The Effect of Algal Extract of Spirulina Platensis, Cladophora Glomerata on Some Positive and Negative Bacterial Isolates of Gram Stain

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Abstract

This study examines the efficacy of the algal alcoholic extract isolated from Cladophora Spirulina platensis and glomerata and testing their efficacy against positive bacteria of Gram stain Staphylococcus aureus and the negative bacteria for Escherichia coli and Klebislla sp. The organic solvent ethanol was used at 95%. The efficacy of the extract was tested against some strains of positive and negative bacteria of Gram stain. The results of the extract showed that the positive bacteria of Gram stain has sensitivity towards the extract higher than the negative bacteria. The highest inhibitory efficacy against Staphylococcus aureus by inhibitive diameter zone of 20 mm and less inhibitive against negative bacteria E.coli by inhibitive diameter of 16 mm.

Key Words: Cladophora, Spirulina, Platensis, Glomerata.

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Introduction

Algae are a group of rootless, stemless, and leafless Thalophyta. They do not have flowers or fruits either. They are considered autotroph because they contain the photosynthetic pigment of chlorophyll, besides carotenoids, xanthophylls and proteins (Athbi, 2011). Algae are all over the world and they play essential roles in nature, both beneficial or harmful role. Among the beneficial roles, they participate in the chemical, pharmaceutical and feed industries, besides the production of antibiotics from various microorganisms. The term Antibiotics is used for chemical compounds produced by some microorganisms during secondary metabolism processes and has an inhibitory efficacy on the growth of germs and other microorganisms (Walker, 1974). Algae is one of the microorganisms with high efficiency in the production of a group of antibiotics that have a direct effect on the removal of many of pathological bacteria. Algae produce two types of active compounds inside their cells; intra cellular products or outside their cells; extra cellular products. Algae extracts have a different nature based on their different chemical composition of amino acids and fatty acids (Arnold and Targett, 2002; Britannica, 2010). Studies have indicated that fatty acids have strong biological activities represented in the ability to kill or stop the growth of bacteria (Desbois et al., 2009). Moreover, therapeutic uses for algae have been discovered since 1950, which led to useful treatments (Amer, 2004; Mendes *et al*., 2016). These active biological compounds are considered antibiotics tested against bacteria using either aqueous extracts or alcoholic algae extracts to prove the efficacy of anti-bacterial extra cellular products (Justo *et al*., 2011).

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The reason for this effectiveness is the presence of fatty acids, acrylic acid, halogens of aliphatic compounds, terpenes, phenols and sulfur, which contain cyclic heterogeneous compounds that have an anti-microbial nature. Studies and research have continuously been carried out on the discovery of many antagonists, (Cardozo *et al*., 2007; Ghasemi *et al*., 2004) after testing its biological effectiveness, diagnosing it and identifying its chemical composition Stephen and Horace, 2000) due to the increased resistance of strains of pathological bacteria to the used antibiotics (Robbers *et al*., 1996). Furthermore, many researchers have studied the antimicrobial effects of different plant extracts and the antimicrobial activity of algae. Among the algae that were used as antibiotics is *Cladophora glomerata*, which is a green algae that include more than 183 species. This genus is rich in phytochemicals that can be used to preserve human and animal health due to a set of secondary receptors. Some species of this group have antioxidant, antidiabetic, antimicrobial, and antitoxic cell activities despite previous extensive research to isolate and identify compounds of pharmaceutical significance of cladophora (Guiry and Guiry, 2011). The other sex is *Spirulina platensis*, which is cyanophyta referred to in many studies that some of its genera have high efficacy when testing its extracts in inhibiting the growth of microorganisms in comparison to the rest of the other algal varieties. They gave positive results against bacteria with wide range of inhibition (Gene, 2010). Meanwhile they are capable of producing 7 and 8-methyl heptadecanes consisting of tetradecane. and heptadecanes, which inhibit the growth of bacterial species (Ladygina *et al*., 2006). *Spirulina platensis* is found in polluted environments, in fresh and salty water, in sewage, laboratories, and factories. It is a simple filamentous alga, spiral in shape, and its cell is almost equal in length and width. The algal cell is often round, and its shape is an important characteristic among the species. Alga is considered important in a nutritional point of view as it changed the world due to its high energy content of nutrients represented by sugars, proteins and fats as well as medical and pharmaceutical materials (Guiry and Guiry, 2011). It is often used as a unicellular protein. This species contains essential amino acids, proteins and fatty acids, and antioxidant dyes and carotenoids. It is classified as a healthy food by the World Health Organization. At present some antibiotics produced by known microorganisms have become useless due to the appearance of severe to antimicrobial symptoms as well as their high cost and side effects such as hypersensitivity and death of essential organisms in the alimentary tract. The decrease in the effectiveness of the antibiotics and the increase of bacterial resistance is due to their ability to change the chemical composition of the antibiotic and the wrong use of antibiotics (Biondi *et al*., 2007). All these factors led to new types of antibiotics in diverse environments where they build new chemical compounds. Algae are considered as one of the rich sources in active substances (Ely *et al*. 2004). Therefore, the current study aims to extract the active substances from both algae *Cladophora glomerata* and *Spirulina platensis* using the organic solvent of ethanol, obtain some pathogenic microorganism isolates from pathological human specimens, and study their sensitivity and resistance to antibiotics. The study also aims to study the anti-effect of algal extracts against microorganisms isolated from pathogenic samples.

