ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 5, pp.3859-3863, May, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF SOIL CYANOBACTERIA *CYLINDROSPERMUM MAJUS*

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ARTICLE INFO

Article History:

Received 5th, April, 2015
Received in revised form 12th, April, 2015
Accepted 6th, May, 2015
Published online 28th, May, 2015

Key words:

Cyanobacteria, *Cylindrospermum majus*, solvent extracts, antimicrobial activity

ABSTRACT

The main objective of this study was to test the antimicrobial activity of various solvent extracts (Aqueous, Chloroform, Ethyl acetate, Hexane and Methanol) of cyanobacterium, *Cylindrospermum majus* (Kutzing ex Born. et Flah.) against four pathogenic bacteria, in which two are Gram-positive *Bacillus subtilis* (MTCC-1427), *Staphylococcus aureus* (MTCC-1430) and two are Gram-negative *Escherichia coli* (MTCC-1302), *Klebsiella pneumoniae* (MTCC-4030) and four fungal pathogens of *Aspergillus fumigatus* (MTCC-4163), *Aspergillus niger* (MTCC-4325), *Mucor* sp. (MTCC- 3340) and *Trichophyton mentagrophytes* (MTCC-8476**).** The cyanobacterial strain *C. majus* was collected from the soil samples of paddy fields of Warangal district, India, and maintained in (BG-11 N^-) medium. Antimicrobial activity was determined by agar disc diffusion method, in which the culture extracts of *C. majus* was exhibited with potential activity against bacterial and fungal growth by expressing various zone of inhibitions. The present results indicates that the culture crude extract of *C. majus* was shown with significant antibacterial activity (17.33 mm) in the solvent of Chloroform against *K. pneumoniae* and in fungal activity the Aqueous extract showed maximum inhibition zone (15.33 mm) against *A. fumigatus* under observation. Thus, the genus *Cylindrospermum majus* proved to be more potential in the bioassay studies against selected bacteria and fungi.

INTRODUCTION

Cyanobacteria also known as blue-green algae, cyanoprokaryotes and cyanophytes, are oxygenic photosynthetic prokaryotes that possess features familiar to both bacteria (prokaryota) and algae (eukaryota). Their special structure and chemical composition of the cell wall are basically the same as those of Gram-negative bacteria. Harmful algal blooms have increased worldwide in fresh, estuarine and coastal marine waters (Smayda, 1990; Hallegraeff, 1993; Van Dolah, 2000; Allen *et al*., 2006). Cyanobacterial metabolites show an interesting and exciting range of biological activities ranging from antimicrobial, anticancer, antiviral, immunosuppressant, insecticidal, anti-inflammatory to proteinase-inhibiting activities which are striking targets of biomedical research (Borowitzka, 1995; Kulik, 1995; Soltani *et al.,* 2005; Tan, 2007; Wase and Wright, 2008; Gerwick *et al*., 2008; Shweta Yadav *et al*., 2011; Jyoti Bala Chauhan *et al*., 2014; Sachin Chauhan *et al*., 2014; Pandey, 2015).

Cyanobacteria are rich source of structurally novel and biologically active metabolites, which are shown to exhibit antibacterial (Ghasemi *et al.,* 2003), antifungal, anticancer or cytotoxic (Kwan *et al*., 2010), antimalarial (Linington *et al*., 2007) and other pharmacological activities. Antimicrobial effects from cyanobacterial aqueous and organic solvent extracts are visualized in bioassays by using selected human pathogens as test organisms (Falch *et al*., 1995). Secondary metabolites with antibacterial activity are widely produced by cyanobacteria. These compounds are effective against Gram-positive and Gram-negative bacteria; however, it has been found that the antibacterial activity of cyanobacteria is mainly directed against Gram-positive bacteria since most Gram-negative bacteria are resistant to toxic agents in the environment due to the barrier of lipopolysaccharides on their outer membrane. Several bioactive metabolites produced by cyanobacteria and algae have been discovered by screening programs, employing target organisms quite un-related to those for which the metabolites evolved (Smith and Doan, 1999). Many cyanobacteria produce compounds are generally considered to be secondary metabolites that compounds are not essential for general metabolites or growth of the organism and are present in restricted taxonomic groups. Cyanobacteria like *Microcystis*, *Nostoc*, *Anabaena* and *Oscillatoria* turn out an excellent kind of secondary metabolites. A variety of vital marine cyanobacterial molecules together with Dolastatin 10, Cryptophycins and Curacin A are discovered and these were either in diagnosing or clinical testing as anticancer agents (Newman and Cragg, 2004). Many secondary metabolites are potent toxins, causing health problems for humans and animals when the producer organisms occur in masses in water bodies. Recently, some of the researchers were also studied on certain members of cyanobacteria with reference to their antimicrobial

