Screening of antimicrobial potential of naturally occurring freshwater filamentous green algae *Cladophora glomerata* collected from Birbhum District, West Bengal, India.

Harisankar Dey* and Subhrajit Ghosh

ABSTRACT

Microalgae have been recognized in the last several years as source of novel anti-viral, anti-algal, antibacterial and antifungal metabolites. *Cladophora glomerata* is a branched filamentous green algae commonly found in freshwater bodies. The aim of the present work is to study the antimicrobial screening of the cell extracts of naturally occurring freshwater green algae *Cladophora glomerata* against clinically significant microorganisms both Gram positive and Gram negative bacteria and pathogenic fungi. In this study four test pathogens were considered, these test bacterial strains were *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and fungal strain was *Candida krusei*. Crude metabolites were extracted using four different solvents namely chloroform, methanol, butanol and petroleum benzene. The antimicrobial activities of crude extracts were evaluated using agar cup diffusion methods. Among the solvents highest antimicrobial activity was detected in methanol extracts against both Gram positive and Gram negative bacteria, i.e. *Bacillus subtilis* (19.66±0.57 mm) followed by *Pseudomonas aeruginosa* (15.66±0.57 mm) and *Staphylococcus aureus* (15.33±0.57 mm) respectively. Moderate activity were detected in chloroform extract against *Bacillus subtilis* (14.00±0.57 mm) and *Pseudomonas aeruginosa* (13.33±1.00 mm) respectively. The less activity was detected in petroleum benzene extract against *Staphylococcus aureus* (10.66±1.00 mm) and no activity was found against fungal pathogen *Candida krusei.* The present finding that may serve as leads for the development of new pharmaceuticals addressing the novel therapeutic needs of mankind. It is concluded that the branched filamentous green algae *Cladophora glomerata* occurring in natural freshwater bodies represents a new source of antimicrobial formulation with stable and biologically active compounds.

Keywords: *Cladophora glomerata*, Freshwater bodies, Solvent extracts, Antimicrobial potential.

MS History: 13.05.2019 (Received) -23.08.2019 (Revised) - 15.12.2019 (Accepted).

Citation: Harisankar Dey and Subhrajit Ghosh. 2019. Screening of Antimicrobial Potential of Naturally Occurring Freshwater Filamentous Green Algae *Cladophora glomerata* Collected from Birbhum District, West Bengal, India. *Journal of Biopesticides,* **12**(2): 186-190.

INTRODUCTION

Microalgae have been recognized in the last several years as source of novel cytotoxic, anti-viral, anti-algal, growth stimulatory, antibacterial and antifungal metabolites as well as specific enzyme inhibitors. A wide range of results of in vitro antibacterial and antifungal activities of extracts of freshwater as well as marine algae have been reported (Naik Ansari *et al.,* 2012). Aquatic organisms are a rich source of structurally novel and biologically active metabolites (Ely *et al.,* 2004). The green algae *Ulva lactuca* commonly known as sea lettuce has long been used as food and as a traditional medicine agent to treat helminthis infections, fever, urinary disease and dropsy (Kim *et al*., 2007). The antimicrobial activity of *Ulva lactuca* was reported to be caused by the acrylic acid commonly found in the algae (El-yamany, 2008). Microalgae represent a very large untapped reservoir of novel compounds, especially secondary metabolites, which are diverse in their structure and physiological

functions. Most of the bioactive metabolites identified so far have been derived from marine microalgae (cyanobacteria), however, freshwater green algae remain less explored for bioactive metabolites. Secondary metabolites with antibacterial effects are one group of allele chemicals produced by prokaryotic microalgae. Recent investigations reveal that antibacterial effects are caused by distinct substances different from the cyanotoxins (Compbell *et al.*, 1994). The use of algae for therapeutic purposes has a long history and the systematic examination of algae for biologically active substances especially antibiotics began in the year 1950 (Prakash *et al.*, 2011). There are numerous reports of macroalgae derived compounds that have a broad range of biological activities, such as antibiotic, antiviral, anti-neoplastic, antifouling, anti-inflammatory, cytotoxic and antimitotic. The investigaton of antibacterial effects of microalgae (*Cladophora glomerata*) was studied against multidrug resistant bacteria pathogens i.e. *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* by Dina *et al.* (2013). The production of secondary metabolites by green algae has an interesting scientific and commercial potential. From a biotechnological point of view cell suspension cultures still seem the most appropriate systems for the production of secondary products on an economically feasible scale (Knutsen and Hansen, 1997). *Cladophora glomerata* is a green branched filamentous microalgae abundantly found in freshwater bodies. There are more than 160 species of *Cladophora* in the world and they are very common in relatively clean eutropic water, developing slimy filamentous green masses. Therefore the present investigation is aim to study the antimicrobial screening of the cell extracts of naturally occurring freshwater green algae *Cladophora glomerata* against clinically significant microorganisms both Gram positive and Gram negative bacteria and pathogenic fungi.

