# Effect of ethanolic and oil extracted from some Cyanobacteri on the growth of bacteria . yeast and filamentous fungi

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**Summary**

Recent study included isolation, identification and purification of three species of algae, belongated to cyanophyta they are : *Nostoc linkia* ; *Calothrix membranacea* and *Hapalosiphon aureus*. from samples related to agricultural soil.

The species: *C.membranacea* Schmidle and *H.aureus* West and West 1897, were recorded for the first time according to check list of the algae in Iraq 1993.

The extracts of algal isolates (Cyanophyta) was testing to clarify their ability on growth inhibition on each of gram +ve and gram –ve bacteria, filamentous fungi and yeasts. The extracts (alcoholic, alkaloid, isolate oil) of species *H.aureus* showed higher inhibition activity in compared with other algal isolates.

Oils were isolated from cyanophyta and tested their biological activity against bacteria and fungi molds. The oil isolated from *H.aureus* was showed higher activity in comparison with other algal isolated, there form the identification of this oil was carried out by column chromatography, and the active group of oil isolate was identified chemically by : TLC ; UV. Spectrum ; IR-spectrum and function group was tested. Many reactive groups were detected by chemical tests and identified as Tetra-terpenoid.

**Introduction :-**

Cyanobacteria are very old groups (are dated back three or four billion years ) of the oldest photoautotrophic vegetation in the word that occur in fresh water ,marin and terrestrial habitats (Ghasemi *et al*,2004).

Blue-green algae (Cyanobacteria) provide a potential source of biologically active primary or secondary metabolite (Kreitlow *et al*, 1999 ; Mundt , *et al*, (2003) have proved that the cyanobacterium *Oscillatoria redekei* produce fatty acid which show antibacterial activity . The lipids of

cyanobacteria are generally esters of glycerol and fatty acid . Some of them are rich in essential fatty acid such as the $C_{18}$ Linoleic acid and $C_{20}$ arachidonic acid (Singh, *et al* ,2002).

Varion strains of Cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal, algicidal and antiviral activity (Abedin and Taha, 2008). The antibacterial agents found in the algae include amino acids, terpenoids, acrylic acids, phenolic compounds, steroids, halogenated ketones and alkanes and fatty acid (Mtolera and Semesi, 1996).

The aim of this study was to isolate and purification some blue –green algae species and show the ability of them to produce oil and test its antimicrobial effect.

**Material &Methods;-**

**1-Microorganisms used for bioassay.**

The bacteria used were *Staphylococcus aureus* ,*Escherichia coli* .The test filamentous fungi and yeast were *Aspergillus fumigatus* and *Candida albicans* .The maintenance media were nutrient agar and nutrient broth ,Muller-Hinton agar for bacteria and sabouraud dextrose agar for fungi .

**2-Collection of algae**

The following three cyanobacteria isolate were screened for biochemical effect in different microorganism above. *Nostoc linkia* ,*Calthrix membranacea* ,*Hapalosiphon aureus* .These algae were collected from moist soil in Basrah city . Unialgal culture and axinic culture were made unialgal culture and axienic were made of microalgae were maintained in Chu-10 medium (Stien, 1973 ; Al-Aarajy ,1996 ) in the laboratory under control conditions. The axinic culture for all algal isolate were harvested during stationary phase (8,day )of *Nostoc* ,and (10 ,day ) of both *Calothrix* and *Hapalosiphon* ,by centrifuge the biomass was freezed dried

**3-Preparation of ethanolic extract**

The dried biomass of algae was extracted with 70 % ethanol by reflux for (16 ) houres . The supernatants of the extraction were concentrated by rotary evaporation at (50 )C°

**4-Qualitative test on the ethanolic extract.**

Several of qualitative test were mede to investigating the chemical characters of ethanolic extract from cyanobacteria (Table , 1)

**5-Primary screening .**

Used plate agar diffution method (Spooner & Sykes ,1972 )for primary screening .So sterilized paper discs (6 mm ) impregnated with extracted ethanol and were placed over the Muller –Hinton agar surface wich inoculated with (0.1 ml )of bacterial suspenion ,then the plates were incubated for (24 h.)and estimation the diameter of inhibition zon by (mm ).

