Global Journal of Pharmacology 8 (2): 228-236, 2014
ISSN 1992-0075

DOI: 10.5829/idosi.gjp.2014.8.2.8369

Antifungal Agent from *Spirulina maxima*: Extraction and Characterization

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Abstract: In the current investigation the cyanobacterium; *Spirulina maxima* exhibited antagonistic activity against *Pencillium oxalicum*. The results indicated that, the active substance produced maximally after 15 days of incubation in aerated culture at 30°C and pH 8 in B-G 11 medium. Dimethyl was the best solvent for extracting the active material. The antagonistic material was purified using thin layer chromatography. It was found that the purified antagonistic agent produced had a moderately toxic level and the LC_{50} was 1027 mg/l. The partially purified agent of *S. maxima* showed a broad spectrum of antifungal activity with an average activity of 26% against five tested human and plant pathogenic fungi compared to the three tested commercial drugs. The most inhibited fungus was *P. oxalicum* (91%) followed by *F. solani* (65%) and *R. solani* (20%) compared to the tested antifungal drugs. The compound showed maximum absorption band at 250 nm. Infrared (IR) indicated presence of (NH_2, OH, NH) groups, C-H aliphatic, carbonyl group (amide), benzene ring and ether linkage and nuclear magnetic resonance (NMR) showed signals at 0.9 (t, 3H, CH_3), 1.4 (t, 2H, CH_2), 2.6 (s, 3H, CH_3), 4.1 (s, 2H, CH_2), 5.0 (s, 2H, NH_2) and 7.6 (m, aromatic protons). Mass spectroscopy indicated that its molecular weight is 383 Dalton.

Keywords: *Spirulina maxima* · Cyanobacterium · Antifungal · Substance · NMR · IR · UV

INTRODUCTION

Pencillium sp. is significant in mammalian disease because of its production of potent mycotoxins [1]. *Pencillium oxalicum* appears to be a common pathogen of greenhouse cucumbers and on animals: It has been implicated in genital diseases of water buffalo. It produces the hepatotoxins: secalonic acid D and oxaline, together with roquefortine C [2]. Contaminated grain fed to young ducklings proved to be toxic [3]. Cyanobacteria have been studied for producing various biologically active compounds. These include antibiotics which in laboratory tests inhibited bacteria and fungi that incite human, plants and fish diseases [4]. Some of cyanobacterial antifungal agents have been used in the treatment of resistant fungal-induced diseases of domestic plants and agricultural crops, such as; majusculamide C; a microfilament depolymerizing agent extracted from *Lyngbya majuscule*, that showed potent fungicidal activity [5]. Some of cyanobacterial antifungal agents have been used in the treatment of resistant fungal-induced diseases by the human pathogenic yeast; *Candida albicans*. For instance, Jaki *et al.* [6] isolated two novel cyclic peptides with antifungal activity against the yeast *Candida albicans* from the cyanobacterium *Tolypothrix byssoidea* (EAWAG195). Also, Ozdemir *et al.* [7] obtained antifungal agents with phenolic nature against *Candida albicans* ATCC 10239. While, Volk and Mundt [8] proved that the exometabolite of the cyanobacterium *Nodualria harveyana* (9 H - pyrido (3, 4-b) indole) and exometabolite of the cyanobacterium *Nostoc insulare* (4.4 - dihydroxybiphenyl) showed high sensitivity against the yeast; *Candida albicans*. Soltani *et al*. [9] isolated six species of cyanobacterial that inhibited the growth of *Candida kefyr ATCC 1140* and one species inhibited the growth of *Candida albicans* ATCC 14053. In such manner, Shanab [10] isolated three *Oscilatoria* species: *O. hamelii*, *O. platensis* and

