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ANTIBACTERIAL STUDY OF *CHLORELLA VULGARIS* ISOLATED FROM FRESH WATER

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Abstract:-

Chlorella vulgaris is growing in either fresh water or sea water. It can provide various other nutrients including proteins, minerals, vitamins, and antioxidants. World production of consumable algae and algae products to be used as foods and medicines have reached thousands of tons per year. In this study, Chlorella vulgaris was collected and isolated from freshwater. The extracted of chlorella vulgaris assay was tested to investigate its efficiency against four bacterial strains (Achromobacter sp (S1), Staphylococcus sp (S2) Escherichia coli (S3), Shigella dysenteriae (S4)), and was determined by disk diffusion method. Different concentration extracts from the microalgae Chlorella vulgaris (25, 50, 75 and 100%) were used. Results showed that the 75% of the extract was highly significant against Escherichia coli and followed by concentration 25% against Achromobacter sp, however, the lowest significant against Staphylococcus sp at the concentration 100%. The antimicrobial activity of the Chlorella vulgaris extract was higher than the antibiotics used against the testes microorganisms.

Keywords:- *Antimicrobial, Identifications, Isolation, Chlorella vulgaris.*

INTRODUCTION

Microalgae exhibit a notable biodiversity; they can in fact be found as individual cells, colonies or extended filaments. These microorganisms account for the basis of the food chain in aquatic ecosystems; they possess the intrinsic ability to take up H_2O and CO_2 that, with the aid of solar energy, are used to synthesize complex organic compounds, which are subsequently accumulated and/or secreted as primary or secondary metabolites. They are ubiquitously distributed throughout the biosphere, where they have adapted to survive under a large spectrum of environmental stress e.g. heat, cold, drought, salinity, photooxidation, anaerobiosis, osmotic pressure and UV exposure [1]. Microalgae grow in most of the natural environments including water, rocks and soil, but interestingly also grow on and in other organisms. Their main habitats are freshwater, brackish and marine ecosystems. Microalgae can be found and collected not only in general aquatic ecosystems such as lakes, rivers and the oceans, but also in extreme environments such as volcanic waters and salt waters [2]. Algae is a rich resource for pharmaceuticals on the basics of the presence of these biologically active primary and secondary metabolic compounds [3]. The secondary metabolites presents in the Algae are in favor of organizing a numerous biological defense systems and they act against predation, herbivores and competes for a space [4]. Microalgal cell-free extracts are already being tested as additives for food and feed formulation, in attempts to replace antimicrobial compounds of synthetic origin currently in use – including subtherapeutical doses of antibiotics employed as a prophylactic measure in animal breeding [5]. Was pharmaceutically important with respect to a first investigation reporting its antibiotic activities. The extensive research reports indicate that it has already been utilized as a traditional medicine, the highlights are of bacteria-static, bactericidal, antifungal, antiviral and antitumor activities. The abilities are due to the presence of fatty acids in these micro algae organisms, which develops antibacterial activity [6]. These remarkable aspects encourage to investigate this phytochemical and its derivatives and validate its effectiveness as antibacterial character that present with *Chlorella vulgaris* a microorganism. As per the reports available in the literature, the antimicrobial substances are unsaturated lactones, cyanogenicglycosides, sulfur containing compounds, phenols, phenolicglycosides, saponins and phytoalexins [7]. The therapeutic use of algae has reported from the 1950s and some of the systematic drug discoveries of algae and biologically active substances of antibiotics were reported. The positive and negative gram bacterial tests were carried out by the aqueous and solvent extracts of this alga [8]. 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. Nowadays, increasing popularity of traditional medicine has led researchers to investigate the natural compounds in plants and algae [9]. Medical Importance important uses, such as algae alga *Chlorella* in the extraction of an antibiotic called chlorellin [10]. The present study, work was assessed antimicrobial activity of green alga *Chlorella vulgaris* against selected pathogenic bacteria.

