Antimicrobial activity of microalgal extracts with special emphasize on *Nostoc* sp.

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Abstract: Microalgae are rich sources of biologically active compounds. In the present study, three cyanobacteria (*Nostoc* sp., *Microcystis* sp. and *Oscillatoria geminata*,) and 2 green microalgae (*Chlorella vulgaris* and *Scenedesmus* sp.) were tested with the agar well diffusion method for their antibacterial and antifungal activity. Four Gram-positive bacteria (*Staphylococcus aureus, Sarcina lutea*, *Bacillus subtilis* and *Bacillus megaterium*), one Gram-negative bacteria *(Klebsiella pneumonia*), and five fungal strains (*Fusarium solani, Fusarium oxysporum, Rhizoctonia solani, Asperigillus niger,* and *Candida albicans*) were examined. The antimicrobial activity was tested using methanol and acetone extracts of cyanobacteria and algal species. The methanol extract was more effective against the studied bacterial strains, inhibited *B. subtilis*, *K. Pneumonia* and *S. aureus* while acetone extract affected *S. aureus* and with less impact on *B. subtilis*. *B. megaterium* was the most resistant strain in the two types of extracts. The antifungal activity of algal acetone extracts was higher than methanol extracts. *C. vulgaris* methanol extract exhibited no antifungal activity to all studies strains. Among all the algal species studied *Nostoc* sp. indicated wide spread spectrum of antimicrobial activity, so its methanol and acetone extracts were analyzed by GC/Mass and the data obtained showed that the most abundant compounds in methanol extract of *Nostoc* sp. were phenols, plasticizer compound, phytol and flavenoids where, in acetone extract were plasticizer compound, phytol, alkenes and esters. These results give an indication of the presence of promising antimicrobial compounds in the algal species under study. Further phytochemical studies are needed to elucidate these compounds structures and activity.
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1. Introduction:

Antibiotic resistance in bacteria and fungi is one of the major emerging health care related problems in the world; it became a greater problem of giving treatment against resistant pathogenic bacteria (Sieradzki, *et al.*, 1999). One approach to antibiotic resistance is the discovery of novel antimicrobial compounds for clinical application (Desbois *et al*., 2008 and 2009). Algal organisms are rich source of structurally novel and biologically active secondary and primary metabolites which may be potential bioactive compounds of interest in the pharmaceutical industry (Ely *et al.,* 2004 and Tuney *et al.,* 2006). Microalgae and cyanobacteria offer numerous advantages for antimicrobial investigations because of their enormous biodiversity and fast growth rate (Pulz and Gross, 2004)

The cell extracts and active constituents of various algae shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria (Borowitzka *et al.,* 1992, Ostensvik *et al.* 1998 and Goud *et al*., 2007). A wide range of *in vitro* anti-fungal activities have also been reported from extracts of green algae, diatoms and dinoflagellates **(**Ely *et al.,* 2004) and from *Nostoc* sp. (Kim, 2008). Extracts from 10 cyanobacteria proved to be active against multidrug-resistant *Mycobacterium tuberculosis*, the causative agent of tuberculosis **(**Rao *et al*., 2007). Najdenski *et al.* 2013 stated that ethanolic extract of *Scenedesmus obliqus*, *Chlorella* sp. and *Nostoc* sp. has antibacterial effect against *Staphylococcus aureus* and *Bacillus cereus.* In the same manner Sanmukh *et al.* (2014) explored bioactive compounds of a group of microalgae with emphasizing on the *Chlorella* sp. which showed antibacterial effect against *Staphylococcus* sp. Beena and Krishnika (2011) tested antibacterial activity of *Scenedesmus* sp. isolated from a natural pond against three pathogenic bacteria with different solvents, the aqueous and methanol extracts gave better results.. Sanmukh *et al*. (2014) explored microalgae for their bioactive compounds and affirmed promising applications encompassing antibacterial, antiviral, and antifungal activities; also he stated that the application of bioactive compounds derived from algae will prove beneficial and much more effective as compared with traditional treatment methods.