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activity (Digamber Rao *et al*., 2010; Digamber Rao *et al*., 2011; Malathi *et al*., 2014 and Digamber Rao *et al*., 2015).

MATERIALS AND METHODS

Collection of samples

Soil samples of cyanobacterium, *Cylindrospermum majus* (Kutzing ex Born. et Flah.) was collected from various locations of Warangal district. All the samples were brought to laboratory in plastic vials and washed with distilled water to prevent potential contaminants.

Isolation and culture conditions

Samples were isolated, identified and photo-graphed under Olympus system attached digital microscope. The cyanobacterium was cultured in a 250 mL flask containing 100 mL of BG-11 (N^-) medium without shaking, for 30 days. The incubation temperature was 28 ± 2°C and illumination at 3000 Lux with a white continuous light and a regime of 16 hr light / 8 hr dark. The cultures were harvested after 30 days by centrifugation at 5000 rpm for 15 min.

Identification of cyanobacteria

For identification of cyanobacterium at generic and species level, the schemes and characters proposed by Desikachary (1959), Pandey (1965), Tiwari (1972), Anand (1989) and Santra (1993) were used.

Preparation of cyanobacterial culture crude extracts

The cyanobacteria 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. 0.5 gram of dried powder of *C. majus* was extracted in 20 ml of Aqueous, Chloroform, Ethyl acetate, Hexane and Methanol to get extract compounds with increasing polarity by shaking overnight for complete extraction. The extracts were filtered and the filtrates were evaporated under reduced pressure at 37-40ºC and the concentration was adjusted for the resultant dried crude extract 1 mg was weighed and dissolved in 1 ml of Dimethyl sulfoxide (DMSO) as stock solution and it was preserved at 4 ºC until it use for further studies. For the bioassay study 50 µg/ml concentration of crude cyanobacterial extract was taken.

Antimicrobial screening activity

Antimicrobial activity of various solvent extracts of *C. majus* was carried out by agar disc diffusion method. In the present study the following bacteria and fungi were used as test organisms. Pure bacterial cultures, *Bacillus subtilis* (MTCC-1427), *Staphylococcus aureus* (MTCC-1430), *Escherichia coli* (MTCC-1302) and *Klebsiella pneumoniae* (MTCC- 4030) and fungal cultures, *Aspergillus fumigatus* (MTCC- 4163), *Aspergillus niger* (MTCC- 4325), *Mucor* sp. (MTCC- 3340) and *Trichophyton mentagrophytes* (MTCC- 8476**)** were obtained from Department of Microbiology, Kakatiya University, Telangana State. The sterilized Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) medium were poured into Petri dishes were allowed to cool and solidify and then 100 µl of bacterial and fungal suspension were spread on MHA and SDA plates with a lawn of cultures. Filter paper discs (6 mm) saturated with 50 µl of the crude extracts dried and placed on Muller Hinton Agar (Bacteria) and Sabouraud Dextrose Agar (Fungi) plates. Plates were incubated for bacteria at 37 ^{0}C for a period of 24 hrs and for fungi at 27 ^{0}C for a period of 48-72 hrs. In the present study Ciprofloxacin 10 µg/disc for bacteria and Nystatin 50 µg/disc for fungi were used as standard control.