MATERIALS AND METHODS

Collection and Maintenance of samples

The sample of *Cladophora glomerata* (Fig 1) was collected from natural freshwater bodies of Kirnahar and adjoining regions of district Birbhum, West Bengal. The samples were collected from freshwater bodies using clean forceps, sampling bottles, polythene bags and planktonic net. All the samples were deposited and maintained at Post Graduate Department of Botany, Ramakrishna Mission Vivekananda Centenary College (Autonomous) Rahara, Kolkata, West Bengal, India.

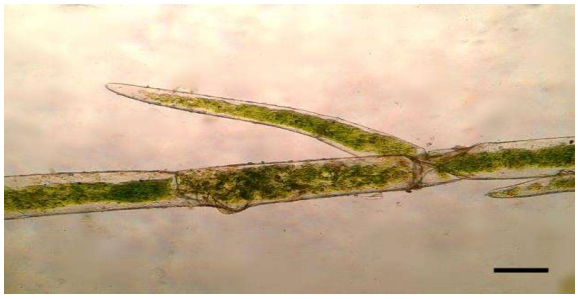

Figure 1. Microscopic photograph of *Cladophora glomerata* (Scale bar 20µm)

Slide Preparation and Identification

The samples were brought to the laboratory and processed immediately. Slide preparation and microscopic observation were done for the identification of sample. The organisms were identified according to their morphological status like cell colours, cell size and shape following the standard literature and available monographs (Anand, 1998).

Cultivation and Extraction of Metabolites

Freshly collected green algal (*Cladophora glomerata*) mats were washed thoroughly in running tap water and then finally wash with sterile distilled water in order to remove the suspended particles. The samples were then shade dried in room temperature and finally ground into powered form for solvent extractions. Extractions were done by using different solvents (methanol, chloroform, butanol and petroleum benzene) following the standard soxhlet extractor methods (Das *et al.*, 2005). The solvents were finally evaporated to yield the crude metabolites. The crude extracts were dissolved into DMSO (Dimethyl sulphoxide). The dissolved crude extracts were put into air-tight sampling bottles and

store at low temperature for antimicrobial bioassay. Pure culture of *Cladophora glomerata* were cultivated in freshly prepared Bold's Basal Medium (BBM) and maintained in algal culture room at 25±2˚C temperature and illumination at 3000 Lux with a white continuous light.

Screening for Antimicrobial Activity

The crude extracts were screened for their antimicrobial activity against some clinically significant microorganisms using agar cup diffusion method (Grammer, 1976). The test organisms include two Gram positive bacteria namely *Bacillus subtilis* (Bs) and *Staphylococcus aureus* (Sa); one Gram negative bacteria namely *Pseudomonas aeruginosa* (Pa) and one pathogenic fungi namely *Candida krusei* (Ck). All the test pathogens were collected from Microbiology Laboratory, Post Graduate Department of Botany, North Orissa University, Baripada, Odisha and maintained on freshly prepared medium. For antimicrobial assay, bacterial pathogens were cultured in Nutrient Broth (NB) and fungal pathogens were cultured in Potato Dextrose Broth (PDB). Nutrient agar plates were then inoculated with the overnight culture suspension of each test bacterial pathogens and potato dextrose agar plates were inoculated with the overnight culture suspension of each test fungal pathogens using glass spreader or cotton swabs. The plates with inoculated organisms were evenly prepared by scooping out the media with a sterile cork borer (8 mm in diameter). The cups were then filled with 200 µl of the crude extract that was already dissolved in DMSO. The plates were then incubated at 36±1˚C for 24 hours and the zone of inhibition was recorded and compared with the control i.e. a cup filled with DMSO solution only (Dey *et al*., 2010).

RESULTS AND DISCUSSION

The antimicrobial activities of crude extracts were evaluated using agar cup diffusion method. Among the solvents highest antimicrobial activity was detected in methanol extract followed by chloroform, butanol and petroleum benzene extracts in case of pathogenic bacteria. But in case of pathogenic fungi no activity was detected almost in the all solvent extract. The methanolic crude extracts showed highest zone of inhibition against *Bacillus subtilis* followed by *Pseudomonas aeruginosa* and *Staphylococcus aereus* respectively. Moderate activity were observed in chloroform extracts against *Bacillus subtilis* followed by *Pseudomonas aeruginosa* and butanol extract of *Bacillus subtilis* respectively. The less activity were detected against *Staphylococcus aereus* of chloroform and butanol extracts each with 11.66±0.57mm followed by petroleum benzene extract also 11.33±0.57mm diameter zone of inhibition in butanol extract of *Pseudomonas aeruginosa* respectively (Fig. 2 and Fig. 3; Table 1).

