STUDIES ON THE SECONDARY METABOLITES AND ANTIMICROBIAL ACTIVITY OF CYANOBACTERIA FROM SOUTH EAST COAST OF INDIA.

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Abstract : In the present investigation suggested that the qualitative bioactive compounds analysis from microalgae *Stigonema* sp. and *Spirullina* sp. with methanol and hexane were individually estimated. The bioactive compounds like Alkaloids, Anthraquinone, Amino acid, Carbohydrate, Flavonoids, Phenols, Protein, Steroids, Saponin, Tannin and Terpenoids were qualitatively analysed from *Stigonema* sp. whereas *Spirulina* sp. was Alkaloids, Amino acid, Carbohydrate, Steroids, Saponin, Tannin and Terpenoids presented in methanolic extract but anthraquinone flavonoids and saponins were absent. The quantitative bioactive compounds like alkaloids aminoacid, carbohydrate, flavonoids, protein steroids, tannin and terpenoids were represented in methanolic and hexane solvent. The methanolic extract of *Stigonema* sp. and *Spirulina* sp. were individually maximum produced when compared with hexane solvent. The screening of microalgae by the effect of antibacterial properties of stigonema sp with different concentration of 25, 50, 75 and 100 µl extract were treated with *Bacillus cereus, Klebsiella pnemoniae, proteus vulgaris, Pseudomonas aeroginosa* and *Staphylococcus aureus* bacteria and 100 µl concentration of *Stigonema* sp. was excellent zone inhibition observed. Whereas hexane extract also moderate zone of inhibition were performed with respective clinical bacteria. The effective antibacterial activity of *Spirulina* sp. higher concentration of methanolic extract was minimum zone of inhibition against some clinical bacteria respectively. The evaluation of antifungal activity of *Stigonema* sp. and spirulina sp with methanol and hexane solvent were performed. The microalgae *Stigonema* sp. was extraordinary antifungal activity than *Sigonema* sp.

Index Terms: *Cyanobacteria, Bioactive compounds, Antimicrobial activity, Stigonema sp. and Spirulina sp*.

I.INTRODUCTION

Cyanobacteria are nature's unique gift to mankind, as they possess several innate properties that make them ideal organisms with potential for multifaceted biotechnological applications. They are large and

morphologically diverse group of unique photosynthetic organisms of great importance because of their very long existence for well over 3.5 billion years and cosmopolitan distribution in terrestrial, freshwater and marine habitats. Cyanobacteria, the blue green algae are an assemblage of gram negative eubacteria widely distributed throughout the world. Cyanobacteria are rich sources of structurally novel and biologically active metabolites. Recent studies indicate the presence of some bioactive compounds in the freshwater blue green algae which are shown to exhibit anticancer, antimicrobial, antifungal, anti-inflammatory and other pharmacological activities[1&2].

Biologically active substances were proved to be extracted from microalgae and various strains of cyanobacteria are known to produce various strains of cyanobacteria are known to produce diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity. Temperature, pH, incubation period and light intensity are the important factors influencing the production of antimicrobial agents[3]. The *invitro* antimicrobial activity of cell extracts of various cyanobacteria against some selected Gram positive, Gram-negative bacteria and pathogenic fungi.

II. MATERIAL AND METHODS

Preparation of cyanobacterial culture crude extracts

The cyanobacterial culture was harvested after 30 days of growth by centrifugation at 5000 rpm for 15 minutes. Then the algal pellet was collected, weighed and used for extraction. Twenty five gram of dried powder of *Stigonema* sp. and *Spirulina* sp. individually was extracted in 20 ml hexane and methanol to get extract compounds with increasing polarity by shaking overnight for complete extraction was preserved at 4 ºC until it use for further studies.

Qualitative and quantitative bioactive compounds

Preliminary bioactive compounds were carried out for the extract as per standard methods described[4], Bioactive compounds screening was carried out to assess the qualitative chemical composition of crude extracts of *Stigonema* sp. and *Spirulina* sp. individually with hexane and methanol solvents using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as alkaloids, anthraquinone, amino acid, carbohydrate, flavonoids, phenols, protein, reducing sugars, steroids, saponin, tannin and terpenoids. General reactions in these analyses revealed the presence or absence of these compounds in crude extract were tested.

