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Determination of Bioactive Compounds and Antibacterial of *Spirulina sp.* Extract

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Abstract

Objective of this study was to determine of bioactive compounds and antibacterial activity of *Spirulina sp* extract. Pathogenic bacteria, including *Streptococcus mutans*, *Staphyloylococcus aureus*, *Morganella morganii*, *Salmonella Sp.*,*Pseudomonas aerogenosa* and *Escherichia coli*, as the active chemical compounds were extracted from this algae using solvents (cold water and methanol alcohol) with qualitative detection of secondary metabolites of the aqueous extract of this algae, which indicated the presence of phytochemicals analysis such as Alkaloids, Phenols, Flavonoids, Glycosides, Terpenoids, Taninns, Saponins and Carotenoids . The chemical compounds were determined using the gas chromatography mass spectroscopy (Gc-mass) technique of the methanolic extract of *spirulina* These compounds were represented Muramic acid, Isopropyl Alcohol, Diglycerol, Neophytadiene, n-Hexadecanoic acid, Heptadecanoic acid, Phytol, 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, Linoleic acid ethyl ester and Floxuridine . The aqueous extract of this algae did not show any bioactivity against all bacterial species under study. It was noticed that the methanolic extract was more efficient in its effect on the bacterial species, as the rates of inhibition zones for this extract were (23.67, 21.33, 20.67, 18.00, 19.56 and 17.00) mm for each of *S. mutans*, *Staph. aureus*, *M. morganii*, *Salmonella Sp. , P. aerogenosa* and *E. coli*, respectively.

Key words: *Spirulina*, Antibacterial activity, GC mass, Bioactive compounds

Introduction

Infectious diseases are considered one of the main causes of high rates of disease and death among humans all over the world, especially developing countries, as the risk of disease has increased dramatically in recent years due to severe infection and disease-causing bacteria have become resistant to common drugs due to the wrong use of antibiotics and bacteria resistance. The fungi of antibiotics are among the main health problems in the world, and the efficacy has decreased and the resistance of pathogens to antibiotics has necessitated the development of new alternatives, thus algae is a rich source of bioactive compounds (Chowdhury *et al*., 2015). The last century witnessed a scientific development to study many of the medicinal properties of secondary ovaries present in all organisms, especially algae, and to benefit from natural products in the treatment of many diseases, as the last focus was on algae because they are available and diverse in many places (Amr, 2009). Primary or secondary algae produce potential biologically active compounds of therapeutic, industrial and agricultural importance (Mugilan and Sivakami, 2016). The main reasons for using algae extracts as antibacterial agents are their natural origin and a low chance of developing

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resistance to pathogens, as they have minimal adverse side effects on humans and animals and lower environmental risks compared to their synthetic alternatives (Kolanjinathan *et al*., 2014).

Materials and Methods

Sample collection and preparation of extraction

The algae samples (*Spirulina sp)* were obtained from the American company Amazon in the form of Powder. The different types of solvent used were absolute methanol 80% and water 100%. All tests were performed at room temperature.

Gas Chromatography mass device.

Some chemical compounds were identified in the methanolic extract of *spirulina* algae using the Gas Chromatography mass device in the Basra Oil Company / Research and Quality Control Department / Nahran Omar Laboratories .

Antibacterial Assay

Streptococcus mietus, Staphlloccus aureas. Morganella morganii. Salmonella Sp., Pseudomonas aerogenosa and Escherichia coli were used in experiment. Mueller Hinton agar was used in antibacterial assay. *spirulina* algae extracts were dissolved in methanol to obtain a concentration of 50, 100 and 200 mg/mL. Antibacterial assays were conducted using the disc diffusion method as previously described by (Kumar2014). Zones of inhibition around the discs were measured in mm. The experiment was repeated in triplicate and the mean of diameter of the inhibition zones was calculated.

Results and discussion

The results shown in Table (1) showed that the aqueous extract of *Spirulina sp.* on Alkaloids, Phenols, Flavonoids, Glycosides, Terpenoids, Tannins, Saponins and Carotenoids.