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*O. rubescen*s that showed antifungal activity against *Candida albicans* and *Aspergillus flavous*. Mo *et al.* [11] isolated Scytoscalarol (1), an antimicrobial sesterterpene bearing a guanidino group from the cultured cyanobacterium *Scytonema* sp. which shows antifungal activities against *Candida albicans* and *Mycobacterium* tuberculosis. The Oscilatoria sp. was found to have antifungal activity on yeasts [12]. Other cyanobacterial strains; *Anabaena* sp. and *Calothrix* sp. were examined against phytopathogenic fungi, such as; *Rhizoctonia bataticola* and *Pythium debaryanum.* The diameter of the inhibition zone was largest when extracellular filtrates of the two cultures incubated at high light intensity (90-100 ìmol photons m^{-2} s^{-1}) and at 40°C [13]. The biological control aptitude of the cyanobacteria; *Anabaena subcylindria, Nostoc muscorum* and *Oscillatoria angusta* filtrates was evaluated by Abo- Shady *et al.* [14] on the growth of the isolated pathogenic fungi from the different organs of *Faba bean*. Their filtrates revealed high efficiency on the control of these fungi. The reduction in fungal mat growth diameter was greater than in that of the fungal dry weight showing inhibited fungal spread by greater rate. The reduction in the fungal dry weight was mostly linear and significantly correlated with the algal filtrate concentrations.

Biondi *et al.* [15] isolated Antarctic cyanobacteria isolated from benthic mats that showed antifungal activity against the filamentous fungus *Aspergillus fumigatus* or the yeast *Cryptococcus neoformans.* Abedin and Taha [16] tested cyanobacterial species; *Anabaena oryza, Tolypothrix ceytonica* and *Spirulina platensis* for antifungal agent production on various organisms that incite diseases of humans and plants (*Aspergillus niger*, *Aspergillus flavous*, *Pencillium herquei*, *Fusarium moniliforme, Helminthosporium* sp. *Allternaria brassicae, Saccharomyces cerevisiae* and *Candida albicans*). They found that *Spirulina platensis* and *Anabaena oryza* had the highest antifungal activity towards the tested fungi. Chen and Huang [17] demonstrated that culture filtrate of five strains of *Clitocybe nuda* displayed various degrees of antifungal activity against plant pathogenic fungus; *Phytophhora capsici.*

Therefore, the current study was suggested to obtain antifungal compounds from *S. maxima* and optimize the antifungal production, purifying the antifungal agents, study the bio-toxicity of the purified antifungal agents and elucidate the chemical structure of the purified antifungal agents

MATERIALS AND METHODS

Isolation and Culturing of *Spirulina Maxima:* The blue green alga *S. maxima* was isolated from soil. The dilution culture technique adopted by Venkatarman [18] was used for isolation and purification. The axcenic culture was obtained using the method recommended by Bolch and Blackburn [19]. The isolated organism was grown autotrophically in BG-11 medium as described by Rippka *et al.* [20], for determination the optimal time at which the highest antagonistic activities of *S. maxima* was attained.

Microorganisms Used for Antagonistic Activity: The test filamentous fungi used in this investigation were *Aspergillus niger, Fusarium solani, Penicillium oxalicum, Rhizoctonia solani* and *Candida albicans* ATCC 14053.

The maintenance of these fungi except *C. albicans* was carried out using a modified Czapek Yeast Extract Agar (CYEA) medium, it Composed of (g/L): Sucrose, 20; $NaNO_3$, 2.0; K_2HPO_4, 1.0; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; Yeast extract, 2.0. The pH was adjusted at 5.0 [21]. While, *C.albicans* was maintained using Sabouraud-dextrose agar (SDA) medium (British Pharmacopoeia) according to Sandven and Lassen [22].

Bioactivity Test of *S. Maxima:* The supernatants of *S. maxima* were applied in 5% using Czapex's agar plates. The 5 mm discs of the tested plant and human pathogenic fungi (*Aspergillus niger, Fusarium solani, Penicillium oxalicum* and *Rhizoctonia solani* were placed separately on the amended prepared agar plates (One disc/plate) and incubated at 28°C for a week. The bioactivity of these crude supernatants to inhibit the growth diameter of these pathogenic fungi was estimated daily by measuring the diameter of the fungal growth on amended agar plates compared to the control (Fungal growth diameter on un-amended agar plates), then the suppression percentage was calculated as follows: (Fungal growth diameter on un-amended agar plates in mm - fungal growth diameter on amended agar plates in mm)/ fungal growth diameter on un-amended agar plates in mm X100% [23]. On the other hand, the bioactivity against *C. albicans* ATCC14053 was carried out as follows: the microalgal supernatants were amended in 5% using SDA agar plates [22]. One hundred microliters of *C. albicans* TCC14053 suspension (OD ~ 1.0) was added to amended and un-amended plates, the incubation was carried out for 48 h at 30°C. The bioactivity of the crude supernatants was estimated by