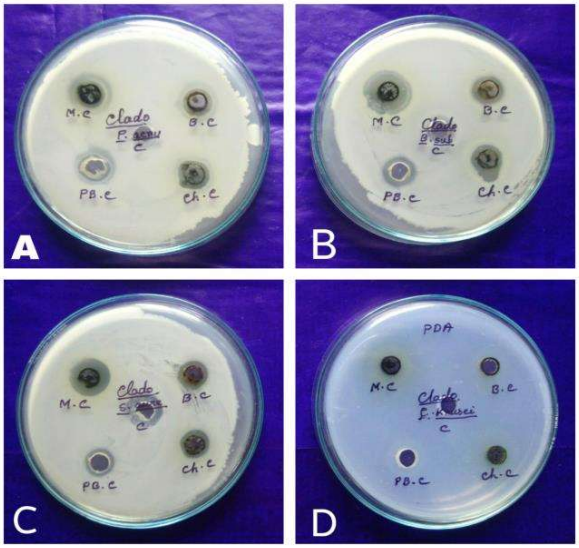

Figure 2. Photo plates showing activity of the crude extracts of *Cladophora glomerata* against different pathogenic bacteria and fungi. A- *Pseudomonas aeruginosa*, B- *Bacillus subtilis*, C- *Staphylococcus aureus*, D- *Candida krusei*, M.C-Methanol *Cladophora* extract; Ch.C- Chloroform *Cladophora* extract; B.C-Butanol *Cladophora* extract; PB.C- Petroleum Benzene *Cladophora* extract and C- Control.

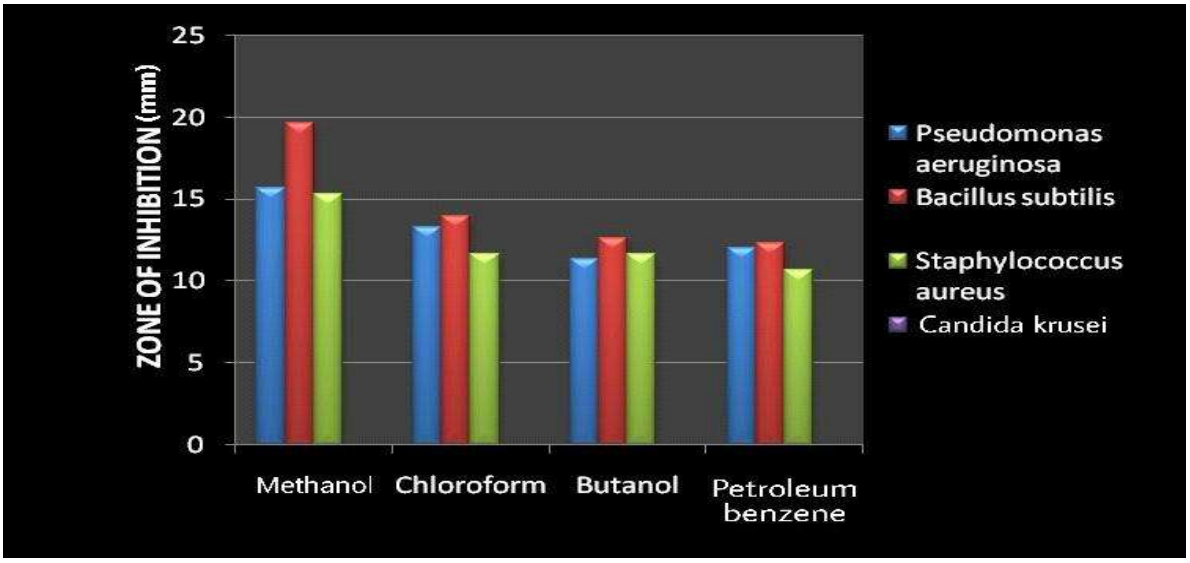

Figure 3. Graphical representation showing the activity of different solvent crude extracts of *Cladophora glomerata* against different pathogenic bacteria and fungi.