At the end of incubation period, the zone of inhibition around the paper disc (6 mm), including (diameter of inhibition zone plus diameter of the disc) the disc was calculated and expressed in millimeter (mm) and compared with standard control of Ciprofloxacin (bacteria) and Nystatin (fungi). The various extracts containing antimicrobial components produced distinct, clear, circular zones of inhibition around the discs and the diameters of clear zones were determined and used as an indication of antimicrobial activity. All tests were performed in aseptic condition in triplicates and their mean and standard errors were presented.

Statistical analysis

The results of the data were statistically analysed and the values are mean ± standard error (SE) of the three measurements (N=3).

RESULTS AND DISCUSSION

The results obtained from the present study deals with the biological activity of the antimicrobial compounds (secondary metabolites) of selected cyanobacterium, *C. majus* against two Gram-positive bacteria, *B. subtilis* (MTCC-1427), *S. aureus* (MTCC-1430) and two Gram-negative *E. coli* (MTCC-1302), *K. pneumoniae* (MTCC- 4030) and four fungal pathogens of *A. fumigatus* (MTCC- 4163), *A. niger* (MTCC- 4325), *Mucor* sp. (MTCC- 3340) and *T. mentagrophytes* (MTCC- 8476**)** were recorded in **Table-1**. It is quite clear from the present study that the diameter of the inhibition zone depends mainly on the type of the algal species, type of the solvent used and the tested bacterial and fungal organisms.

Antibacterial activity

The cyanobacterial culture of *C. majus* was taken and extracted using five different solvents namely Aqueous, Chloroform, Ethyl acetate, Hexane and Methanol respectively. The antimicrobial potential of the cyanobacterial strain with different extracts were shown in **Table -1**. The results indicates that the maximum antibacterial sensitivity measured in terms of zone of inhibition (17.33 mm) against Gram-negative bacteria *K. pneumoniae* was noticed in the Chloroform culture extract, followed by (15.66 mm) against *K. pneumoniae* in the Hexane culture extract and (14.00 mm) against *B. subtilis* in the culture extract of Methanol. A moderate inhibitory effect (13.00 mm) was shown by Ethyl acetate extract against *S. aureus* and (12.00 mm) against *S. aureus* in the Aqueous extract.

Table 1 Antimicrobial activity of *Cylindrospermum majus*

Zone of inhibition (diameter in mm)								
	Bacterial species used				Fungal species used			
Solvent extracts	*B.subtilis* MTCC-1427	*S. aureus* MTCC-1430	*E. coli* MTCC-1302	*K. pneumoniae* MTCC-4030	*A. fumigatus* MTCC-4163	*A. niger* MTCC-4325	*Mucor* sp. MTCC-3340	*T. mentagrophytes* MTCC-8476
Aqueous	7.33±0.33	12.00±0.57	--	--	15.33± 0.33	--	9.00±0.57	13.66±0.88
Chloroform	8.33±0.88	8.00±1.00	9.33±0.88	17.33±0.66	--	--	--	--
Ethyl acetate	8.33±0.66	13.00±0.57	8.66±0.66	--	--	14.00±0.57	9.33±0.33	9.00±1.00
Hexane	9.33±0.66	7.66±0.66	12.00±0.57	15.66±1.20	14.33±0.88	--	--	10.33±0.66
Methanol	14.00±1.15	7.66±0.33	8.33±0.33	--	--	12.33±0.33	9.33±0.66	--
Ciprofloxacin (10µg/disc)	25.33±0.33	24.66±0.66	28.33±0.33	29.66±0.33				
Nystatin (50 µg/disc)					23.33±0.33	22.00±0.57	21.33±0.88	21.66±0.66

" --" No inhibition zone
Diameter of the inhibition zone including disc diameter (6 mm).
Values were with mean ± SE of three separate experiments (n=3).

The low inhibitory effect (9.33 mm and 8.66 mm) were found in the culture extracts of Hexane against *B. subtilis* and Ethyl acetate extract against *E. coli*. The minimum zone of inhibition (7.33 mm) was noticed in the culture extract of Aqueous against *B. subtilis*. However, antibacterial activity was not found in the Aqueous extracts of cyanobacteria against *E. coli* and *K. pneumoniae*, similarly same result was found in Ethyl acetate and Methanol extract against *K. pneumoniae*, respectively.