counting the colonies of *C. albicans* ATCC14053 on the amended agar plates compared to the control (Un-amended agar plates), them the suppression percentage was calculated compared to the control.

Purification of the Antifungal Agent (s) Using TLC

***Preparation of the Plates*:** Glass plates (20X20 cm) were cleaned and air dried. About 40 g of silica gel G60 were suspended in 100ml of distilled water. The suspension was spread over the plates with a thickness of 0.1 cm.The plates left for air dryness then activated by heating at 120°C for one hour. The Rf of the active components was determined using 100ml of different solvent systems preformed in vol. /vol. as follows: Benzene: chloroform, 50:50, v/v. Benzene : acetone, 90:10,v/v. Benzene : ethyl alchol : formic acid,75:24:1,v/v. Butanol: acetic acid : water,50:40:10,v/v. Toluen : chloroform: acetone, 40:25:35, v/v. Chloroform : methanol, 50:50, v/v. Dichloromethane : methyl alcohol: water, 50:30:20, v/v. and Dichloromethane: methyl alcohol: water, 65:32:3, v/v.

Bioactivity Test of the Developed Spots: In each case the chromatograms were detected under a UV lamp. Each spot was removed and dissolved in dimethylsulfooxide. Silica gel was removed by centrifugation at 5000rpm for 15min. The supernatant was tested for biological against the pathogenic fungi.

Bio-toxicity of the Purified Antifungal Agent (s): The toxicity bioassay of *S. maxima* was carried out according to Meyer *et al.* [24] using 24h old neuplii of *Artemia salina* as a bio-toxicity biomarker. Different concentrations of the purified extract (100, 200, 1000, 1500, 2500 and 5000 mg/l) were made and distributed separately using clean and dry glass vials (20 ml) then completed to a total volume of 10 ml using sterile seawater. Ten live neuplii of *A. salina* were transferred to each vial. The number of the viable biomarker was counted after 24 h of application. The mortality percentages and the half lethal concentration (LC_{50}) were determined using the probit analysis method [25].

Comparison of the antifungal activity of the partially purified agent of *S. maxima* to some commercial antifungal agents

The bioactivity of the partially purified antifungal component was estimated compared to three commercial antifungal drugs Diflucan, Flocoral and Fungimycin. 10mg of each tested agent was dissolved in 1 ml of dimethylsulfooxide (DMSO) and then a solution of 5% concentration was prepared and used separately to amend the culture medium. The suppression growth % of the tested pathogenic fungi was detected compared to the control (Untreated fungi).

Partial Chemical Characterization of the Purified Antifungal Agent of *S. Maxima*

***UV Spectrum* :** The UV spectrum of each obtained spot was determined using a solution of DMSO in quartz cuvette. The wave length was ranged from 0-500 nm. The spectrum was achieved using spectrophotometer UV-visible Jenway 6800, The Marine chemistry lab, National Institute of Oceanography and Fisheries, Alex. Egypt.

Infrared spectrum (IR): The Infrared spectrum (IR) was obtained using Peak Find-Memory -27 spectrophotometer, in the Microanalysis Center, Cairo University, Egypt. The molecular structure of each antagonistic material (A) was partially identified and compared to known antibiotics. The method makes use of small discs made from the mixture of about 1 mg of the tested material and 300 mg of pure dry KBr, followed by pressing into a disc. The measurements were carried out at infrared spectra between 400-4000 nm.

Mass spectrum (MS): A mass spectrophotometer (DI Analysis Shimadzu Qp-2010 plus) was used in the Microanalysis Center, Cairo University, Egypt. The product subjected to a steam of high energy of electrons at elevated temperature up to 100°C cleavage fragments were yielded which can be characterized by mass/charge from mass spectra data.