Materials and Methods

Sample Collection

The samples were obtained from the College of Agriculture / University of Kufa

The Culture Medium for Bacteria

An amount of 38 grams of Mueller Hinton Agar medium is dissolved in 1000 ml of distilled water. Then the mixture is placed in an Autoclave until it boils at a temperature of 121 ° C and at 15 atm for 30 minutes for sterilization. After that, the mixture is shaken well and left for a short period of time to cool before pouring into Petri dishes.

Algae Identification

Division: Chlorophyta
Class: Ulvophyceae
Order: Cladophorales
Family: Cladophoraceae
Genus: Cladophora glomerata
Division: Cyanophyta
Class: Cyanophyceae
Order: Oscillatoriales
Family: Oscillatoriaceae
Genus: Spirulina Platensis

Preparation of Algal Extracts

The method described by [Taskin et al., 2007] was used to extract the active substances from the filtrate of the liquid algal culture medium as follows:

1. The filtrate of the culture medium for the studied algae was dried by an electric oven. Then the dry material was weighed and kept at -4 ° C.
2. An amount of (3-10) grams of dehydrated alage was weighed and placed in a cylindrical container made of porous paper material called *Thimble*.
3. The Thimble was placed in the assigned place in the soxhlet extractor, 250 ml of 95% ethanol was added to it and left for a period of time for the powder to be saturated with the solvent.
4. After that, the extraction process was carried out in the soxhlet extractor for eight hours until a colorless filtrate was obtained.
5. The filtrate was dried by an electric oven at a temperature not exceeding 40 ° C.
6. The product of the extraction was weighed.

Determination of the Inhibitory Efficacy of the Algae Extract towards the Bacteria

The bioactivity of the anti-bacterial positive and negative algal extract of Gram stain was determined by adopting the Well method and Agar diffusion method [Harley, 2002]. The bacteria were activated by taking small *colonies* from them by loop and placed in tubes containing 5 ml of nutrient broth medium and incubated at 37 ° C, for 24 hours. After that 1 ml of the nutrient solution was taken and added to a tube containing 5 ml of normal saline, shaken well and measured by a spectrophotometer. The bacteria were planted on the nutrient medium Mueller Hinton Agar, prepared by dissolving 38 grams in a liter of distilled water. Then it was dissolved by heating and sterilizing the autoclave for 30 minutes at a 121 ° C and a pressure of 15 atm, then it was cooled and poured into sterile dishes and left to solidify and the bacteria were grown on the solid medium using cotton swab. The whole plate was planned to ensure an equal distribution of bacteria on the medium. Then three pits with a diameter of 6 mm were made in each plate using the cork borer and 0.2 ml of extract was added to each hole. The dishes were incubated at a temperature of 37 C° for 24 hours and the diameter of the inhibition zone was measured in millimeters, using the graded ruler.

Results and Discussion

In the current study, the biological efficacy of the extracts was determined by the method of diffusion in solid medium (AGAR) which is a common method for testing the efficacy of algal extracts against germs (Al-Aarajy & Al-Deelami, 1997). This method gives distinct results for the efficacy of extracts because of the concentration of the active compounds around the disk sides according to the results of Vlachos et al. (1996).

