ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com
Journal DOI:10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

USE OF SPIROGYRA SP. EXTRACT AGAINST MULTIDRUG RESISTANT BACTERIAL PATHOGENS.

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Manuscript Info

Manuscript History:

Received: 12 May 2016
Final Accepted: 23 June 2016
Published Online: July 2016

Key words:
Spirogyra sp. ,antimicrobial, active compound, extracts.

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Abstract

The antibacterial effect of the crude hot Chloroform extracts and purified fractions of Spirogyra sp. (Green algae) against multidrug resistant human pathogen were isolated from burn and bound. The test bacterial strains were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli*. Hot Chloroform extracts (128 and 256 mg/mL) of *Spirogyra sp.* inhibited growth of all the test organisms. Primary detection of active compounds showed that (*Spirogyra sp.*) containing Terpenoid, Flavonoids ,Phenols Saponins and Alkaloids. Gas Chromatography-Mass Spectrometry was used to know the compounds which responsible of antibacterial activity and they wereNonadecane (44.5%) and Eicosane(19.2%) are alkane hydrocarbon Alkanes .While, Pentadecane represented(10.2%).Octadecane(8.3%),Tetradecanedihydroxyl (4.2%) Hexadecane 2-hydroxyl(2.1%) and Hexadecane(1.3%) from the crud hot extract of Spirogyra sp.These findings suggest the possibility of using the *Spirogyra sp.* as a novel source of natural antimicrobial agents in pharmaceutical industries.

Introduction:-

Spirogyra sp. is a genus of filamentous unbranched green algae that forms free-floating mats in shallow waters. It widely occurs in stagnant waters, such as ponds and canals, in shaded littoral zones of lakes, and in slow streams.[1,2] Which belong to class (Chlorophyta) and order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus .Currently, Spirogyra is gaining interest and considered as an ingredient or supplement for cosmetics, antioxidants or in foods, as well as in pharmaceutical products.[3, 4]

There are numerous reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, anti-neoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic [5,6]. In this investigation, antibacterial effects of *Spirogyra sp.* was studied againstmultidrug resistant bacterial pathogens (*Klebsiellaspp , Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa*and*Streptococcus faecalis*).

Materials and methods:-

Collection and Preparation of Sample:-

Spirogyra sp. specimens was collected from Tigris river in Baghdad city, Iraq. Which located on longitude 33°36'01.94"N and latitude44°20'19.41"E, during summer 2015. Samples of Spirogyra sp. was collected manually .The harvested algae were stored in plastic bags for transportation to the laboratory. *Spirogyra sp*.samples washed with water to clean the dirt, and then dried for three days in the sun.

Preparation of alcoholic extract:-

The alcoholic extract was prepared by soxhelet extraction according to Davis [14]. In this process the dried powders form of algae material extracted by using (100%) Chloroform. The concentrated active constituents from algae were kept in sterilized test tubes stored in refrigerator till further use. The traces of methanol were removed by keeping the tubes at 50°C for 1 h.

Bacterial Strains:-
Bacterial strains used in this study were obtained from the Department of biology, Collage of Science University of Mustansiryah, they were*Klebsiellaspp,Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris* all these strains were isolated from burns and wounds.

Antibacterial Assay:-
Antibacterial tests of algal extracts were performed in vitro using the disc diffusion method [7], in Petri dishes. Sterile disks of 6 mm in diameter were impregnated with 25 µL of algae extract, deposited on the surface of agar medium (Mueller-Hinton Agar, pH 7.4 ± 0.2 at 25 °C) previously inoculated with bacteria strains and incubated at 37 °C for 24 h [15]. The results are expressed by measuring the diameters in millimeter of the inhibition halos of bacterial growth around the disk. Chloroforme (100%) without algae extract was used as negative control. All tests were performed in triplicate, and clear halos greater than 10 mm were considered as positive results [16], experimental in comparison data represent mean ± SD of each sample.

Gas Chromatography-Mass Spectrometry:-
For GC-MS analysis, a high-temperature column (Inert cap 1MS; 30 m × 0.25 mm id × 0.25µm film thickness) was purchased from Agilent Technologies (SHIMADZU—Japan), by employing a high-temperature column. Derivatization of each sample was eliminated. The injector and detector temperatures were set at 280°C while the initial column temperature was set at 100°C. A 5 µL sample volume was injected into the column and ran using split (1:10) mode After 1 min, the oven temperature was raised to 225°C at a ramp rate of 12.5°C/min (hold time 4 min). The oven temperature was then raised to 300°C at a ramp rate of 7.5°C/min (hold time 5 min). The helium carrier gas was programmed to maintain a constant flow rate of 17.5 mL/min and the mass spectra were acquired and processed using both Agilent GC-Mass. Solution (SHIMADZU—Japan) and postrun software. The compounds were identified by comparison of their mass with NIST library search and authentic standards.

Qualitative estimation of active compounds from the *Spirogyra sp*:-
The presence of active compounds from algae of the studied were determined by adopting standard protocols.[14]

Results and discussion:-

Bacterial Strain:-
Sensitivity to antibiotics for all bacteria were tested **Table 1** results were measured in millimeters.
Table 1:- Sensitivity test for all bacteria were used in millimeter (Inhibition zone was measured to the nearest millimeter).

Test organism	AM	CE
Klebsiellaspp	**12**	**16**
S. aureus	**13**	**12**
P. vulgaris	**17**	**21**
E. coli	**15**	**20**
P.aeraginosa	**17**	**18**

AM: ampicillin 10 µg; CE:cefazolin 30 µg.

