

# CONCLUSION

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In India, floodplain wetlands are conspicuous features where these water bodies support livelihood to thousands of people through collecting edible plants, agriculture, water transport, irrigation, subsistence and commercial fisheries, besides harbouring rich biodiversity. These valuable natural resources contribute immensely to the country's food basket through fisheries but these wetlands are being destroyed at an alarming rate across the developed and developing world. Fish mortality due to widespread fish diseases is the prime cause to immense economic loss to fish farming. The three prime reasons behind these are:

- Numerous counts of aqueous pathogens.
- Low resistance of fish stock.
- Unsatisfactory ambience of aquatic ecosystem.

These could threaten the long-term sustainability of the ecosystems and contribution of their fisheries to food supply. In order to alleviate these problems it has now become crucial to reduce pathogen level in water bodies which demands the need for a bio-disinfectant and an antimicrobial agent readily soluble in aqueous solution. The allelopathic compounds with antimicrobial property could be used to address such problems. The major difference between terrestrial and aquatic ecosystem is the resource variable “soil” and non-resource variable “water”. All these factors when unrevealed have a cumulative contribution in deterioration of water body environment. Long term economic viability and integrity of these worthy resources depends on management and usage.

Allelo-chemicals released by aquatic plants need to be sufficiently hydrophilic and to reach the destined organism in effective concentration after huge dilutions.

This study addresses these issues by isolating phyto-chemicals or allelochemicals from the aquatic macrophytes and applying them as antimicrobial agents. The associated

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biological activity which encompasses any aquatic ecosystem such as anti-algal activity, duckweed toxicity and brine shrimp lethality test has also been assayed to draw a conclusive inference on the plant samples. The plant samples have also been purified and their chemical structures are elucidated so as to provide prospect of synthesis in terms of biodiversity loss. In cases of synergistic activity such as in CrM and CrP fractions of *C. rotundus* and VsF7 fraction of *V. spiralis*, the major constituents have been identified via GCMS. The in-vivo application via formulated feed has been tested on induced infection in Pangus fish and an affirmative result is comprehended. Some new observations have been made during the study which is stated below.

### 18hrs magnitude

The plant extracts (crude fraction and semi-pure component) obtained under this research manifested as a potent antimicrobial agent or as toxic agent, articulated 18hrs time interval as vital for its biological class inference. Time interval bound experiments showed an unusual phenomenon at/from 18hrs interval. It demands for time dependent rather than concentration dependent application of the lead compound for persistence of results. In this context, *Cyperus rotundus* methanol, chloroform and petroleum ether fraction (CrM, CrCh and CrP) used for brine shrimp lethality test displayed 100% mortality at higher concentrations from 1hr to 18hrs time interval with absolute detained movement but after 18hrs the shrimps invigorated in the test with only 20% mortality in test samples.

### Synergism

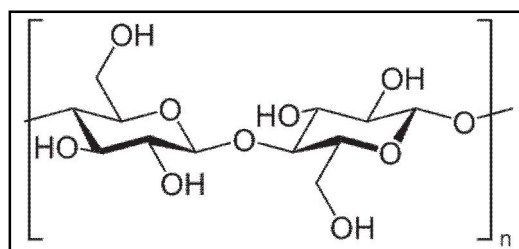
In the pursuit of a bioactive single pure compound, we isolated three crystalline compounds but when compared the efficacy (antibacterial, antioxidant, and cytotoxicity) of the crude and the final product, a sharp decline was vivid in all the parameters. Following this the remaining semi-pure fraction was assayed and the result

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was not much satisfactory. This observable fact repeated in every case. Hence, the search of bioactive crude plant fraction assured with bio-safety is far more economic and within the participation spell of fish farmers than the passive single pure crystal. The principal factor is the plant must be invasive in growth without much specific requirements as crude fraction cannot be synthetically produced in lab as reverse is the case for single pure crystal.

### Agar Diffusion Antibacterial Assay – Well Vs Disc

The disc diffusion assay is one of the recognized procedures (CLSI Antimicrobial Susceptibility Testing) for antibacterial test as compared to the punched well diffusion but disc diffusion does not work well with plant extracts especially with the polar fraction. The chemical structure of the cellulose content of the discs is hydrophilic in nature trapping the fraction intact and hinders smooth diffusion in the agar medium and bacterio-static or cidal activity, if contained, is masked.



