ACTIVITY DETERMINATION OF PLANT EXTRACTS IN THE CONTROL OF CYANOBACTERIA.

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ABSTRACT

Some Iraqi plants which belonging to different families were extracted in 80% Ethanol and the ability of these extracts to control and growth of Cyanobacteria was evaluated. Results indicated that *Artemisia campestris, Achillia santolina, Artemisia herba-alba* and *Centurea khotchii* extracts were very active in inhibiting the growth of the test Cyanobacterial species. However, other plants extracts in this study did not show any promising algicidal potencies.

INTRODUCTION

A variety of problems, such as, dermatitis diseases, fish toxicity, amenity of water …etc.were considered due to the presence of algal blooms in general and Cyanobacteria species in particular in the environment (Ridley, 1970; Lund, 1972; Edwards, 1972; Gentile, 1971) .

However, research to find ways of controlling their growth was encouraged. Plants have many chemical constituents, some of which proved to have ability to control the growth of many pathogenic bacteria and Candida species (Jawad, *et al*.1988(a); Ikram, 1984; Aysen *et.al*.2003; Jose et.al.2002).A few plants which contain saponins or tannin compounds were exhibited a promising ability to attack Cyanobacterial blooms (Jawad, 1998).The plants under investigation were known to contain many different compounds but their algicidal properties were not evaluated. Therefore, due to the economic and hygienic importance of the biological control of algae, the determination of algicidal activity from plant extracts will be proceeded in this laboratory as an attempt to find methods of controlling algal growth.

MATERIALS AND METHODS

Plants materials

Achillea santolina L.(Compositae); *Artemisia campestris* L.(Compositae); *Artemisia herba-alba* L.(Compositae); *Centurea Khotchii* L. (Compositae); *Tagetes patula* L.(Compositae); *Althaea officinalis* L. (Malvaceae); *Antirrhinum majus* L.(Scrophulariaceae) and *Elettaria cardomomum maton* L. (Zingeberaceae) were collected from different environs of Iraq. These plants were identified and authenticated at her barium of college of Science University of Baghdad. They

were air dried at room temperature and grounded to powder form.

Extraction

The powdered plant material (100g) of each plant was extracted with 80% ethanol using Saxhlet apparatus until exhausting .The cooled solution was evaporated down to dryness under reduced pressure at 45ºC and the resulted crude extracts were evaluated for their algicidal activity.

Test cyanobacterial species and culture media

The cyanobacterial species were isolated from different habitats in Baghdad. The isolates were purified and identified according to authentic keys of Prescot, (1973) and Desikachary, (1959). They were grown in Allen's media (Allen, 1968), pH 7.6-7.8, and stocked in the laboratory at 4ºC illuminated refrigirator. The species selected for this study were a representative of filamentous and unicellular blue-greens. They were , *Anabaena azollae* St. Rasburger; *Anabaena constricta* Szafer; *Anabaena cylindrica* Ghose&Randh; *Nastoc carneum* Ag.; *Nostoc muscorum* Ag.; *Myxosarcina spectabilis* Geitler and *Entophysalis granulosa* Kutz.

Determination of algicidal activity

Allen's medium with agar for each cyanobacterial species was made and allowed to cool down to approximately 45ºC with continuous swirling. Aliquots of known volume of homogenous algal suspension was then added and mixed rapidly before pouring into sterilized plates. These plates were kept at normal temperature for 2 hours in order to allow the agar to solidify. Wells (9mm in diameter)were made using cork borer and vacuum to pull out agar pillets. The total extracts were dissolved in 80% ethanol and aliquot 0.2 ml of each extract (20 mg /l) were added in triplicates, then incubated in illuminated cooled incubator at 26º±1º C for 5 days. Ethanol (80%)and streptomycin sulphate (0.5 mg/ l)were used as experimental control in this study.

Chlorophyll determination

Different concentrations of each extract (5,10,20 mg/l)were added in triplicates to the liquid culture of each algal species and then incubated as previously for 5 days. The chlorophyll content was determined everyday for 5 days by the methanol extraction technique described by Golterman *et al*. (1978).

