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Effect of Chlorella vulgaris Alcoholic Extract to Limit the Growth of (Staphylococcus aurous) That Couse Food Poison

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Abstract
The present study was planned to investigate and conducted to evaluate the antibacterial activities of the green algae crud extract of Chlorella vulgaris Staphylococcal food poisoning is a common cause of foodborne illness worldwide. The growth phases of algae were determined and the cultures were harvested at the end of logarithmic phase .The cultures of green algae gave 0.2gm/L. The lipid content of C. vulgaris cell was measured and it was 6.3% of dry weight then the fatty acids were analyzed using the high perform liquid chromatography HPLC technique system. The pathogenic bacteria was isolated and identified from different parts of body including swab from skin, expectorant of lung and from sputum of gum , then 5 different concentration of crude extract of alga C. vulgaris, 100%, 50%,25%,12.5% and 6.3% were tested against pathogenic bacteria Staphylococcus aureus .The resistance of bacterial isolates was examined against all algal extract concentrations. The results showed that Staphylococcus aureus was sensitive to 6.3% concentration of the crude extract of alga C. vulgaris which is the lowest concentration of the algal crude extract. The study concluded that microalgae especially fresh water algae represent a very important source for many active compounds and can be used as antimicrobial agents.

Introduction

Algae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, including antimicrobial, anticancer, antioxidant, and anti-inflammatory effects (1, 2). Microalgae are microscopic algae, typically found in freshwater and marine systems They are unicellular species which exist in dividedly, or in chains or groups. depending on the species, their sizes can range from a few micrometers (μm) to a few hundreds of micrometers. Unlike higher plants, microalgae do not have roots, stems and leaves. Microalgae, capable of performing photosynthesis which is very important for life on earth; they produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically.(3)

Most efforts were devoted to the study of antibiotic resistance in bacteria for several reasons:
(i) Bacterial infections are responsible for most community-acquired and nosocomial infections
(ii) The large and expanding number of antibacterial classes offers more diverse range of resistance mechanisms.
(iii) The ability to move bacterial resistance determinants into standard, well-characterized bacterial strains facilitates more detailed studies of the underlying molecular mechanisms(4)

The growing resistance of some bacterial strains arising from the widespread and essentially unrestricted use of antibiotics in cattle handling, and by domestic consumers use via self-prescription (5) However, a key factor for their eventual economic feasibility, which are able to produce biomass and metabolites to sufficiently high level (6),(7). Some microalgae species such as Chlorella, pirulina and Dunaliella species have been used in several areas in nutraceutical, pharmaceutical, cosmetics, nutrition and functional quality of foods(8). Cyanobacteria like Anabaena and Spirulina and green microalgae like Chlorella and Scenedesmus were tested incompliance with the agar well diffusion method for their antibacterial and antifungal agent production on various organisms that incite diseases of humans and plants(9). Fatty acids are widely occurring in natural fats and dietary oils and they play an important role as nutritious substances and metabolites in living organisms (10). Many fatty acids are known to have antibacterial and antifungal properties (11).

Aim and study

Collect, isolate, optimize the growth and Lipid analysis of fresh water sample of Chlorella sp. Also study the bacterial susceptibility testing using algal extracts by MHA assay.

Materials and methods

Sampling of algae

Fresh water Algal samples were collected from Tigris River at Baghdad-Iraq, The algal sample was cleaned and necrotic parts were removed. Then the sample was rinsed with sterile water to remove any associated debris. The cleaned culture of the sample was incubated in sterile container. Modified Chu-10 table (1) was used for the algal growth.(12), (13). Isolation of algae Streaking on plate agar techniques were used for algae isolation and purification in this study the samples were incubated under 200 μE/m²/s, 16:8 light: dark and 25± 2 C°, with a PH of 8.2±1.for 14 days. (14), (15).

