STUDY OF ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF SOME OF ALGAE[+]

Hussain Yousif Khalaf Al-Rekabi[*]

Abstract

The present study carried out to determine the antibacterial activity of aqueous and alcoholic extracts of three different algae which are dominant in local habitats: *Spirulina maxima ; Oscillatoria amoena* [Cyanophyta (Blue green algae)] , *Cladophora fracta* [Chlorophyta (Green algae)], extracts tested against (6) species of pathogenic bacteria. Alcoholic extracts of all algae were more active to inhibit growth of bacteria in comparison with aqueous extracts.

***Spirulina maxima* alga extracts were more effective on bacteria from other studied algae extracts.**

المستخلص

أجريت الدراسة الحالية لتحديد الفعالية ضد البكتيرية للمستخلص المائي والكحولي لثلاثة أنواع مختلفة من الطحالب الشائعة في بيئتنا المحلية وهي : *Spirulina maxima ;Oscillatoria amoena* (الطحالب الخضر)، ضد (٦) ستة أنواع من البكتريا *Cladophora fracta* الخضر المزرقة) و المرضية ، المستخلص الكحولي لجميع الطحالب كان اكثر تأثيرا على أنواع البكتريا مقارنة بالمستخلص المائي لتلك الطحالب. وكانت مستخلصات طحلب *Spirulina maxima* اكثر تأثيرا على البكتريا من مستخلصات بقيه الطحالب المدروسة.

INTRODUCTION

Algae which are widely spread in the world, are playing important roles in the nature. Their role may be useful or harmful, They may be considered as primary producers for organic and inorganic materials ((like enzymes, pigments and vitamins)) in aquatic ecosystem [1], The importance is unlimited on structural materials in the cells or their walls , however, that include the extracted materials too [2],others are involved in the chemical, pharmaceutical, feed and food industries [1 ; 3].

Antibiotics were produced from different microorganisms. [4 ; 5] ,at the same time the bacterial infections are still the major problem in the world today, because these bacteria which causes disease will eventually develop ways to resist the drugs as well., to help preventing and to treat these illnesses, many researchers have studied the antimicrobial effects of various plants extracts, as well as antimicrobial activity

[+] **Received on 2006/3/15 , Accepted on 2007/4/29**

[*] **Assistant Prof, Community Health Dept., Technical Institute /Al-Nassiriah**

of algae [6 ; 7], and the scientists referred to that seaweed (macroalgae) defends itself from specific pathogens (seaweed surprise) by chemical defence. Some of studies isolate a potent antimicrobial compounds from alga *Lobophora variegata* ,for example, these substances inhibit growth and germination of sea fan pathogen *Aspergillus sydowii*,that may explain why macroscopic algae are rarely infected [8].

There are numerous reports on compounds derived from algae with a broad range of biological activities , such as antibiotics , antiviral , antitumors and anti-inflammatories [9 ; 10], for example ,the *Porphyra* used as a drug in traditional Chinese medicine by using extracted polysaccharides[1]., Egorov [11] refered to a certain connection between the quantity of algae growing in a given water body and the number of bacterial organisms, the greater quantity of algae the less amount of bacteria , this indicates that antagonistic relation between algae and bacteria .And also there is more interesting to study the possibility of their use in food preservation for substituting chemical preservative in food industry [3].

Horikawa et al., [12] reported significant anti-methicillin – resistant *Staph. aureus* (MRSA) activity by crude methanol extract from (11) species of algae in Japan, and they isolate four bromindoles from the alga *Laurencia brongniartii* as antimicrobial substances . Ten species of algae were screened for the production of antimicrobial compounds against Gram positive and Gram negative bacteria, all these studied species were active against Gram positive bacteria, whereas only three species were active against Gram negative bacteria (seaweeds or macroalgae) [3].

Cyanobacteria (Blue-Green Algae) (BGA) are prokaryotic, photosynthetic microorganisms that are rich in biologically active secondary metabolites may inhibit or stimulate their own growth or that for other microorganisms like different groups of bacteria or fungi which may provide competitive advantages to bacteria in their interactions with fungi [13 ; 8].

Harder [14] first showed that *Nostoc punctiforone* (BGA) produced some antibiotics inhibit the growth of other microorganisms. Proctor [15] found evidence of substance produced by (BGA) which were toxic to fungi and other algae

Kubanek et al., [8] refer to a relationship between shrimp embryos and Cyanobacteria which covers it and produces the broad-spectrum antimicrobial compound (isatin) and this compound protects the embryos from pathogenic fungus *Lagenidium callinectes* .

