

## Evaluation of Antibacterial Competence of *Cladophora glomerata* and *Lyngbya diguetii*

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### ABSTRACT

Two filamentous species of algae collected from GC University Lahore were tested against two bacterial species. Extracts of algal species were prepared in three solvents, methanol, ethanol and acetone. Two different concentrations (w/vol.) 1/10 g/ml and 1/100 g/ml were made in each of the above mentioned solvents. Extracts were loaded on agar plates, containing test bacteria, *Bacillus subtilis* and *Streptococcus mutans*. Methanol and ethanol found to be the best solvents for making extracts that showed good zone of inhibition in bacterial species maximum up to 2.05 cm than the acetone which was up to 0.7 cm. Methanolic and ethanolic extracts could be the alternate of antibacterial agents as both showed results very close to the antibiotics used as control. The mechanism of fate of microbial inhibition must be analyzed in later microbial and phyecological researches.

**Key Words:** *Cladophora glomerata*, *Lyngbya diguetii*, antibacterial competence

### INTRODUCTION

The use of algal species for medicine has been well known and their analysis begun from 1950 in medical industry. The antibacterial action was an indication that the algae have potential to synthesize vital bioactive secondary metabolites (Gonzalez *et al.*, 2001; Smit, 2004; Christobel *et al.*, 2011). Members of green, brown and red algae were analyzed and their extracts in ethanol were found antibacterial especially red algae showing maximum antibacterial action (Valachos *et al.*, 1997). Long-term practices of antibiotics create resistance in bacterial strains and therefore the use of natural compounds encourages minimizing the growth of parasitic bacteria. The growth of unscrupulous bacteria in liquid media has been reduced by microalgae extracts in various organic solvents (Salvesen *et al.*, 1999). Microalgae produce secondary metabolites in the growth media (Borowitzka, 1995). Many compounds have antibacterial action from algal specimen as fatty acids (Desbois *et al.*, 2009), Terpenoids, Carbohydrates (Duff & Bruce, 1966), Peptides, Polysaccharides and Alkaloids (Borowitzka, 1995). Seaweeds have been known to produce antibacterial action against gram positive and gram negative bacteria (Kandhasamy & Arunachalam, 2008). Microalgae have been reported to synthesize compounds like antibiotics which are effective against fish and human pathogen bacteria (Das *et al.*, 2005). Antiseptic activity of *Spirolina platensis* was analyzed *in-vitro* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*,

*Salmonella typhi* and *Klebsiella pneumoniae* (Kaushik & Chohan, 2008).

Plant species and numerous herbs including algae and fungi were screened for their antibacterial, antifungal and antioxidant potential. It is reported that antimicrobial resistance is due to the presence of certain novel secondary metabolites, i.e. alkaloids, flavonoids, coumarins, lignans, anthocyanins and catechins in plants including algae and fungi (Ajaib *et al.*, 2013; Crasta, 1997). Keeping in view the significance of freshwater and marine algae the present work was carried on *Cladophora glomerata* and *Lyngbya diguetii* as there is no such detailed work on these two algal species.

### MATERIALS AND METHODS

The samples were collected in May to June, 2013 from Botanic Garden, GC University Lahore and the test bacterial species (*Bacillus subtilis* and *Streptococcus mutans*) were obtained from Institute of Agricultural Sciences, and Department of Pharmacy, University of the Punjab, Lahore. The following strategy was adopted for analysis: Preparation of algae extracts in organic solvents methanol, ethanol and acetone. Estimation of antibacterial activity by zone of inhibition method.

The finely ground samples were weighed and 5 g were mixed with 250ml of various solvents (1:50, w/v); 100% ethanol, 100% methanol, 100% acetone. The mixtures were kept for four days at room temperature and mixed at regular intervals. After four days the samples were filtered using

Whatman filter paper No. 1 to separate the filtrate and the extracts were freed from solvent by rotary evaporator.

Algal cultures were centrifuged and the pellets were collected, weighed and used for extraction of antibacterial agents. About half gram of each of the algae bits was used to prepare extract in 10 ml of acetone, ethanol or methanol. All the extracts were stored at 4°C. Two dilutions (1/10 and 1/100) of each solvents were prepared by weight/volume. Mueller Hinton Agar Medium was prepared after sterilizing at 121°C and 15lbs pressure for 15 minutes. 10 ml of the sterilized media was poured into a Petri dish and solidified at room temperature.