While we used cork borer (6 mm) for testing of fungi and yeast .The sterilized Petri

dishes cotaining Sabouraud dextrose agar was inoculated with test fngus and yeas then made a hole by cork borer .in each hole , about (40µ) from the ethanolic extract was added and after inocubation at (25)C° for (3-5) days , examined for zone of inhibition

**6-Isolation and purification of oil from algae ;**

The oil isolate from all algal isolate were prepared by solvent extraction method using soxhlet ( continuously extraction ) for ( 16 ,h.) N-hexane was used as solvent . The extracts were kept for evaporation at (40) C° to remove the solvent and concentrated the extract and stored in a refrigerator for further usage , (Zarzuelo , *et al* .1991 ) .

Purification of oil was made by using column chromatography .The column size. (1.6 x 32) cm filling with silica gel G60, mesh (230-400) .Chloroform the fraction elunet was used . The flow rate is (0.1 )ml ⁄ mn. Then estimation the $R_f$ of all fraction by TLC.Liberman –Burchard test were measured , The color of blue –green refered to containd the Tri- terpenoid.(Harbone , 1984 )

**7-Chemical determination of active compound isolated from oil of cyanobacteria :**

**A-Infrared spectra (IR ) :**

Using (FTIR )infrared spectrophotometer, according to (E-Sheekh ,*et al* , 2006 ).

**B-Ultra violate spectra (UV ):**

The UV- spectra of the test active fraction isolated from oil were determind using UV-visible spectrophotometer. The wave lenth ranged from ( 200- 500 )nm.

**C-Function groups test of active fraction isolate from the oil :**

Two types of method were made to investigating the function groups of oil isolated from algae . The first by testing the double bond ,according to (Shriner ,*et al.* 1980 ). The second method by using carboxylic acid test .

**Results ;-**

**1-Morphlogical characterstic of algal isolate ;**

Three filamentous cyanobacteria were isolated from moist soil in Basrah city , ( picture-1 ).

The alga *N. linkia* uniseriate unbranched trichomes of globose shaped of cells. Trichomes without basul-distal differentiation (Prescott, 1975). Heteroegst subspherical to avate, some time gelatinous, blue-green to violet, or blackish green brown, (Desikachary, 1959).

*C. membrunacea* trichomes tapering from basal heterocyst to fine point. Heterocysts subglobos or hemispherical, basal, (Prescott, 1975). Cell hulf as long as broad to subquadrata, (Desikachary, 1959) *H.aureus* is filamentous algae branching in one side (rarely from both) that equall to or less than main filaments in diameter of its cell, (Prescott, 1975).

**2-Qualitative chemical test and primary screening for ethanolic extract:**

Ethanolic extracts of all algal study obtained free-amin group , charbohydrate and alkaloid (table, 1) in addition , there were no containing glycosides ,cumarine , tannine and sabonines .

The primary screening of ethanolic extract was shown in ( picture-2) and (table 2) .*N.linkia* and *H.aureus* showed strong inhibition on yeast test (picture-2) While showed slight inhibitory activities on both Gram positive or Gram negative bacteria .

**3- Bioactivity test of oil isolated from cyanobacteria**

The bioassay was estimated as the diameter of the inhibition zone formed as a result of disc assay method in case of bacteria and hollow well technique method in case of fungi . Table , (3) present the results of the antimicrobial assy of oil isolated from algae against a wide range of test organisms , show that the oil isolate from *H. aureus* was highest activity against all test microorg anism examined .

According to the results of primary screening test the isolate *H. aureus* (because of its good antimicrobial activity )was selected for the isolation of bioactive compound .

**4- Separation , qualitative test and bioactivity test on oil component isolated from *H.aureus* :**

Column chromatography method was used for separation of oil component isolated from *H.aureus* . Results in fig, (1) reveald that the component was separated in five fraction which were tested for inhibition activity (table 4). The most active fraction (1) fig, (2) was nominate (active component )with $R_f$ equal to (0.92 )( picture 8 ). Additional , the Liberman – buchard test reveald to tri – terpenoid in this fraction (table, 4)

**5- Chemical identification on active component from oil isolated from *H.aureus* :**

Further information on the structure of the active component ($R_f$= 0.92 ) from oil isolated from cyanobacterium *H.aureus* was showght by spectroscopic techniques .