Generally, the studies on BGA producing novel secondary metabolites such as antibiotics are scanty, and the local studies are very few except [16].

The objective of present study focused on the investigation of the antibacterial activity of extracted materials from algae dominated in local aquatic ecosystem ,and screening the specific properties of this compounds.

MATERIAL AND METHODS

1-Isolation, purification, growth and mass culture of studied algae

Algae were isolated from the eutrophic Al-Ashar canal in Basrah city, south of Iraq, by streaking method and classified by use[17] were: *Spirulina maxima ; Oscillatoria amoena , Cladophora fracta* .Purification and growth of algae was done according to criteria described by [17], the cultures maintained in a dilute Chu-10 medium in illuminated cabinet. The mass cultures produced using a procedure described in [18],the mass production was lyophilized by freeze-drier and well ready for extraction.

2-Extraction

(5) gm of each freeze dried algae were mixed with (50) ml distilled water in blender for (15)min. , mix still on magnetic stirrer for (10) hrs. in (40)° c ,and then placed in centrifuge at (5000) rpm for (25)min., take the supernatant and filtered by sterilized seitz filter (pore size 0.45μ), collect the filtrate in dark bottle [19], that is considered as a aqueous extracts for algae in (1:10), this procedure was repeated for alcoholic extracts by use(95%)Ethanol alcohol.

3-Determination of antibacterial activity

Six species of pathogenic bacteria were used to study antibacterial activity of algae extracts by using:

a)determination of growth inhibition zones for (5) concentrations (100,250,500,750 and 1000 μg /ml) from crude extracts by agar diffusion technique [20].

b)determination of minimal inhibitory concentrations (MICs) for many series concentrations for each extract, by a tube dilution procedure [21].

c)determination of minimum bactericidal concentration (MBC), by procedure explained in [21].

4-TOXICOLOGICAL EFFECTS:

Use (65) albino mice (28 ±5)g weights, in (4 – 5)months old, divided into (13) groups ,one considered as a control group injected with water by mouth, the others(4 groups for each alga) injected with different concentrations of crud aqueous extracts (250, 500 ,1000 and 1500) mg/kg, the animals monitored for (96)hrs. to detect the mortality [22].

5-Specific tests of aqueous and alcoholic extracts .

many specific tests were carried out on the principle chemical compounds of both extracts as explained briefly in [23] which were :

a)alkaloid test [19], by using the Dragendroff reagent ;Mayers reagent ;Wagners reagent and Marqus reagents

b)Flavonoids test [24], by using of Magnesium Turings test

c)Glycosides test includes before glycoside hydrolysis by using Benadicts reagent[19]
and after glycoside hydrolysis [24].

d)Carbohydrates test by Molishs test [25] .

e)Peptides and Free Amine group test by using Ninhydrine reagent [19] .

f)Saponins test [26] ,by using of aqueous mercury chloride (5%).

g)Tannins test [27], by using of aqueous lead acetate (1%) [28].

RESULTS

1- Antibacterial activity of algae extracts (Inhibition zones determination)

The study showed the activity of aqueous and alcoholic extracts of studied algae (especially BGA) by inhibition of growth both G+ ve and G– ve germs. These activities are varied according to the species of algae and type of extract (Table 1-3).

The extracts of *Spirulina maxima* and *Oscillatoria amoena* (Cyanophyta, Blue Green Algae) inhibits the growth of all germs isolates approximately , in comparison with the extracts of *Cladophora fracta* (Chlorophyta, Green Algae) , with significant obvious superiority of alcoholic extract in comparison with aqueous extract ($p < 0.01$). The biggest diameter of growth inhibition zone was of the alcoholic extracts of *Spirulina maxima* against (*E. coli and Enterobacter* spp.) was (19)mm in 1000 µg /ml (Table 1).

2-Minimum Inhibitory Conc. (MIC) and Minimum Bactericidal Conc. (MBC)

The MIC and MBC of algae extracts which appeared activity against germs in (Sec.1 above) were significantly varied according to studied algae species and type of extracts (Table 4).

