THE EFFECT OF SOME FRESH WATER ALGEA EXTRACTS IN THE INHIBITION OF THE GROWTH OF SOME MICROORGANISIM THAT CAUSE FOOD SPOILAGE

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ABSTRACT

This study was carried out to isolate, identified and study two types of Iraqi fresh water algae *Scenedesmus sp*. and *Oscellatoria sp* also determine some of their active compounds that play an important role as antioxidants and antimicrobial againsts some bacteria. Three types of bacteria *Salmonella sp, Escherichia coli and Staphylococcus sp* that cause spoilage for food were tested. Antimicrobial activity of ethanolic extracts for both algae were examined toward tested bacteria. Four treatments were prepared which were [S.Ex(T1) ,O.Ex(T2), S.Ex: Of*(T3), 1:1 O.Ex: Of*(T4) 1:1],and (C) as control treatment with the antibiotic only (Oflaxacin). Disk diffusion method was used to test the antibacterial activity. Some active compounds indicate and high performance liquid chromatography (HPLC) technique used to determine some of them . Both algae contain some active ingredients that consider to be the reason for their ability as antimicrobials. The results of this study showed that the highest inhibition zones were in the treatments T3 and T4 compared to control treatment that means the synergistic activity for both antibiotic and the algae extract as alternative sources gave a better result for health and food protection.

Key words: Fresh water algae, Algae extracts, Food spoilage.

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تاثير مستخلصات بعض طحالب المياه العذبة في تثبيط نمو بعض الاحياء المجهرية المسببة لتلف الاغذية

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المستخلص

شملت هذه الدراسة عزل وتشخيص ودراسة اثنان من طحالب المياه العذبة العراقية هما *Scenedesmus sp.* و*Oscellatoria sp* وايضا الكشف عن بعض المركبات الفعالة والتي تلعب دورا مهما كمضادات للاكسدة ومضادات مايكروبية ضد ثلاث انواع بكتيرية هي بكتريا السالمونيلا وبكتريا المكورات العنقودية وبكتريا القولون البرازية *Salmonella sp, Escherichia coli and Staphylococcus sp* والتي تسبب تلف اوفساد الاغذية. اختبرت الفعالية المضادة للمستخلص الكحولي لكلا النوعين من الطحالب تجاه البكتريا. حضرت اربع معاملات وتم اختبارها تجاه البكتريا وهي المعاملة الاولى والثانية والثالثة والرابعة وكالاتي([S.Ex(T1) ,O.Ex(T2), S.Ex: Of*(T3), 1:1 O.Ex: Of*(T4) 1:1]ومعاملة السيطرة كانت المضاد الحيوي الاوفلاكسين Oflaxacin(C). استعملت طريقة الانتشار للاقراص المشبعة لاختبار الفعالية المضادة للبكتريا. بعض المركبات الفعالة شخصت و استخدمت تقنية كروماتوغرافيا الســـــائل عالي الاداء (HPLC) لتحديد بعضها كلا النوعين من الطحالب يحتوي بعض المجاميع الفعالة والتي اعتبرت بانها السبب لقابليتها كمضادات مايكروبية. النتائج في هذه الدراسة بينت بان اعلى اقطار للتثبيط كانت للمعاملتين الثالثة والرابعة بالمقارنة مع معاملة السيطرة مؤكدا" بان الفعالية التازرية للمضاد الحيوي ومستخلص الطحالب كمصادر بديلة اعطت نتيجة افضل لحماية الصحة والغذاء.

الكمات المفتاحية: طحالب المياه العذبة، مستخلصات الطحالب، تلف او فساد الاغذية.

INTRODUCTION

Eukaryotic microalgae and cyanobacteria (blue-green) algae have been reported to produce a different biological and unimp - ortant active compounds that inhibit the growth of different bacteria and fungi in laboratory tests against various microorg - anisms that cause food spoilage and human diseases(1).Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption. The spoilage of food and presence of food poisoning organisms in food are very important from the point of food safety. Today the emphasis is on total quality of food which means that not only food should be nutritionally balanced but should be microbiologically safe too. Due to changes in sensory characteristics. Spoiled foods may be safe to eat, i.e. they may not cause illness because there are no pathogens or a toxin present, but changes in texture, smell, taste, or appearance cause them to be rejected (2). Various group of algae especially, cyanob - acteria, green algae have received great attention as producers of a diverse and biologically active compounds as well as implications for environmental health (3). A lot of active substances with antibacterial, antiviral, fungicide, enzyme inhibiting, immun osuppressive, cytotoxic and algaecide activity has been isolated from algal biomass (4,5).Moreover, algae are promising organ - isms for providing essential compounds for human nutrition (6).The current application of chemical compounds isolated from diverse classes of algae is enormous. Recent trends in drug research from natural sources suggest that algae are a promising group to furnish novel biochemically active substances (2).