**A-Infrared spectra :**

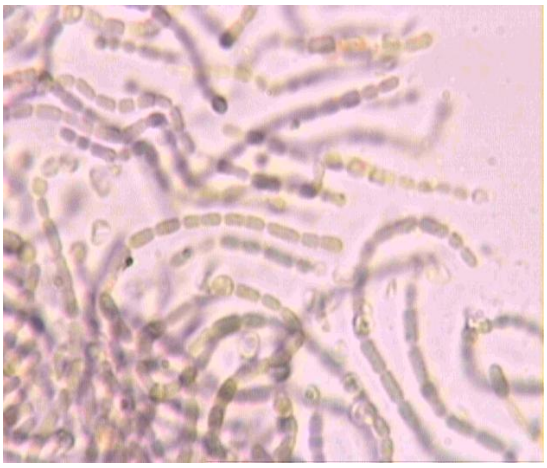
Infrared spectra of active component from oil were obtained using dried powders dispersed in kcl disc . the spectra (fig, 3) showed peaks indicating the present of CH ,OH. CH3, C=O and C=C groups in oil isolated from *H. aureus* (table, 5)

**B-UV. Spectra :**

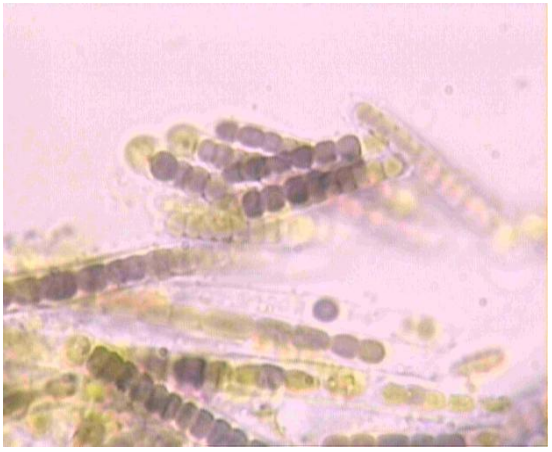
The UV and visible spectra of active component from oil showed high obsorption in ((237 )nm which equal to (0.397) (fig, 4).

**C-Function groups :**

Table (6) indicated the present of carpoxylic acid and tow bands due to the C=C groups in oil isolated from *H.aureus* .