Material and Methods

Isolation and Cultivation of *Chlorella vulgaris*

Local freshwater microalgae, *Chlorella vulgaris*, isolated from spring water in the city of Shahhat. It has been isolated in the laboratory under suitable culture conditions. The medium used throughout the maintenance and experimental studies was medium (MBL) [11]. 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 micronutrient 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 carried out using the moist plate method recommended by [12].

Microalgae culture:

The Isolated *Chlorella vulgaris* was cultivated with 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. Light was provided by cool-white fluorescent lamps at 9000 Lux with a dark/light cycle of 16:8 h for 14 days.

Harvesting of cultures for analyses:

After period the culturing the cells of *Chlorella vulgaris* were harvested by centrifugation at 5000 r p m for 30 min using angle rotor centrifuge. The supernatants were discarded and the remaining pellets were used to study the effect of their extract some strains of bacteria [13].

Extraction 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 50 mg/ml [14].

Bacterial Strain

The pathogenic bacteria (*Achromobacter* sp, *Staphylococcus* sp, *Escherichia coli*, *Shigella dysenteriae*) Obtained from Department of microbiology El- Beyda Hospital was used for this study.

Determination of Antimicrobial Activity:
The test organisms subject to determination of antimicrobial activity are swabbed on the air dried nutrient agar plates by using sterile cotton swabs [15 and 16]. The sterile discs are loaded with varying concentration of *Chlorella vulgaris* (25%, 50%, 75%, and 100 %). The flame sterile disc loaded with 150 µl of sample extracts are placed with the surface of nutrient agar plates and swabbed bacterial cultures. The discs loaded with proper solvents as prescribed and controls are incorporated in order to maintain the culture at the incubation temperature of 37°C at lease for 24 hours. The antimicrobial activity is to be 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.

RESULTS:
The antimicrobial activity of *Chlorella vulgaris* was carried out to determine inhibition against some of the common pathogen *Achromobacter sp*, *Staphylococcus sp*, *Escherichia coli*, *Shigella dysenteriae*. Which are pathogenic to humans and vector diseases. The results illustrated Figure (1) showed that the pathogenic bacteria was the highest value in concentration 75% against *Escherichia coli* more than another concentrations by determining the inhibition zone (1.5cm), followed by concentration 25% against *Achromobacter sp*, while the smallest value in the concentration100% against *Staphylococcus sp* (0.4cm).

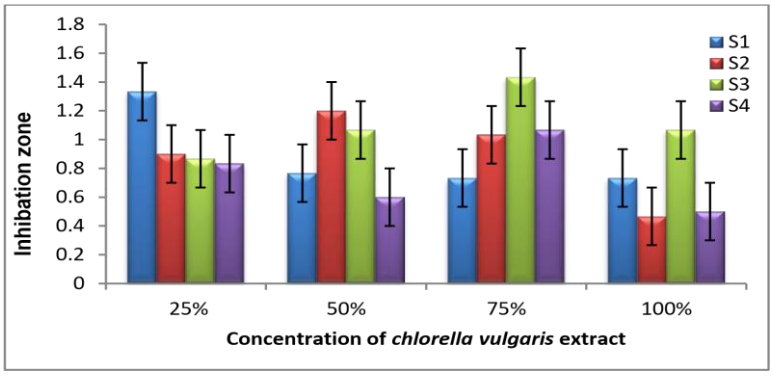

Figure (1) Effect of different concentrations of Chlorella vulgaris extract on the strains of bacteria.

The results also showed that the effect of two kinds of antibiotic (AMP and OXA) on the same types of bacteria was the highest in AMP on S4 recorded (1.6 cm) followed by OXA on the same bacteria (1.4cm) while no effect on S2and S3. Figure (2).