RESULTS

The yield of 80% ethanolic extracts obtained from plants under investigation were ranged from 14-30.6% and the highest yield was achieved from *Tagetes patula* and *Althaea officinalis* (Table1). In this study the algicidal activities of 80% ethanolic extracts (20mg/l) were determined everyday for 5 days of inhibition and visible clear inhibition zone were observed. The inhibition zone was increased dramatically and the highest zones of inhibition were considered after 5 days (plate 1) The anticyanobacterial potency of the crude extracts were varied

and *Artemisia campestris, Achillea santolina, Artemisia herba-alba* and *Centurea khotchii* were the most active as shown in table (2).The results achieved from other plant extracts were considered as a non significant algicidal potencies .Samples from inhibition zones in the cyanobacterial lawns which had been caused by the activity of the ethanolic extracts were removed at different times during lawn growth and observed by light microscopy. It was found that the vegetative cells of the cyanobacteria had lysed in the inhibition zones, but the heterocysts and akinetes appeared free but intact(plate 2&3).The effect on the growth of *Anabaena cylindrica and Anabaena constricta*, after the addition of different concentration of ethanolic extract(5,10,20 mg/l)to each culture, from *Artemisia campestris* were shown in fig.(1&2).The results indicate that the growth was more effected in *Anabaena cylindrica* and *Anabaena constricta* after 3-5 days. However the growth of *Nostoc carneum* and *Nostoc muscorum* were less effected.

DISCUSSION

The major chemical constituents reported to be present in these plants were indicated in table 3 (Rizk, 1986).Some of these plant extracts were evaluated for their ability to inhibit the growth of pathogenic bacteria and Candida species (Jawad, *et al*. 1988_b).These results indicated that *Antirrhinum majus* and *Achillea santolina* extracts were active against bacteria. Since the flavonoids and terpenes were the major chemical constituents in the active plants, therefore, the algicidal activity might be due to the presence of one or both compounds. Some of the plants as indicated in table 3 have the same chemical compounds, but they did not show algicidal activity, despite the large quantity of these constituents. This might be attributed to the absence of specific flavonoids or terpenes which could be important to cause the growth inhibition. Authors reported that the susceptibility of cyanobacteria to antibiotics such as mytomycin C, neomycin sulphate, rifamycin and streptomycin(Kumar, 1964; Rodriguez-Lopez, *et.al*.1970).However, only few reports were noticed on the use of plants chemical compounds in the control of algal blooms(Hussein-Ayoub, *et al*.1982). Finally the results obtained prompted further work on screening other plants in addition to identification of the active algicidal agents.

Table (1): The yield of 80% ethanolic extracts from plants under this study.

Plant species	Family	%Yield
Althaea officinalis L.	Malvaceae	23.5
Tagetes patula L.	Compositae	23.8
Elettaria cardamomum maton L.	Zingberaceae	16.0
Antirrhinum majus L.	Scrophulariaceae	30.6
Artemisia herba-alba L.	Compositae	19.5
Artemisia campestris L.	Compositae	17.4
Achillea santolina L.	Compositae	14.0
Centurea khotchii L.	Compositae	18.0

Table(2): Algicidal properties of some Iraqi plant extracts

Plant species	Cyanobacterial species						
	A.a.	A.c.	A.con.	N.c.	N.m.	M.s.	E.g.
Althaea officinalis	14	12	14	15	13	15	16
Tagetes patula	14	12	14	15	16	14	14
Elettaria cardamomum	14	-	15	15	16	14	13
Antirrhinum majus	13	-	15	17	13	14	14
Artemisia herba-alba	40	33	27	25	15	15	17
Artemisia campestris	40	35	30	23	15	-	18
Achillea santolina	40	35	30	17	17	20	20
Centurea khotchii	35	35	28	15	17	22	20
Streptomycin sulphate 0.5mg/ml	32	20	-	30	28	15	-
Ethanol 80%	11	11	11	-	11	-	11

Inhibition zones were determined in mm.

