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Full Length Research Paper

Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound

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The concept of biological control for health maintenance has received widespread attention during the last few years. Therefore, the main objective of this work was to look for active substances that could be used as antibacterial agents. To achieve this target, two different extract (Methanol and Acetone) from *Spirulina platensis* was examined. The algal extracts were tested *in vitro* for their antibacterial effects against (*Staphylococcus aureus, Salmonella typhimurium*) using Agar well diffusion method and Paper disc diffusion method and concentration from 250 ppm up to 7000 ppm was taken and observed all these bacteria showed inhibition in growth by these extracts. During gas chromatography mass spectrometry (GC-MS) analysis it was observed that mostly fatty acid compounds are present in crude extract which are associated with the antibacterial properties.

Key words: *Spirulina platensis*, antibacterial activity, agar-well diffusion method, paper-disc diffusion method, gas chromatography-mass spectrometry (GC-MS) analysis.

INTRODUCTION

Pharmaceutical drug discoveries, for most of the past 40 years, have depended heavily on the process of empirically screening of large number of pure compounds to provide new leads. *Spirulina platensis* or its extract show therapeutic properties, such as the ability to prevent cancers, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals and toxic metals and provide protection against the harmful effect of radiation. Cyanobacteria are a very old screening method of cyanobacteria for antibiotics, and other pharmacologically active compounds have recently received considerable attention (Borowitzka, 1995). The search for cyanobacteria with antimicrobial activity has gained importance in recent years due to growing worldwide concern about alarming increase in the rate of infection by antibiotic-resistant micro-organisms. Various active substances with antibacterial, antiviral, fungicide, enzyme inhibiting, immunosuppressive and cytotoxic and algicide activity have been isolated from cyanobacterial biomass (Knubel et al., 1990; Mule et al., 1991; Gerwork et al., 1994; Jaki et al., 1999). Microalgae, such as *Ochromonas* sp., *Prymnesium parvum*, a number of blue green algae produce toxins that may have potential pharmaceutical application. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, of which many are based on their uses in traditional medicine. *S. platensis* produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Pathogen resistance to synthetic drugs and antibiotics that are already in use makes search for plants with antimicrobial activity more important, as they can substitute for synthetic antibiotics and drugs. Characteristics of plants that inhibit microorganisms have been investigated in laboratories since 1926. The past decade has witnessed a significant increase in the prevalence of resistance to antibacterial and antifungal agents. Resistance to antimicrobial agents has important implications for morbidity, mortality and health care costs in U.S. hospitals, as well as in the community. These developments and the associated increase in bacterial infections intensified the search for new, safer, and more

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efficacious agents to combat serious bacterial infections. All over the world phycologists studied the different types of natural products occurring within marine algae. A variety of fatty acids (both saturated and unsaturated), sterols, terpenes and sugars have been isolated from them. A very limited amount of phycochemical knowledge is available about freshwater algae, in comparison with the detailed work carried out on seaweeds, which includes not only the isolation of fatty acids but also a complete phycochemical analysis showing the types of sterols, terpenes, glycosides, polyols, halogenated compounds as well as new and novel metabolites. These developments and the associated increase in bacterial infections intensified the search for new, safer, and more efficacious agents to combat serious bacterial infections.

MATERIALS AND METHODS

Algal source

The blue-green alga, *S. platensis* were collected from Jal Mahal Lake, Jaipur and was maintained in Zarrouk's medium at 30°C with 500 lux light intensity for 30 days. Samples were then shade dried and ground in pulverization to get coarse powder. Subsequently, the powdered samples stored in refrigerator.

Preparation of organic algae extract

Microwaves are electromagnetic waves with frequency between 300 MHz to 300 GHz. The principle of microwave heating is dependent on ionic conductance and dipole rotation. During microwave heating, if moisture is present in the algal material, it will evaporate and cause pressure that will rupture the algal cell wall. So the active constituents from the algal cell leach out in the solvent used.