The MIC for the alcoholic extracts of *Spirulina maxima* ranged between (1-5) µg /ml , for *Oscillatoria amoena* between (1-25) µg /ml, and for *Cladophora fracta* (10-75) µg /ml. While for the aqueous extracts were (1-50), (5-75) and (50-100) µg /ml for algae respectively.

The MBC values were higher than MIC values in the most cases (Table 4).

Toxicological tests showed there's no mortality happened among experimental animals after (96) hours of injection.

Table (1) :Antibacterial activity (inhibition zone) of different concentrations of *Spirulina maxima* extracts against different bacteria.

Organisms	Extract	Concentrations(µg/ml)				
		100	250	500	750	1000
		Inhibition zone (mm)				
Eschrichia coli	Alco.	3	8	12	15	19
	Aque.	2	6	7	8	12
Enterobacter sp.	Alco.	3	7	14	16	19
	Aque.	1	5	7	10	15
Klebsiella pneumonia	Alco.	1	3	8	15	17
	Aque.	0	2	5	9	11
Streptococcus faecalis	Alco.	3	7	7	11	14
	Aque.	1	5	6	7	10
Bacillus subtilis	Alco.	1	1	3	9	12
	Aque.	0	1	1	6	9
Staphylococcus aureus	Alco.	1	2	5	10	15
	Aque.	1	1	2	7	11

Alco. : Alcoholic extract Aque. : Aqueous extract

Table (2):Antibacterial activity (inhibition zone) of different concentrations of *Oscillatoria amoena* extracts against different bacteria.

Organisms	Extract	Concentrations(µg/ml)				
		100	250	500	750	1000
		Inhibition zone (mm)				
Eschrichia coli	Alco.	2	6	9	12	15
	Aque.	2	5	6	8	11
Enterobacter sp.	Alco.	2	5	11	13	15
	Aque.	1	3	5	8	13
Klebsiella pneumonia	Alco.	2	5	6	9	10
	Aque.	0	2	3	6	9
Streptococcus faecalis	Alco.	2	3	5	9	11
	Aque.	2	4	4	6	8
Bacillus subtilis	Alco.	1	1	2	8	10
	Aque.	0	1	1	4	8
Staphylococcus aureus	Alco.	2	5	9	11	12
	Aque.	1	1	1	5	9

Table (3) :Antibacterial activity (inhibition zone) of different concentrations of *Cladophora fracta* extracts against different bacteria.

Organisms	Extract	Concentrations(µg/ml)				
		100	250	500	750	1000
		Inhibition zone (mm)				
Eschrichia coli	Alco.	1	4	6	10	12
	Aque.	0	2	3	3	8
Enterobacter sp.	Alco.	1	3	6	11	13
	Aque.	0	1	3	4	9
Klebsiella pneumonia	Alco.	0	0	3	10	11
	Aque.	0	1	2	4	7
Streptococcus faecalis	Alco.	1	3	4	6	9
	Aque.	0	2	2	4	6
Bacillus subtilis	Alco.	0	0	1	3	9
	Aque.	0	0	1	2	5
Staphylococcus aureus	Alco.	0	0	2	5	10
	Aque.	0	0	1	3	8

Table(4):Minimum Inhibitory Concentrations(MIC) (µg/ml) and Minimum Bactericidal Concentrations MBC) (µg /ml) of studied algae extracts.

			Organisms					
Algae	Extract		*Eschrichia coli*	*Enterobacter sp.*	*Klebsiella pneumonia*	*Streptococcus faecalis*	*Bacillus subtilis*	*Staphyloccoccus aureus*
S. maxima	Alco.	MIC	1	1	0.1	1	0.1	5
		MBC	5	5	5	5	1	25
	Aque.	MIC	5	25	50	1	50	5
		MBC	25	50	100	5	>100	25
	Alco.	MIC	5	5	25	5	5	1

O. amoena		MBC	10	10	50	10	5	5
	Aque.	MIC	5	50	50	5	75	50
		MBC	25	75	100	25	100	75
C. fracta	Alco.	MIC	25	50	75	25	10	10
		MBC	25	75	100	50	25	50
	Aque.	MIC	50	75	100	75	75	75
		MBC	75	100	>100	>100	>100	>100

Table (5):Specific tests of alcoholic and aqueous extracts of studied algae.

algae / Tests	*S. maxima*		*O. amoena*		*C. fracta*	
	Alco.	Aque.	Alco.	Aque.	Alco.	Aque.
Alkaloids	+	+	+	+	-	-
Flavonoids	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	+
Peptides	+	+	+	+	+	+
Saponins	+	+	+	+	-	-
Tannins	+	+	+	+	+	+
Phenoles	+	+	+	+	-	-
Glycosides (pre-analysis)	+	+	+	+	+	+
= (post-analysis)	+	+	+	+	+	+

DISCUSSION

Algae secrete some of compounds with a broad range of biological activities for example as antibiotics .