- =No inhibitory action; A.a.= Anabaena azollae, A.c.= Anabaena cylindrica, A.con.=Anabaena constricta , N.m=Nostoc muscorum N.c=Nostoc carneum; M.s.=Myxosarcina spectabilis, E.g.=Entophysalis granulosa .

Table (3): The major chemical constituents in the plants used in this study

Plant species	major constituents
Althaea officianalis	polyphenolic compounds + mucilage
Antirrhinum majus	Flavonoids
Artemisia campestris	Flavonoids + Terpenes
Achillea santolina	Flavonoids + Terpenes
Tagetes patula	Flavonoids
Elettaria cardamomum	Terpenes
Artemisia herba-alba	Flavonoids + Terpenes
Centurea khotchii	Flavonoids + Terpenes

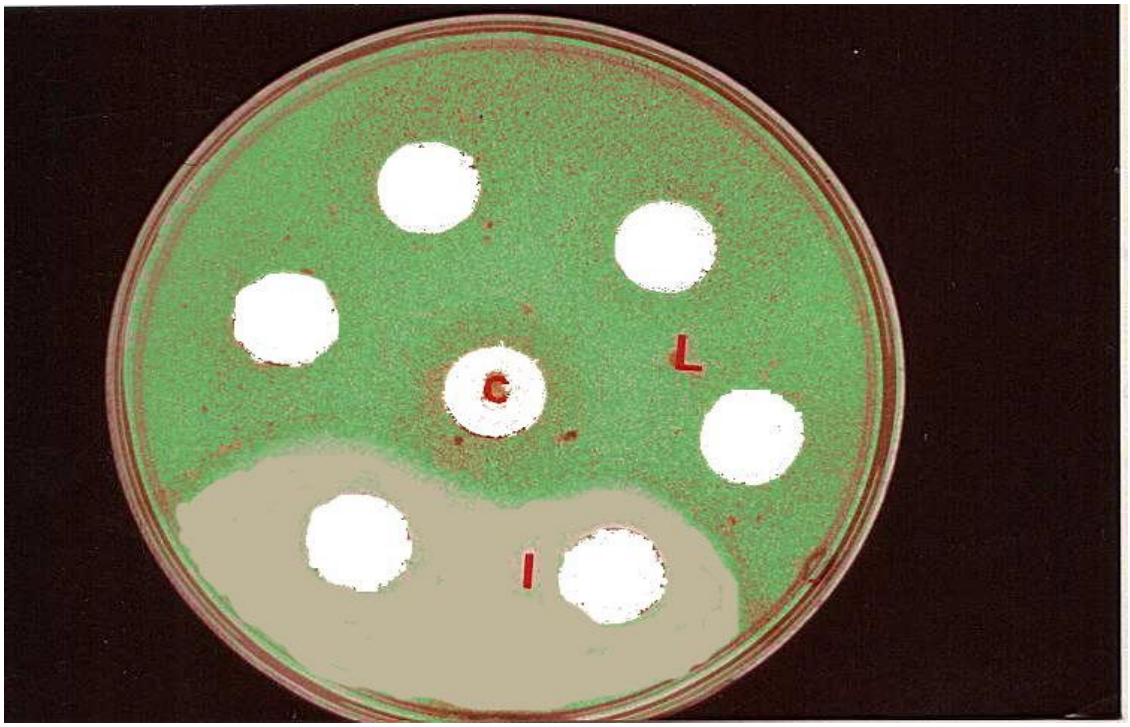

Plate (1) The inhibition zones observed after 5 days of incubation C= control , I = inhibition zone, L = The lawn