The great diversity of all algal organisms can be a source of enrichment compounds gro - wing at various levels of conditions producing bioactives(7).Microalgae have proven to be potential sources of specially and important compounds(8) .Enterobacteriaceae are gram-negative, facultatively anaerobic bacteria that include a number of human pathogens (*Salmonella, E. coli, Shigella, Yersinia*) and also a large number of spoilage organisms. These bacteria are widespread in nature in soil, on plant surfaces and in digestive tracts of animals and are therefore present in many foods.

MATERIALS AND METHODS

Collection of algae

Algal were collected from ponds in the local gardens of Baghdad, then two species where selected and isolated as study organisms *Scenedesmus sp*. and *Oscillatoria sp.*, the samples washed first with tap water and then with sterile distilled water to remove all the debris and waste particles (9).

Culturing the algae samples

Algae samples were aseptically grown in liquid culture and conditions were carred out. The green algae *Scenedesmus sp*. cultured in Chu-10 media and the blue-green algae cultured in BG-11 media. All the culture plates or flasks broth were kept under specific growth conditions which were maintained at temperature; 30°C, light intensity; 200 µmole /m2/sec, pH; 6.0 (10).

Algal extracts

The completely dried algal sample was crushed in mortar. The powdered algal material was extracted in Soxhlet apparatus using ethanol as organic solvent for 24 h. The resulting extract was evaporated and the final concentrated extract was stored in refrigerator at 4°C(11).

Table 1. Treatments of ethanolic extracts for both algae and the antibiotic Oflaxacin

S.Ex	*(T1)*	*Ethanol extract of the microalgae Scenedesmus sp*
O.Ex	*(T2*	*Ethanol extract of the microalgae Oscillatoria sp*
S.Ex: Of 1:1	*(T3*	*Ethanol extract of Scenedesmus : Oflaxacin extract solution*
O.Ex: Of 1:1	*(T4*	*Ethanol extract of Oscellatoria : Oflaxacin extract solution*
C (control)		*Antibiotic Oflaxacin used as control or positive result , to compare with treatments*

Active compound analysis and HPLC Chromatographic analysis

Chemical compounds analysis of the extract was carried out using chemical methods according to the methodology proposed by Harborne (12).A binary gradient high-pressure liquid chromatography (Shimadzu HPLC) with two LC–10 AT VP pumps, variable wavelength programmable UV/ Visible SPD 10 AVP were used. The C-18 (5 micron 25 cm×4.6 mm) column. Chromat - ographic runs were performed at a flow rate was 1.2 ml/min, the wavelength of detection was 264nm, the injection volume was 10 µl (13).

Bacterial cultures

Three pathogens ,were tested *Salmonella* isolated from Egg, *Staphylococcus* isolated from meat , *Escherichia coli* were isolated from salad ,all these bacteria were identified in Market researchs center and consumer protection laboratories according to (14).

Evaluation of antibacterial activity

Disc diffusion method was employed for this purpose. The algal extract and Oflaxacin stocks were also made at 30 µg \ml the concentration the Oflaxacin served as positive controls. Autoclaved discs were loaded with 10 µL of the respective algal extract or Oflaxacin only and 1:1 (v: v) of both extracts and Oflaxacin were prepared then all disks air dried for 5 minutes. The nutrient agar plates were spread with 100 µL of respective culture with the help of glass spreader and the loaded discs were placed onto the surface of agar. The plates were left to dry for 5 min and kept in incubator at 37°C for 24 h. The results were seen as zone of inhibition which was measured in millimeters with the aid of transparent ruler. Samples of The experiment was done in triplicates (15,9).

RESULTS AND DISCUSSIONS

Identify of algae

Two isolated algae were obtained success - sfully, and they were identified according to Prescott (16).

***Scenedesmus* sp.**

A green algal that can exist as unicells; they are also frequently found in coenobia of four or eight cells inside a parental mother wall. Various coenobial architectures have been described, including linear, costulatoid, irregular, alternating, or dactylococcoid pa - tterns.