One gram of algae powder was added to 50 ml of respective solvent. The extracts obtained after microwave heating (at 720 W, for 300, 120, 50, 70, 180 s, with intermittent cooling to avoid overheating) were cooled, centrifuged at 10,000 rpm for 15 min and filtered through Whatman #1 filter paper (Whatman International Ltd., England). The supernatant were evaporated at the respective boiling points of the solvents. Dried extracts were then reconstituted in respective solvents.

The targeted bacteria

The micro-organisms used in antimicrobial assays were supplied by Institute of Microbial Technology (IMTECH). The bacterial species taken for the study are (*Staphylococcus aureus* MTCC-96, *Salmonella typhimurium* MTCC-98). A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these were streaked on to the appropriate culture slants and incubated at 37°C for 24 h.

Antibacterial testing

Agar-well diffusion method

It was done as described by Shanmuga et al. (2002). Briefly, 1×105 spores/ml of different bacteria was prepared and 0.2 ml spore suspension was spread over the agar surface of the plates. The plates were placed at 27±2°C for 30 min in order to make the agar surface dry. Different conc. of the algal extract was added into the well with the help of sterilized micropipette. The plates were kept in an upright position in an incubator until the extracts diffused in the agar at least for 3 to 4 h. These plates were then inverted and further incubated at 27°C for 3 to 5 days. The plates were observed for zone of inhibition (mm) around the wells.

Paper-disc diffusion method

Antibacterial activity by disc diffusion method (Okigbo et al., 2005) was observed. Whatmann Filter paper discs, saturated with different extracts of varying concentrations were placed on SDA medium supplemented with the test organism. Disc fed with solvent alone serve as control. The plates were incubated at 27°C and were observed for zone of inhibition after 3 days.

Chemical composition (GC-MS analysis)

The Gas chromatography mass spectrometry (GC-MS) analysis of the samples isolated from the acetone and methanol extract of *S. platensis* was performed using a Shimzdu Mass Spectrometer-2010 series system (AIRF, JNU, New Delhi) equipped with a AB inno-wax column (60 m × 0.25 mm id, film thickness 0.25 µm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a flow rate of 1.2 ml per minute. Injector and mass transfer line temperature were set at 270 and 280°C. The compounds were identified by comparing them with the standards, or the mass spectra were matched with the in built library (NIST/Wiley).

RESULTS

The antibacterial activity of *S. platensis* was evaluated in different concentration of solvent extract (Methanol, Acetone) against targeted bacterial species (*S. aureus* and *S. typhimurium*). Toxicity of the solvent extract has been tested by Agar-well diffusion method, Paper disc diffusion method.

Assessment of antibacterial activity of *S. platensis* solvent extract by pathogenic bacteria

Agar-well diffusion method

The results obtained from the present study concerning the antibacterial activity of *S. platensis* extracted with different solvents against different species of bacteria are recorded in. It is clear from study that the diameter of the inhibition zone depends mainly on the type of the solvent used and the tested anti-bacterial activity. *S. platensis* extract concentration was prepared from 250 ppm upto 7000 ppm in both the solvent acetone and methanol solvent to check the antibacterial activity. In case of *S. aureus*, acetone solvent extract showed maximum inhibition of (21.5 mm) at 5000 ppm but maximum inhibition in case of methanol was observed at 6000 ppm of (19.0 mm) and as the concentration increases to 7000 ppm its inhibition decreases to (18.5 mm) in case of

Table 1. Results of antibacterial activity of *Spirulina platensis* in organic solvent extracts against *S. typhimurium and S. aureus.*