Nuclear Magnetic Resonance Spectra (NMR): Each spot of antagonistic agents (A) was dissolved in durated dimethylsulfooxide. The different functional group could be identified using NMR (JEOL ECA 500), the Microanalysis Center and Cairo University, Egypt.

RESULTS

Effect of Different Incubation Period on Growth and Antifungal Activity of *S. Maxima:* The results in Figure (1) indicated that, the antimicrobial activity increased as the culture age increased reaching their maximal values at 15th day of incubation against *P. oxalicum* about 40%. Thereafter, the antimicrobial activity of *S. maxima* against *P. oxalicum* decreased reaching 23%.

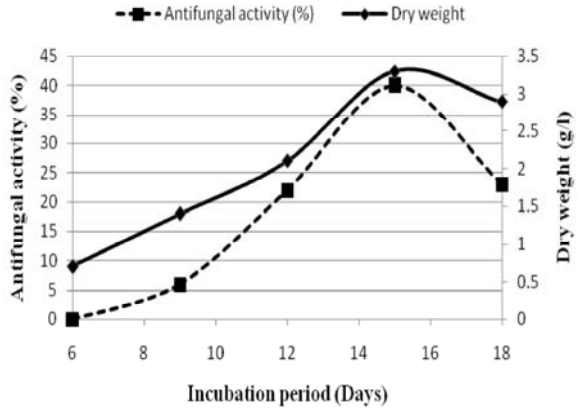


Fig. 1: Effect of different incubation period on the growth and antifungal activity of S. maxima against P. oxalicum

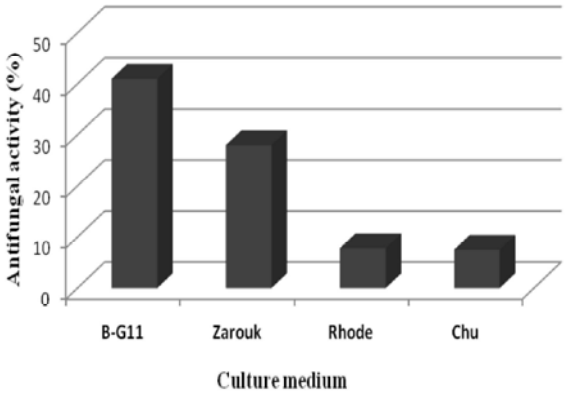


Fig. 2: Effect of different culture media on the growth and antifungal activity of S. maxima against P. oxalicum.

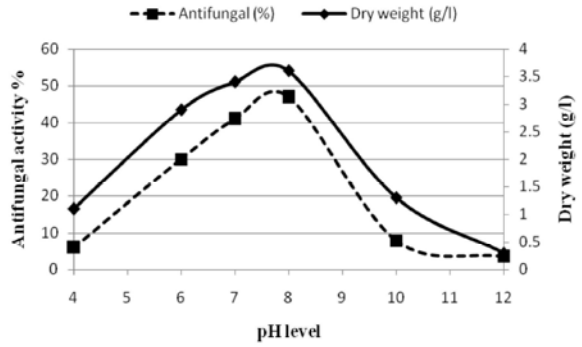


Fig. 3: Effect of different pH on the growth and antifungal activity of S. maxima against P. oxalicum

Effect of Different Culture Media on Growth and Antifungal Activity of *S. Maxima*: Four different types of culture media (B-G 11, Zarouk, Rhode and Chu) were used. The results indicated that the highest growth value was obtained in BG-11 medium 3.4 g/l where the lowest value of growth was obtained in Chu medium 2.4 g/l (Figure 2). The results also indicated that *S. maxima* exhibited the highest antimicrobial activity when being cultured in BG-11 medium and the antimicrobial activity against *P. oxalicum* was 41%, wherever *S. maxima* exhibited the lowest antimicrobial activity when being cultured in Chu medium and the antimicrobial activity against *P. oxalicum* was 7.5%. As a result of this experiment, the BG-11 medium was used throughout the subsequent experiments (Figure 2).

