Study the effect of some algae extracts against activity of some fungi

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Abstract

Study is carried out to investigate the effect of algae extracts on the activity of some fungi (*Trichophyton rubrum ; Aspergillus flavus ; Microsporium canis* and *Cryptococcus neoformans*) .The minimal inhibitory concentrations(MIC) of algae against fungi were for *Oscillatoria irrigua* (blue green algae) (150-250µg/ml) less than its for *Scenedesmus quadricauda* (green algae) (400 µg/ml). Also the minimal cidal concentrations (MCC) were at the same above trend for algae. The inhibition zones diameters were for *Oscillatoria irrigua* extract varied between (5-33)mm and for *Scenedesmus quadricauda* ranged between (2-24)mm, results showed no mortality occur in experimental animals after injected by high concentrations of algae extracts. Generally, study concluded the effects blue-green algae extracts on fungi activity were higher than its for green algae extracts; and the *Cryptococcus neoformans* (yeast) was significantly ($p<0.01$) more sensitive somewhat to algae extract then other studied filamentous fungi.

دراسة تأثير مستخلصات بعض الطحالب ضد فعالية بعض الفطريات

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قسم صحة المجتمع ، المعهد التقني في الناصرية ، ذي قار/العراق

الملخص

أجريت الدراسة الحالية لمعرفة تأثير مستخلصات بعض الطحالب (الطحالب الخضر والخضر المزرقه) على فعالية بعض الفطريات وهي:

***Trichophyton rubrum ، Aspergillus flavus ، Microsporium canis* و *Cryptococcus neoformans*.**

ا لتركيز المثبط الأدنى (MIC) لمستخلصات الطحالب ضد الفطريات كان لطحلب *Oscillatoria irrigua* (الطحالب الخضر المزرقة) يتراوح بين (١٥٠-٢٥٠) مكغم/مل وهو أقل منه بالنسبة لطحلب *Scenedesmus quadricauda* (الطحالب الخضر) وهو (٤٠٠) مكغم/مل ، كما أن التركيز القاتل الأدنى (MCC) كان بنفس النمط أعلاه بالنسبة للطحالب. أما أقطار مناطق التثبيط لتلك المستخلصات فكانت في مستخلص طحلب*Oscillatoria irrigua* **تتراوح بين (٥ – ٣٣) ملم ولطحلب *Scenedesmus quadricauda* بين (٢ – ٢٤) ملم ،كما أشارت نتائج الدراسة الى عدم حصول هلاكات بين الحيوانات المختبرية التي حقنت بتراكيز عالية من مستخلصات الطحالب. يمكن الأستنتاج من نتائج الدراسة أن تأثيرات مستخلص الطحلب الأخضر المزرق على نشاط الفطريات كان أعلى منه معنويا ($p<0.01$) بالنسبة لمستخلصات الطحلب الأخضر، كذلك فأن(*Cryptococcus neoformans* الخمائر) كانت نوعا ما أكثر حساسية لمستخلصات الطحالب من بقية الفطريات الخيطية المدروسة.

Introduction

Aquatic plants [macrophytes and microphytes(such as algae)] are considered as sources for drugs used in many civilizations (Yan,2004), it takes over important place in industrial and agricultural production, and are considered essential sources for other active materials which enter in manufacturing drugs.

The attention in finding drugs (or its primary materials) from aquatic ecosystems started from 1970s (Yan,2004); the search for antimicrobial agents has taken a definite direction in developed countries (Concepcion *et al*.,1994), about (300) patents on bioactive natural products have been issued between 1969 and 1990 in China alone, so for more than (10 000) compounds have been isolated from aquatic ecosystem organisms in these period (Proksch,2002).

These importance come from the fact, the natural products are considered to be saved and less harmful, mainly because they have higher biodegradability and are often active at lower doses (Sexena and Pandey,2001).

Algae occur in many different habitats, in fact, these microorganisms comprise most of the world′s biomass. A number of algae produce various biologically active compounds, So, it′s an important source of novel antimicrobial compounds, these include antifungal compounds which in laboratory tests inhibited some of fungi that incite diseases of humans,animals and plants (Kulik,1995).