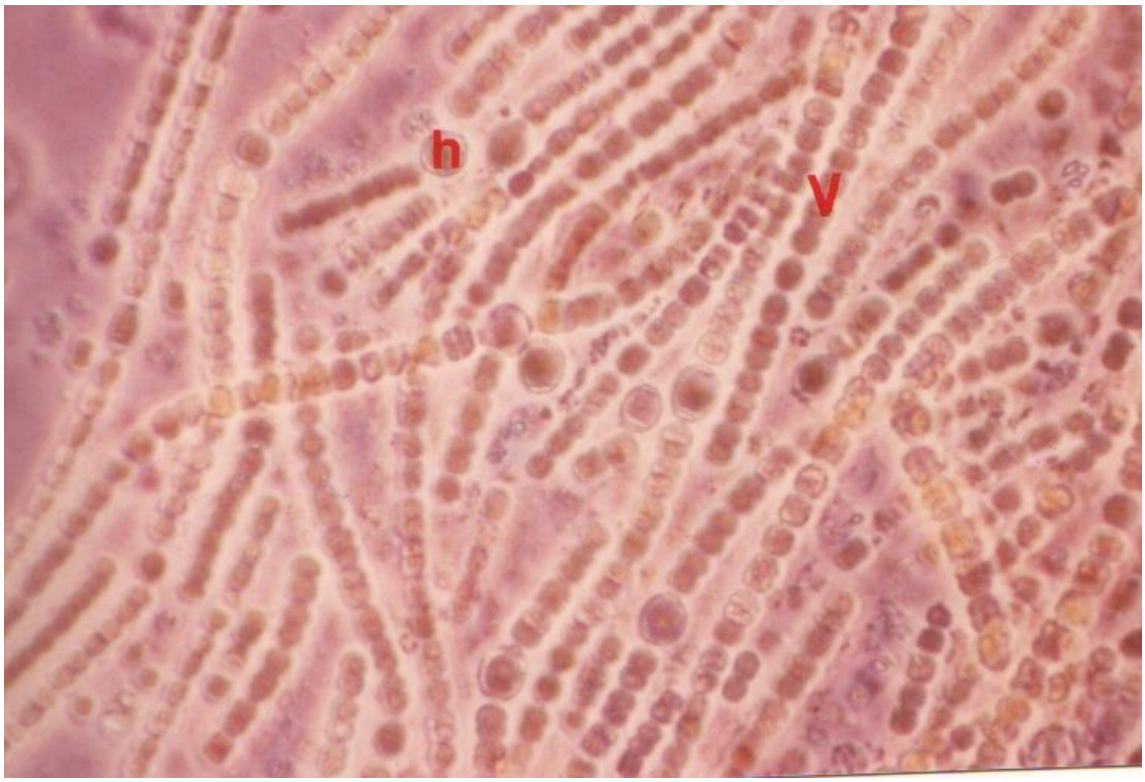

Plate (2) Typical unaffected filaments of *Anabaena cylindrica* X2000, showing intact heterocysts(h)and vegetative cells(v)