Effect of Different PH on Growth and Antifungal Activity of S. Maxima: The data in Figure (3) indicated that, the optimum pH value for maximum growth and antimicrobial activity of *S. maxima* was 8. The growth of *S. maxima* was achieved 3.5 g/l at pH 8, where higher and lower pH values caused reduction in the growth of the organism The antimicrobial activity of *S. maxima* exhibited the highest antimicrobial activity against *P. oxalicum* 47%, where the higher and lower pH values were less effective. As a result of this experiment, cultures have been adjusted at pH 8 throughout the subsequent experiments.

Determination of the Best Solvent for Extraction of the Antifungal Agents (A) from *S. Maxima*: The results in Figure (4) indicated that, the highest extraction of the antagonistic material had obtained when DMSO was used as a solvent followed by methyl alcohol, ethyl alcohol and chloroform, while, N-butanol was less effective in extraction of the antagonistic material

Partially purification of the antifungal agents of *S. maxima* using TLC: Only one spot was developed when the sonicated cells were eluted in a solvent system formed of methyl alcohol: chloroform, (60: 40; v/v). Spot (A) showed a Rf value equal 0.55.

The results indicate that of the partially purified agents (A) of *S. maxima* show increase of the antifungal activities against *P. oxalicum* compared to the crude product used. It was observed the spot (A) showed increase in the antifungal activity about 25% compared to the crude material.

Bio-toxicity Test of *S. Maxima* Purified Extracts Using *Artemia Salina* as Biomarker: The bio-toxicity of *S. maxima* purified extracts was carried out by using *Artemia salina* as biomarker. As shown in (Figure 5) that different

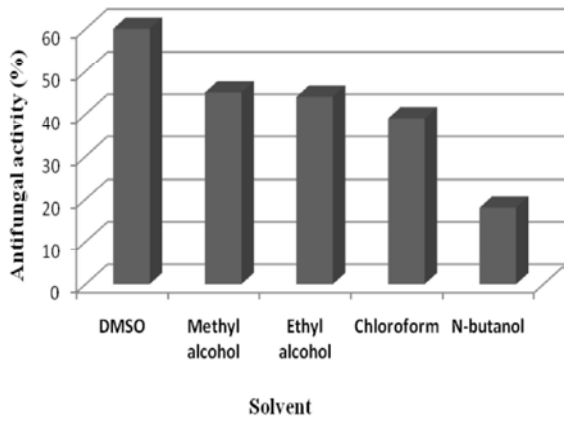


Fig. 4: Determination of the best solvent for extraction of the antifungal agent from S. maxima

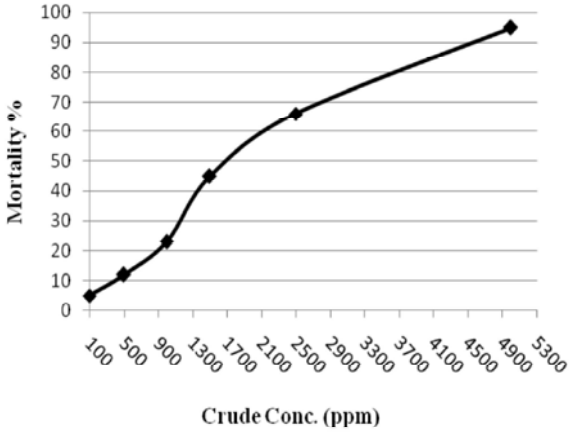
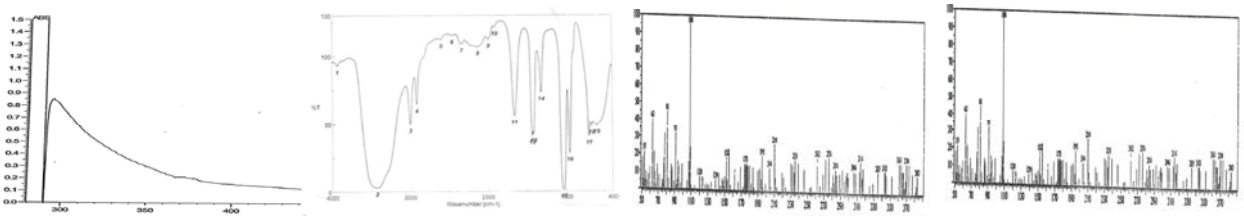


Fig. 5: Bio-toxicity of different concentrations of S. maxima purified antifungal agent using Artemia salina as a biomarker.