Phylum: Chlorophyta \ Class: Chlorophyceae \ Order: Sphaeropleales \ Family :Scened - esmaceae \ *Scenedesmus sp.*

2- *Oscillatoria*

A cyanobacteria algal that is important because it can conduct photosynthetic activities. It has a long un-branching filame - ntous morphology and is color green due to the chlorophyll it contains.

Phylum: Cyanophyta \ Class: Cyanophyceae \ Order: Oscillatoriales/ Family: Oscillatoriaceae \ *Oscillatoria sp.*

Active compounds

The results of active compound screening of ethanolic extracts of, *Oscellatoria sp. and Scenedesmus sp*. revealed the presence of flavonoids, saponins, tannins, carbohydrates and phenolics as in Table 2. .Gallic acid was determined as a very important compound of phenolics that reveled to be one of a very important antioxidants which was identif - icated by using high performance liquid chromatography(HPLC)(13).

Table 2. The active compounds of *Scenedesmus sp*. and *Oscellatoria sp.*

Antimicrobial compounds	*Scenedesmus sp.*	*Oscellatoria sp.*
Flavonoids	++	+
Saponins	+	+
Tannin	+	+
Carbohydrates	++	+
Phenolic	++	++

Many researches suggested that all these active compounds specially phenolic comp - ounds play important role as antioxidants, such activity that based on the physiological functions of polyphenol polymers so could be the explanation for inhibition ability against microorganisms that cause food spoilage or poisoning(17,18).

Antimicrobial activity: The results of anti - microbial activities of ethanolic extracts of

Scenedesmus sp. and *Oscellatoria sp.* are presented in Table 3. T1 and T2 were algal extract only and the biological activation were inhibiting the three bacteria *Salmonella*, *Staphylococcu*s and *E. coli* with inhibition zones of 12.5mm, 6.5mm, 11 mm and 11.5mm,8mm, 9mm, respectively and these results were less than inhibition zones at(C) control treatment. But in T3 and T4 the results show a great change at the inhibition zones that were 20mm, 13.5mm, 16.5mm and 17mm, 15mm, 14.5mm respectively. The results of this study were in agreement with many researchs as (19,20). Some studies have found that the efficacy of antimicrobial agents can be improved by combining them with crude plant extracts against different pathogens. These results consider to be very important because the synergic activity was noticed very strongly from the inhibition zone of each treatment also using the mixed treatment from the extract and the drug was more active than using the antibiotic only or algal extracts only. It was noted from the tables that the diameter of the inhibition zone depends on the type of extract algal species, type of active compound worked and the tested bacterial organism.All microorganisms were sensitive for the antibiotic Oflaxacin, but the inhibition zone of all the bacteria was increased specially at the (T3),(T4) because both algal extract and antibiotic have a great action or activity. This fact was in agree with a study by Olgica and Comic . 2012 (21) which included that observed enhancement of antibiotic activity could be explained by the presence of biologically active compounds in these extracts and at another side The mechanism governing the joint action of plant extract compounds and antibiotics is still unknown. Some scientist suggests that phytocompounds disturb cell wall or increase permeability of the cytoplasmic membrane or inhibit proteins and this results were agree with present study and that facts were showen in (2,22,23). This study was showing the importance of algae extracts as antioxidant and antibacterial activity together with other unidentified compounds to be future explor - ation of antibacterial potential and open new horizons for food and health protection**.**

Table 3. The inhibition zone of antimicrobial activities of ethanolic extracts of *Scenedesmus sp.* and *Oscellatoria sp. at different treatments*

Algal extracts (treatments) and the control treatment	*Inhibition zone(mm)* of Salmonella sp.*	*Inhibition zone(mm) of Staphylococcus sp.*	*Inhibition zone(mm) of E. coli*
Scenedesmus sp. (T1)	*12.5*	*6.5*	*11*
Oscellatoria sp. (T2)	*11.5*	*8*	*9*
Scenedesmus : Oflaxacin (T3) 1: 1	**20**	**13.5**	**16.5**
Oscellatoria : Oflaxacin (T4) 1: 1	**17**	**15**	**14.5**
Oflaxacin (C)	*15*	*12.5*	*13*