Table 1. Antimicrobial activity of solvents extract of *Cladophora glomerata* against different human pathogenic microorganisms (Zone of inhibition in mm ± SD, n=3)

Pathogens	Control (DMSO) (mm)	Methanol (mm)	Chloroform (mm)	Butanol (mm)	Petroleum benzene (mm)
Pseudomonas aeruginosa	8.33±0.57	15.66±0.57	13.33±1.00	11.33±0.57	12.00±0.57
Bacillus subtilis	9.33±0.57	19.66±0.57	14.00±0.57	12.66±0.57	12.33±0.57
Staphylococcus aureus	10.33±0.57	15.33±0.57	11.66±0.57	11.66±0.57	10.66± 1.00
Candida krusei	-	-	-	-	-

Recent investigation reveals that freshwater filamentous green algae could also be a potential source of important bioactive metabolites for therapeutic applications. The finding revealed that pattern of inhibition varied with the algal strains, nature of extraction solvents and the pathogenic microorganisms tested. In such cases, the present work with antimicrobial activity of filamentous green algae *Cladophora glomerata* occurring in natural freshwater bodies of Birbhum, West Bengal would generate important bioactive metabolites of pharmaceutical importance. Considering the present health problem of drug resistant microbes and emerging of new diseases, research priority should be directed to screen microorganisms from diverse habitats for production of novel bioactive molecule to solve the growing health problems. Since filamentous green algae have been identified as repository of bioactive metabolites, research investigations on less explored filamentous green algae occurring in freshwater bodies could be very promising in acquiring novel metabolites.

ACKNOWLEDGMENTS

The authors are thankful to the Principal, Ramakrishna Mission Vivekananda Centenary College (Autonomous), Rahara, Kolkata, West Bengal for providing necessary laboratory facilities.

REFERENCES

Anand, N. 1998. Indian Freshwater Microalgae. *Bishen Singh Mahendra Pal Singh*. Dehradun, India. 1-94 **PP**.

Compbell, D. L., Lawton, J. A., Beattie, K. A. and Codd, G. A. 1994. Comparative assessment of the specificity of the brine shrimp and microtox assays to hepatotoxic (microcystin-L-containing) Cyanobacteria. *Environmental Toxicology Water Quality* **9**: 71-77.

Das, B. K., Pradhan, J., Pattnaik, P., Samantaray, B. R. and Samal, S. K. 2005. Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). *World Journal of Microbiology and Biotechnology*. **21**: 45-50.

Dey, H. S., Tayung, K. and Bastia, A. K. 2010. Antimicrobial Evalution of some freshwater cyanobacteria collected from local rice fields of Orissa, India. *Journal of Pure and Applied Microbiology*. **4:** 589-597.

Dina, M., Dwaish, A., Ghaidaa, A. A. and Latif, J. A. 2013. Use of Cladophora glomerata Extract Against Multidrug Resistant Bacterial Pathogens. *Journal Environmental Science and Engineering*. **2**(8A):495-500.

Ely, R., Supriya, T. and Naik, C. G. 2004. Antimicrobial activity of marine organisms collected from the coast of South East India. *Journal of Experimental Marine Biology and Ecology*. **309**: 121-127.

El Yamany, K. N. M., 2008. Studies on some marine macroalgae isolated from Red Seashores (Quseir, Marsa Alam), Egypt. M.Sc. Thesis, Faculty of Sci., Beni-Suef University, Beni-Suef, Egypt.

Grammer, A. 1976. Antibiotic sensitivity and Assay test. In Microbiological method, Collins, C. H. and Lyne, P.M. (Eds), Butterworths, London. 235**P**.

Kim, I. H., Lee, D. G., Lee, S. H., Ha, J. M., Ha, B. J., Kim, B. J. and Lee, J. H., 2007. Antibacterial activity of *Ulva lactuca* against Methicillin- resistant *Staphylococcus aureus* (MRSH). *Biotechnology and Bioprocess Enggineering*. **12:** 579–582.

Knutsen, G. and Hansen, K. 1997. Search for bioactive substances from marine microalgae and cyanobacteria. In Le Gal Y, Muller-Feuga A (eds), Marine microorganisms for industry. Editions Ifremer, Plouzane, 169-178**PP**.

Naik Ansari, A., Hemavani, C. and Thippeswamy, B. 2012. Evalution of antimicrobial property of *Spirogyra* species. *International Multidisciplinary Research Journal*. **2**: 13-15.

Prakash, J. W., Marimuthu, J. and Jeeva, S. 2011. Antimicrobial activity of certain fresh water microalgae from Thamirabarani river, Tamil Nadu, South India, *Asian Pacific Journal of Tropical Biomedicine*, **S**: 170-173.

Harisankar Dey* and Subhrajit Ghosh

P.G. Department of Botany, Ramakrishna Mission Vivekananda Centenary College (Autonomous), Rahara, Kolkata-700118, West Bengal, India.

***Corresponding author**

E-mail harisankardey@gmail.com