Some of antifungal molecules derived from algae [especially cyanobacteria (blue-green algae)] have been patented for agricultural use, but research on this topic has not been continued (Biondi *et al*.,2004). From the other hand, because many of algae produce a large number of antibacterial and antifungal materials, are almost never a threat to the environment ,and many can be grown in quantity of mass cultures (Kulik,1995).

In 1990,researchers isolated a novel depsipeptide from blue green algae of the genus *Nostoc* (cyanobacterium) that showed potent activity against filamentous fungi and yeasts, mainly of the genus *Cryptococcus* (Schwartz *et al*.,1990) . Biondi *et al*. (2004) used methanolic extracts obtained from *Nostoc* strain ATCC53789 and tested against a variety of (12) species of fungi.

Generally, antifungal natural products could be grouped into four major categories: (1)polyketide, such as Aurantoside (Sata *et al.*,1999); (2)Macrolide, such as phorboxazole A ;Halishigamide A; Halichondramide (Kobayashi *et al*.,1997); (3) alkaloids, such as Fascaplysin ; Meridine ; Ptilomycalin A ; Haliclonadiamine (Kashman *et al*., 1989 ; McCarthy *et al*.,1992) ; (4)Fatty-acid ester, such as Bengazole A. (Yan,2004)

In recent years, the attention was increased in study of the fungal infections which are produce by a various fungi species, and used multi type of chemical compounds against the fungi, and because of the excessive uses for these chemicals in treatments, the fungi have a type of auto resistance for these compounds, so the researchers oriented to find or synthesis other new sources for antifungal agents (Al-Mowali *et al*.,2002) .

So, the present study carried out to study the activity of extracts from some dominant algae in local aquatic ecosystems against some fungi.

MATERIAL &METHODS

STUDIED ALGAE:

algae were isolated from the local aquatic ecosystem by streaking method (Stein,1973);classified by use (Prescott,1975) were : *Scenedesmus quadricauda* (Turp.) de Bre bisson (green algae) and *Oscillatoria irrigua* (blue green algae). Isolates were purified and cultured as described in (Stein,1973) by use Chu-10 medium, the mass cultures produced by a procedure which described in (Al-Rekabi,2003), the produced algae lypholized by freeze-drier (lyophilizer) that become ready for extraction.

EXTRACTION:

(5) gm of freeze dried algae take place in conical flask , add (50) ml of distilled water, the mixture place on magnetic stirrer at room temperature for (24)hrs.; precipitate the mixture by centrifuge (5000)rpm for (25) min., take the supernatant and filtered by sterilized seitz filter (pore size (0.45 µ),collect the filtrate by special freeze drying flask and freeze dried under pressure, collect the dried samples, take (0.5) gm of it and add (5) ml sterilized distilled water(Harborn,1984), the produced solution is considered as stock solution (100% concentration) and prepare the following dilutions (50,100, 150,250,400,600,1000,1250 and1500) µg/ml by use sterilized distilled water, and stored in refrigerant.

STUDIED FUNGI:

Studied fungi include : *Trichophyton rubrum ; Aspergillus flavus ; Microsporium canis* and *Cryptococcus neoformans.* classified by use (Ellis, 1994). grown on Sabouraud Dextrose agar for (7) days at (28) °c. added distilled water to cultures and mix well for produce the spore suspension and filtered by sterilized cotton and washed (3) times by distilled water, the total number of spores were 10^6 /ml (Ellis,1994).

MINIMAL INHIBITORY CONCENTRATIONS (MIC):

Algae extracts added to Sabouraud Dextrose medium for produce the concentrations (50,100, 150,250,400,600,1000,1250 and1500) µg/ml ,and distilled water added to control dishes. the dishes inoculated by (0.1) ml of spore suspension and incubated at (28) °c, the results showed after (3) weeks (Begum *et al*., 1996).