Antimicrobial activity depends on both algal species and the solvents used for their extraction (Prakash *et al.,* 2011 and Radhika *et al*., 2012). The antimicrobial activity of algae extracts is generally assayed using various organic solvents which always

provide a higher efficiency in extracting compounds for antimicrobial activity (Cordeiro *et al*., 2006 and Tuney *et al*, 2006). Analytical methods play important roles in the discovery, development and manufacture of bioactive molecules (Mariswamy *et al.*, 2011).

The aim of the present study was to study the antimicrobial activity of various cyanobacteria (*Nostoc* sp, *Oscillatoria geminata* and *Microcystis* sp.) and green microalgae (*Chlorella vulgaris* and *Scenedesmus* sp.) methanol and acetone extracts against some pathogenic bacterial and fungal strains. Furthermore, GC/MS autogram for *Nostoc* sp extracts was employed for preliminary detection of active constituents.

2.Materials and methods

Sample collection and culture characterization

The algal strains (*Nostoc* sp., *Microcystis* sp., *Scenedesmus* sp., *Chlorella vulgaris*) isolated from the soil and River Nile in Helwan and Menoufia regions –Egypt, during June 2010, in addition to *Oscillatoria geminate* an isolate from Wadi Elnatroun and taken from National Research Centre (NRC). Samples were grown in BG-11 medium under (16 h light \ 8h dark) at 28±2°C and 30 µmol photon m^{-2} s^{-1} light intensity. Blue green and green strains were harvested at their exponential phase of growth which is 27th day for *Chlorella,* 24th day for *Scendesmus* sp.*,* 14th for *Microcystis* sp. and at 30th day for *Nostoc* sp. and 14th for *O. geminata.* Harvesting took place by centrifugation at 4000 rpm for 15 min. The isolated strains were identified according to Prescott (1982) and Komárek and Anagnostidis (2005).

Extraction of bioactive metabolites

The dried biomass was sonicated with liquid nitrogen and then was extracted with 95 % methanol and 95% acetone (Cowan, 1999 and Zheng *et al.,* 2011). The extracts were centrifuged at 4000 rpm for 10 min, and were further concentrated in vacuum under reduced pressure, the stock solutions of extract were prepared in DMSO at 50 mg ml^{-1} for the evaluation of antimicrobial activity (Hussain *et al*., 2011 and Jelodarian *et al*., 2013).

Antimicrobial bioassay

The microbial pathogenic strains were 4 Gram positive (*Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis* and *Bacillus megaterium*) and one Gram- negative *(Klebsiella pneumonia*). In addition to five pathogenic fungi (*Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Asperigillus niger*, *Candida albicans*). The strains were obtained from NRC. Antimicrobial activity of methanol extracts was evaluated using agar well diffusion method as described by Patra *et al.* (2009). The wells were then filled with 100 µl of extract, and DMSO was used as negative control. Plates were incubated 24 hours at 37 ± 1 ºC for bacterial strains and 72 hours at 25 ± 2 ºC for fungal strains. The diameter of inhibition zones was measured in triplicates and the average was calculated.

GC/MS Analysis of *Nostoc* sp. crude extract:

Methanol and acetone extracts of *Nostoc* sp. was analyzed by GC/MS at NRC, Cairo, Egypt.

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (15 m, 0.251mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1mL/min. The injector and MS transfer line temperature was set at 280 °C. The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

3.Results

The methanolic extracts of the five algal species (*Scenedesmus* sp., *C. vulgaris, Nostoc* sp.*, Microcystis* sp., and *O. geminate*) were tested for their antimicrobial activity against 4 Gram positive (*S. aureus*, *Sarcina*, *B. subtilis* and *B. megaterium*) and one Gram- negative *(K. pneumonia*). In addition to five pathogenic fungi (*F. solani*, *F. oxysporum*, *R. solani*, *A. niger* and *C. albicans*). *C. vulgaris* extract showed antibacterial activity against *B. subtilis*, *S. aureus,* and *K. pneumonia* with inhibition zones 17.5, 17 and 14.5 mm respectively.