(a) (b) (c) (d)

Fig. 6: UV/Vis. spectrum (A), IR spectrum (B), mass spectrum (C) and NMR (D) spectrum of the antifungal agent produced by S. maxima

concentrations (From 100 to 5000 ppm) of *S. maxima* purified extract were tested and the mortality percentage was estimated. The purified antifungal agent produced by *S. maxima* was found to have a moderately toxicity level and the $L.C_{50}$ of that agent (The concentration at which 50% of the biomarker individuals die) was estimated from the best fit line obtained by using the profit analysis method, it was found to be 1027 mg/l

Comparison of the Antifungal Activity of the Partially Purified Agents of *S. Maxima* to Some Commercial Antifungal Drugs: The data obtained in Table (1) show that the antifungal activities of the partially purified antifungal agents produced by the *S. maxima* were estimated in comparison with three different commercial antifungal drugs including; Diflucan, Flocoral and Fungican. It has found that *S. maxima* showed a broad spectrum of antifungal activity with an average activity of 26% against five tested human and plant pathogenic fungi compared to the three tested commercial drugs; they showed average inhibition percentage of 48 %, 49% and 54%, respectively. Moreover, the most inhibited fungus was *P. oxalicum* (91%) followed by *R. solani* (20%) compared to the tested antifungal drugs; they showed average inhibition percentage of 30% and 55%, respectively. On the other hand, the produced marine microbial by product showed no activity against *C. albicans* ATCC 14053(0%) compared to the tested antifungal drugs; they showed average inhibition of 77%.

Partial Characterization of the Purified Antifungal Agent (A) of *S. Maxima* : The chemical characterization presented in Figure (6) showed the UV-Vis, infra-red (IR) and Mass spectra of the purified antifungal agent. The UV spectrum of the compound (Figure 6A) resulted in a single peak appeared at 250 nm proved that the compound (B) is an aromatic compound. The IR spectrum showed five absorption bands (Figure 6B); the first band appeared at

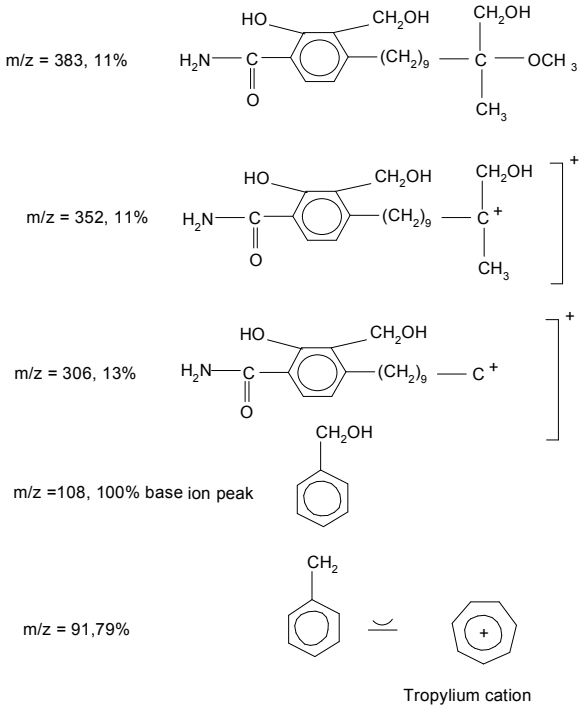


Fig. 7: The suggested chemical structure for the purified antifungal agent produced from S. maxima

Table 1: Antifungal activities (%) of the partially purified antifungal of S. maxima compared to some commercial antifungal drugs