Bacteria	Concentration	Agar-Well diffusion method		Paper–Disc diffusion method	
		Methanol	Acetone	Methanol	Acetone
S. aureus	7000 ppm	18.75	18.5	17.25	17.5
	6000 ppm	19.0	19.0	15.50	15.0
	5000 ppm	17.5	21.5	15.25	14.50
	4000 ppm	17.0	21.0	13.75	14.0
	3000 ppm	15.5	19.0	12.75	13.75
	2000 ppm	13.75	16.5	12.25	13.50
	1000 ppm	12.0	13.3	11.25	13
	500 ppm	11.75	12.5	10.25	11.5
	250	10.0	13.0	10.0	24.0
	Positive control	24.0	27.0	20.00	Nil
	Negative control	Nil	Nil	Nil	NIL
S. typhimurium	7000 ppm	16.25	17.00	17.0	16.5
	6000 ppm	17.50	17.00	17.25	15.5
	5000 ppm	16.5	17.50	16.0	12.50
	4000 ppm	15.0	15.30	15.75	14.00
	3000 ppm	15.5	14.50	14.25	16.0
	2000 ppm	14.0	14.00	14.0	16.0
	1000 ppm	13.25	13.50	12.75	15.5
	500 ppm	12.25	12.00	12.50	13.75
	250	12.25	11.75	12.0	22.00
	Positive control	21.00	29.00	22.0	Nil
	Negative control	Nil	Nil	Nil	

acetone and 18.75 mm in methanol solvent extract. The minimum inhibition was observed at 250 ppm of (10.0 mm) in methanol solvent and 13.0 mm in acetone solvent. The negative control (Solvent) showed no inhibition whereas positive control (Antibiotic, chloramphenicol) showed an inhibition of (24.0 mm) in methanol and 27.0 mm in acetone.

In case of *S. typhimurium,* maximum inhibition 17.50 mm was observed at 6000 ppm in methanol solvent extract and at 5000 ppm in acetone extract of (17.50 mm). As the concentration increases to 7000 ppm inhibition decreases to (16.25 mm) in methanol solvent extract but in case of acetone decreases to (17.0 mm) at 6000 ppm and remain same at 7000 ppm. Least inhibition was observed at 500 ppm in methanol of (12.25 mm) and (11.75 mm) in acetone. The negative control (Solvent) showed no inhibition whereas positive control (Antibiotic, chloramphenicol) showed an inhibition of (21.0 mm) in methanol and (29.0 mm) in acetone as showed in Table 1.

Paper disc diffusion method

In case of *S. aureus*, methanol showed maximum inhibition of (16.50 mm) at 6000 ppm and (16.0 mm) acetone solvent extract and as the concentration increases to 7000 ppm its inhibition decreases to (15.25 mm) in methanol and 15.5 mm in acetone solvent extract. The minimum inhibition was observed at 250 ppm of (12.0 mm) in methanol solvent and 13.75 mm in acetone solvent. The negative control (Solvent) showed no inhibition whereas positive control (Antibiotic, chloramphenicol) showed an inhibition of (20.0 mm) in methanol and 24.0 mm in acetone. In case of *S. typhimurium,* maximum inhibition observed at 6000 ppm of (17.25 mm) in methanol solvent extract and at 5000 ppm in acetone extract of (17.50 mm). As the concentration increases to 7000 ppm inhibition decreases to (16.0 mm) in methanol solvent extract but in case of acetone it increases up to 7000 ppm of 16.5 mm. Least inhibition was observed at 500 ppm in methanol of (12.25 mm) and (11.75 mm) in acetone. The negative control (Solvent) showed no inhibition whereas positive control (Antibiotic, Chloramphenicol) showed an inhibition of (22.0 mm) in methanol and (22.0 mm) in acetone as showed in Table 1.