MINIMAL CIDAL CONCENTRATIONS (MCC):

(0.1) ml of spore suspension mix in series test tubes with (0.9) ml of diluted algae extracts for produce (1) ml of the following concentrations (50,100, 150,250,400,600,1000,1250 and1500) µg/ml. and inoculate the solid Sabouraud medium by loop from these tubes and incubated the dishes at (28) °c for (3) weeks (Begum *et al*., 1996).

DETERMINATION OF INHIBITION ZONES:

Inhibition zone determined according to Bauer method as described in (Al-Rubayea,2001) by use the fungal suspension and sprayed it by L-shape glass road on medium, and place the algae extract (400, 600, 1000 and 1500 µg/ml) in a pit (hole) in media (diameter 6 mm), incubate the dishes and detect the antifungal activity of extracts by measurement the diameter of inhibition zones as (mm).

TOXICOLOGICAL EFFECTS:

Use (54) albino mice(male) (28 ±5)g weights, in (4 – 5)months old, divided into (9) groups (n=6) ,(orally treatment was used),first group considered as a control group treated with water , the others (4 groups for each alga extract) treated with different concentrations of extracts (250, 500 ,1000 and 1500) mg/kg, the animals monitored for (96)hrs. for detect the mortality (Waynforth, 1980).

RESULTS

Results showed the minimal inhibitory concentration (MIC) for studied algae extracts were varied according to algae and fungi species , the MIC for *Oscillatoria irrigua* was (150) µg/ml for *Cryptococcus neoformans* fungi and (250) µg/ml for others fungi (Table 1); for *Scenedesmus quadricauda* alga were (400) µg/ml for all fungi (Table 2).

Also ,from above results ,the minimal cidal concentrations (MCC) for *Oscillatoria irrigua* were (1000) (µg/ml) (Tables 1), for *Scenedesmus quadricauda* were (1250) (µg/ml) against all fungi (Tables 2).

For determination the inhibition zone diameter, that′s varied between the algae extracts , for *Oscillatoria irrigua* varied between (5-33)mm (Table 3) , and for *Scenedesmus quadricauda* between (2-24)mm (Table 4) against studied fungi.

For toxicological tests ,there′s no mortality occur between experimental animals(mice) after (96) hours of treatment.

Table 1: Antifungal activity (as MIC) of *Oscillatoria irrigua* alga extract.

Fungi	Concentrations (µg/ml)									
	0 (cont.)	50	100	150	250	400	600	1000	1250	1500
Trichophyton rubrum	+	+	+	+	–	–	–	– *	–	–
Aspergillus flavus	+	+	+	+	–	–	–	– *	–	–
Microsporium canis	+	+	+	+	–	–	–	– *	–	–
Cryptococcus neoformans	+	+	+	–	–	–	–	– *	–	–

***: Minimal cidal concentrations (MCC)**

Table 2 Antifungal activity (as MIC) of *Scenedesmus quadricauda* alga extract.

Fungi	Concentrations (µg/ml)									
	0 (cont.)	50	100	150	250	400	600	1000	1250	1500
Trichophyton rubrum	+	+	+	+	+	–	–	–	–*	–
Aspergillus flavus	+	+	+	+	+	–	–	–	–*	–
Microsporium canis	+	+	+	+	+	–	–	–	–*	–
yptococcus neoformans	+	+	+	+	+	–	–	–	–*	–

***: Minimal cidal concentrations (MCC)**

Table 3: Inhibition zone diameter (mm) of *Oscillatoria irrigua* algae extract against fungi.

Fungi	Concentrations (µg/ml)			
	400	600	1000	1500
Trichophyton rubrum	6	9	20	29
Aspergillus flavus	6	10	22	30
Microsporium canis	5	6	19	24
Cryptococcus neoformans	7	12	28	33

Table 4: Inhibition zone diameter (mm) of *Scenedesmus quadricauda* algae extract against fungi.