Algae identification and cultivation

Chlorella vulgaris Bejerinck Based on the morphological identification the culture was identified under the microscope according to algal classification reference (16). For algae cultivation, 25 ml of isolated culture was added to a flask containing 250 ml of Chu-10 media and incubated under same factors for 14 days, then transported to 1000 ml of media and incubated for 14 days.

Table (1) The components concentration of modified Chu-10 medium and the concentration of each component

Number of stock solution	Chemical formula of each salt	Concentration g/l
1	$MgSo_4.7H_2O$	10
2	K_2HPO_4	4
3	$NaNO_3$ $CaCl_2$	8 16
4	$FeCl_3$	0.32
5	EDTA-Na2	4
6	NaCl	30
7	Na_2CO_3	8
8	$MnCl_2.4H_2O$ $(NH4)\ 6Mo_7O_{24}.4H_2O$ $ZnSO_4.7H_2O$ $CuSO_4.5H_2O$ $COCl_2.6H_2O$ H_3BO_3	0.02 0.028 0.224 0.08 0.004 0.288

Determination of Growth Curve:

The growth curve was determined for the alga Chlorella vulgaris. Microalgae concentration was determined daily by optical density (OD) measurements at 540 nm by a UV-Vis spectrophotometer (17).

Harvesting of Algae for Lipid Extraction:

Microalgae culture was harvested at the beginning of the stationary phase., C. vulgaris harvested in the tenth day (18) , The cells were harvested by centrifugation at 3000 rpm for 5min., then the algal pellet was collected and dried at 50ºC for 48hrs.(19)

Preparation of crude extract

A volume of 2 g from algal has been put on filter paper (Whatman No.1) and extracted in a soxhlet apparatus with 200 ml of solvent (mixture from methanol and hexane 1:1) had been used and the process taken three-four hours. The solvent was evaporated under vacuum at room temperature.(20), (21).

Antibacterial assay

The crude extract was tested for antibacterial activity by agar diffusion assay (22). Wells of 6mm were made in agar plates, and about0.1 ml of bacteria was spreading on agar surface. . Indicator microbe was spread on muller-hinton agar plates. This was allowed at least 5 minutes to dry before cutting the wells of 6 mm. The crude extract was tested against different concentration of algal extract samples of pathogenic bacteria Staphylococcus aurous taken by swab from different parts of human body, Staph 1 swab from skin, staph 2 from expectorant of lung, Staph 3 from sputum of gum , then 5 different concentration of crude extract 100%,50%, 25%, 12.5% and 6.3% and about 50µl of each extract was pipetted into the wells. The control run using the same volume solvent only (methanol and hexane 1:1) The plates were incubated at 37ºC for 24hr, then the inhibition zone was measured (19). (23), (24).

Lipid Analysis:

High Performance Liquid Chromatography (HPLC) system, model Shemadzo was used for lipid analysis. HPLC consists from a mobile phase which is polar mixture of solvents such as water: methane (60:40), while the stationary phase is a column stainless steel s, the column is discovery HSC: 18, dimension (25cm× 4.6mm × 5µm), injection flow is 1 ml/min, the absorption at UV 210 nm, and 30 C° temperature.

The results

Isolation and Identification of micro algae Chlorella sp. is an important microalgae which involved in the aquatic food chain, and rich in protein content it it can be easily grown in chemically defined media and in sewage. (25). Chlorella sp that isolated from the collected freshwater was spherical, chloroplast a parietal cup which nearly fills the cell , sometimes without a paranoid ; cells 2-4.5 µm in diameter.(26).