Antimicrobial screening activity

Antimicrobial activity of various solvent extracts of *Stigonema* sp. and *Spirulina* sp. was carried out by agar well diffusion method. Bacteria and fungi were used as test organisms. Pure bacterial cultures were

Bacillus sp. *Klebsiella pnemoniae, Protease* sp., *Pseudomonas aeroginosa* and *Staphylococcus aureus* and fungal cultures like *Aspergillus flavus, Aspergillus fumigatus, A.ochraceus, A. terreus* and *Trichoderma viride* was introduced for antimicrobial study. The sterilized Nutrient Agar (NA) and Potato Dextrose Agar (PDA) medium were poured into Petri dishes were allowed to cool and solidify and then 100 µl of bacterial and fungal suspension were spread on NA and PDA plates with a lawn of cultures separately. Plates were incubated for bacteria at 37 ˚C for a period of 24 hrs and for fungi at 27 ˚C for a period of 48b hrs. At the end of incubation period, the zone of inhibition was measured.

III. RESULTS

In the current investigation stated that the qualitative and quantitative analysis of bioactive compounds from *Stigonema* sp. and *Spirulina* sp. were performed with two different solvents like methanol and hexane for extraction of microalgae. The methanolic extraction of bioactive compounds like alkaloids, aminoacid ,carbohydrate, flavonoids, protein, steroids, tannin and terpeniods were strongly indicated when compared with hexane extraction of bioactive molecules (Table 1).

The microalgae of *Stigonema* sp. was extraordinary quantity production of compounds than the *Spirulina* sp. However, the microalgae is very important microorganisms for the source of product among the other microbes (Table 2).

In the recent study of effect of antibacterial properties were *invitro* experimentally analysed against bacteria *Bacillus cereus, Klebsiella pnemoniae, Proteus vulgaris, Pseudomonas aeroginosa* and *Staphylococcus aureus* and fungi like *Aspergillus.flavus, A.fumigatus, A. ochraceus, A. terreus* and *Trichoderma viride* introduced. Among the bacteria inhibition in *Bacillus* sp was maximum suppression than the other bacteria in both microalgae and also higher concentration was excellent inhibition when compared with low concentration of extract. Whereas fungi the *T. viride* was maximum inhibition than the other fungi due to the bioactive compounds has enormous antifungal properties were proved from the experiments. (Table 3, 4, 5 and 6).

Table 1. Qualitative analysis of bioactive compounds from microalgae

Name of the bioactive compounds	*Stigonema* sp.		*Spirulina* sp.	
	Methanol	Hexane	Methanol	Hexane
Alkaloids	+	+	+	+
Anthraquinone	-	-	-	-
Amino acid	+	+	+	+
Carbohydrate	+	+	++	+
Flavonoids	+	+	+	+
Phenols	-	-	-	-
Protein	++	+	+	+

Steroids	+	+	+	+
Saponin	-	-	-	-
Tannin	++	+	+	+
Terpenoids	+	-	+	-

Absent (-), Present (+), Strongly present (++)

Table 2. Quantitative analysis of bioactive compounds from potential microalgae

Name of the bioactive compounds	**Quantity (ug/ml)**			
	***Stigonema* sp.**		***Spirulina* sp.**	
	Methanol	**Hexane**	**Methanol**	**Hexane**
Alkaloids	0.99±0.08	0.91±0.03	0.45±0.04	0.34±0.02
Amino acid	0.98±0.07	1.68±0.48	1.12±0.14	0.98±0.07
Carbohydrate	0.88±0.04	1.75±0.02	1.12±0.74	1.09±0.28
Flavonoids	0.78±0.14	0.24±0.17	0.44±0.98	0.24±0.74
protein	1.54±0.03	0.42±1.08	0.20±0.98	0.74±0.08
Steroids	1.09±1.14	0.98±0.14	0.13±0.14	0.12±0.12
Tannin	1.14±0.04	1.02±0.08	1.04±0.12	0.64±0.09
Terpenoids	0.78±0.08	-	0.67±0.06	-