Fungi	Concentrations (µg/ml)			
	400	600	1000	1500
Trichophyton rubrum	3	5	10	22
Aspergillus flavus	2	4	10	20
Microsporium canis	2	4	8	20
Cryptococcus neoformans	4	8	13	24

DISCUSSION

The minimal inhibitory concentrations (MIC) is defined as a lesser concentration of contrary which inhibits the fungal growth under optimum test condition (Al-Mowali *et al*.,2002) ; from the other hand, the fungi are eukaryotic organisms and similar to it's eukaryotic hosts in structure and metabolism, So, the antifungal agents work on inhibit (or kill) the pathogenic fungi and in same time may be effects on host tissues , therefore, the study includes the MIC tests to detect the lesser concentrations inhibit the growth fungi *In vitro* .

The results of MIC tests showed there is a somewhat variation in sensitivity of fungi against algae extracts, the *Cryptococcus neoformans* (yeast) was more sensitive for *Oscillatoria irrigua* extract than other filamentous fungi ,that's may be due to the difference of fungi nature and to variation in metabolism trend of fungi which effects on the resistance of filamentous fungi against antifungal agents in extent differ from yeasts (Al-Mowali *et al*.,2002).

Also, above results showed the extract of blue- green alga *Oscillatoria irrigua* was significantly ($p<0.01$) more effects in the inhibition of fungi growth in comparison with that's of green alga *Scenedesmus quadricauda*, that's may be due to the nature of blue-green algae bodies which are poor (or lacked) in structural elements like chitin and cellulose which consider more difficult in extraction processes in comparison with green algae bodies which are rich in these substances (Venkataraman and Becker, 1985), that means easy of extraction the antifungal substances from blue-green alga and increase concentration these compounds in solution in comparison with green alga.

At the same time, from the results in (tables 1 &2) we showed the minimal cidal concentrations (MCC) for blue-green algae extracts were less than its for green algae, also, that's may be due to the same causes for MIC which is mentioned above(Venkataraman and Becker, 1985).

For the results of determination the inhibition zones diameters (Table 3 &4),the diameters of inhibition zones were varied between the studied algae extracts,thus proven from the results the sensitivity of *Cryptococcus neoformans* (yeasts) to the extracts was significantly ($p<0.01$) more than the others filamentous fungi.

The results showed that inhibition zones diameter for blue-green alga *Oscillatoria irrigua* extracts were higher than its for green alga *Scenedesmus quadricauda*, against all fungi approximately, that's may be due to the blue –green algae contain phenolic compounds more than in green algae (Venkataraman and Becker, 1985 ; Al-Rekabi,2004)), and these compounds contain multi free hydroxilic groups have ability to formation hydrogenic bounds with carbohydrates and proteins which found at cell wall and its inside living cells, or to these phenolic compounds may be linked with active sites of enzymes and change its nature and precipitate the enzymes as complex mixture that's causes inhibition of essential metabolism reactions for reproduction and growth of fungi (Reed, 1995).

Thus ,that's may be due to the easy of extraction the active substances from blue-green algae as explained above; and the alkaloids and semi alkaloids which found in blue green algae more than its in green algae(Al-Rekabi,2004) have a similar effects for phenols work but less than it as inhibitional ability against growth of microorganisms (Venkataraman and Becker, 1985 ; Baker, 1997).

The experiments of toxicological tests for algae extracts, showed the extracts were untoxic for experimental animals and there are no mortality after (96) hours from treatment its by different high concentrations of extracts,that's may be confirm the studied algae uncontained any toxicants or other harmful substances .

Finally, in spite of abundance the active compounds in alcoholic extracts in comparison with aqueous extracts (Al-Shamaa,1989 ; Al-Jobory and Al-Rawi,1994) because of the nature of compounds which dissolve in alcohol better than in water ,however, the present study depended on the extraction by aqueous solution because that's in agreement with options of most people in the extraction of compounds from weeds by aqueous solutions; also its easy preparation and use ;low costs; less side effects and in agreement with concept of World Heath Organization in the search about save substitutes ;less costs ;more effective in treatment and easy in production (Venkataraman and Becker, 1985).

From above, may be conclude that's aqueous extracts of algae in general have more effect on the activity and growth of fungi (include pathogenic fungi)and yeasts, in especially, approve the effect of blue-green algae extract was significantly ($p<0.01$) more than of green algae extract , and these extracts were safe (from toxicological view) and from others side effects for experimental animals at least.

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