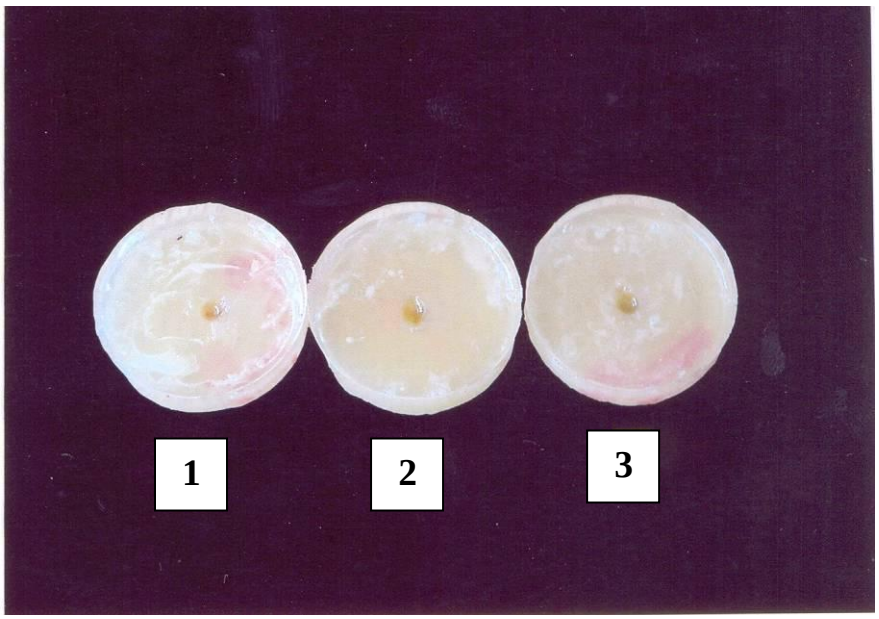
***N.linkia***



***C.membranacea***

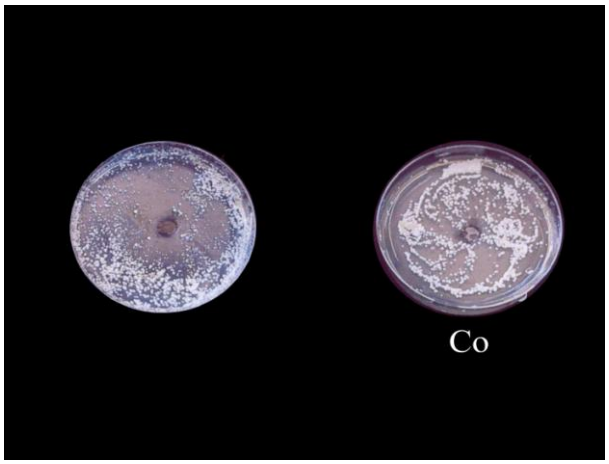
***H.aureus***

**Picture (1): Morphological characters of cynobacteria species isolates.**

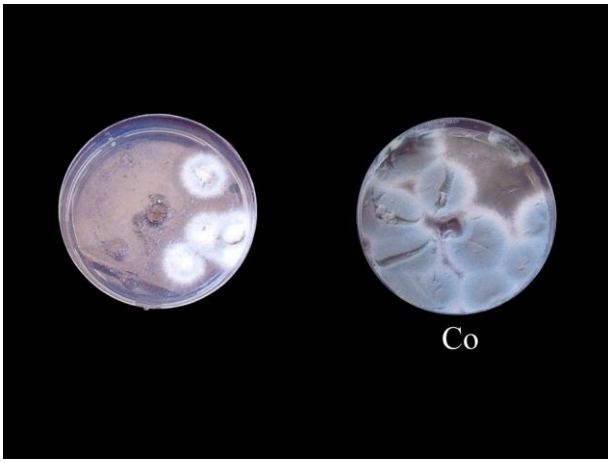



**Picture (2): the inhibition reactive of alcohol extracted from species: 1- *C. membranacea* 2- *H. aureus* 3-*N. linkia* against *C. albicans***

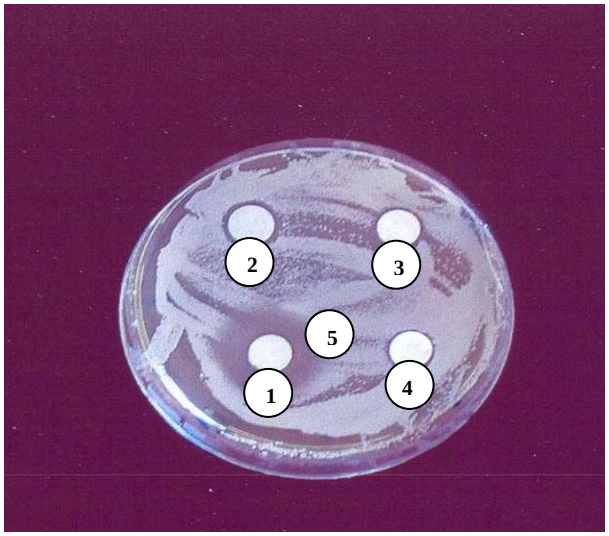
A




B




C




**Picture (3): The inhibition reactive of oil (active component) isolated from *H. aureus***

**Agoinst A: *C. albicans* B: *A. fumigates* C: *E. coli***

**Table (1): Qualitative test on alkahol extracted from cynobacteria.**

| *H.aureus* | *C.membranacea* | *N.linkia* | **Algal isolate / test** | |
|---|---|---|---|---|
| + | + | + | Ninhydrine | Free-amin group |
| - | - | + | biurate | Protein |
| + | + | + | Molish | Carbohydrate |
| - | - | - | Mayer | Alkaloid |
| + | + | + | wagner | |
| + | + | + | Dragendorff | |
| - | - | - | Before analysis | Glycoside |
| - | - | - | After analysis | |
| + | + | + | Folin | Phenol |
| - | + | + | Magnesium | Flavonide |
| - | + | - | KOH alkohol | |
| - | - | - | 5% NaOH | Cumarin |
| - | - | - | 1% $FeCl_3$ | Tannin |
| - | + | - | خلات الرصاص | |
| - | - | - | 5% $HgCl_2$ | Sabonin |