Antifungal activity

The Aqueous culture extract expressed with the significant inhibition zone (15.33 mm) against *A. fumigatus*, followed by the Hexane culture extract (14.33 mm) against *A. fumigatus*, Ethyl acetate extract (14.00 mm) against *A. niger* and the Aqueous extract (13.66 mm) against *T. mentagrophytes* under study. The moderate zone of inhibition (12.33 mm) was expressed in the culture extract of Methanol against *A. niger* and (10.33 mm) against *T. mentagrophytes* in the culture extract of Hexane. The low inhibitory effect (9.33 mm) was noticed against *Mucor* sp. in the culture extract of Ethyl acetate and Methanol. The minimum zone of inhibition (9.00 mm) was found in the culture extract of Aqueous and Ethyl acetate against *Mucor* sp and *T. mentagrophytes* under observation. The fungal pathogens such as, *A. niger* and *Mucor* sp. have not shown any zone of inhibition in the culture extracts of Hexane. The fungal species like *A. fumigatus* was not shown any kind of antifungal activity in the culture extracts of Ethyl acetate. The fungi, *A. niger* did not respond to the Aqueous culture extracts of cyanobacteria. Similarly, the culture extract of Methanol also did not exhibit any antifungal activity against *A. fumigatus* and *T. mentagrophytes*. The results were also clearly indicates that Chloroform extract of *C. majus* has not expressed inhibition against all tested fungi. The antimicrobial activity of the test microorganisms against standard control Ciprofloxacin 10 µg/disc (bacteria) and Nystatin 50 µg/disc (fungi) were mentioned in the **Table-1**. In conclusion the results of all the culture extract were found with less inhibition zones against tested pathogenic bacteria and fungi when compared with the standard control under investigation.

The earlier reports published by different authors are in agreement with our present observations. Evaluation of the antimicrobial activity of aqueous and methanolic extracts of *Synechococcus elongatus* against pathogenic bacteria (Safari *et al.*, 2015) and antimicrobial activity of extracts from aquatic algae isolated from salt soil and fresh water in Thailand (Hind and Juntawong, 2014), antimicrobial activity and carbon sequestration capability (Padhi *et al.*, 2014), *in vitro* antimicrobial activity along with biomass production in waste water by cyanobacteria, *Spirulina platensis* (Suman Das, 2014), Cyanobacterial extracts of *Anabaena variabilis* and *Synechococcus elongatus* have shown significant antibacterial proportion towards *E. coli*, *Enterococcus* sp. and *Klebsiella* (Archana *et al*., 2013). Extracts of *Spirulina platensis* obtained by different solvents exhibited different degrees of antimicrobial activity on both Gram-positive and Gram-negative organisms (Rania and Abedin Hala Taha, 2008). Prashantkumar *et al*., (2006) studied antimicrobial activity in organic extracts of six species of marine algae against different bacterial strains. A variety of solvents (Water, Methanol, Ethanol, Acetone, Petroleum ether and Hexane) used as solvent to study the antibacterial agents in which methanol was found to be the best over other solvents (Challouf *et al*., 2011).

CONCLUSION

It is quite evident from the present investigation that the preliminary investigation of biological studies of *C. majus* has shown antimicrobial activity in different solvent extracts like Chloroform and Aqueous. It is concluded that the antimicrobial activity of cyanobacterial strains depends on the individual solvent used for making the extracts from the different cyanobacterial strains. Therefore work warrants for further research to identify and purify natural products from the selected cyanobacterium against the Pathogenic bactevia and fungi.

Acknowledgments

Authors are thankful to the Head, Department of Botany, Kakatiya University for providing research facilities to carry out the present investigation.

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How to cite this article:

B. Digamber Rao *et al*., Antimicrobial Activity Of Soil Cyanobacteria Cylindrospermum Majus. *International Journal of Recent Scientific Research Vol. 6, Issue, 5, pp.3859-3863, May, 2015*

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