Standard deviation ± error

Table 3. Screening of microalgae by the effect of antibacterial activity of *Stigonema* sp. against bacteria

Name of the bacteria	**Zone of inhibition (mm)**							
	Methanol				**Hexane**			
	25µl	**50µl**	**75µl**	**100µl**	**25µl**	**50µl**	**75µl**	**100µl**
Bacillus cereus	6.67± 1.89	8.00± 2.67	10.00± 3.34	11.00±3 .67	6.00± 2.00	6.00± 2.00	8.00± 2.67	10.00± 3.34
Klebsiella pnemoniae	3.00± 1.00	5.67± 1.89	05.68±1 .89	07.00±2 .34	3.00±1 .00	4.00± 1.34	3.34± 1.12	04.34±1. 45
Proteus vulgaris	4.00± 1.34	5.67± 1.89	07.00±2 .34	08.00±2 .67	2.34±0 .78	3.00± 1.00	3.67± 1.23	04.67±1. 56
Pseudomonas aeroginosa	4.02± 1.32	5.34± 1.78	06.34±2 .12	10.00±3 .34	2.00±0 .67	3.00± 1.00	3.34± 1.12	04.67±1. 56
Staphylococcus aureus	3.00± 2.00	0.06±2.0 0	07.00±2 .34	08.00±2 .67	3.00±0 .67	2.00± 0.67	4.00± 1.34	03.67±1. 23

Standard deviation ± error

Table 4. Screening of microalgae by the effect of antibacterial activity of *Spirulina* sp.

Name of the bacteria	Zone of inhibition (mm)							
	Methanol				Hexane			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
Bacillus cereus	7.00± 2.31	5.00± 1.67	6.00± 2.00	7.67± 2.56	2.00± 0.67	2.67± 0.89	3.07± 1.23	3.17± 1.25
Klebsiella pnemoniae	2.67± 0.89	3.00± 1.00	4.00± 1.34	5.00± 1.67	1.67± 0.56	3.00± 1.00	3.67± 1.23	4.00± 1.34
Proteus vulgaris	3.00± 1.00	4.00± 1.34	5.00± 1.67	6.00± 2.00	1.34± 0.45	1.34± 0.45	2.67± 0.89	3.67± 1.23
Pseudomonas aeroginosa	2.00± 0.67	2.00± 0.67	2.67± 0.87	4.00± 1.34	2.00± 0.67	1.34± 0.45	3.00± 1.00	3.67± 1.23
Staphylococcus aureus	3.00± 0.01	2.00± 0.67	3.67± 1.23	5.00± 1.67	0.00± 0.00	0.00± 0.00	0.00± 0.00	3.34± 1.12

Standard deviation ± error

Table 5. Effect of antifungal activity of *Stigonema* sp. against fungi

Name of the fungi	Zone of inhibition (mm)							
	Methanol				Hexane			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
Aspergillus.flavus	1.67±0.56	2.00±0.67	2.00± 0.67	3.00± 1.00	1.67± 0.56	1.67± 2.56	2.00± 0.67	4.00± 1.34
A. fumigatus	0.00±0.00	0.00±0.00	2.00± 0.67	1.67± 0.56	2.00± 0.67	2.00± 0.67	3.00± 1.00	3.67± 1.23
A.ochraceus	1.67±0.55	2.00±0 67	2.00± 0.66	3.00± 1.00	2.00± 0.67	2.00± 0.67	3.00± 1.00	4.00± 1.34
A. terreus	2.00±0.67	2.00±0 67	2.67± 0.89	3.34± 1.12	2.67± 0.89	3.00± 1.00	3.34± 1.12	4.00± 1.34
Trichoderma viride	5.34±1.78	6.00±2 .00	7.67± 2.56	8.00± 2.67	1.67± 0.56	2.00± 0.67	3.00± 1.00	3.00± 1.00