Each algal extract was dissolved in 5 ml of the corresponding solvent and 1 mg was applied to sterile filter paper disc. The discs were placed on to the agar plates inoculated with an 18 hour culture of the test pathogen in nutrient broth. A disc load with a certain commercial antibiotic, such as Erythromycin, Tetracycline, and amoxilin was used as control. The plates were incubated for 24 hours at 37°C. The zone of inhibition of bacteria around the disc was measured and the assay was scored positive. Nutrient broth medium was used to grow bacterial strains. Erythromycin, Tetracycline and Amoxicillin were used in control group while 10 µL of algal dilutions (1/10 and 1/100) of each algal species in three solvents (methanol, ethanol and acetone) were laden on Petri plates by micropipette. The negative control of solvents was also determined which was almost negligible. The plates were incubated at 37 °C for 24. After a period of incubation, the diameter of zone of inhibition was determined by agar well diffusion method.

## RESULTS AND DISCUSSION

Extracts of algal species were prepared in three solvents, methanol, ethanol and acetone. Two different concentrations (w/vol.) 1/10 g/ml and 1/100 g/ml were made in each of the above mentioned solvents. Extracts were loaded on agar plates, containing test bacteria, *Bacillus subtilis* and *Streptococcus mutans*. Methanol and ethanol found to be the best solvents for making extracts that showed good zone of inhibition in bacterial species maximum up to 2.05 cm than the acetone which was up to 0.7 cm (fig 1). Methanolic and ethanolic extracts could be the alternate of antibacterial agents as both showed results very close to the antibiotics used as control (fig 2). The antibiotics are synthetic chemicals and may have side effects are now time to replace with

natural antibiotic source (Ajaib *et al.*, 2015a & 2015b). It is necessary to make natural extracts that are equally effective as the artificially prepared antibiotics. There are chances that bacteria become resistant against the used antibiotic which is serious threat in biological treatments.

According to figure 2, *B. subtilis* showed maximum zone of inhibition. On analysis of the data in Fig., 1 it was clear that extracts in ethanol of both algae with 1.85 and 1.65 cm of zone of inhibition were closer than others.

Zone of inhibition of erythromycin was more than any other antibiotics used as control (Fig., 2). Comparison of both algal extracts concluded that methanolic and ethanolic extracts of *Lyngbya diguetii* had inhibition range of 2.05, 2.0 cm which were closer with control.

*Bacillus subtilis* was best inhibited by erythromycin as in control (Fig., 2). On the other hand methanolic and ethanolic extracts of *L. diguetii* showed good inhibition results against *S. mutans* 2.05 and 2.00 cm (Fig., 1) and *Cladophora glomerata* with 1.85 cm zone of inhibition would be natural antibacterial agent. *Streptococcus mutans* had shown 2.5 cm zone of inhibition against erythromycin but against methanolic extract of *L. diguetii*, it showed 2.05 cm zone so it would be helpful in developing antibacterial agent from natural source.