Antifungal Agent	Antifungal activity (%)				
	F. solani	R. solani	C. albicans	A. niger	P. oxalicum
S. maxima agent	20	10	0	10	91
Diflucan	55	51	78	26	28
Flucoral	57	52	80	27	29
Fungican	70	64	75	25	35

3426 cm^{-1} due to (NH_2, OH, NH) groups, the second band appeared at 2912 cm^{-1} due to C-H aliphatic, the third band appeared at 1657 cm^{-1} due to carbonyl group (amide), the fourth band appeared at 1413 cm^{-1} due to benzene ring and the last band appeared at 1029 cm^{-1} due to ether linkage. Moreover, the obtained Mass spectrum of this compound (Figure 6C) showed the appearance of a molecular ion peak at m/e = 383 which indicate the molecular formula of (A) compound is 383 Dalton. The proton NMR spectrum of the compound under investigation (Figure 6D) showed signals at 0.9 (t, 3H, CH_3), 1.4 (t, 2H, CH_2), 2.6 (s, 3H, CH_3), 4.1 (s, 2H, CH_2), 5.0 (s, 2H, NH_2) and 7.6 (m, aromatic protons).

The suggested chemical structure was presented in Figure (7) according to the obtained molecular weight and the obtained spectrophotometric results.

DISCUSSION

The algal extracts natural products may have potential for the management of fungal diseases in sustainable agriculture such as organic farming. In the past decades, food scientists have been searching for natural alternatives to replace synthetic antioxidants. The well-studied carotenoids and phenolic compounds contribute significantly to the antioxidant capacity of microalgae [26].

The present study is an endeavor towards the production of antifungal agents by the blue green alga *S. maxima*. It has been screened for its biological activity against different species of fungi and it was found that it has high biological activity against *P. oxalicum*.

A directed search for biologically active compounds as well as their production by algal culture requires some understanding of the culture conditions favoring their production. Almost all of the biologically active compounds of interest are secondary metabolites and thus are usually most abundant in stationary phase or in slow-growing cultures [27].

The algal isolate which was identified as *S. Maxima* and grown on B.G- 11 medium and then screened for their antifungal activities against some plant and human pathogenic fungi, it was found that *S. maxima*, was most potent acting against the pathogenic fungus, *P. oxalicum*, the antifungal activity was 35%. The average of antifungal activities was 7% against the used plant and human pathogenic fungi.

Cosoveanu *et al.* [28] tested the biological activity of *Alaria esculenta, Fucus vesiculosus, Fucus sp. Spirulina platensis* and *Ecklonia maxima* was in vitro against *Fusarium roseum, F. oxysporum, Alternaria alternata, A. dauci, A. longipes, Trichoderma viride, Botrytis cinerea, Aspergillus niger, Penicillium expansum*. Their potential toxic effects were evaluated on mycelial growth. Results are presented as effective concentration which inhibits mycelial growth by 50% and 90%.

The current investigation initiated an optimization program aimed to maximize the production of antifungal agent produced by *S. maxima* and optimum conditions for extraction. *S.maxima* was subjected to further investigations in order to detect the culture conditions under which they produced the highest antifungal activity. Also, many investigators observed the great relationship between the nutritional culture conditions and antagonistic activities of microorganisms which affect the production of the bioactive compounds [29,30].

The results indicate that *S. maxima* produced the antagonistic material optimally at the stationary phase when it cultured under aerated conditions and reaches the maximal values after 15th day of incubation. The results obtained indicated that the antimicrobial activity was higher in aerated than in static cultures and this results in agreement with Piccardi *et al*. [31] who found high antibacterial and antifungal activities produced from *Nostoc* sp. when incubated in an orbital shaker flushed with a mixture of (air : CO_2,95 :5 V:V).

Our results are in agreement with Bloor and Engl [32] who found that the highest antimicrobial activity of *N. muscorum* achieved by day 14 of the cultivation. In addition, Gromov *et al*. [33] found that the antibiotic cyanobacterium LU1 from *Nostoc linckia* is synthesized throughout the growth cycle. Patterson and Bolis [34] pointed out the production of tolytoxin in *Scytonema ocellatum* is also unusual for a secondary metabolite in that it is produced throughout the cell cycle. Oufdou *et al.* [35] obtained that the extracellular and intracellular products released by the cyanobacterium *Pseudanabaena* sp. in the stationary growth phase, reduced the survival of *E. coli*, *Salmonella* sp. *S. aureus* and *C. albicans*.