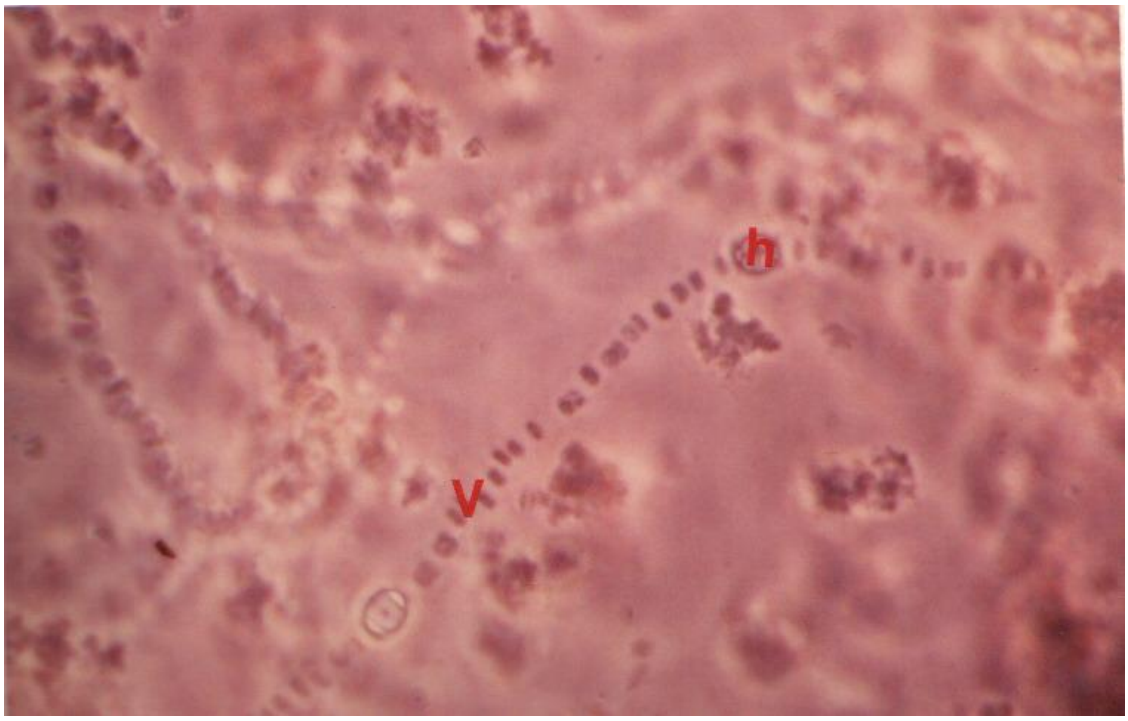

Plate (3) Lysed filaments of *Anabaena cylindrica* X2000, after treatment with plant ethanolic extract.The vegetative cells (v)were lysed but the heterocysts (h) remained intact

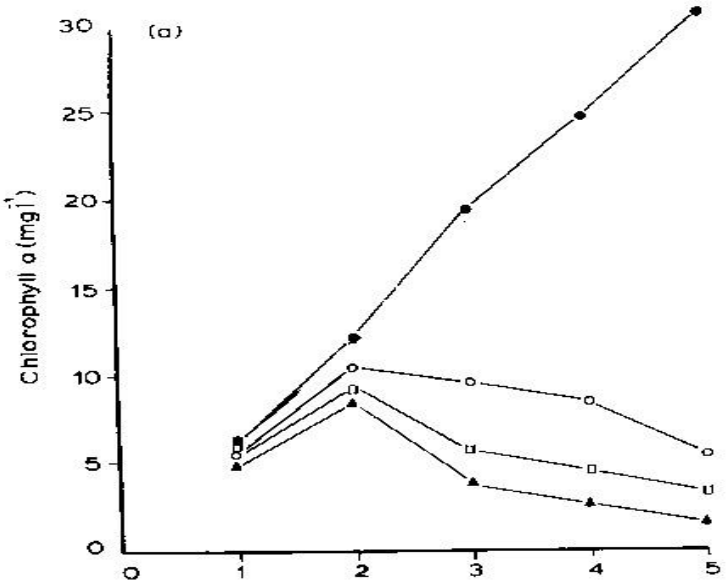

Fig. (1) The effect of different concentration Of *Artemisia campestris* ethanolic extract on the growth of *Anabaena cylindrica*.

▲ 20mg /l, □ 10 mg/l , o 5 mg/l.

● Ethanol was used as a control

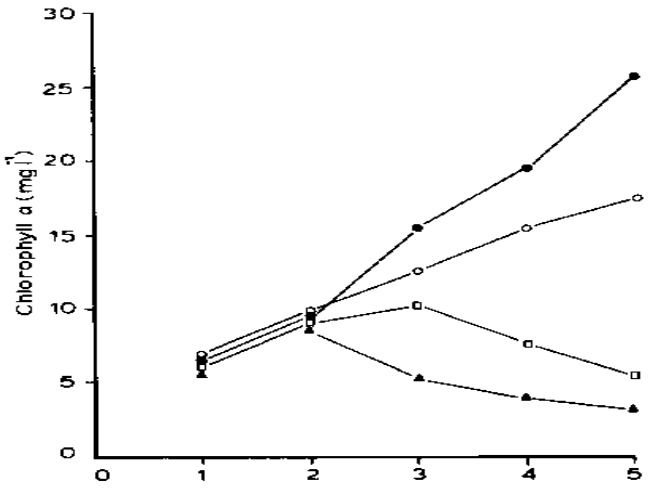

Fig.(2) The effect of different concentration of *Artemisia campestris* ethanolic extract on the growth of *Anabaena constricta*

▲ 20mg/l , □ 10mg/l, o 5mg/l, ● Ethanol was used as a control

REFERENCES

Allen,M.M.(1968) Simple condition for growth of unicellular blue-green algae on plates. J.Phycol.,4:1-4.