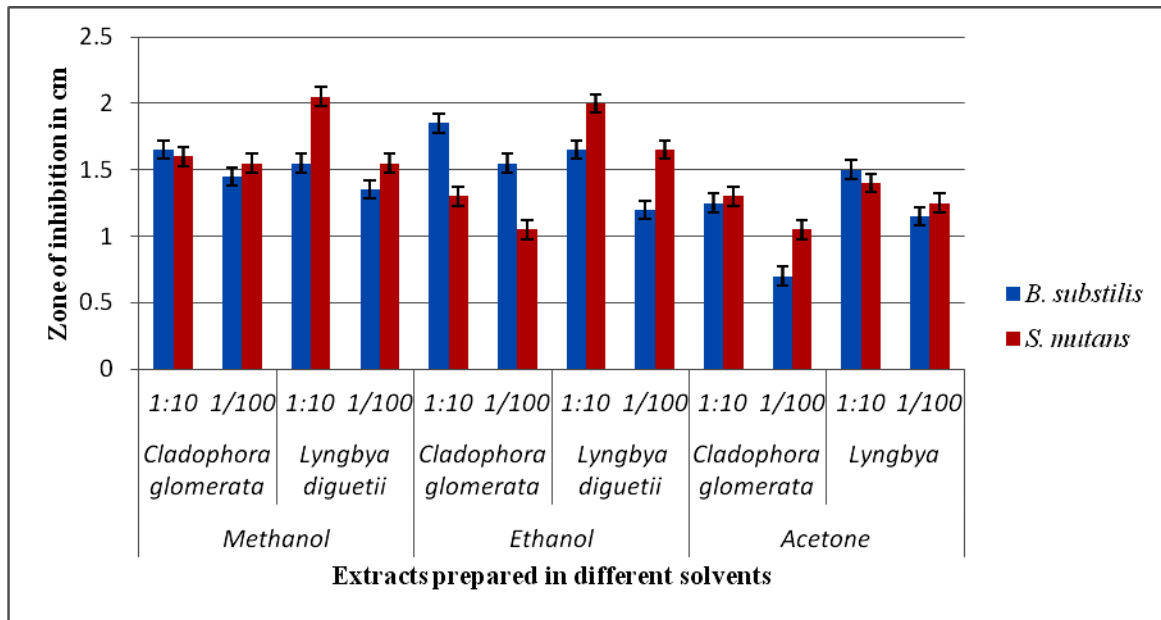
Algae had proven a good source for bacterial resistance. Arun *et al.* (2012) proved that methanolic extracts of *Spirulina platensis*, *Chlorella pyrenoidosa* and *Nostoc muscorum* were good against the human pathogenic bacteria and fungi.

Algae based antibiotics were equally good against plant pathogenic microbes. Ansari *et al.* (2012) tested bioactive compounds from *Spirogyra* sp. against many plant microbial species with positive results.

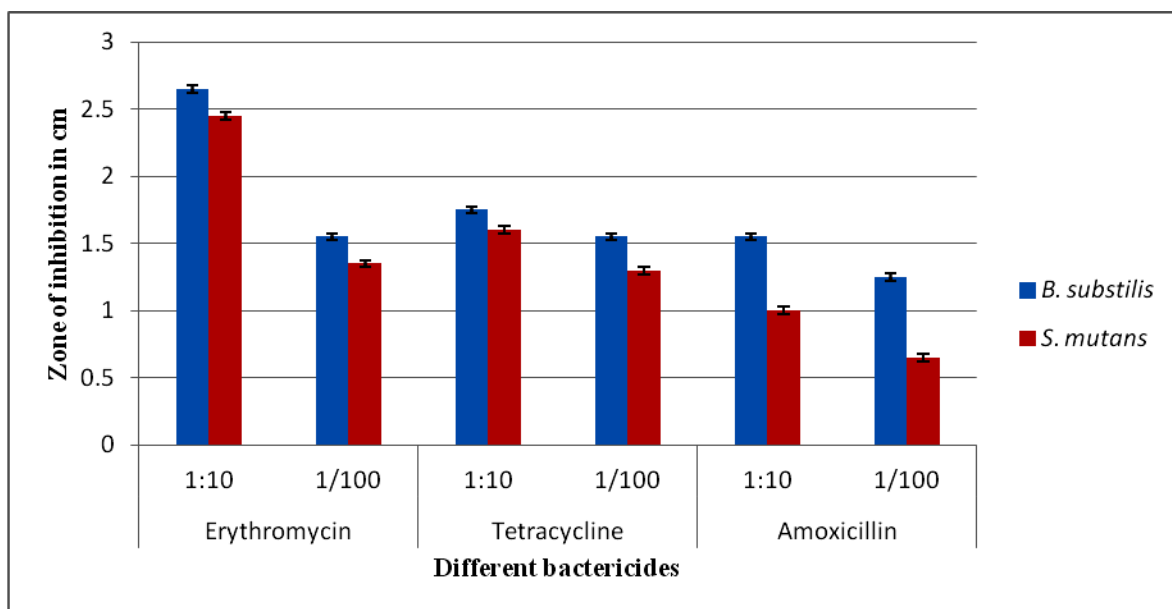
## CONCLUSION

It could be concluded from this experimental work that algae with some exceptions have both antibacterial chemicals that may be used in pharmaceutical industries. Natural antibacterial agent must be used rather than synthetic agents that cause several side effects.

