Antibacterial activity of *Anabaena circinalis* isolated from fresh water

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Abstract

Cultures of the blue green algae (cyanobacteria) *Anabaena circinalis* were identified and isolated from freshwater and their antimicrobial effect was studied. The extract of *A. circinalis* was tested to investigate its efficiency against four bacterial strains (*Achromobacter xylosoxidans*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteria*e. Antimicrobial test was determined by disk diffusion method. Different concentrations of algal extracts (25, 50, 75 and 100%) were tested. Results showed that the highest level of antimicrobial activity was recorded against *S. dysenteriae* at 100% concentration followed by 25% extract concentration against the same bacteria. In comparison with two antibiotics Ampicillin (AMP), oxacilina (OXA), AMP was the most effective on *S. dysenteriae* followed by OXA. *S. aureus* and *E.coli* were resistant to both antibiotics while they were sensitive to *A. circinalis* extracts at even at low concentrations (25% and 50%). Thus the present study revealed that extracts of *A. circinalis* extract is would be a promising natural source, for novel antibiotics, hence worthy for more investigations.

Key words*: Anabaena circinalis*, Antimicrobial, Algal extract, Ampicillin, oxacilina**.**

Introduction

Algae are important organisms in the aquatic ecosystems and are the primary source of food. Algae are also one of the richest sources of bioactive compounds, including antibacterial and antifungal compounds [1, 2, 3]. Secondary metabolites obtained from algae have important properties. In recent years, the interest in biological activities obtained from cyanobacteria molecules has increased [4, 5]. In addition, cyanobacterial secondary metabolites have been shown to have hypocholesterolemic properties, enzyme inhibiting, 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 an excellent sources for investigation by the ecologists, physiologists, biochemists, pharmacists and molecular biologists. Biologically active substances were extracted from cyanobacteria [7, 8]. Microalgae have meanwhile been found to produce antibiotics: a large number of microalgal extracts and/or extracellular products have proven antibacterial, antifungal, antiprotozoal and antiplasmodial [9,10]. The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons [11,12]. 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, and are potentially useful as biochemical tools, and as herbicidal or biocontrol agents [13]. 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, the increasing popularity of traditional medicine has led researchers to investigate the natural compounds [14]. Phenolic compounds in particular are considered as one of the most important classes of natural antioxidants. These molecules are formed by one or more aromatic rings with one or more hydroxyl groups. Chemically, polyphenols can be divided into several classes, such as phenolic acids, flavonoids, isoflavonoids, stilbenes, lignans, and phenolic polymers, Bioavailability within polyphenols differs considerably, as far as some compounds are concerned, it also depends on their form in their respective dietary sources, Generally, their primary function in plants is as protectants against ultraviolet radiation and pathogens [15] Phenolic

compounds found in algae also include the phlorotannins which are found in brown algae and in lower amounts in some red algae and reported with a wide array of biological activities (anticancer, antioxidative, antibacterial, anti-allergic, anti-diabetes, anti-aging, anti-inflammatory and anti-HIV activities) [16,17]. Our research was designed to investigate the antibacterial activity of blue-green alga *A. circinalis* extract against four selected pathogenic bacteria.

Material and Methods

Isolation and culture of *Anabaena circinalis* :

Algae samples were taken from spring water in the city of Shahat. The medium used throughout the maintenance and experimental studies was MBL medium [18]. MBL medium consists of stock solutions of macro and micronutrients as given in Table 1.

Table 1: Composition of MBL medium

Macronutrient stock solutions (each g/L distilled water)		**Micronutrient stock solution (all g/L distilled water)**	
$CaCl_2$. $2H_2O$	36.76	Na-EDTA	4.36
$MgSO_4$	36.97	$FeCl_3.6H_2O$	3.15
$NaHCO_3$	12.60	$CuSO_4.5H_2O$	0.01
K_2HPO_4	8.71	$ZnSO_4.7H_2O$	0.022
$NaNO_3$	85.01	$CoCl_2.6H_2O$	0.01
Na_2SiO_3. $9H_2O$	28.42	$MnCl_2.4H_2O$	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 was carried out using the moist plate method according to (Jurgensen, M.F. and C.B. Davey, 1968) [19].

Microalgae culture

A. 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 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.

After culturing, the cells of *A. circinalis* were centrifugated at 5000 rpm for 30 min using angle rotor centrifuge. The supernatants were discarded. The remaining pellets were then used to test the effect the algal extracts on some bacteria strains [20].