GC-MS analysis

During GC-MS analysis of crude extract of *S. platensis,* it

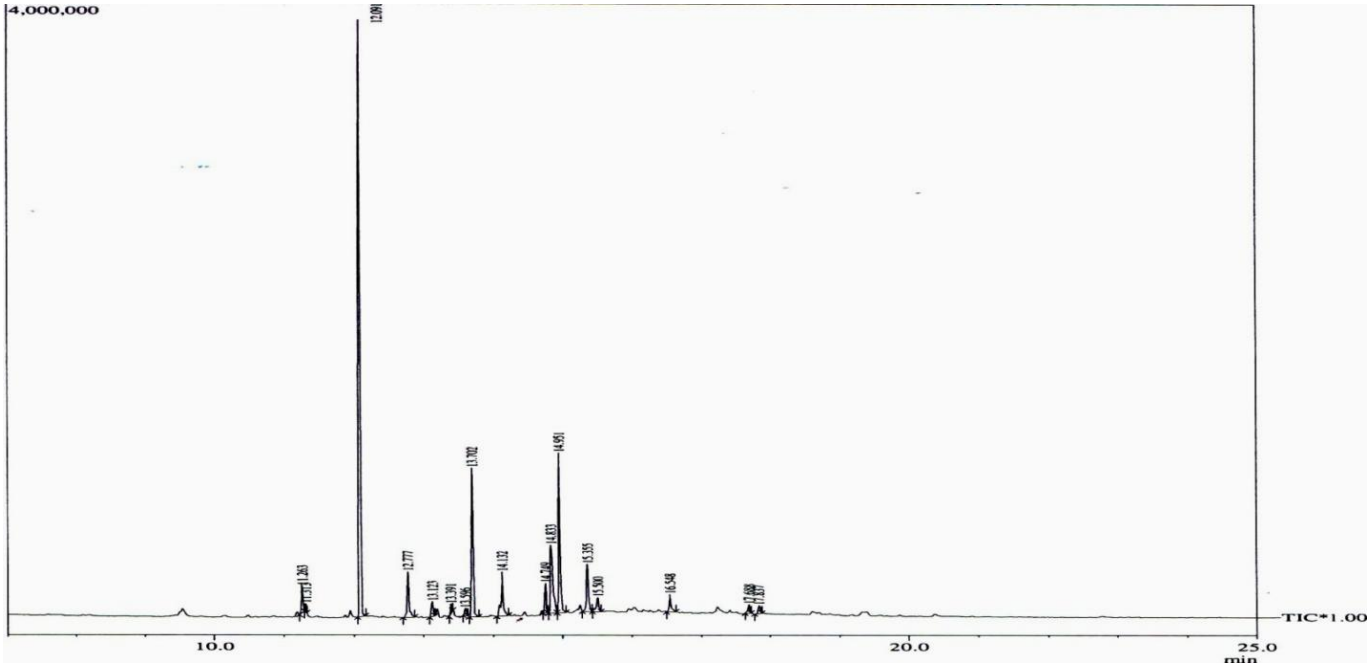

Figure 1. GC-MS Spectrum of acetone extract of *Spirulina platensis.*

was observed that acetone showed 17 (1-Hexadecene, 1-Hexadecene, Heptadecane, Heptadecane, Eicosane, 1-Octadecene, 2,6,10-Trimethyl, 14-Ethylene1, 4-Pentadiene, Allyl N-Octyl Ether, Methyl 9-Octadecenoate, Hexadecanoic Acid Methyl Ester, E-15-Heptadecenal,Hexadecatrienoic Acid, Methyl Ester, 10,13-Octadecadienoic Acid, Methyl, Phytol, 2-Bromopropionic Acid, Pentadecyl Ester,Alpha.-D-Glucopyranoside, Methyl 2,3,4,1-Docosene, 1,2-Benzenedicarboxylic Acid, 1-Tetradecanol) compounds (Figure 1) and Methanol extract showed 16 (1-Tridecene, Sulfurous acid, 2-ethylhexyl isohexyl ester, Heptadecene, 1-Pentadecene, Octane, 9-Octadecenoic acid (Z), Methyl Ester, Hexadecanoic acid, Methyl Ester, 3-Eicosene, (E)-,6,9,12-Octadecatrienoic acid, 11,14-Eicosadienoioc acid, Methylester, Oxirane, 1,2,3,4-Tetrakis 0 (Trimethylsilyl), 2-Undecene, 9-methyl, Tetracosanoicacid, methyl ester,1,2-benzenedicarboxylic acid) (Figure 2) active compounds associated with the antibacterial properties. Some of the compound is present in both the solvents showed in Table 2 and 3. The maximum amount of compound present is Eicosane of 42.11% which are responsible for antibacterial activity because it is a fatty acid.