Extracts obtained from methanol and ethanol had greater anti-bacterial activity than tetracycline and amoxilin. Hence, these species might be useful for drug development after isolation and synthesis of particular chemical compounds which inhibit the growth of bacteria.



**Fig., 1:** Antibacterial activity of *Cladophora glomerata* and *Lyngbya diguetii*



**Fig., 2:** Zone of inhibition in cm shown by different bactericides (control)

## REFERENCES

- Ajaib, M., Khan, K.M., Perveen, S. & Shah, S., 2015b. Antioxidant and Antimicrobial Activities of *Helinus lanceolatus*. *J. Chem. Soc. Pak.*, **37**(1):139-143.
- Ajaib, M., Khan, Z., Abbasi, M.A. & Riaz, T., 2013. Antimicrobial screening of *Iris aitchisonii* (Bakar) Boiss. *Biologia*, **59**(1): 51-55.
- Ajaib, M., Mati-ur-Rehman, A., Khan, K.M., Perveen, S. & Shah, S., 2015a. *Pulicaria undulata*: A Potential Phytochemical, Antimicrobial and Antioxidant Source. *J. Chem. Soc. Pak.*, **37**(3):559-566.
- Ansari, A., Hemavani, C & Thippeswamy, B., 2012. Evaluation of antimicrobial property of *Spirogyra* species. *Int. Multidis. Res. J.*, **2**(2): 13-15.
- Arun, N., Gupta, S. & Singh, D. P., 2012. Antimicrobial and antioxidant property of commonly found microalgae *Spirulina platensis*, *Nostoc muscorum* and *Chlorella pyrenoidosa* against some pathogenic bacteria and fungi. *Int. J. Pharma. Sci. Res.*, **3**(12): 4866-4875.
- Borowitzka, M.A., 1995. Microalgae as sources of pharmaceuticals and other biologically active compounds. *J. App. Phy.*, **7**: 3-15.
- Christobel, G. J., Lipton, A. P., Aishwarya., M.S., Sarika, A. R. & Udayakumar, A., 2011. Antibacterial activity of aqueous extract from selected Macroalgae of Southwest coast of India. *Seaw. Res.*, **33**(2): 67-75.
- Crasta, J., 1997. Antimicrobial screening of some marine algae of Southwest Coast of India. *Ind. J. Marine Sc.* 26: 201-205.
- Das, B.K., Pradhan, J., Pattnaik, P., Samantaray, B.R. & Samal, S. K. 2005. Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). *W. J. Microbiol. Biotech.*, **21**: 45-50.
- Desbois A.P., Mearns-Spragg, A. & Smith, V.J. 2009. A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including multi resistant *Staphylococcus aureus*. *Mar. Biotech.*, **11**: 45-52.
- Gonzalez, D.V., Platas, G. & Basilio, A., 2001. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int. Microbiol.*, **4**: 35-40.
- Kandhasamy, M. & Arunachalam, K. D., 2008. Evaluation of *in vitro* antibacterial property of seaweeds of Southeast coast of India. *Afri J. Biotech.*, **7**(12): 1958-1961.
- Kaushik, P. & Chauhan, A., 2008. *In vitro* antibacterial activity of laboratory grown culture of *Spirulina platensis*. *Ind. J. Microbio.*, **48**: 348-352.
- Salvesen, I., Skjermo, J. & Vadstein, O., 1999. Growth of turbot (*Scophthalmus maximus* L.) during first feeding in relation to the proportion of r/K strategists in the bacterial community of the rearing water. *Aquacul.*, **175**: 337-350.
- Smit, A.J. 2004. Medicinal and pharmaceutical uses of seaweed natural products. *A Review J. Appl. Phycol.*, **16**: 245-262.
- Valachos, V., Critchley, A.T. & Holy, A.V. 1997. Antimicrobial activity of extracts from selected Southern African marine macroalgae. *South Afri. J. Sci.*, **93**: 328-332.

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