Algal extraction

Dried algae biomass was mixed in a glass flask with methanol: acetone: diethyl ether 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 air dried to dryness and the residue was re-dissolved in 2 ml distilled water to form a stock solution at 50 mg/ml [21].

Microorganism strains:

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

Antimicrobial activity test

Indicator bacteria is swabbed on the air dried nutrient agar plates by using sterile cotton swabs [22, 23]. Sterile discs (Whatman filter paper) are loaded with varying concentration of *A. circinalis* (25%, 50%, 75%, 100%), Alga extract was diluted in distilled water. The sterile disc loaded with algal extracts were placed on the nutrient agar plates. The discs were loaded with 150 µl only and the appropriate solvents were used as controls. Plates are incubated at 37°C for 18-24 hours. Experiments were carried out under aseptic conditions. The antimicrobial activity was evaluated by measuring the inhibition diameter zone (in mm) around the disc [24].

Determination of total phenolic content

Aliquots of the extracts (1 ml) 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 [25].

RESULTS:

A. circinalis extracts showed the highest level of antimicrobial activity against *Shigella dysenteriae* with an inhibition zone of 1.7mm. The alga extract at a concentration of 100 % was the most effective against indicator bacteria, followed by the 25% concentration with a zone of inhibition of 1.2mm against the same bacteria. On the otherhand, *Staphylococcus aureus* was inhibited by the extract at a concentration of 25% (zone of inhibition of 0.1mm) and *Achromobacter xylosoxidans* at a concentration of 50% (zone of inhibition of 0.2mm). This indicated that these two pathogens were the most resistant microorganisms. The results are illustrated in figure (1).

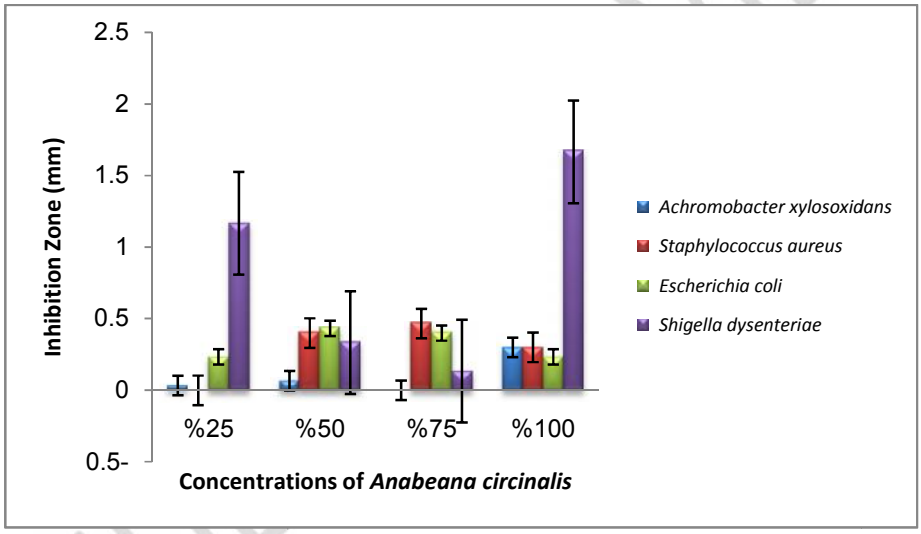

Figure 1: Inhibition zone diameter values of the *Anabaena circinalis* extracts on test microorganisms

The results also showed that out of AMP and OXA, the effect on *Shigella dysenteriae* was highest for AMP while OXA was more effective than AMP against *Achromobacter xylosoxidans*. However, *Staphylococcus aureus* and *Escherichia coli* were resistant to both antibiotics tested (Fig. 2).

