

SUMMARY

Summary

Despite numerous studies on terrestrial ecosystem, methodological impasses and the complexity of the aquatic environment are the frequently cited hindrances for the evaluation of allelopathic interaction of the aquatic plants as fish curatives and their précised role in an aquatic ecosystem. In various perspectives, these interactions determine the fish populace in a particular aquatic environment. The commercial fisheries largely depends on extensive use of need specific synthetic chemicals to maintain a fish friendly ambience for high yield, which not only deselected certain fish species over others but simultaneously declines the physiological aspects of the water body leading to environmental hazards. Bio-intensive aquaculture techniques add to *Good Aquaculture Practices*. The plant resources serve as an unsurpassed replacement for synthetic chemicals nullifying their adverse impact.

In this study, the CrM and CrCh fraction extracted and purified from the inflorescence extract of *C. rotundus* are possible natural resources of antioxidants with median scavenging parameter $IC_{50} < 0.1$ mg/ml. The GCMS results obtained from this study reveals that the active fraction CrM of *C. rotundus* contains 52.52% peak area with Mycorradicin, a carotenoid cleavage product which is produced by plants infected with fungal attack. The second highest peak area is of cinnamyl cinnamate which is a standard phenol and a known disinfectant constituent. The Annosquamosin A found with 14.43% area is a terpenoid with known variants of antimicrobial, anti-parasitic and insect repellent property.

The raphide crystals ($IC_{50} < 0.3$ mg/ml) isolated and identified from the F2a_{1a} of *I. aquatica*. The extensive use of synthetic disinfectants in aquatic ecosystem which claims to possess antioxidant activity has shown to cause biomagnifications of antibiotic sediments in fish populace which causes serious human health issues. The study also shows that the phenolic compounds found in the leaf extracts of *V. spiralis*

Summary

and *I. aquatica* attributes to the homomorphism of the graph for phenols and antioxidants. The statistical evaluation shows the coefficient of determination ($R^2 = 0.993$) for the methanol fraction of *C. rotandus* (CrM) to be most proficient which also shows restrained toxicity (LC50 -237.2 μ g/ml to 21.04 μ g/ml) and strong antimicrobial activity against major fish pathogens which could be compared to commercially available antibiotics. The statistical models are exponential in nature as one of the variables is constant and can have a maximum value of 100 and relative to the other. A statistical correlation is witnessed within the free radical scavenging potential and the concentration of the plant samples.

Additionally it is worth stating that the anti-dandruff activity of the VsE80 of *Vallisneria* leaves suggests justifies the fact that lathering agents like Cocamide MEA or Cetyl Alcohol are added to commercially available hair cleanser to make the lather rich, creamy and easy to spread across your scalp and throughout your hair (Rieser *et al.*, 1996; Boekhout, 2010). *Malassezia globosa* is commonly known as smut plant pathogen or dandruff fungus with a significant MIC < 200 μ g/ml (Amend, 2014). Being a foremost mycobiota in human dermatitis, *Malassezia* is now reported widespread in marine ecosystem (Bouayed, 2011) which presents possibilities of scale lesions in marine colonising as biotopes. The FT-IR (Fourier transform Infra red) spectra of the isolated fraction of the *V. spiralis* leaves explains its fungicidal property with three most important characteristic bands at 3642.10 cm^{-1} for free hydroxyls (OH), 1665.22 cm^{-1} for carbonyls (C=O) and 1360 cm^{-1} for sulphoxides (S=O). The free hydroxyl groups which are usually a part of phenols, imparts to free radical inhibition trait to a compound when positioned in ortho or para bonds. But the fraction was found to show high cytotoxicity against shrimps hence undermines its application in aquatic habitat.

Summary

However utilizing its toxicity trait, *V. spiralis* leaf extract, VsF7 and CrM of *Cyperus* is found to retarded the growth of harmful bloom causing algae at concentrations 0.03mg/ml-0.5mg/ml, *Microcystis aeruginosa* under laboratory conditions. The mode of action suggests its anti-algal property more as therapeutics then preventives. (Hemanth *et al.*, 2013; Hovatta *et al.*, 2010; Pal and Dutta, 2006; Kuete and Efferth, 2010). The GCMS of the VsF7 fraction reveals the main constituent to be plant hydrocarbons viz; 1-Nonadecene (100%), 1-Tridecene (99.04%), 1-Hexacosene (25.22%). They are reported to be the prime content of aromatic medicinal plants.

