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ANTIBACTERIAL ACTIVITY OF FIVE SPECIES OF MARINE MICROALGAE

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Marine microalgae, Ethanolic extract, Methanolic extract, Antibacterial activity, Inhibition zone

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ABSTRACT: Antibacterial activity of five marine microalgae *Isochrysis galbana, Chaetoceros calcitrans, Tetraselmis suecica, Nannochloropsis oculata, Aphanocapsa* sp. was studied from Visakhapatnam. The ethanolic extract of *T. suecica, C. calcitrans,* and *N. oculata* s howed inhibitory activity against *V. harveyi* and *S. Aureus* with inhibition zone between 11.5 and 20mm. The ethanolic extract of *I. galbana* showed inhibitory activity against *S. aureus* only with an inhibition zone of 15mm. Among the four extracts, *T. suecica* showed maximum inhibitory activity against *V. harveyi* with an inhibition zone of 20mm. The methanolic extract of *T. suecica* moderately inhibited the growth of *V. harveyi* and *S. aureus* with inhibition zones of 11.5 and 11 mm respectively, whereas the extracts of *I. Galbana* and *C. Calcitrans* inhibited only *V. harveyi* with inhibition zones of 10 and 12 mm respectively, but no activity was found against *S. aureus, A. hydrophila* and *E. coli.* So, the studied microalgae could be used as a potential source to extract, bioactive marine natural compounds with antibacterial activity.

INTRODUCTION: Natural products from marine organisms have recently acquired importance in the pharmaceutical and pesticide industries. Marine organisms could be a potential source of bioactive secondary metabolites that represent useful leads in the development of new pharmaceutical agents [1, 2]. Many chemically distinct marine compounds with various biological activities have been isolated to date, and a number of them are being investigated and/or developed as new pharmaceutical products [3-5]. More than 10,000 compounds have been isolated from marine organisms so far, with hundreds (or) more being discovered every year [6, 7].

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Decades of research have demonstrated that marine organisms provide tremendous opportunities for harvesting anti-microbial substances and providing clues for their laboratory synthesis. Microalgae have recently received much attention due to their diverse phytometabolic content with various chemical structures and biological activities [8]. Algal biomass and its derivatives have many potential applications, ranging from animal feed and aquaculture to human nutrition and health products [9, 10].

Because of their phototropic existence and constant exposure to high oxygen and radical stresses, microalgae also have a high ability to produce a variety of efficient protective chemicals against oxidative and radical stressors [11]. With the dawn of molecular biology, the screening of microalgae for antibiotics and other bio-active compounds has received significant attention since Pharma-

cological properties were detected in their extracts. Several researchers have investigated a variety of algal-derived substances with bacteriostatic and bactericidal properties [12]. Microalgae have become a prominent source of antibacterial compounds and offer numerous advantages for antimicrobial studies due to their huge biodiversity and rapid growth rate [13].

Bioactive substances with antitumor, antileukemia, antibacterial, and antiviral properties have been reported all around the world. The demand for effective and non-toxic antibacterial therapeutics has increased. Antibacterial activity has been reported for a variety of compounds such as fatty acids [14], terpenoids, carbohydrates [15], peptides, polysaccharides, and alkaloids [16].

The production of secondary metabolites from microalgae is usually higher during stressful conditions [17]. In the larval rearing of marine fish, crustaceans, and bivalves, cultured microalgae are commonly used as a live feed.

It has been observed that the addition of microalgae has a positive effect on the larval rearing systems by decreasing the number of opportunistic bacteria [18]. The larvae's enhanced survival rates in tanks supplemented with microalgae were observed in the rearing of many marine fish species [19, 20].

The positive effect of microalgae introduced to larval rearing tanks has been attributed to the stabilization of the nutritional value of live food organisms added to the tanks [21], as well as to non-nutritional aspects such as the stimulation of the larvae's digestive system and immune system and the effect of the bacterial communities associated with the microalgae [22-24]. In light of the above, the present investigation focused on the antibacterial activity of five marine microalgal species *viz*. *Isochrysis galbana*, *Chaetoceros calcitrans*, *Tetraselmis suecica*, *Nannochloropsis oculata* and *Aphanocapsa* sp. (Cyanobacteria) against selected bacterial pathogens *Staphylococcus aureus* (Gram+ve), *Vibrio harveyi*, *Aeromonas hydrophila* and *Escherichia coli* (Gram -ve), which cause diseases in human and aquaculture species. This study was undertaken in the dept. of Marine Living Resources, Andhra University, Visakhapatnam, India during 2017-18.