**Table (2): Zone of inhibition of alkohol extracted from cynobacteria against test pathogens**

| **Inhibition zone (mm)** | | | | **Algal isolate / Microorganism** |
|---|---|---|---|---|
| **Control** | ***H.aureus*** | ***C.membranacea*** | ***N.linkia*** | |
| - | 7 | 7 | 6 | *E.col* (ATCC 25922) |
| - | 7.5 | - | 6.5 | *S.aureus* (ATCC 25923) |
| - | - | - | - | *A.fumigatus* |
| - | 20 | 14 | 20 | *C.albicans* |

Control = Alkohol 70%

**Table (3): Zone of inhibition of oil isolated from cyanobacteria against some bacteria and fungi.**

| Inhibition zone(mm) | | | Algal isolate Microorganism |
|---|---|---|---|
| *C.membranacea* | *N.linkia* | *H.aureus* | |
| - | - | 13 | *E.coli* (ATCC 25922) |
| - | - | 11 | *S. aureus* (ATCC 25923) |
| - | - | 3.Colony | *A.fumigatus* |
| 10 | - | 26.5 | *C.albicans* |

**Table (4): Qualitative test and bioactivity on oil isolated from *H. aureus***

| Inhibition zone (mm) *E.coli* | Test Liberman-buchard | $R_f$ of oil isolate | No. |
|---|---|---|---|
| 11 | +ve | 0.92 | 1 |
| 7 | +ve | 0.69 | 2 |
| 7 | +ve | 0.4 | 3 |
| 6 | -ve | 0.23 | 4 |
| 6 | -ve | 0.2 | 5 |
| - | - | - | Control |

Control= chloroform

**Table (5): absorption and chemical groups in infrared of active component of oil isolated from *h. aureus*.**

| Groups active | Number + ($cm^{-1}$) |
|---|---|
| OH St. | 3421 |
| C – H St. | 2954 |
| $CH_2$ او $CH_3$ | 2848 - 2916 |
| C = O St. | 1735 |
| C = O St. Conjugated | 1641 |
| C = C b. | 667 |

St. = Stretching ، b = bending

**Table (6): Qualitative test on active component of oil isolated from *H. aureusi***

| Result | Test |
|---|---|
| passing of the red color of bromine | Double bond |
| Occurrence of bubbles | $NaHCO_3$ 10% |


**Fig (1): continent of oil isolated from: 2: *H. aureus* ; *3: N. linkia 4: C. membranacca* by TLC in chloroform solution.**

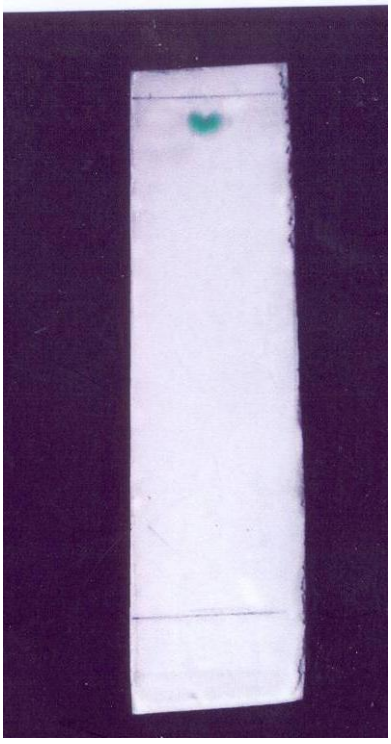


**Fig (2): TLC of testing of purity oil ((active compoment)) isolated by colomn chromatography from *H. aureus* in chloroform solution by $H_2So_4$ 40%**