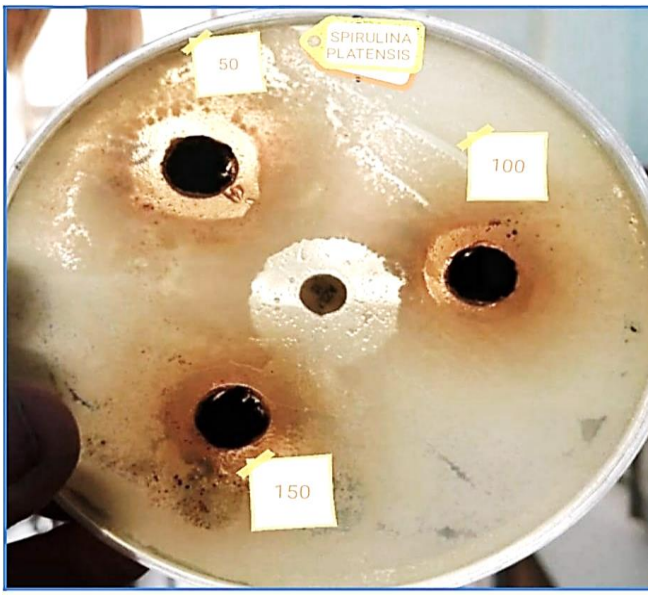

Figure 1. The inhibitory activity of algae towards some bacterial isolates

The results in (Fig. 2) show a significant effect on the activity of the extract against the pathogen. It reached the highest inhibitory diameter in Spirulina platensis which is 20 mm, at a concentration 150 ml / liter, in Staphylococcus aureus, and the minimum inhibitory diameter was 16.0 mm, at a concentration of 50 ml / liter against Klebsiella sp.

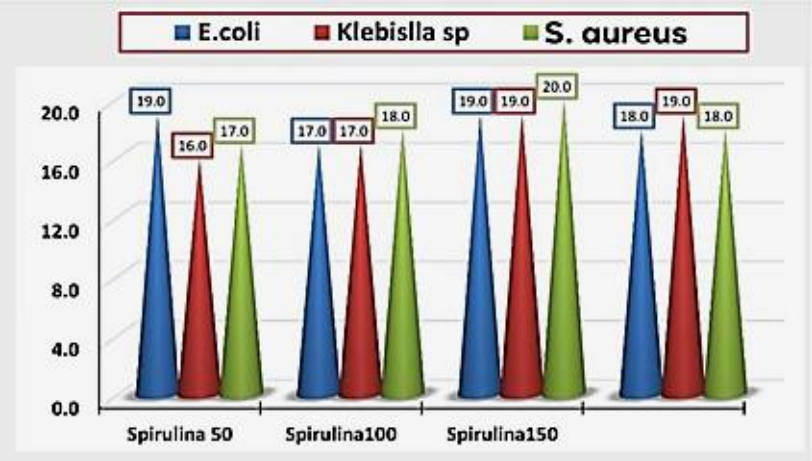

Figure 2. The effect of the algae extract, Spirulina platensis, against some bacterial isolates

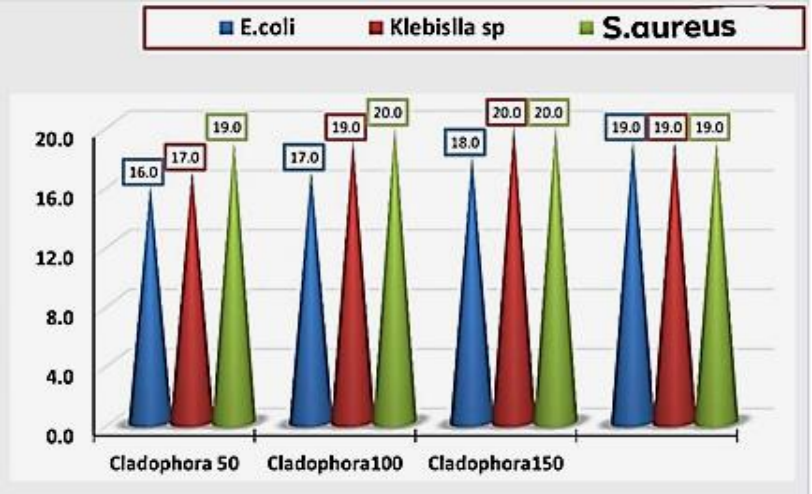

Figure 3. The presence of a significant effect of Cladophora glomerata extract

The highest percentage of inhibition is with a diameter ranging from the highest inhibition of 20 mm at 100 and 150 ml / liter against Staphylococcus aureus to the lowest inhibition of 16.0 mm at 50 ml / liter against Escherichia.coli.

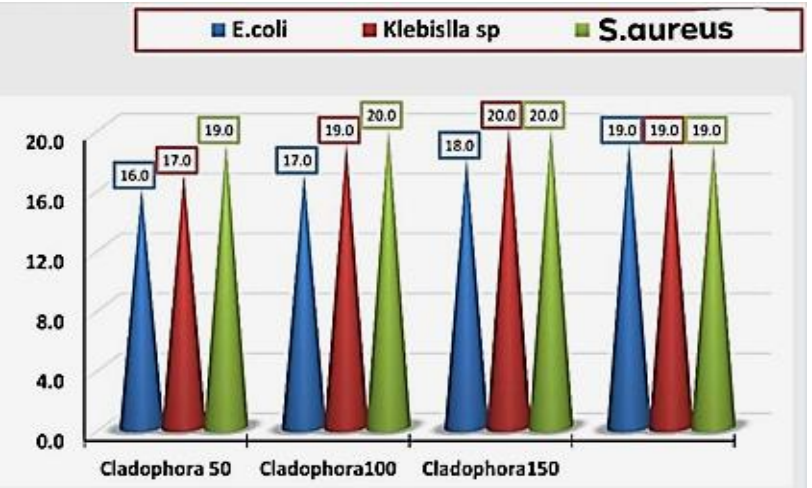

Figure 3. The effect of Cladophora glomerata extract against some bacterial isolates

