Exploring Microalgal lipids as anti-virulent Agents Targeting MDR *Vibrio Cholerae* infection: A Step Toward Developing Herbal Oral Rehydration Salt (ORS) Formulations

Sweta Jaiswal
CSIR-Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

Nikita Vadadoriya
CSIR-Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

Abdul Nasir
CSIR-Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India

Ramalingam Dineshkumar
CSIR-Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India
Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Neeraj Khatri
CSIR-Institute of Microbial Technology, Chandigarh
Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Sachin Raut
CSIR-Institute of Microbial Technology, Chandigarh
Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Saumya Ray Chaudhuri
CSIR-Institute of Microbial Technology, Chandigarh
Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Shruti Chatterjee*
Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Soumya Haldar*
CSIR-Central Salt & Marine Chemicals Research Institute, Bhavnagar, Gujarat, India
Academy of Scientific and Innovative Research, Ghaziabad, Uttar Pradesh, India

Abstract:

Cholera, a life-threatening diarrheal disease caused by *Vibrio cholerae*, remains a major global health issue. Rising antibiotic resistance, especially in developing countries, poses a significant threat to effective treatment. Novel anti-virulence strategies can address this gap. Our study investigated the potential antivirulent effect of *Chlorella variabilis* (CV) and *Chlorococcum sp.* (CCM) microalgae to inhibit cholera toxin (CT) production.

Using a multidrug-resistant *V. cholerae* strain (SRK-19), lipid extracts and crude freeze-dried biomass were tested *in vitro* for their effects on CT production via ELISA and bacterial viability. For *in vivo* validation, rabbit ileal loop assay was performed to measure the fluid accumulation (FA) ratio, colony-forming units (CFU), and CT levels.

Results indicated that both lipid extracts and crude biomass significantly reduced CT production in a dose-dependent manner, with CV and CCM biomass achieving reductions of over 90% in vitro and 70% in vivo. Crucially, the *in vivo* study demonstrated reduced FA ratios without affecting bacterial growth, indicating that bacterial viability was uncompromised.

These findings provide a compelling proof of concept that extracts from CV and CCM possess potent anti-virulence activity without promoting antimicrobial resistance, supporting the development of microalgae-based oral rehydration formulations as a promising alternative to conventional antibiotic therapy.

Keywords:

Cholera Toxin, Microalgae, Antibiotic Resistance, In-Vivo Study, Anti-Virulence.