Study results showed ,the extracts of studied algae have effects on the growth of some of G +ve and G –ve germs species, these effects are varied according to the species and type of extract.

Generally, pathogenic germs showed different responses and sensitivity, The G +ve (in most cases) have responses more than G –ve ($p<0.01$), these differences may be due to genetic or chemical structure of microbe [6];also may be due to the nature of cell membrane of these germs which contains very high level (90-95%) of peptidoglycan and contains lipopolysaccharides and phospholipids (5-10%), that to find suitable medium to reaction and entrance the antigerms factors (Bactericidal and Bacteriostatic agents) to inside the G +ve cell and destroy the cell membrane or protein biosynthesis units (DNA and RNA) in comparison with G –ve germs which their membrane consists of two layer (outer and inner membrane) separate by periplasmic space, the outer membrane consists of phospholipids ,lipoprotein and mucopolysaccharides while the inner membrane consists of peptidoglycan(glycopeptide) (5-10%); that means the membrane of G –ve cell contains high level of lipids (90-95%),which does not assist and does not find suitable medium to reaction and entrance the antimicrobial agents and then reduces of its effects on the germs [23].

The alcoholic extracts were more effective on growth some of germ species in comparison with aqueous extracts ($p<0.01$) , that may be due to the difference of the two solvents in extraction efficiency of active substances from the studied algae cells, and also may be due to the semi-alkaloids (which find in present study) are difficult solubility in water that leads to its concentration in aqueous extract less than in alcoholic extract [29 ; 30].

The results of present study showed that effects of extracts of blue-green algae BGA (Cyanobacteria) on the germs growth were more than of green algae(Chlorophyta) ($p<0.01$), that's may be due to effect of essential oils which are found in (BGA) more than in Green algae [18] ;or other compounds like acroline and crotonaldehyde [6];also may be due to the nature of membrane of BGA are thin and that facilitates the extraction process in comparison with the membrane of green algae which contains more cellulose , and to the extracts of BGA contain semi-alkaloids, saponins and phenols more than its in green algae extracts [1] .

Experiments of toxicological tests for algae extracts, showed the extracts were untoxic for experimental animals and there is no mortality after (96) hours from its injection by different high concentrations of aqueous extracts

.REFERENCES

[1]Venkataraman,L. and Becker,E*Biotechnology and utilization of algae- the indian experience*. New Delhi and Central Food Technology Research Institute, Mysore,India, ,1985

[2]Bold,H.C. and Wynne,M.J. *Introduction to the algae.* Prentice-Hall,Inc., Englewood Cliffs, New Jersey , 1985

[3]Nezha,S.;Mohamed,L.;Mohamed,F. and Khadija,F. "Inhibition of growth and mycotoxins formation in moulds by marine algae *Cystoseira tamariscifolia"*. *Afrrican Journal of Biotechnology.* Vol. 3, No. 1, pp.71-75, 2004.

[4]Scheuer,P. *Bioorganic marine chemistry, Vol. 1-3*. Springer,Berlin Heidelberg New York. .1987.

[5]Faulkner,D."Marine natural products chemistry: introduction". *Chem.Rev*. vol.93, pp.1671- 1673.1993. [6]Caccamese,S.;Azzolina.R.;Furnari,G.;Cormaci,M.and Grasso,S. "Antimicrobial and antiviral activities of some marine algae from eastern Sicily" *Botanica Mar* Vol.1.pp.365-367,1981.

[7]Bennamara,A.;Abourriche,A.;Berrada,M.;Charrouf,M.;Chaib,N. and Boudouma,M. "Methoxybifurcarenone:an antifungal and antibacterial meroditerpenoid from the brown alga *Cystoseira tamariscifolia" .Phytochemistry*. vol.52, pp.37-40, 1999.

[8]Kubanek,J.;Jensen,P.;Keifer,P.;Sullards,M.;Collins,D. and Fenical,W.. "Seaweed resistance to microbial attack A targrted chemical defense against marine fungi" *PNAS*. Vol.100, No.12, pp.6916-6921, 2003.