Evaluation of Antibacterial Activity:-
The antibacterial activity of *Spirogyrasp* crude chloroform extracts are shown in the **Table 2.** The antibacterial activity was ranged between (11-19 mm) the highest was in *S. aureus*at concentration 256 mg/ml and the lowest was in *E. coli* at concentration 128 mg/ml .

Table 2:-Crude extract antibacterial activity of Spirogyra (inhibition zone was measured to the nearest millimeter).(mean ±S.D).

Test organism	Crude extract (128 mg/mL)	Crude extract (256 mg/mL)	Negative control chloroform
Klebsiellaspp	**14.8±0.2**	**17±0.1**	**0.00±0.00**
S. aureus	**16 ±0.9**	**19±0.3**	**0.00±0.00**
P.vulgaris	**12±0.4**	**14±0.1**	**0.00±0.00**
E. coli	**11±0.1**	**13±0.2**	**0.00±0.00**
P. aeruginosa	**15±0.4**	**17±0.5**	**0.00±0.00**

Antibacterial activity of crude extract with concentration (128 and 256 mg/mL) has a great potential for the discovery of lead compounds that could be used against infectious diseases and parasites [8]. Among crude chloroform extract, the green algae showed high inhibiting activity against *S. aureus.* Similar observation was made in a methanol extract of green seaweed Ulvalactuca (500 µg/mL) which showed high inhibiting activity against *S.aureus* [9]. In the present study, the minimum inhibitory of chloroform extract of was found against Gram-negative pathogens, *E. coli* and *P. vulgaris*. Earlier studies showed that methanol extracts of seaweeds *Enteromorphaintestinalis*and*Gracilariacorticata* were active against Gram positive bacteria [10]. In the present study, we observed that chloroform extracts of green seaweed Spirogyra was active against Gram-positive bacteria. Many species of seaweeds were screened and found that members of red algae exhibited high antibacterial activity [11,12]. The variation of antibacterial activity of our extract might be due to the presence of antibacterial substances, which varied from species to species [13]. Previous reports showed that Gram-positive bacteria were more effectively controlled by the extracts of algae used in their study in comparison to Gram-negative bacteria [14,15]. This may be probably due to the presence of more complex cell wall structure in Gram negative bacteria [16,17]. In addition to that, the resistance displayed by the pathogens might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract [18]. Interestingly, in the present study, it was observed that seaweedwhich was used in this work exhibited good antibacterial activity to all Gram-positive pathogens tested except a few. Conflicting reports were observed on the presence of bioactive compounds in the algae related to the seasonal variation, as well as the method of extraction and organic solvents used for bioactive compounds extraction and differences in assay methods.

Qualitative Estimation of Active Compounds from *Spirogyra sp*:-

Results from current study show during analysis of the hot alcoholic extracts revealed that, Terpenoid, Flavonoids ,Phenols Saponins and Alkaloids are generally present in alcoholic extract of *Spirogyra sp*. other metabolites such as Glycosides and Tannins were absent in the extracts as show in table (3) .This result supports the findings of many authors[3,19, 20] they screened the most active compounds in macro-algae , biochemical analysis are being undertaken to determine the structure and nature of compounds responsible of the bioactivity of the extracts with high antibacterial activity. Not only the presence of a particular compound which makes these organisms, interesting but also their huge diversity and the possibility of not only harvesting them but also of growing them at different conditions, leading to an enrichment of some bioactive compounds.[21,22,,23,24,25]

Table 3:-Presence or absence of active compounds in *Spirogyra sp*. extract.

Active compounds	Presence Or Absence
Glycosides	**-**
Tannins	**-**
Terpenoid	**+**
Flavonoids	**+**
Phenols	**+**
Saponins	**+**
Alkaloids	**+**

The results reported in current study show in the table (4) that 6-major compounds were found in hot alcoholic crud extract of *Spirogyra sp*. ,these were: Nonadecane (44.5%) and Eicosane(19.2) are alkane hydrocarbon Alkanes .While, Pentadecane represented(10.2%) from the crud hot extract of *Spirogyra sp*. the alkane hydrocarbon the generic name for the group of aliphatic hydrocarbons Cn-H_2n^{+2} ,which represented reactive groups.[26, 27,28]

Materials in this group may be incompatible with strong oxidizing agents like nitric acid. Charring of the hydrocarbon may occur followed by ignition of unreacted hydrocarbon and other nearby combustibles. In other settings, aliphatic saturated hydrocarbons are mostly unreactive.

Table 4:-The major identified compounds of crud alcoholic (Chloroform) extract (*Spirogyra sp*.) by using GC-Mass spectrophotometer.

Rt	Compound	Area%
12.98	Pentadecane	10.2
13.78	Eicosane	19.2
14.11	Nonadecane	44.5
16.61	Tetradecanedihydroxyl	4.2
16.75	Octadecane	8.3
18.94	Hexadecane 2-hydroxyl	2.1
21.77	Hexadecane	1.3

The result presumes that the long chain hydrocarbons may act as potential bioactive substance and can be exploited in pharmaceutical preparations.[27,28] The cultivable nature of seaweeds is an added advantage for mass production of potential antibacterial products, our finding agreed with[29] how reported the most similarly compound in macro-algae where isolated from green algae[30,31]The main reasons for using algal extract as antifungal agents is their natural origin and low chance of pathogens developing resistance and less environmental hazards (eco-friend).[28,32]

Conclusions:-

The present study provides data to show the appreciable antibacterial activity of seaweed *Spirogyra sp*.crude extract and purified fractions against Gram-negative and positive human pathogens.

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