DISCUSSION

The antibacterial activity of algal compounds extracted from algae depends upon the type of solvent used for extraction. The present study revealed that the use of organic solvents in the preparation of algal extracts provide more consistent antimicrobial activity. This observation clearly indicates that the polarity of antimicrobial compounds make them more readily extracted by organic solvents and using organic solvent does not negatively affect their bioactivity against antibacterial and antifungal species. Many investigations mentioned that the methanol extracts of *Nostoc muscorum* revealed antibacterial activity on *Sclerotinia sclerotiorum* by Ishida et al. (1997). Also the methanolic extract of a blue green alga has been investigated by Kumar et al. (2006) for *in vitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger, Aspergillus* flavus and *Rhizopus nigricans* using agar cup diffusion method. The antimicrobial activity of methanolic extract of *S. platensis* was also explained by Demule et al. (1996) due to the presence of γ- Linolenic acid and compound was also present in the methanol extract in the present study as observed by GC-MS analysis. Previous publications reported that the compounds such as 1-Octadecene, 1-Heptadeceane were found in both algae and plants show anticancer, antioxidant and antimicrobial activity (Lee et al., 2007; Mishra and Shree 2007). Antimicrobially active lipids and active fatty acids are present in a high concentration in this alga. It was hypothesized by Lampe et al. (1998) that lipids kill microorganisms by leading to disruption of the cellular membrane as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible

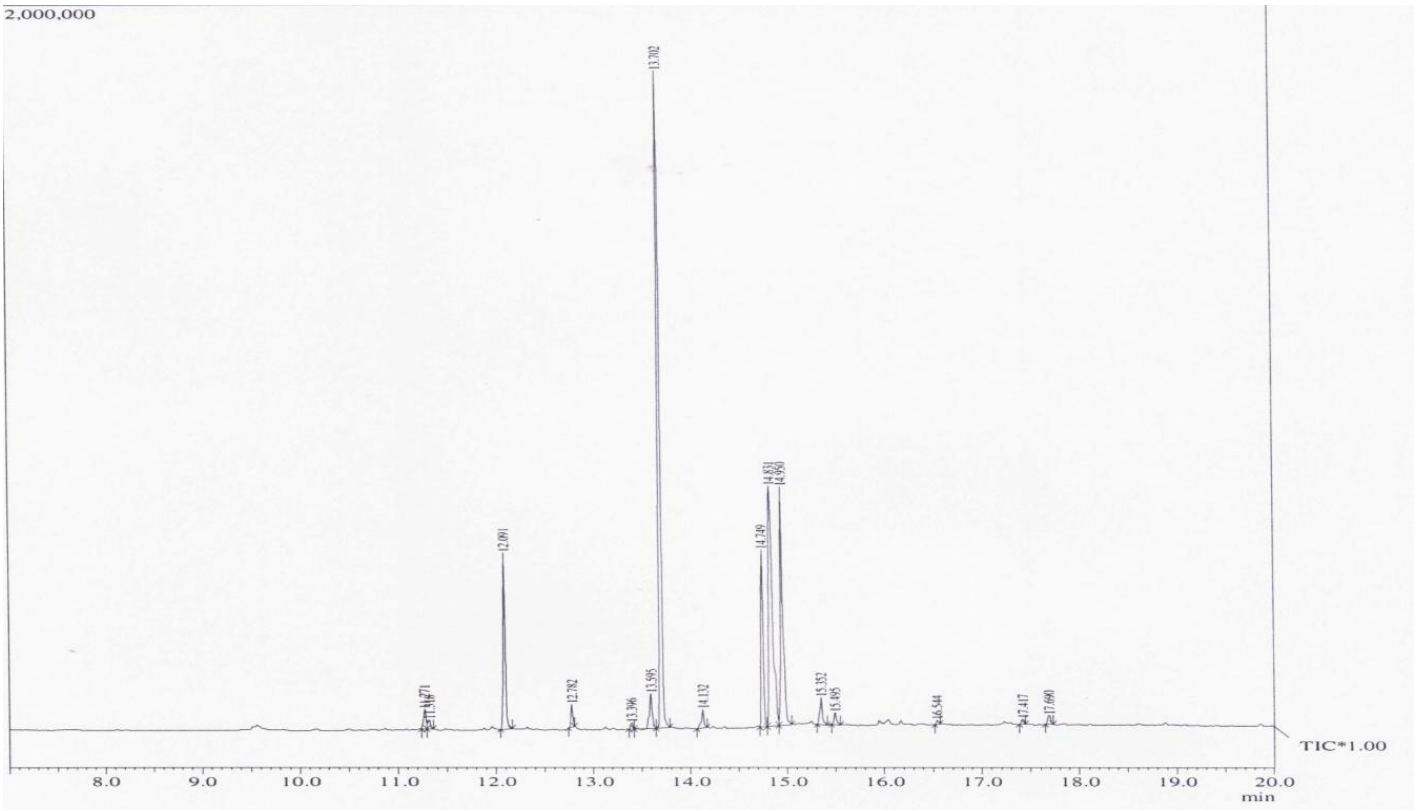

Figure 2. GC-MS Chromatogram of crude extracts of *Spirulina platensis*.

Table 2. GC-MS analysis of different compounds in acetone extract of *Spirulina platensis.*

Peak	R. Time	Area	Area (%)	Name
1	11.263	307359	2.20	1-Hexadecene
2	11.313	78489	0.56	Heptadecane
3	12.091	5872713	42.11	Eicosane
4	12.777	588557	4.22	1-Octadecene
5	13.123	163985	1.18	2,6,10-Trimethyl,14-Ethylene1,4-Pentadiene
6	13.391	88099	0.63	Allyl N-Octyl Ether
7	13.596	83299	0.60	Methyl 9-Octadecenoate
8	13.702	1467801	10.53	Hexadecanoic acid, Methyl Ester
9	14.132	627607	4.50	E-15-Heptadecenal
10	14.749	286274	2.05	Hexadecatrienoic acid, Methyl Ester
11	14.833	1235624	8.86	10,13-Octadecadienoic acid, Methyl
12	14.951	1789267	12.83	Phyto
13	15.355	6322269	4.53	2- Bromopropionic acid, Pentadecyl Ester
14	15.500	240224	1.72	.Alpha.-D-Glucopyranoside, Methyl 2,3,4
15	16.548	256602	1.84	1-Docosene
16	17.688	118699	0.85	1,2-Benzenedicarboxylic acid
17	17.837	108176	0.78	1-Tetradecanol

changes and reach the bacterial membrane leading to its disintegration. Present investigations is contradictory with the results of other studies (Kaushik and Chauhan, 2008; El-Baky et al., 2008) may be due to the production of bioactive compounds related to the seasons, method, organic solvents used for extraction of bioactive

Table 3. GC-MS analysis of different compounds in methanol extract OF *Spirulina platensis.*

Peak	R. time	Area	Area (%)	Name
1	11.271	106819	1.31	1-Tridecene
2	11.318	41868	0.51	Sulfurous acid,2-Ethylhexyl Isohexyl Ester
3	12.091	762878	9.37	Heptadecene
4	12.782	91362	1.12	1-Pentadecene
5	13.396	27954	0.34	Octane
6	13.595	196469	2.41	9-Octadecenoic acid (Z), Methyl Ester
7	13.702	2688125	33.01	Hexadecanoic acid, Methyl Ester
8	14.132	95738	1.18	3-Eicosene, (E)-
9	14.749	721661	8.86	6,9,12-Octadecatrienoic acid
10	14.831	1875273	23.03	11,14-Eicosadienoioc acid, Methyl Ester
11	14.950	1249522	15.35	Phytol
12	15.352	138676	1.70	Oxirane
13	15.495	57437	0.71	1,2,3,4-Tetrakis-0-(Trimethylsilyl)
14	16.544	17590	1.22	2-Undecene, 9-Methyl
15	17.417	25200	1.31	Tetracosanoic acid, Methyl Ester
16	17.690	45750	0.56	1,2-Benzenedicarboxylic acid

compounds.