Scendesmus inhibited only *B. subtilis* and *S. aureus* with inhibition zones 15 and 16.25 mm, respectively. *Nostoc* showed the highest inhibition zone (24 mm) against *K. pneumonia*, followed by 18 and 17 mm inhibition zone with *S. aureus* and *B. subtilis*, respectively. *Oscillatoria* inhibited only *S. aureus* with 17.5mm inhibition zone and *K. Pneumonia* with 16 mm. *Microcystis* affected on *S. aureus*, *K. Pneumonia* and *B. subtilis* with inhibition zones 18, 16 and 15 mm, respectively. On the other hand acetone extract of *Chlorella* sp. inhibited the growth of *S. lutae*, *B. subtilis*, *S. aureus* and *K. pneumonie*, with inhibition zone diameters 30, 20, 18 and 16 mm respectively, without any effect on *B. megaterium* (Table 2).

Microcystis acetone extract inhibited the growth of *B. subtilis* and *S. aureus* with inhibition zone 18 and 16 mm. *Scenedesmus*, *Oscillatoria* and *Nostoc* have antibacterial effect on *S. aureus* only with 16, 15 and 14 mm inhibition zone, respectively.

It was clear from our results in table 3 that all the algal species have antifungal activity except *Chlorella*. The methanol extract showed antifungal activity on *A. niger, R. solani* and *F. oxysporum.* *Nostoc* extract was the most effective; it inhibited *R. solani* and *A. niger* with clear inhibition zones of 35 and 22 mm.

Microcystis extract inhibited the growth of *R. solani* with inhibition zone of 40 mm, *Scendesmus* inhibited the growth of *F. oxysporum* (35.5 mm), and finally *Oscillatoria* gave 12 mm inhibition zone with *R. solani*.

Algal species acetone extracts affect only on *A. niger* and *F. oxysporum* (Table 4). *Nostoc* sp. inhibited the growth of *A. niger* and *F. oxysporum* with 21 and 27 mm inhibition zones, while *Oscillatoria* gave 22 and 18 mm inhibition zones with *A. niger* and *F. oxysporum,* respectively.

Microcystis inhibited the growth of *A. niger* with 19 mm inhibition zone. The green algal species *Chlorella and Scenedesmus* inhibited the growth of *F. oxysporum* with inhibition zones of 45 and 35.5 mm, respectively.

GC/MS Analysis of *Nostoc* sp. methanol crude extract:

The chemical composition of *Nostoc* methanol extract determined by GC/MS was illustrated in figure 1 and table 5. The spectrum analysis revealed the presence of 4 distinct peaks.

4-Methyl-2, 6-ditert-butylphenol (44.5%) was the highest percentage in the extract followed by Diisooctyl phthalate (plasticizer compound) with 30.42 % followed by phytol giving 8.71% the last peak (1.4) was represent Quercitin 7,3',4'-trimethoxy, which was type of flvenoids. Finally we can say that; the most abundant compounds in methanol extract were phenols, plasticizer compound, phytol (acyclic diterpene alcohol) and flavenoids.

The spectrum analysis resulted from GC/MS for *Nostoc* sp. acetone extract revealed the presence of 6 distinct peaks (Fig 2). Acetone extract gave Diisooctyl phthalate Plasticizer compound 56.97**%** followed by Distearyl acid phosphate ester 10.53%, Heptadecane 12.56**%** Butanoic acid, trimethylsilyl ester 7.34% and 2-Butenoic acid ,tert-butyldimethylsilyl ester 3.98% (Table 6)

The most abundant compounds in acetone extract were plasticizer compound, phytol (acyclic diterpene alcohol), alkenes and ester.