Standard deviation ± error

Table 6. Effect of antifungal activity of *Spirulina* sp. against fungi

Name of the fungi	Zone of inhibition (mm)							
	Methanol				Hexane			
	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
Aspergillus flavus	0.00± 0.00	2.00± 067	2.00± 0.67	4.00± 1.34	1.34± 0.45	1.67± 0.56	2.00±0 .67	2.00±0. 67
A.fumigatus	2.00± 0.67	3.00± 1.00	3.34± 1.12	8.00± 2.66	2.00± 0.67	3.00± 1.00	3.66±1 .22	4.00±1. 34
A.ochraceus	1.67± 0.56	2.00± 0.67	3.00± 1.00	7.34± 2.45	2.34± 1.12	2.00± 1.34	3.00±1 .34	5.00±1. 67
A.terreus	1.67± 0.56	2.00± 0.67	2.00± 6.67	3.00± 1.00	3.67± 1.23	3.34± 1.12	3.34±1 .12	4.00±1. 34
Trichoderma viride	3.67± 1.22	4.00± 1.34	4.67± 1.56	6.34± 2.12	-	-	-	-

Standard deviation ± error

IV. DISCCUSSION

The bioactive analysis of acetone, methanolic, etheric, dichloromethalonic and hexanic extracts of *Spirulina platensis and Chlorella pyrenoidosa* revealed the presence of flavanoids, saponins, tannins, carbohydrates, phenolics, terpenes and cardiac glycosides. Steroids and alkaloids were absent in all the extracts. Tannin, sterols, terpenoids and quinonic substances were absent in all the extract. Phenolic compounds and flavonoids were present in all the extract. Alkaloids are present only in acetonic and methanolic extracts[5].

In addition, the highest value of total flavonoid was noted in *Chlorella* (37.12± 0.94 mg/g) then *Spirulina* (15.35± 0.54 mg/g). The higher concentration of phycocyanin was in *S. platensis* sample and the higher concentration of Chlorophyll in *Chlorella.* These results are agreement with those reported[6] in which they observed that *Chlorella* sp. and *Scenedesmus obliquus* presented higher phenolic and carotenoid contents. Microalgae contain a variety of phenolic classes but they were different from many other plant species like vegetables, fruits and medicinal plants. The microalgae could contain different antioxidant compounds compared to other plants[7].

The antibacterial activity of microalgae and cyanobacterial species,[8] isolate an antibacterial substance from *Chlorella* sp was followed by *invitro* antimicrobial activity along with biomass production in waste water by cyanobacteria, *Spirulina platensis*[9] antibacterial activity of two blue-green algae against pathogenic bacteria, *Proteus vulgaris, Bacillus cereus, E. coli*[10]. Blue-green algae against pathogenic bacteria *Staphylococcus*

aureus[11], *Phormidium*, and *Lyngbya* extracts against pathogenic bacteria *Staphylococcus aureus, S. epidermis, Bacillus cereus, B. bravis*[12].

The increasing resistance of pathogenic bacteria against a significant number of antibiotics, with consequences for human health, has been a great concern for the past decades and has forced the efforts to find new antibacterial substances[13,14&15]. Some bacteria may infect and cause serious diseases in humans and some others can also provoke foodborne illness inducing moderate to severe nausea, vomiting and diarrhea which demonstrated the activity of the green alga *Chlorella* against several Gram-positive (G+) and Gram-negative (G^-) bacteria [9] the interest for antibacterial compounds from microalgae has been identified.

Large screening programs have thus been conducted to assess the potential antibacterial activity of various microalgal extracts against pathogenic and foodborne bacteria. Numerous microalgal species from distinct taxonomical groups originating from various areas[16&17] mainly from marine environment[18&19], but also from freshwater environment[20&21] or even from the soil[22] were showed to have potent antibacterial activity against both (G+) and (G^-) bacteria. As screening studies can sometimes include hundreds of different microalgae[23,24&20], only presents the microalgae with the highest antibacterial activity or the wider spectrum of activity from these screenings.

V. CONCLUSION

It can be concluded that the analysis of bioactive compounds from cyanobacteria and its importance of extraordinary performance against human pathogens. The cyanobacteria are important components of the ecosystem and theirs distribution may indicate the health of the environment and contributing to the society.

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