Santoyo *et al*. [36] extracted the supercritical fluid and fractionation of *Spirulina platensis* in order to obtain functional extracts with antioxidant and/or antimicrobial activities.

Results conducted also that the BG-11 medium proved to be the best for the production of antifungal agent. In such manner, Bloor and England [32] found that BG-11 medium is the best medium for antibiotic production from *N. muscorum* and they elucidated and optimized the medium constituents of BG-11medium constituents controlling antibiotic production from the cyanobacterium *N. muscorum*. Piccardi *et al*. [31] found that BG-11 medium is the best medium for antibiotic production from 38 *Nostoc* strains.

Moreover, Abd El-Baky *et al*. [37] illustrated that the enhancing process of phenolics synthesis in *Spirulina maxima* grown in Zarrouk's medium supplemented with different concentration of $NaNO_3$ and/or combined with phenylalanine (L-PA). Their results revealed that the levels of $NaNO_3$ and L-PA in growth medium had positive effects on the production of biomass (34-64 mg/day), total phenolics (4.51-16.96 mg/g d.w) and flavonoids (1.32-5.12 mg/g d.w) contents. The highest levels of these compounds were obtained in Zarrouk's medium containing 3.77 g/L $NaNO_3$ and 100 mg/L L-PA.

The relationship between the production of antibiotic or antagonistic substance and the producer isolate seem to depend largely on pH value of the culture medium. So, the optimum pH value for productivity and growth was 7.0 for our strain. Knubel *et al*. [38] have reported that, the optimum pH for production of indocarbazoles by *Nostoc sphaericum* was 7.8.

In the extraction step, it was found that DMSO was the best solvent for extraction of the antifungal agent. Kaushik and Chauhan [39] used hexane, ethyl acetate, dichloromethane and methanol to obtain the phenolic extracts from *Spirulina platensis* and they found, on countrary, that the methanolic extracts had the best results. Also, Vinay *et al.* [40] studied the effectiveness of *Spirulina platensis* in different solvent (Petroleum ether, chloroform, acetone, methanol) extract against three dermatophytic fungi namely *Aspergillus fumigatus, A. niger, Candida albicans.* The methanolic extract only of *S. platensis* showed significant activity against *A. fumigatus*. While, Cosoveanu *et al.* [28] observed that the ethanolic algal extracts showed almost an antifungal activity.

On the other side Medina-Jaritz *et al.* [41] evaluated the antimicrobial activity of *Arthrospira maxima* different concentrations of aqueous and methanolic extracts were tested by the agar diffusion technique against *Proteus vulgaris, Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Candida albicans.* The aqueous extracts showed antibacterial activity against all organisms tested, except to *Bacillus subtilis,* while methanol extract showed antimicrobial activity against all microorganisms, even to *Staphylococcus aureus*.

Santoyo *et al*. [36] found the most active fraction against all the microorganisms tested, was the one collected in the second fraction in the experiment performed at 220bar and 26.7 °C with 10% of ethanol. They tested antimicrobial activities of microalgae extracts against four different microbial species, including a gram positive bacterium (*Staphylococcus aureus*), a gram negative bacterium (*Escherichia coli*), a yeast (*Candida albicans*) and a fungus (*Aspergillus niger*). Abd El-Baky *et al.* [37] explained that the HPLC-DAD profile of all phenolic extracts of *Spirulina* showed the presence of large numbers of phenolic acids and flavonoids, in variable levels. Gallic, chlorogenic, cinnamic, pinostrobin and *p*-OH-benzoic were found as the most abundant constituents among different extracts.

ACKNOWLEDGEMENT

We appreciate Prof. Abd El-Metaal M.A. -Chemistry Department, Benha University, Egypt, for his great effort in the elucidation of the chemical structure of the produced antifungal agent in this study.

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