[9]Sreenivasa,P. and Parekh,K. "Antibacterial activity of indian seaweed extracts*"* *Botanica Marina.* Vol. 24, pp.577-582, 1981 .

[10]Hellio,G.;Bremer,A.;Pons,G.;Cottenceau,Y. and Le,G. "Antibacterial and antifunga activities of extracts of marine algae from Brittany France. Use as antifoulimg agents" *.Appl. Microbiol.Biotechnol.* Vol.54, No.4, pp.543-549, 2000.

[11]Egorov,N. *Antibiotics a scientific appraoch* . MIR publishers Moscow. .1985.

[12]Horikawa,M.;Noro,T. and Kamei,Y.. "*In vitro* anti-methicillin –resistant *Staphylococcus aureus* found in extracts of marine algae indigenous to the coastline of Japan". *J. Antibiotics.* Vol.52, pp.186-190 , 1999.

[13]Fogg,G. " Extracellular products of algae in fresh water" . *Arch.Hydrobiol. Belh. Ergegn. Limnol* Vol. 5, pp.1-25, 1971.

[14]Harder,R. *Blue green algae. In physiology of antarctic fresh water algae*. Vol.2, pp.632- 638 Academic press .London. . 1917. (cited by Mehdi and Al- Mousawi , 1999).
[15]Proctor,V. " Studies of algae antibiosis using *Haematococcus* and *Chlamydomonas " Limnol Oceanogr.* Vol. 2, pp.125-139 , 1957.
[16]Mehdi,K. and Al-Mousawi,N. " Isolation and activity of an antibiotic agent produced by *Oscillatoria amoena* " . *Marina Mesopotanica*. Vol.14, No.2, pp.257-264 ,1999.
[17]Stein,J.*Hand book of phycological method*. Cambridge Univ.Press. Cambridge,1973.
[18]Al-Rekabi,H.Y.. *Utilization of mass cultures of certain microalgae in nutrition of chicks* Ph.D.Thesis, Univ. Basrah. Iraq. 2003.
[19]Harborn,J. *Phytochemical Methods: A Guide to modern Techniques of plant analysis*. 2nd ed. Chapman an Hall, London, UK. 1984.
[20]Cruickshank,R.;Duguide,G.;Marmion,B. and Swain,R.. *Medical microbiology*. Vol. 2 12th ed. Churchill, Living ston,Edinburgh,London. 1975.
[21]Begum,S.; Usmni,S.; Siddigui,B.; Saeed,S.; Farnaz,S.; Ali Khan,K.; Ahmed Khan,S.; Khalid, S. and Zia,A.." Chemistry and biological activity of a tryptamine and B- carboline series of bases". *Arzneim-Forch./Drug Res*.Vol. 46,pp. 1163-1168 , 1996.
[22]Waynforth,H.*Experimental and surgical techniques in the Rat*. Acad. Press. London, p. 7-9. 1980.
[23]Al-Samary,I. " A study of antibacterial activity of aqueous extract of *Allium sativum* L. (Liliaceae)" . *Eastern Mediterranean health J*. Vol. 5, No.4 , pp.803-810 , 1999.
[24]Al-Khazragi,S. *Biopharmacological study of Artemisia herba alba* .M.Sc. Thesis. Univ. Baghdad. Iraq. 1991.
[25]Hawk,P.; Oser,B. and Sumerson,H.. *Practical physiological chemistry*. 13th ed. ,McGraw- Hill Co., Int. New York,U.S.A. 1954.
[26]Hadad,D. *The chemistry of vegetable drugs*. Part 2 pp.1-27. Cairo University Press, Cairo,Egypt. 1965.
[27]Geisman,T. *Chemistry of flavonoid compounds*. MacMilon Co., Ney York. U.S.A. 1962.
[28]Jawad,A. *Ethological studies in assessing the anti-aggressive effects of some iraqi plants on laboratory mice*. A thesis in physiology ,college of Education, Univ. Basrah. Iraq.1997.
[29]Al-Shamaa,A. *Drugs and Chemistry of medical plants*. Al-Hikma house. Basrah Univ. Iraq. (In Arabic). 1989.
[30]Al-Jobory,A. and Al-Rawi,M.. Natural Pharmacology. Books and Documents House . Baghdad. Iraq. (In Arabic). 1994