MATERIALS AND METHODS:

Microalgal Culture: The stocks of five marine microalgal species *I. galbana, C. calcitrans, T. suecica, N. oculata* and *Aphanocapsa* sp. were used in this study procured from the regional center of Central Marine Fisheries Research Institute (CMFRI), Visakhapatnam, Andhra Pradesh, India. The microalgae are cultured in Conway [25] medium at salinity 34 ppt and at 23±2 °C temperature under 12:12 light-dark cycle with a light intensity of 80 µmol photons. $m^{-2}.s^{-1}$.

Biomass Harvest: At the end of the exponential phase, the algal culture was kept at 1-4° C, where the water could be in a minimum cooling point without freezing for 2-5 days until all the cells settled down. The period was varied according to cell size and density, *i.e*. larger and high-density cells settled early. Then the upper clear culture medium was discarded, and the biomass was washed twice with distilled water and dried in a hot air oven at 45-50° C for 48h.

Preparation of Crude Extract: Individually dried microalgal samples were powdered finely and soaked for 48 h in two solvents, *i.e*. ethanol and methanol, separately in a ratio of 1:8 (w/v). The solvent was decanted and then concentrated using a rotary evaporator (Heidolph G3). The final concentration was adjusted to 100mg/ml.

Antibacterial Activity: The concentrated crude extract thus obtained was tested against four pathogenic bacteria of the shrimp, fish, and human *viz*. *Vibrio harveyi* (MTCC: 3438), *Staphylococcus aureus* (ATCC: 11632), *Aeromonas hydrophila* (MTCC: 1739), and *Escherichia coli* (MTCC: 1678). Antibacterial activities of the extracts were analyzed using the agar well diffusion technique followed by [26].

The isolates *viz*. *S. Aureus* (gram +ve), *V. harveyi* (-ve), *A. hydrophila* (-ve) and *E. coli* (-ve) were inoculated individually into sterile nutrient broth taken in four different test tubes and incubated at 37° C for 18 h. Young cultures aforementioned were swabbed onto the surface of 3.8% Muller's Hinton Agar (MHA) plates separately. In each of these plates, wells of 6 mm diameter were made using a sterile cork borer. Exactly 50 µl of each crude extract was filled in respective wells and

allowed to diffuse at room temperature for 2 h. The respective solvents were used as controls. Then plates were inverted and incubated at 37°C for 24 h. Antibacterial activity was expressed in terms of zone of inhibition diameter (mm) using the Kirby-Bauer scale [27].

Sensitivity of Bacterial Pathogens to Commercially Available Antibiotics: Simultaneously the inhibitory activity of six commercially available antibiotics *viz*. Chloramphenicol, Gentamycin, Tetracycline, Erythromycin, Furozolidone and Streptomycin were tested against *S. aureus, V. harveyi, A. hydrophila* and *E. coli* in question for comparative study by using agar disc diffusion method.

The antibiotic discs (6mm) were placed on the MHA plates. Antibacterial activity was expressed in terms of zone of inhibition diameter (mm) using the Kirby-Bauer scale [27].

RESULTS:

Ethanol Extract: The ethanolic extract of *T. suecica* highly inhibited the growth of *V. harveyi* and *S. aureus* with inhibition zones of 20 and 13 mm respectively, but no activity was found against *A. hydrophila* and *E. coli,* whereas the extracts of *C. calcitrans* and *N. oculata* also inhibited *V. harveyi* and *S. aureus* with inhibition zones of 13 and 12 mm & 13 and 11.5 mm respectively, but showed no inhibitory activity against *A. hydrophila* and *E. coli.*

The extract of *I. galbana* highly inhibited the growth of *S. aureus* only with an inhibition zone of 15 mm. The extract of *Aphanocapsa* sp. showed no inhibitory activity against all four tested pathogenic bacteria **Table 1 Fig. 1** and **2**.

Methanol Extract: The methanolic extract of *T. suecica* moderately inhibited the growth of *V. harveyi* and *S. aureus* with inhibition zones of 11.5 and 11 mm respectively, but no activity was found against *A. hydrophila* and *E. coli*, whereas the extracts of *I. galbana* and *C. calcitrans* inhibited only *V. harveyi* with inhibition zones of 10 and 12 mm respectively, but no activity was found against *S. aureus, A. hydrophila* and *E. coli.* Extracts of *Aphanocapsa* sp. and *N. oculata* showed no inhibitory activity against all four tested pathogenic bacterial strains **Table 2, Fig. 3** and **4.**

Sensitivity of Bacterial Pathogens with Commercially Available Antibiotics: Six selected antibiotics were tested against four bacterial pathogens, but results presented here were only against two bacteria *i.e*. *V. harveyi* and *S. aureus*, as there was no inhibitory activity with microalgal extracts on *A. hydrophila* and *E. coli*.