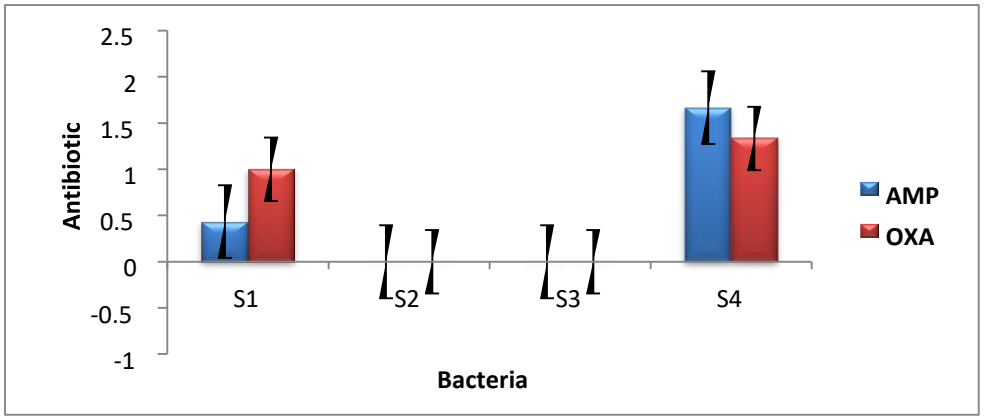

Figure (2): The effect of antibiotics AMP, OXA on four strains of bacteria.

Discussion
Were tested against various strains of bacteria S1, S2, S3, and S4. The extracts showed maximum antibacterial activity was at concentration 75% against S3 than another concentrations, the zone of inhibition were ranged from 1.5 to 0.4 cm against bacterial strains. The inhibitory activity of against bacterial strains depended on concentration and the type of bacteria. These results may due to the antibiotic activity of *Chlorella vulgaris* and the antibiotic Chlorellin obtained from *Chlorella* inhibits the growth of some species of bacteria [17]. The antimicrobial activity of *Chlorella vulgaris* was carried out to determine inhibition against some of the common pathogen like *E. coli*, *Klebsilla sp*., *Bacillus sp*. and *Pseudomonas sp*. Which are pathogenic to humans and considered to cause water borne and vector diseases and is causing drastic impacts with huge impacts [18 and 19]. The same results recorded by [20]. The antimicrobial ability appears to derive either from interference with chlorophyll and protein syntheses [21], or because of changes in membrane permeability coupled

with dissociation of phycobilin assemblages in the thylakoid membranes – thus leading to leakage across the cell wall [22]. Microalgae are rich source antibacterial agent and provide a safer and cost effective way of treating bacterial infections like *Euglena, Chlorella, Chroococcus*. Pressurized (liquid) ethanol extracts from *Haematococcus pluvialis* in its red stage possess antimicrobial activity against a Gram negative bacterium, *E. coli*, and a Gram positive bacterium, *S. aureus;* this was once again associated with the presence of short-chain fatty acids, namely butanoic and methyl lactic acids[23].[24] Found the two algal (*Dunaliella salina* and *Pseudokirchneriella subcapitata*) extracts showed interesting antimicrobial properties, which mostly inhibited the growth of isolated *S. aureus, P. aeruginosa, Escherichia coli*, were these results agreed with our results. As regards isolated the first antibacterial compound from a microalga, *Chlorella*; a mixture of fatty acids, viz. chlorellin, was found to be responsible for that inhibitory activity against both Gram+ and Gram- bacteria.

Conclusion

The results of the current study indicate that the *Chlorella vulgaris* has had an effect on some pathogenic bacteria of humans and this inhibitor has been effected more on bacteria compared to some antibiotics. This inhabitation efficiency due to the presence of stable biologically active compounds. The bio active compounds presents in this system will have some specific chemical structures which enables to functions, it as a beneficial substance and creates the effects of inhibition of harmful pathogens, and therefore the present research enables to know the ability of this micro-algae species to promote as an antimicrobial.

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