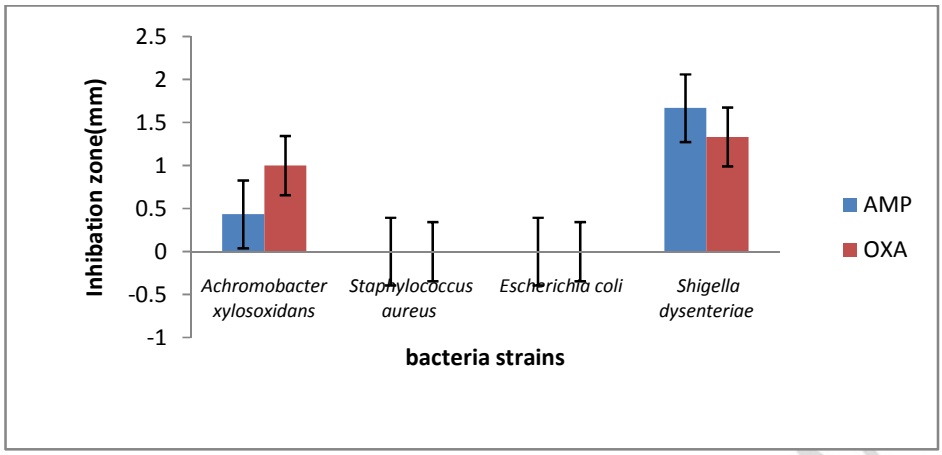

Figure (2): Inhibition zone diameter values of the AMP, OXA on test microorganisms

In addition, our observations suggested that the content of total phenolic compounds in *A. circinalis* was 28.76ppm.

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 [26]. Sensitive bacterium develops 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. Bacterial strain can also acquire resistance (Extrinsic factors) either by the uptake of exogenous genes or by mutation. The use of antimicrobial substances beyond the therapeutic dosage is one of the most common reasons for bacteria resistance [27]. Because of the growing bacterial resistance against the commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity is of increasing importance [28, 29]. Most previous studies on antimicrobials from natural sources have been focused on various Cyanobacteria species such as *Spirulina platensis*, *Chrococcus* sp, *Oscillatoria* sp, *Synechocystis aquatilis, Anabaena* sp, *Oscillatoria limosa*, *O. limnetica* (Synonymous *Pseudoanabaena limnetica*), *Phormidium tenue*, and *Spirulina major* [30, 31]. In our study, *A. circinalis* extract at 100% concentration, was the most active against *Shigella dysenteriae* compared with other concentrations. Whereas the same extract at 25% concentration showed the lowest activity against the same bacteria. In agreements with (Farag *et al* 2014) revealed that, aqueous and ethanol extracts of the blue green alga *A. circinalis* exhibited antibacterial activity against *Serratia marcescens* and *Escherichia coli* [32]. Algae are a source

of many biologically active substances, that include phenolic compounds, therefore considerable amounts of total phenolic content could be found in algal species. However, it is needed to keep in mind that all algal polyphenols are responsible for the health benefits. Previous study revealed that *A. circinalis* and *Nostoc entophytum* isolated from benthic and pelagic habitats at Yeşilırmak River (Turkey) the most effective cyanobacteria species in terms of antimicrobial activity[33]. Sánchez-Saavedra *et al* [34] demonstrated that all extracts of algae (*A. circinalis* and *N.entophytum*) inhibited the growth of *Bacillus subtilis*. In the present study, antibacterial activity may vary with bacterial strain and algal extract concentration. In line with these findings, Jyotrimayee p et al(2014) the mechanism of action of antimicrobial agents was based on bacteria structure [35]. Similar results were reported by (P. S. Syed Shabudeen et al 2013 and Jyotrimayee p et al 2014) [35] , [36] for *Chlorella vulgaris* these authors the antimicrobial activity was carried out to determine inhibition against some of the common pathogen like *E. Coli, Klebsilla sp*., *Bacillus sp*. and *Pseudomonas sp*. 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 [37]. Regard that algae is a very interested natural source of new compounds and many of them possess antioxidant, antimicrobial and antiviral activities. Algae are very interested natural source of new compounds [38]. They are able to produce a wide range of biologically active substances with antibacterial, antiviral, antifungal, enzyme inhibiting immunostimulant cytotoxic and antiplasmodial activities [38, 39]. In this study, the effect of OXA and AMP antibiotics, was less interesting than that of algal extracts. Addition using these antibiotics may develop resistance by 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 (Jyotrimayee p *etal* 2014). So that A. circinalis may also be a viable candidate for the production of compounds [35].

Conclusion

This study found that there is an inhibition effect of the alga extract on some pathogenic bacteria compared with some antibiotics. In some cases, this effect was stronger than the effect of the tested antibiotics. This effect may be due to the presence of effective compounds against tested bacteria and recommended study follow-up research in this area to identify the composition of these materials.

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