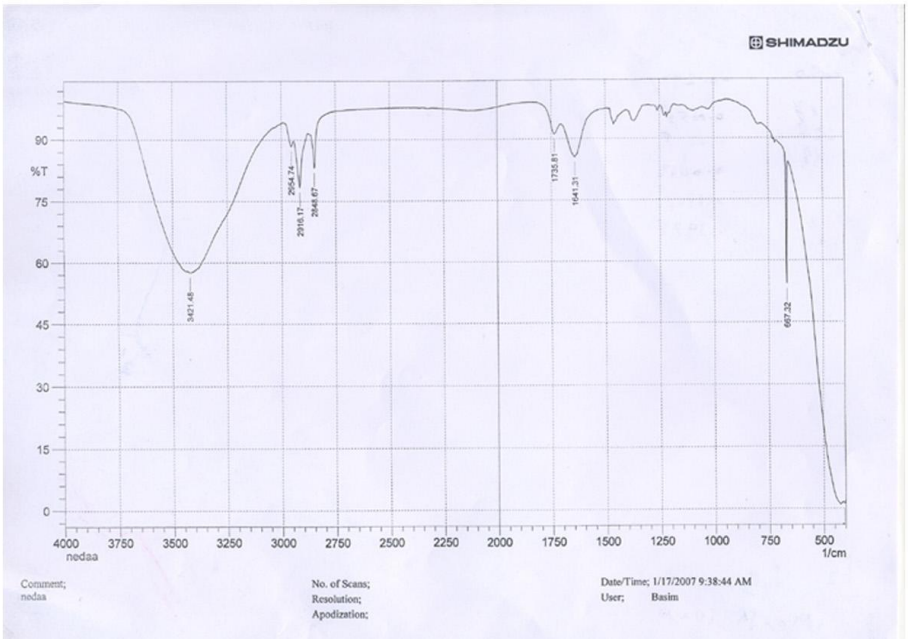



**Fig (3): IR spectra of oil (active component) isolated from *H. aureus***

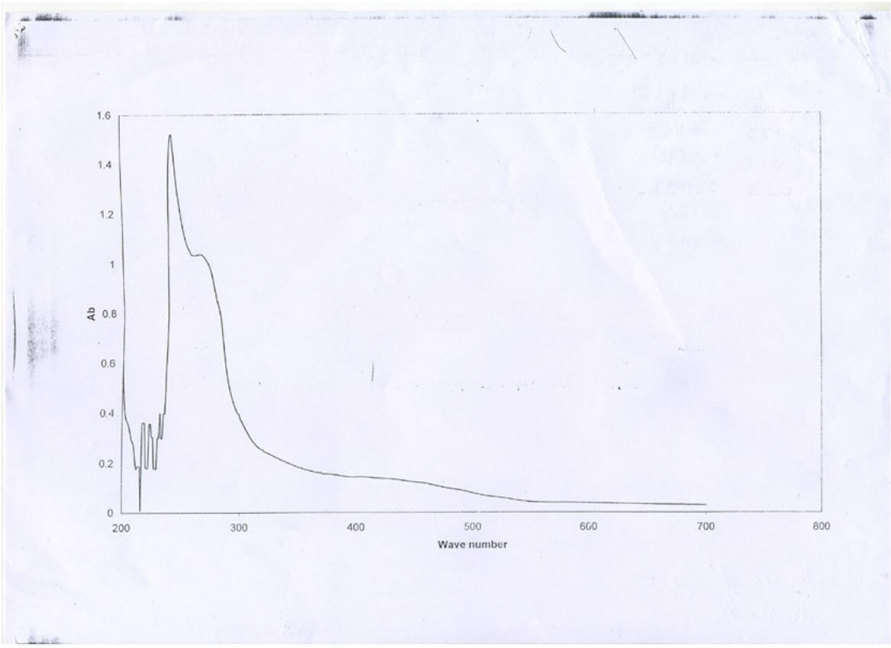



**Fig (4): UV. Of active component of oil isolated *H. aureus***

**Discussion :**

Its known that the cyanobacteria are widely distribution in environment . Thay are a potential source of new active compound for medicine and pharmacy and numerous active compounds have been isolated (Bloor & England , 1991 , El-Sheekh, 2006 ).

According to the results of isolating and purification of algae the cyanobacterium *C.membranacea* and *H.aureus* was the first

recorded in Iraq (Maulood *et al* .1993 ) ,(picture 1) .

In the present work ethanolic extract of *H.aureus* inhibited most of the test bacteria .table(2) .Its clear that the inhibition zone depends mainly on type of the algal species ,type of the solvent used and the tested microorganism ,(Abedin& Taha 2008 )

This results agreement with Abedin& Taha (2008) which found the ethanol extracts of *Anabaena oryzae* gave the highest biological activity against *Bacillus* . The results recorded in picture (2) showed that the antifungal activity of the extracts of both *N.linkia* and *H. aureus* towards the *C. albicans* gave the inhibition zone of both equal to (20) mm .