Division :Chlorophyta
Class: Chlorophyceae
Order: Chlorococcales

Family :Chlorococcaceae
Genus: Chlorella Beyerinck 1890
Species: Chlorella vulgaris Beyerinck

Growth Curve

The growth density was measured to identify the growth curve and the phases that the alga culture were going throw. C. vulgaris spent in lag phase four days and seven days in log phase and entered the stationary phase at the day 12,and the decline phase was began from the day 20.(27)

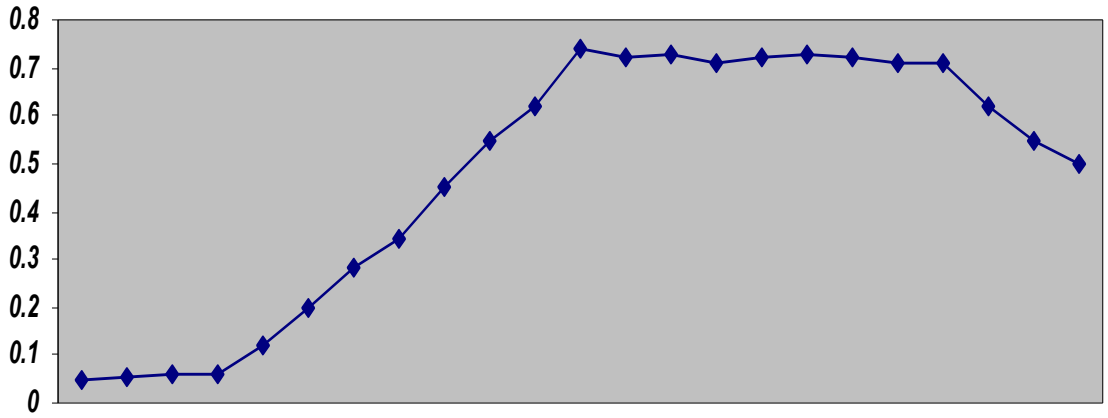

Figure (1) Growth curve of C. vulgaris

Lipid and Fatty Acids

The lipid content for C. vulgaris was measured and it was 6.3% of dry weight then the fatty acids were analyzed using the HPLC, Stearic acid (18:0) and Oleic acid(18:1).Stearic acid for C. vulgaris was 0.81% of total lipid and Oleic acid0.048 % of the total lipid .(28)

Microorganisms Growth and algal extract activity

Staphylococcus aureus bacteria were employed as test organism which is Gram positive bacteria.

Antibacterial activity of five different concentration extract was assayed using the diffusion method (Bauer et al., 1996). sub cultured and routinely maintained on Muller-hinton agar MHA. Antimicrobial activity was evaluated using the agar diffusion technique in petri dishes.

Different concentration of algal extract was added to the respective wells on the MHA plates. After incubation for 24 hours at 30°C, a clear zone round a wells were evidence of antimicrobial activity. The indication whether test organisms is resistant (No inhibition zone) or sensitive (clear or inhibition zone) to the algal extract was observed. (30).

Effect of algal extraction as Antibacterial activity

The activity of crude extract of algae against gram positive bacteria agreed with most studies used algae extract as source for active compounds against bacterial growth(19), (31). Different concentration of the extract of C. vulgaris against the bacteria Staphylococcus aureus shows different inhibition zone as is shown in table (2).

The highest effect of extract was recorded in staph1 at the concentration 6.3% the inhibition zone was 9.0 mm, while the lowest inhibition zone was at concentration 50%, 100%. It is observed that the lowest extract of the algal had the maximum activity against staphylococcus aureus with an inhibition zone compared to other concentration of algal extract. (32). It is clear that using organic solvent always provides a higher efficiency in extracting compounds for antibacterial activities. (23).

Table (2): The inhibition zone diameter (mm) of different concentration of crude algal extract against samples of Staphylococcus aureus bacteria

Bacteria	6.3%	12%	25%	50%	100%
Staph 1	9.0	5.0	3.0	0.0	0.0
Staph 2	6.0	7.0	3.0	0.0	0.0
Staph 3	5.0	6.0	5.0	3.0	2.0

In this study C. vulgaris was rich in fatty acid, protein, and pigment concentration the lipid showed that the extracts were rich in fatty acids, in particular saturated fatty acids (SFA), which may indicate their probable role in the antimicrobial activity (33), (34). Fatty acids are potent inhibitors of diverse enzymes and unsaturated fatty acids USFA usually have greater inhibitory activity than (SFA).(35), (36).

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