Table 1: Antibacterial activity of microalgae and cyanobacteria methanol extracts (50 mg ml^{-1}) against Gram-positive and Gram-negative bacteria as presented by inhibition zone diameter(mm)

Algal species	Inhibition zone diameter of bacterial strains				
	B. subtilis	***B. megaterium***	***K. pneumonia***	***S. aureus***	***S. lutae***
Chlorella	17.5	0	14.5	17	0
Scenedesmus	15	0	0	16.25	0
Nostoc	17	0	24	18	0
Oscillatoria	0	0	16	17.5	0
Microcystis	15	0	16	18	0

Table 2: Antibacterial activity of microalgae acetone extract (30 mg ml^{-1}) against Gram-positive and Gram-negative bacteria as presented by inhibition zone diameter (mm)

Algal species extract	Inhibition zone diameter of bacterial strains				
	B. Subtilis	***B. megaterium***	***K. pneumonia***	***S. aureus***	***S. utae***
Chlorella	20	0	16	18	30
Scenedesmus	0	0	0	16	0
Nostoc	0	0	0	14	0
Oscillatoria	0	0	0	15	0
Microcystis	18	0	0	16	0

Table 3: Antifungal activity of microalgae methanol extract (50 mg/ml) against fungal strains as presented by inhibition zone diameter (mm)

Algal species	Inhibition zone diameter of fungal strains				
	A. niger	***C. albicans***	***F. oxysporum***	***F. solani***	***R. solani***
Chlorella	0	0	0	0	0
Scendesmus	0	0	35.5	0	0
Nostoc	22	0	0	0	35
Oscillatoria	12	0	0	0	0
Microcystis	0	0	0	0	40

Table 4: Antifungal activity of microalgal acetone extract (30 mg/ml) against fungal strains as presented by inhibition zone diameter (mm)

Algal species extract	Inhibition zone diameter of fungal strains				
	A. niger	*C. albicans*	*F. oxysporum*	*F. solani*	*R. solani*
Chlorella	0	0	45	0	0
Scendesmus	0	0	35.5	0	0
Nostoc	21	0	27	0	0
Oscillatoria	22	0	18	0	0
Microcystis	19	0	0	0	0

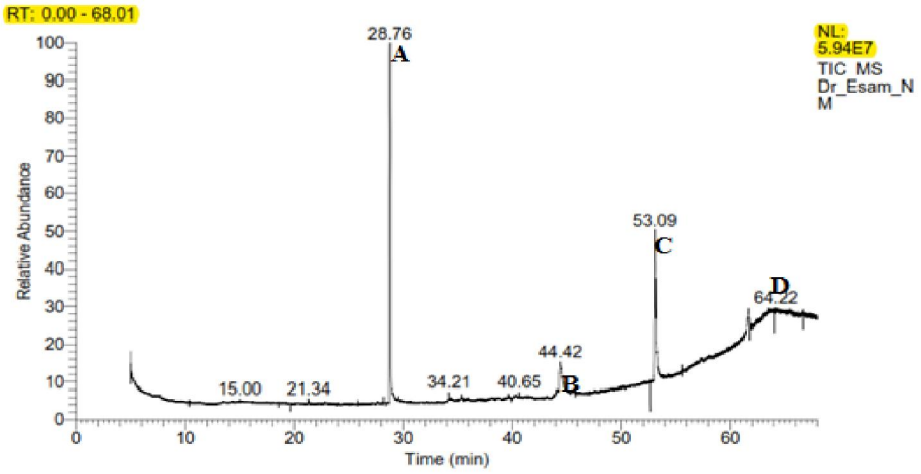

Figure 1: Gas chromatographic profile of the major constituents of *Nostoc* sp. methanol extract.