These activities of algal may be due to alkaloid or ⁄ and phenolds , flavonid compounds ,table (1) .

The results proved also that n- hexan was the best solvent for extrating the oil from *H. aureus*, the table (4) clearly indicated that gave a positive Liberman- buchard reaction and 40 % $H_2SO_4$ test that refered to Terpenoid compounds and fatty acid respectively, and conteind carboxylic acid, table (6) the present result in table (3) indicated that the oil isolated from *H.aureus* was higher activity than other algae ,therefore we using silica gel column to further purification of this oil, its cleare that there are five fraction fig, (1). The 0.92 $R_f$ fraction was active one , picture (3) reduced the growth of E.coli picture (3) fig ( c) and fungi , fig (A ,B ) picture (3) .

Mundt ,*et al* ,(2001 )reported that the n-hexan extracted from cyanobacterium *Limnothrix redekei* was fractionated and identified by NMR and MS as α-Linolenic acid , this polyunsaturated Fatty acid is well known as active compound .

The TLC technique fig, (2) refered to the active compound have $R_f$ =0.92. The infrared spectroscopy indicated the presence of function grops in the active compound isolated from *H.aureus* as follow: C=O (1730 $cm^{-1}$ ) ,(1641 $cm^{-1}$ ) ʿC=C ( 667 $cm^{-1}$ ) CH2 and CH3 (2848- 2916 $cm^{-1}$ ), table (4), fig (3).

According to (fig, 4) ultraviolate spectra of active compound isolated from *H.aureus* was characteristic with maximum absorbance at ( 237 ) nm .So it revealed to type of ( $\pi - \pi^*$) transition .

In the present work , we used UVand IRspectra data to elucidate the chemical composition of the active compound isolated from *H.aureus* according to this data we can say the active compound may be a Tetraterpenoid compound .

More chmical analysis like ( GC ) must be carried out to elucidate the complete structure of the purified active compound from our isolates .

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# التأثيرات الحيوية للمستخلصات الكحولية و الزيتية المعزولة من بعض الطحالب الخضر- المزرقة على نمو بعض البكتريا و الخمائر و الفطريات الخيطية

**موسى جاسم الاعرجي** **نداء جاسم الموسوي** **اقبال جاسم الاسدي**

**الخلاصة**

تضمنت الدراسة الحالية عزل وتشخيص وتنقية ثلاثة انواع من الطحالب الخضر- المزرقة وهي ؛ *Nostoc linkia* ؛ *Calothrix membranacea* ؛ ؛ *Hapalosiphon aureus* عزلت هذه العزلات من الترب الزراعية.

تسجل الأنواع. *C.membranacea* Schmidle 1901 و *H.aureus* west and west 1897 لأول مرة اعتماداً على قائمة الأنواع الطحلبية في العراق (Maulood *et al*.,1993).

حضرت المستخلصات الكحولية و الزيتية من كل عزلة طحلبية بصورة نقية ثم اختبرت هذه المستخلصات لإظهار قابليتها على تثبيط نمو الجراثيم السالبة لصبغة كرام والموجبة لها والفطريات الخيطية والخمائر ، وأظهرت المستخلصات أعلاه للطحلب *H.aureus* فعالية مثبطة عالية مقارنة مع بقية العزلات الطحلبية .

عزلت الزيوت من الطحالب الخضر –المزرقة و اختبرت فعاليتها الحيوية تجاه الجراثيم و الاعفان الفطرية و قد الزيت المعزول من الطحلب الاخضر المزرق *H. aureus* فعالية عالية مقارنة مع بقية العزلات الطحلبية لذا تم فصل مكونات الزيت الخام للطحلب اعلاة باستخدام تقنية كرماتوكرافيا العمو و حصل على خمس مكونات منة و شخصت المكونة الاكثر فعالية كيميائا باستعمال تقنية كروماتوغرافيا الطبقة الرقيقة و بعض الاطياف مثل طيف الاشعة فوق البنفسجية و طيف الاشعة تحت الحمراء كما اجريت بعض الاختبارات مثل كشف المجاميع الفعالة و قد شخصت هذة المكونة على انها تربين رباعى.