The results also indicate that the efficacy of the extract was close to that of the antibiotic used in the study; Azithromycin. The results also reveal that all the extracts used in this study affected the positive bacteria and the negative bacteria of Gram stain.

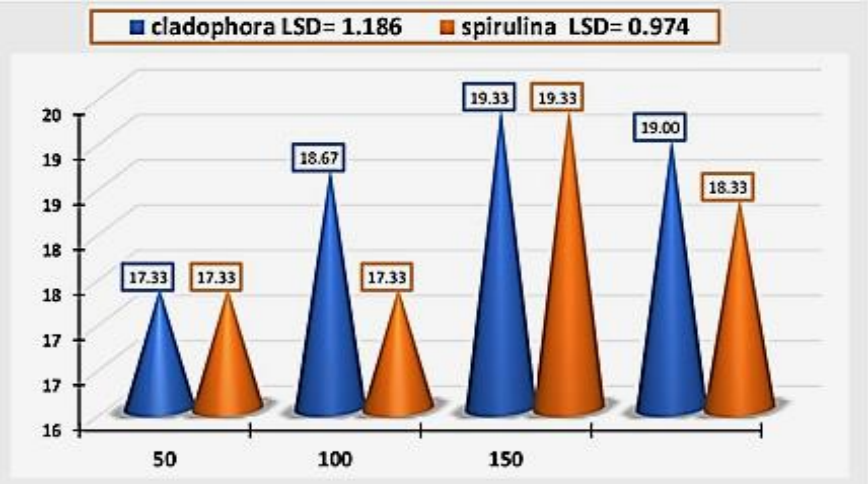

Figure 4. The average inhibitory diameter of the bacterial species

The inhibitory activity depends on the type of alga studied, the efficiency of the extraction method, the active substances, and the type of host used (Lima-Filho et al., 2006). The inhibitory activity of the studied algal extracts against pathogenic bacteria is also attributed to the fact that they contain a complex and mixed amalgamation of multiple chemical compounds, and the active substances can be small quantities (Kumar et al., 2009; Bansemir et al., 200). There is a set of biological factors, such as the algae growth phase and the studied part, associated with abiotic factors such as seasonal changes and geographic location, all of which affect the extraction of secondary substances and the anti-activity of algal extracts against pathogenic bacteria [Vlachos et al., 1996].

The results indicate the presence of this anti-bacterial activity and confirms the presence of compounds that have the ability to inhibit the bacteria. This is consistent with the studies that indicated the ability of algae to result in compounds produced by the effective secondary metabolism with the ability to control the growth of bacteria (Faulkner, 2002) Thes include alkaloids, which are natural compounds produced as secondary metabolites in microorganisms. They are believed to have a role in defending the cells of the organisms that produce them. They are characterized by their therapeutic efficacy and medical significance. These compounds are linked to the DNA of the microbes, which give them a high inhibitory ability against many positive and negative germs of Gram stain. The variation and difference in the efficacy of the extracts as antibiotics result from the method used and the solvents used, as well as the separation in which the samples were collected (Kandhasamy and Arunachala, 2008). Sources indicated that some algal extracts have antibacterial activity (Biondi et al., 2004; Burja et al 2001; Issa, 1999)

It is necessary to notice that types of cyanobacteria have a high capacity to produce effective compounds of medicinal significance. Hence, the primary screening process is one of the important methods to identify organisms capable of producing antibiotics (Burja et al., 2001; Ehrenreich, 2003; Soltani and Tabatabaei, 2005). The obtained results are in agreement with many studies which confirm the efficacy of algal extracts against bacterial pathogens and thus the possibility of using them as alternative natural products in the pharmaceutical industries (Kaushik et al., 2009). This latter study concluded that the methanolic extract of Spirulina platensis has inhibitory efficacy against positive and negative bacteria of Gram stain (Tuney et al., 2006 and Ghosh et al., 2008). The extract isolated from algae can be considered a broad spectrum active ingredient due to its effect on the negative and positive bacteria of Gram stain. It is believed to break down the cell membrane of the microorganism, thus changing the permeability of the membrane [El-Sheekh et al., 2006]

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