Aysen , O. T.; Meral, Y. ;Merih, K.and Hayrettin, T.(2003) Antimicrobial activity of extracts of the lechen *Cetraria aculeate* Desik-achary, T,V.(1959) Cyanophyta Indian Council of Agricultural Research, New Delhi.

Edwards,R.W.(1972) Report by Departm-ent of applied Biology, University of Wales,Institute of science and Technology.

Gentil,J.H.(1971) Blue-green and green algal toxins. PP.59,In: Microbial toxinsVol.7, Edited by Solomon,K.; Alex,C. and Samuel,J.A. Academic press, New York.

Golterman,H.L.,Clymo,R.S.and Ohnst-ead, M.A.M.(1978) IBP Handbook No.8 Methods for physical and chemical analysis of fresh waters. Blackwell Sci.Pub.Oxford, London.

Hussein-Ayoub,S.M. and Yankov,L .K.(1982) Algicidal properties of *Acacia nilotica* Fitoterapia,53:175-177,.

Ikram,M. Inamul-Haq(1984)Screening of Medical plants for antimicrobial activities Fitoterapia,55:62-64,

Jawad,A .L. M.; Jaffer, H.J.; Al-Naib, A.(1988a) Antimicrobial activity of some Iraqi plants.Fitoterapia, 59:130-133,.

Jawad,A.L.M.(1998).Algicidal properties of crude extracs and total isolated saponins from different Iraqi plants.7th April Univ.J.Vol(1).

Jawad,A.L.M.; Jaffer,H.J.; Al-Naib, A. (1988_b). Antimicrobial activity of sesquiterpene lactone and alkaloid fractions from Iraqi plants.Int.J.of Crude Drug Res. 26:185-188,

Jose Vitro M.Lima-Filho, Ana F.F.U. Carvalho (2002) Antimicrobial activity of extracts of six Macroalgae from the North-eastern Brazilian Coast. Brazilian J.of Microbiology, 33:311-313.

Kumar,H.D.(1964). Streptomycin and pencillin induced inhibition of growth and pigment production in blue-green algae and production of strains of *Anacystis nidulans* resistance to these antibiotics. J.Exp.Bot., 15:232-250,.

Lund,J.W.G.(1972).Eutrophication Proc. Soc. London B.180:371-382.

Prescot,G.W.(1973). Algae of the Western Great Lakes area,WM.C.Brown company publishers U.S.A..

Ridley, J.E.A.(1970)The biology and management of eutrophic reserve-oirs. Proc. Soc. of water treatments and examination, 19:374-399,

Rizk,A.M.(1986).The phytochemistry of the flora of Qatar.The Scientific and Applied Research Centre , Univ.of Qatar,.

Rodriguez-Lopez, M.; Munoz, M. and Vazquez, D. (1970).The effects of the Rifamycin antibiotic on algae. Febs.Letters,9:171-174,.

تقييم فعالية المستخلصات النباتية في السيطرة على نمو الطحالب الخضر المزرقة

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الخلاصة

تم جمع بعض ألنباتات ألعراقية والتي تعود إلى عائلات مختلفة ومن ثم اختبار فعالية المستخلص الكحولي لهذه النباتات في منع نمو ألطحالب ألخضر المزرقة. ألنتائج أظهرت أن المستخلصات الكحولية للنباتات *Achillia santolina,Artemisia campestris, Artemisia herba-alba* بألاضافة إلى نبات *Centurea khotchii* ذات قدرة عالية في إيقاف نمو ألطحالب المستخدمة في هذا ألبحث، أما مستخلصات ألنباتات ألأخرى فلم تظهر أي فعالية تذكر أو كانت غير مشجعة على الإطلاق مقارنة بالنباتات ألمستخدمة اولاً.