Table 5: GC mass analysis of *Nostoc* sp. methanol extract

	RT	Name of compound	Group	Molecular formula	Molecular weight	Peak area %
A	28.76	4-Methyl-2,6-ditert-butylphenol	Phenol	C15H24O	220	44.5
B	44.42	Phytol	Acyclic diterpene alcohol	C20H40O	296	8.71
C	53.09	Diisooctyl phthalate	Plasticizer compound	C24H38O4	390	30.42
D	64.22	Quercitin 7,3',4'- trimethoxy	Flavonoids	C18H16O7	344	1.40

RT=Retention time

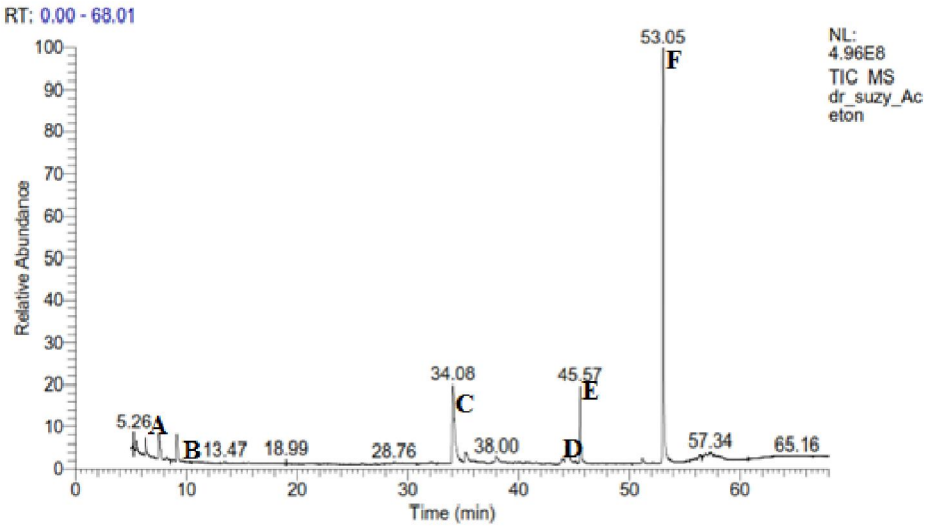

Figure 2: Gas chromatographic profile of the major constituents of *Nostoc* sp. acetone extract.

Table 5: GC mass analysis of acetone crude extract of *Nostoc sp.*:

	RT	Name of compound	Group	Molecular formula	Molecular weight	Peak area %
A	7.53	Butanoic acid,trimethylsilyl ester	Ester	C7H16O2Si	160	7.34
B	9.12	2Butenoic acid,tert-butyldimethylsilyl	Ester	C10H20O2Si	200	3.98
C	34.07	Heptadecane	Alkane	C17H36	240	12.56
D	44.32	Phytol	Acyclic diterpene alcohol	C20H40O	296	1.97
E	45.57	Distearyl acid phosphate	Ester	C36H75O4P	602	10.53
F	53.05	Diisooctyl phthalate	Plasticizer compound	C24H38O4	390	56.97

RT=Retention time

4.Discussion

The present study indicated that the antibacterial properties of the five algal species against the selected strains of bacteria vary depending upon the species and the organic solvent used for extraction. The data of methanol extract showed that the most sensitive bacteria were *S. aureus* and *B. subtilis*, which inhibited by 4 algal species. On the other hand *B. megaterium* and *S. lutae* were the most resistant bacteria. The acetone extract showed that *S. aureus* was the most affected bacteria, inhibited by all algal species, followed by *B. subtilis* inhibited by *Chlorella* and *Microcystis*, while *B. megaterium* was the most resistant bacteria. These results were compatible with the study of Prakash *et al*. (2011) on the antimicrobial potential of *Oscillatoria sancta* and *Lyngbya birgei* against *S. aureus*. *Scenedesmus* exhibited antibacterial activity against *S. aureus* in methanol and acetone extracts in accordance with Guedes *et al.* (2011). In addition, *Scenedesmus* and *Chlorella* had clear antibacterial activity against *B. subtilis and S. aureus* in agreement with Ördög *et al*. (2004), Ghasemi *et al*. (2004) and Desbois *et al.* (2009). *Nostoc, Microcystis,* and *Oscillatoria* methanol extract affected on S. *aureus*, *B. subtilis* and *K. Pneumonia* these results in agreement with Ostensvik *et al.*, 1998 who observed that aqueous extracts of *Microcystis aeruginosa* inhibited *B. subtilis,* and Rao *et al*. (2007) showed that *Oscillatoria* sp. have antibacterial activity. From the previous results we can say that the methanolic extract was more effective against the studied strains, where they inhibited *B. subtilis*, *K. Pneumonia* and *S. aureus* while acetone extract affect on *S. aureus* and with less impact on *B. subtilis*.