* **mm:millimeter**

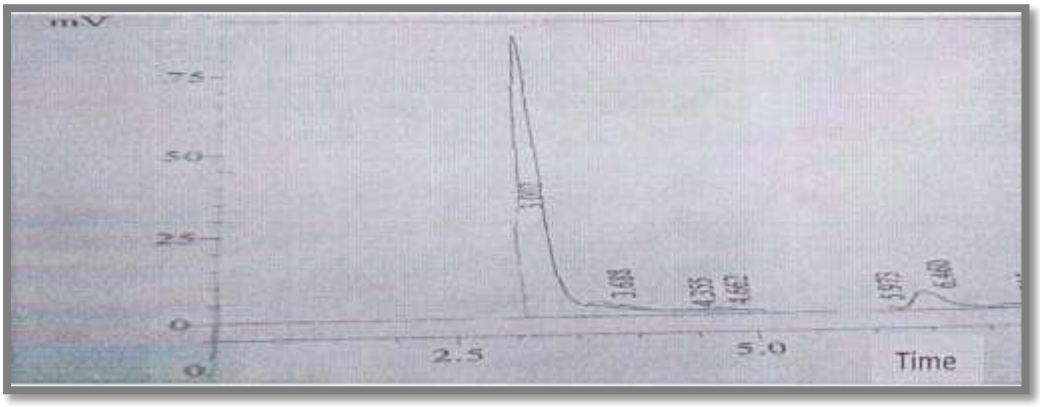

Figure 1. Stander of Gallic acid analyzed by HPLC . Retention Time of Gallic acid (RT) is 3.00

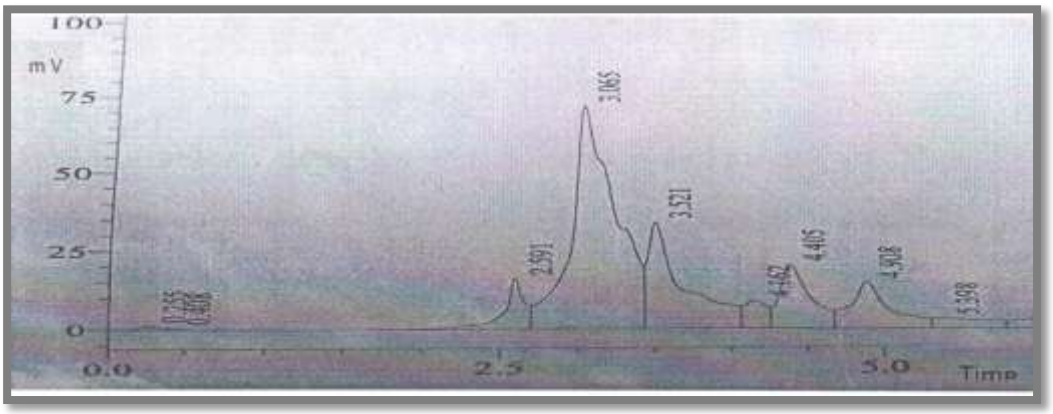

Figure 2. Gallic acid of *Scenedesmus sp*. analyzed by HPLC . Retention Time of Gallic acid (RT) is 3.06

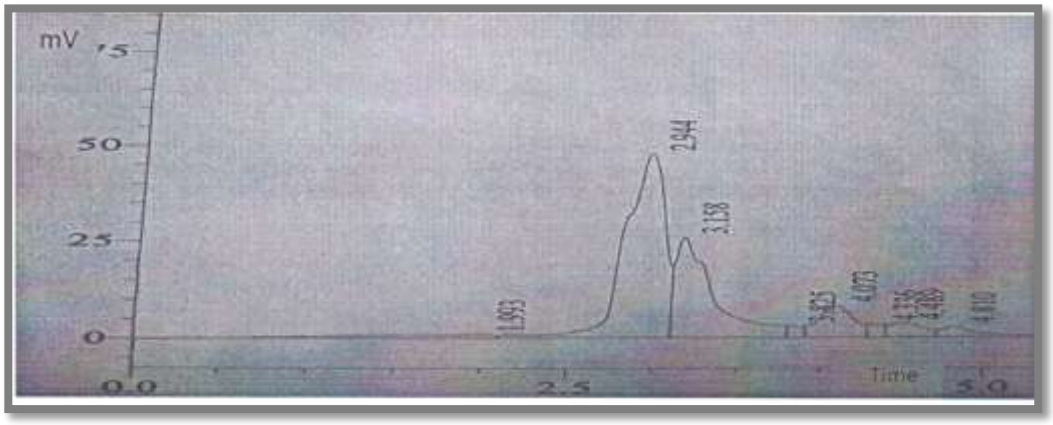

Figure 3. Gallic acid of *Oscellatoria sp.* analyzed by HPLC . Retention Time of Gallic acid (RT) is (2.94)

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