Conclusion

It is concluded from the study that extracts of algal strain used in the present investigation showed better antibacterial activity against the pathogens used, but further researches should be made to identify and purify natural product against antibacterial and antifungal. The enhanced antibacterial activity expressed in sequential extraction might be due to the fact that both hydrophobic and hydrophilic bioactive compounds were extracted. An improved knowledge of the composition, analysis, and properties of *S. platensis* with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application of this cyanobacteria.

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REFERENCES

Ishida K, Matsuda H, Murakami M, Yamaguchi K (1997). Kawaguchipeptin B. and antibacterial cyclic undecapeptide from the cyanobacterium *Microcystis aeruginosa.* J. Nat. Prod., 60: 724-726.

Kumar P, Angadi S, Vidyasagar G (2006). Antimicrobial activity of blue Green and green algae. Ind. J. Pharm. Sci., 68: 647-648.

El-Baky H, Baz K, Baroty G (2008). Characterization of nutraceutical compounds in blue green alga *Spirulina maxima*, J. Med. Pl. Res., 2: 292-300.

Kaushik P, Chauhan A (2008). In vitro antibacterial activity of laboratory grown culture of *Spirulina platensis*. Ind. J. Microbial., 4: 348-352.

Knubel G, Larsen LK, Moore RE (1990). Cytotoxic antiviral indolocarbobazoles from a blue-green alga belonging to Nostocales. J. Antibiot., 43: 1236-1239.

Mule MCZ, Caire GZ, Cano MS, Halperin DR (1991). Bioactive compounds from *Nostoc musorum* (cyanobacteria). Cytobios., 66: 169-172.

Borowitzka MA (1995). Microalgae as source of pharmaceuticals and other biologically active compounds. J. Appl. Phycol., 7: 3-15.

Gerwork WH, Roberts MA, Proteau PJ, Chen JL (1994). Screening cultured marine microalgae for anticancer type activity. J. Appl. Phycol., 6:143-149.

Jaki B, Orjala J, Sticher O (1999). A novel extracellular diterpenoid with antibacterial activity from the cyanobacteria *Nostoc commume*, J. Nat. Prod., 62: 502-503.

Shanmuga PK, Gnanamani A, Radhakrishnan N, Babu M (2002). Antimicrobial activity of *Datura alba.* Indian Drugs, 39: 113-116.

Okigbo RN, Mbajiuka CS, Njoku CO (2005). Antimicrobial potentials of (UDA) *Myopias aesthetical* and *Acetum gratissimum* L. on some pathogens of man. Int. J. Mole. Med. Adv. Sci., 1: 392-397.

Demule MCZ, Decaire GZ, Decano MS (1996). Bioactive substances from S*pirulina platensis.* Int. J. Exp. Biol., 58: 93-96.

Lampe MF, Ballweber LM, Isaacs CE, Patton DL, Stamm WE (1998). Killing of *Chlamydia trachomatis* by novel antimicrobial lipids adapted from compounds in human breast milk. Antimicro. Agents Chemo., 45: 1239-1244.

Lee YS, Kang MH, Cho YS, Jeong CS (2007). Effects of constituents of *amomum xanthioides* on gastritis in rats and on growth of gastric cancer cells. Arch. Pharm. Res., 30: 436-443.

Mishra PM, Sree A (2007). Antibacterial Activity and GCMS Analysis of the Extract of Leaves of *Finlaysonia obovata* (A Mangrove Plant). Asi. J. Pl. Sci., 6: 168-172.