The antifungal activity showed differences between methanol and acetone extracts and also between species. *Chlorella* extract gave the highest inhibition zone against *F. oxysporum* in agreement with Ghasemi *et al.* (2004) which reported that *C. vulgaris* spp have antifungal activity against *A. niger.* and *C. albicans*, and Abedin and Taha (2008) showed also that *C. pyrenoidosa* have antifungal activity against the two fungal strains. On the other hand *Chlorella* methanol extract have no antifungal activity against the five fungal strains, so we can say that the solvent type and algal species affect the extract activity against the pathogens. *Scenedesmus* methanol and acetone extracts had antimicrobial activity on *F. oxysporum* and have no effect on *A niger*, and *C. albicans*, incontrast with Ghasemi *et al*. (2004), which found that *S. obliquus* gave antimicrobial activity against *A niger.* and *C. albicans* and Abedin and Taha (2008) which reported that *S. quadricauda* inhibits the growth of the two fungal strains. This shows that the species has a significant impact on the antifungal activity.

R. solani was the most inhibited strain, by methanol extract. The highest value of inhibition zone was obtained by *Microcystis* extract. This result agree with Pranita *et al*. (2011) where they found a marked reduction in growth (52%) of *R. solani* recorded on the plates supplemented with *M. aeruginosa* extract. *Nostoc* was the most effective algal species it inhibited 2 fungal strains (*A. niger* and *R. solani*). These results agree with Rizk (2006) and Kulik (1995) where they reported that the growth of *R. solani* was significantly inhibited by using *N. muscorm* extract. Also, Biondi *et al*. (2004) showed that mycelia growth of *R. solani*, were inhibited by the methanol extracts of the cyanobacterium *Nostoc*. In case of acetone extract all algal species extracts affected only on *A. niger* and *F. oxysporum. R. solani* was not inhibited by the acetone extracts of all algal species. These results were disagreeing with Kulik (1995) and Rizk (2006). In general, the antifungal activities of algal species acetone extracts were higher than methanol extract.

GC mass analysis indicated the presence of 9,12,15-Octadecatrienoic acid (alpha Linolenic acid)

which is poly unsaturated n-3 fatty acid, so it may be one of the proving antimicrobial effects of *Nostoc*. Desbois *et al.*, 2008 detected the presence of poly unsaturated fatty acid in the methanol extract of microalgae and reported that a polyunsaturated fatty acid hexdecatrienoic acid n-4 is active against Gram +ve and Gram -ve bacteria and is highly active against multidrug resistant *S. aureus*. Tuney *et al.* (2006) also indicated that antimicrobial effect can be related to volatile compounds in the samples such as hydrogen peroxide, terpenoid, and bromo-ether, volatile fatty acids compounds. The most abundant compounds in methanol extract were phenols, plasticizer compound, phytol (acyclic diterpene alcohol) and flavenoid, and in acetone extract were plasticizer compound, phytol, alkenes and ester. Results were compatible with Kannan *et al*. (2010) who explained that the important compounds identified as antimicrobial from *Enhalus acoroides* are fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols. These results give an indication to the presence of promising antimicrobial compounds in the of methanol and acetone extract of these algal species under study. Further phytochemical studies are needed to elucidate the components responsible for antimicrobial activity of these extracts against bacteria and fungi. Also the development of the algal culturing methods and the use of modern technology in breeding will decrease the cost of microalgae production