Cellulose [ $\beta$ -(1-4) linked glucose monomers (Source: Google images)

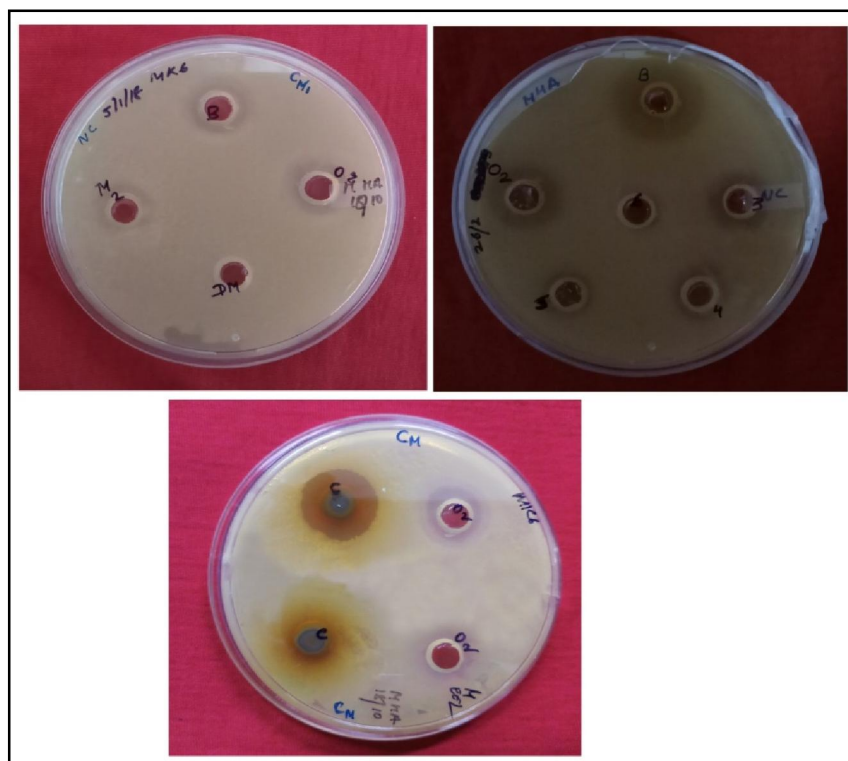
The chemical structure of the disc reveals free hydroxyls present on the sugar moiety which imparts a hydrophilic property to the substance and hence adsorb cationic natural compounds. Accordingly, well agar diffusion method is a much assured and convenient diffuser for polar compounds.

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### Combination Therapy

A combination therapy of commercial antibiotic, oxytetracyclin Hydrochloride (95%) (OTC HCl) and the bioactive compound from methanol fraction of the inflorescence of *Cyperus rotandus* (CrM, CrM1 & CrM2) was tested at various concentrations on *Aeromonas hydrophila* by well diffusion method. An ideal combination of 50ug/ml OTC HCl (O<sub>2</sub>) + 1000ug/ml bioactive compound [1<sub>A</sub>:20<sub>N</sub> OTCLC] = (C) recorded an inhibition zone (ZI) of 23mm diameter & 50ug/ml OTC HCl (O<sub>2</sub>) +500ug/ml bioactive compound [1<sub>A</sub>:10<sub>N</sub> OTCLC] = (B) with ZI 21mm. The combination therapy aims at:

- Prevention of developing drug-resistant pathogen
- Eco-system affable.



**Fig. 78:** Comparative Zone of Inhibition of CrM, OTC & CrM + OTC against *A. hydrophila*

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The expected deliverables of this study are thumb nailed below

- Way to good aquaculture practices in regards to fish disease management.
- Antimicrobial agents from aquatic plants for use as disease preventive for fish.
- Bio-algaecide for removal of algal bloom.
- Maintaining a hygienic and fish friendly water body.
- The isolated pure compound facilitates the scope for synthetic designing for aquaculture system.
- Identification of plants bearing antioxidant properties and capable of being used in pharmaceutical industry.