Table (1) Phytochemicals analysis of *Spirulina sp.* extract

Phytochemicals	***Spirulina sp.* extract**
Alkaloids	+
Phenols	+
Flavonoids	+
Glycosides	+
Terpenoids	+
Tannins	+
Saponins	+

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Carotenoids	+

+ = Presence of constituent; - = Absence of constituent

This is in agreement with Al-Okayli (2019) study on the bluish green alga *Spirulina platensis*. The results shown in Table (2) showed the chemical compounds diagnosed by the technique of gas chromatography (GC - mass) for methanolian extract of *spirulina*, as a number of active chemical compounds were found in the methanolate extract of this algae, and among these compounds that occupied the largest area of the total total area of the diagnosed compounds represented by Diglycer The one that occupied an area of (29926205) which is a tertiary alcohol and has an anti-bacterial activity and may be attributed to it because it occupied the largest part of the total area (Wu *et al*., 2017), followed by the compound n-Hexadecanoic acid, which occupied an area of (18132086) which is one of the solids. Carboxylic activity has antioxidant and antibacterial activities (Saravanakumar *et al*., 2018). As for the compound 9,12-Octadecadienoic acid (Z, Z) - which is called Linoleic acid, which is one of the basic fatty acids that the body cannot manufacture and which occupies an area of (11757585) as studies indicated that it has anti-bacterial activity (Abd-Elnaby *et al*., 2016).

Table (2): Chemical compounds diagnosed with GC - mass for methanolic extract of *spirulina*.

The name of the chemical compound	Molecular formula	Molecular weight Gram / mol	Retention time	Area
Muramic acid	$C_9H_{17}NO_7$	251	9.517	10916002
Isopropyl Alcohol	C_3H_8O	60	10.565	1123882
Diglycerol	$C_6H_{14}O_5$	166	11.983	29926205
Neophytadiene	$C_{20}H_{38}$	278	20.564	861434
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	21.923	18132086
Heptadecanoic acid	$C_{17}H_{34}O_2$	270	22.45	593591
Phytol	$C_{20}H_{40}O$	296	23.186	2191516
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	23.483	11757585
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	23.535	8638884
Linoleic acid ethyl ester	$C_{20}H_{34}O_2$	306	23.609	3261871
Floxuridine	$C_9H_{11}FN_2O_5$	246	26.402	349734

The results showed that the aqueous extract of this algae at all concentrations did not show any biological activity against all the bacterial species under study, and that result is consistent with the findings of Al-Ghanayem, (2017). On the blue-green algae *Spirulina platensis*, this finding can be traced back to the fact that most of the antibacterial active ingredients identified are not soluble in water (Stirk *et al*., 2007). The results shown in Table (3) showed the effect of the different

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concentrations of methanolic extract of *spirulina* algae against the bacterial species under study, as three concentrations (50, 100 and 200) mg / ml were used, and it is clear from the table that there is a clear inhibition against the bacterial species under study in general. There is a direct relationship between the concentrations of algal extract and the average area of inhibition of the bacterial species under study, as when the concentration of algae extract is high, the area of inhibition of the bacterial species is larger, and the results of the study also showed that the higher inhibitory effect is more pronounced against the bacteria positive for the stain of Kram compared to the bacteria negative for the stain of Kram stain.

Table (3) The effect of different concentrations of methanolic extract of *spirulina* algae against the bacterial species under study.

Bacterial species	**Concentration mg/ml**			**Mean**
	50	**100**	**200**	
Streptococcus mietus	16.00±1.00	24.00±2.00	31.00±2.00	23.67
Staphlloccus aureas	14.00±2.00	23.00±2.00	27.00±1.00	21.33
Morganella morganii	14.00±1.00	22.33±2.08	25.67±2.08	20.67
Salmonella Sp.	13.33±1.15	19.00±2.00	21.67±0.57	18.00
Pseudomonas aerogenosa	13.00±1.00	20.33±1.53	25.33±1.53	19.56
Escherichia coli	12.00±1.00	16.67±1.53	22.33±1.15	17.00
Mean	13.72	20.89	25.50	

L.S.D = 2.14 ($P \leq 0.05$)

This is in agreement with what many researchers have stated. The high sensitivity of Gram positive bacteria to algae extracts may be due to differences in the bacterial wall structure. The Gram-negative bacteria wall contains more lipids than the Gram-positive bacteria, and these fats prevent the penetration of the active compounds into the bacteria and thus affect their inhibition (Salem *et al*., 2011). There are many factors that affect the nature of the results obtained by the researcher in the tests related to the effectiveness of algae extracts against the activity of bacterial growth, there may be differences in the results reached by researchers for the same type of algae, and this difference may be attributed to the regions, time of collection and methods Preserving the samples used in the test before extraction, the environmental factors and the stage of algae growth about the farm harvest, the type of solvent in the extraction and the extraction method.

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