References

1. Abedin RMA and Taha HM. Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of *Spirulina platensis*. Global Journal of Biotechnology and Biochemistry, 2008**;** 3:22-31.
2. Adams RP. Identification of essential oil components by gas chromatography/ quadrupole mass spectroscopy. Journal of the American Society for Mass spectrometry, 2001; 16(11): 1902-1903.
3. Beena B. Nair and Krishnika A. Antibacterial activity of freshwater microalga (*Sc enedesmus* sp.) against three bacterial strains. J. Bio sci. Res., 2011; 2(4):160-165.
4. Biondi N, Piccardi R, Margheri M C, Rodolfi L, Smith G D and Tredici M R. Evaluation of *Nostoc* strain ATCC 53789 as a potential source of natural pesticides. Appl. Environ. Microbiol., 2004; 70: 3313-3320.
5. Borowitzka, M A and Borowitzka L J. In: Microalgal Biotechnology, Cambridge University Press, Great Britain, pp. 1992. 179.
6. Cordeiro RA, Gomes VM, Carvalho AFU and Melo VMM. Effect of Proteins from the Red Seaweed *Hypnea musciformis* (Wulfen) Lamouroux on the Growth of Human Pathogen Yeasts. Brazilian Archives of Biology and Technology, 2006; 49(6): 915-921.
7. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiological Reviews, 1999; 12: 564-582.
8. Desbois AP, Lebl T, Yan L and Smith VJ. Isolation and structural characterisation of two antibacterial free fatty acids from the marine diatom, *Phaeodactylum tricornutum*. Applied Microbiology and Biotechnology, 2008; 81:755-764.
9. Desbois A, Spragg A M., Smith VJ. A fatty acid from the diatom *Phaeodactylum tricornutum* .Is antibacterial against diverse bacteria including multi-resistant *Staphylococcus aureus* (MRSA). Marine Biotechnology, 2009; 11:45-52.
10. Ely R, Supriya T, and Naik CG. Antimicrobial activity of marine organisms collected off the coast of South East India. Journal of Experimental Marine Biology and Ecology, 2004; 309(1): 121-127.
11. Ghasemi Y, Tabatabaei Yazdi M, Shafiee A, Amini M, Shokravi S and Zarrini Parsiguine G. A novel antimicrobial substance from *Fischerella ambigua.* Pharm. Biol., 2004; 42: 318-322.
12. Goud MJP, Seshikala D, and Charya M. Antibacterial activity and biomolecular composition of certain fresh water microalgae from River Godavari (India). Sci World J., 2007; 2(3): 19-23.
13. Guedes A C, Catarina R, Barbosa H M, Amaro C I, and Pereira F X M. Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. International Journal of Food Science and Technology, 2011; 46(4): 862-870.
14. Hussain T, Arshad M, Khan S, Sattar H and Qureshi MS. *In vitro* screening of methanol plant extracts for their antibacterial activity. Pakistan Journal of Botany, 2011; 43(1): 531-538.
15. Jelodarian S, Abdolrasoul Haghir E, and Fereshteh J K. Evaluation of antimicrobial activity of *Malus domestica* fruit extract from Kashan area. Avicenna Journal of Phytomedicine, 2013; 3(1): 1-6.
16. Kannan RRR, Arumugam R, and Anantharaman P. *In vitro* antioxidant activities of ethanol

