]: Give reference

Comment [u23]: Author name?

Comment [u24]: Give result found by the authors (reference 28)

Comment [u25]: Write author name

al. [28], freshwater microalgae contained bioactive antimicrobial compounds.

The algal extract harmless or safer than the antibiotic because of their limiting side effects these agreement with an a study on antibacterial activities of freshwater algae described some fresh water microalgae which contains bioactive compounds for antimicrobial activity [28]. As well as the pharmacological effects of these compounds ought to be investigated further through more comprehensive studies as a service to humanity.

Comment [u26]: Please delete this sentence and replace it by a conclusion relative to your own finding, highlighting, the relevant results obtained

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