The antimicrobial activity of *Ipomoea* leaf extracts, F3 & F2a_{1a}, against *Edwardsiella tarda* (MIC < 100µg/ml) and *Citrobacter freundii* (MIC < 50µg/ml) suggest the fact that antioxidant contents are directly proportion to fish antimicrobial defence. Regardless of the fact that the non-polar fraction of *Ipomoea* was found to be antibacterial only against *Bacillus safensis* (MIC < 100µg/ml) but it had very minimal antioxidant content (19.82%) compared to the polar fraction (74%). This further infers that the antioxidants are the responsible agents for Gram (-) bacterial inhibition but their presence is not crucial for prevention of Gram (+) bacterial growth. In this study, 1.6 % of calcium oxalate crystals were extracted from the aqueous fraction of the dried *I. aquatica* leaves and an endeavour has been made to explore the alternate applications of the inorganic salt crystals beyond the producer plant. The crystals were screened for their free radical scavenging capacity which was found to be strong with antioxidant activity index (AAI) of 1.38, however IC₅₀ at 0.144 mg/ml is considered to be moderate (Emelda, 2015). This was however found to be strong. In contrary there are reports where excess of calcium oxalate crystals in mammals have induced lipid per oxidation and free radical generation (Abhirama and Shanmuga Sundaram, 2018). This also explains that calcium oxalate crystals in excess serves versatile task in fish causing

Summary

oxalosis. Direct bio-autography on a silica (SiO₂) strip demonstrated a swift, economical and sensitive technique for objective based fractionation and isolation of the bioactive compound. The crystals displayed a strong antibacterial activity against fish pathogens, *C. freundii* with MIC at 31.25 ppm. *C. freundii* is a gram negative bacteria causing generative anemia, leukopenia, lymphocytosis, and leukoblastosis (Thi *et al.*, 2013; Junior; *et al.*, 2018). The atomic size of the crystals measuring 4-5 nm as revealed by the atomic force microscopy assumes that the antimicrobial potency of the crystal is apparently due to bacterial cell membrane disruption. In addition to the known traditional values of *I. aquatica*, this study further contributes towards its virtue of beneficial weed. The isolation & structural characterization of this compound was generated by nuclear magnetic resonance and infra-red spectroscopy. The polar fractions obtained with prospective MIC reading, is frequently used as phyto-genic products as commercial feed additives for food-producing animals (Yang *et al.*, 2015) and fungicides for plant protection.

The extracts of the inflorescence of *Cyperus rotandus* L. were found to be having strong antimicrobial property. The remarkable zones of inhibition by the chloroform fraction of the extract against fish disease bacteria viz; *Aeromonas hydrophila*, *A. popoffi*, *Pseudomonas putida* and *Bacillus safensis* could be utilized as disinfectants in fish farming by chemical bath with very short exposure. But utilizing for chemical bath would require a lot of the product and also it would contain a mixture of compounds.

All the three fractions of *Cyperus* was effective antimicrobial against *Aeromonas* species. However, the zone of inhibition against *A. hydrophila* and *Edwardsiella tarda* by CrM (methanol fraction) with MIC 15.625µg/ml is note worthy. Also the fraction expressed a uniformity of 18hrs time interval as vital for its biological class inference which suggested its repetitive application instead of sustained release as disinfectant in aqua-farming. The

Summary

anti-algal fraction, CrM also acted as root stimulant of *Spirodela polyrhiza* within 24hrs of root excision. The observation supports the fact that roots exudation of duckweeds might behave antagonistically against algal blooms. The mechanical or chemical ways of bloom removal do not assure sustained resolution and instead deteriorates the ecosystem in many other ways. Nevertheless, extracting phyto-chemicals and introducing them in water bodies is a convenient tool and with least retardation of natural phenomenon. Considering its dual exhibition of inhibition as well stimulation, the present study also brings forward a *combination therapy* of commercial antibiotic, Oxytetracyclin Hydrochloride (95%) (OTC HCl) and the bioactive compound (CrM1, column fraction) from *Cyperus rotandus* was tested at various concentrations on *Aeromonas hydrophila* by well diffusion method. An ideal combination of 50ug/ml OTC HCl + 1000ug/ml [1_A:20_N OTCLC] bioactive compound recorded an inhibition zone of 22mm diameter. From immediate application perspective, OTC HCl is easily available in market and *Cyperus* is a widespread, invasive and aggressive weed causing much nuisance in agriculture, hence its procurement is priceless and obtainable. Presently, most commonly used antimicrobial agent is KMnO₄ (0.6-0.8gm in 8Lts-10Lts of water) for dip method of treatment. The OTCLC has natural product as a major constituent hence the amount is enhanced to 5 - 6gms in 10Lts. This will most importantly prevent resistance forming microbial strains which would fail to mimicry the versatile structure. The CrP dispersed a pleasant aroma which mainly constituted of fatty acids methyl esters of linoleic acid (100% peak area) and stearic acid (10.25%). The Methyl Linoleate is reported to show antifungal activity against *P. brasiliensis* which causes dermatitis infection to people who earn their livelihood on fishing and hunting. Eicosanoic Acid, methyl ester (5.12%) serves as an antifungal against *C. albicans* which is a disease causing agent in Tilapia, *Danio rerio* and *Oncorhynchus mykiss*. The Silicic acid is a natural plant growth factor which occupied the least area of

Summary

0.28%. The bioactive fraction showed remarkable inhibition against *Pseudomonas putida*, *Bacillus safensis* and *Citrobacter freundii*.

The in-vivo application of CrM and F2a_{1a} on induced *Aeromonas hydrophila* infection of *P. hypophthalmus* showed curative results in the histopathological examination of the liver and kidney tissues. The infected fish fed with formulated feed had almost the same histopathology like the uninfected fish samples with only exception of cytoplasmic vacuoles. The intact triad and the proportionate hepatocytes of the liver were seen. The kidney tissue samples were marked with prominent and integrated Bowman's capsule.