extract from *Enhalus acoroides*. Asian Pac J Trop Med. 2010; 3(11): 898-901.
17. Kim J, Kim JD. Inhibitory effect of algal extracts on mycelial growth of the tomato-wilt pathogen, *Fusarium oxysporum* f. sp. *lycopersici*. Mycobiology, 2008; 36(4): 242-248.
18. Komárek J. and Anagnostidis K. Cyanoprokaryota. Oscillatoriales. Süsswasserflora von Mitteleuropa 2005; 19: 752-759.
19. Kulik M M. The potential for using cyanobacteria (bluegreen algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur. J. Plant Pathol., 1995; 101: 585-599.
20. Mariswamy Y, Gnaraj WE, Johnson M. Chromatographic finger print analysis of steroids in *Aerva lanata* L by HPTLC technique. Asian Pacific Journal of Tropical Biomedicine, 2011; 1(6): 428-433.
21. Najdenski H M, Gigova Liliana G, Iliev Ivan I, Pilarski Plamen S, Lukavsky Jaromir, Tsvetkova Iva V, Ninova Mariana S and Kussovski Vesselin K. Antibacterial and antifungal activities of selected microalgae and Cyanobacteria. International Journal of Food Science and Technology, 2013; 48: 1533-1540.
22. Ördög V, Stirk WA, Lenobel R, Bancirova M, Strand M and Van Standen J. Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. J. Applied Phycol., 2004; 16: 309-314.
23. Ostensvik O, Skulberg OM, Underdal B, Hormazabal V. Antibacterial properties of extracts from selected planktonic fresh water cyanobacteria—a comparative study of bacterial bioassays. Journal of Applied Microbiology, 1998; 84: 1117-1124.
24. Patra JK , Patra AP, Mahapatra NK, Thatoi N, Das S, Sahu, RK and Swain GC. Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India. Malaysian Journal of Microbiology, 2009; 5(2): 128-131.
25. Prakash JW, Johnson M and Solomon J. Antimicrobial activity of certain fresh water microalgae from Thamirabarani Asian Pacific Journal of Tropical Biomedicine, 2011; 1(2):170-173.
26. Pranita J, Radha P and Pawan K S. Characterization of the biocide spectrum of extracellular filtrates of *Microcystis aeruginosa* Indian Journal of Microbiology 2011; 51(4): 509-514.
27. Prescott G W. Algae of the Western Great Lakes area (2nd Ed.). Dubuque: WM Brown & Co. 1982.
28. Pulz O and Gross W. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol., 2004; 65: 635-648.
29. Radhika D, Veerabahu C, and Priya R. Antibacterial activity of some selected seaweeds from the Gulf of Mannar Coast, South India. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5(4): 89-90.
30. Rao M, Malhotra S, Fatma T, Rattan A. Antimycobacterial activity from cyanobacterial extracts and phytochemical screening of methanol extract of Hapalosiphon. Pharmaceutical Biology, 2007; 45:88-93.
31. Rizk M. A. Growth activities of the sugarbeet pathogens *Sclerotium rolfsii Sacc. Rhizoctonia solani* Kühn. and *Fusarium verticillioides* Sacc. Under cyanobacterial filtrates stress. Plant Pathogol. J., 2006; 5:212-215.
32. Sanmukh S, Bruno B, Ramakrishnan U, Khairnar K, and Swaminathan S. Bioactive compounds derived from microalgae showing antimicrobial activities. Journal of Aquaculture Research and Development, 2014; 5(3): 224.
33. Sieradzki K, Robert RB, Haber SW, Tomasz A. The development of vanomycin resistance in patient with methicillin resistant *S. aureus*. The New England Journal of Medicine, 1999; 340: 517-523.
34. Tuney I, Cadirci BH, Unal D and Sukatar A. Antimicrobial activities of the extracts of marine algae from the coast of Urla (izmir, Turkey). Turkish Journal of Biology, 2006; 30: 171-175.
35. Zheng H, Yin J, Gao Z, Huang H, Ji X and Dou C. Disruption of *Chlorella vulgaris* Cells for the Release of Biodiesel-Producing Lipids: A Comparison of Grinding, Ultrasonication, Bead Milling, Enzymatic Lysis, and Microwaves. Applied Biochemistry and Biotechnology, 2011; 164:1215-1224.

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