Hence, the comparison has been confined to *V. harveyi* and *S. aureus* only. Out of six antibiotics, four showed sensitivity on *V. harveyi* and *S. aureus.*

Furazolidone and streptomycin showed activity against *V. harveyi* but no activity against *S. aureus*. Among six antibiotics tested, tetracycline showed the highest sensitivity of 22 mm inhibition zone against *V. harveyi* and *S. aureus* **Table 3, Fig. 5.**

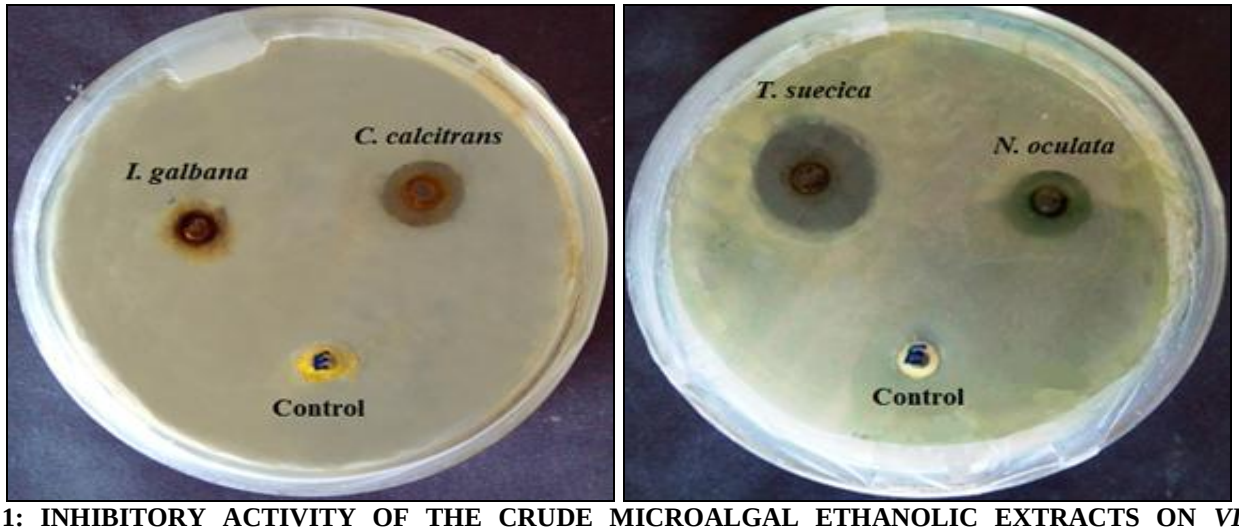


FIG. 1: INHIBITORY ACTIVITY OF THE CRUDE MICROALGAL ETHANOLIC EXTRACTS ON *VIBRIO HARVEYI*

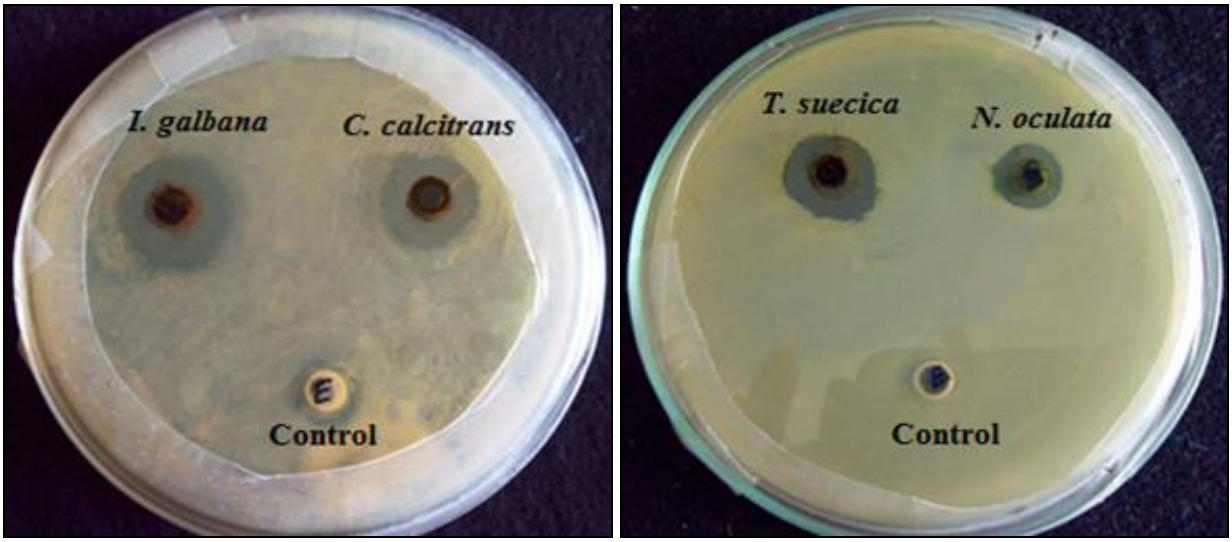


FIG. 2: INHIBITORY ACTIVITY OF THE CRUDE MICROALGAL ETHANOLIC EXTRACTS ON *STAPHYLOCOCCUS AUREUS*

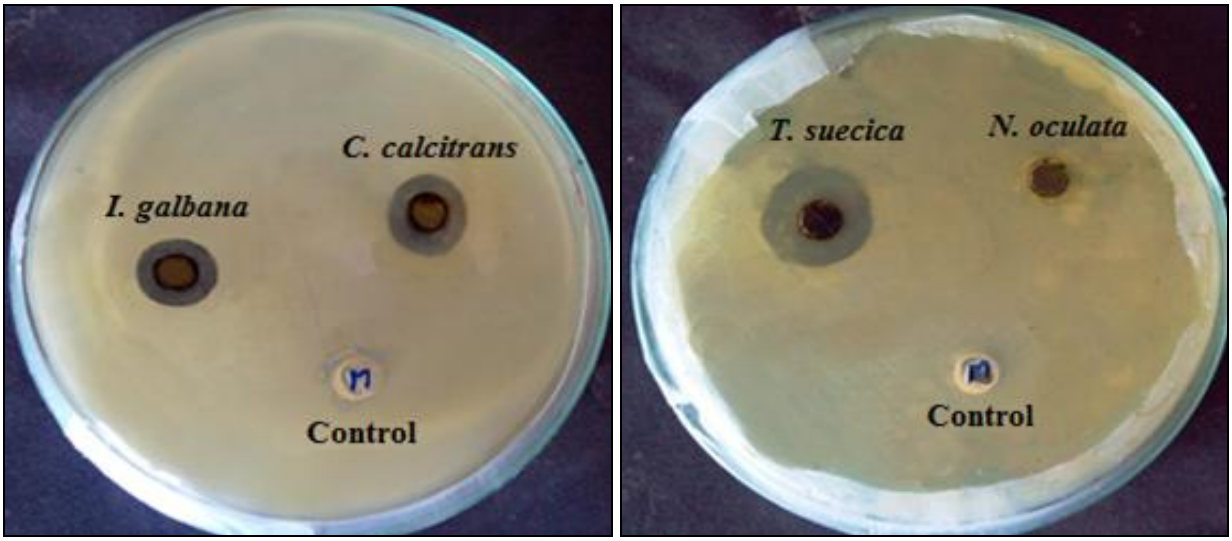


FIG. 3: INHIBITORY ACTIVITY OF THE CRUDE MICROALGAL METHANOLIC EXTRACTS ON *VIBRIO HARVEYI*

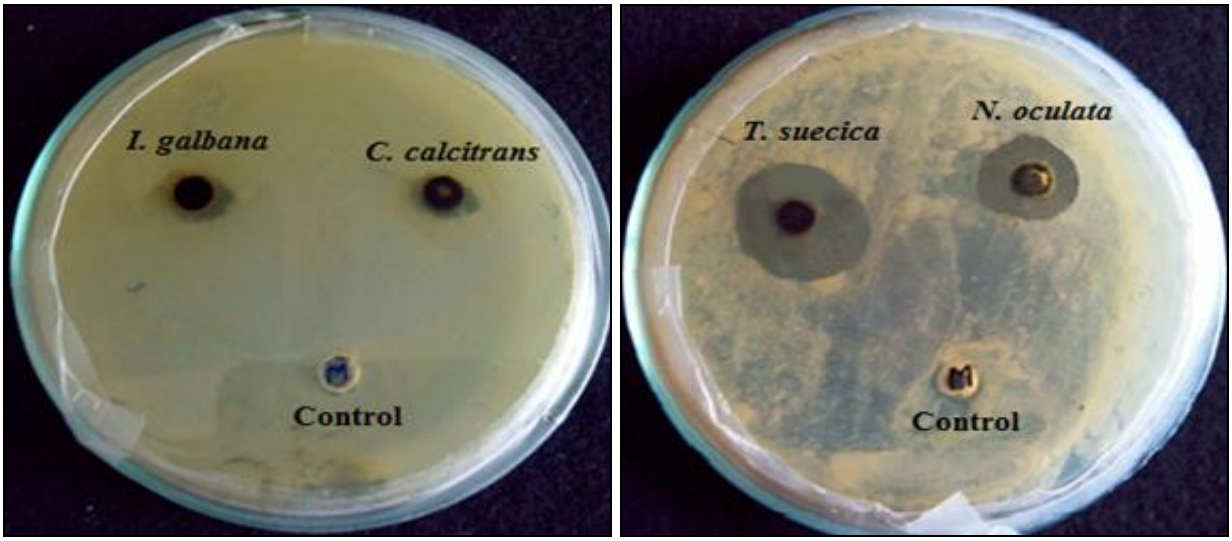
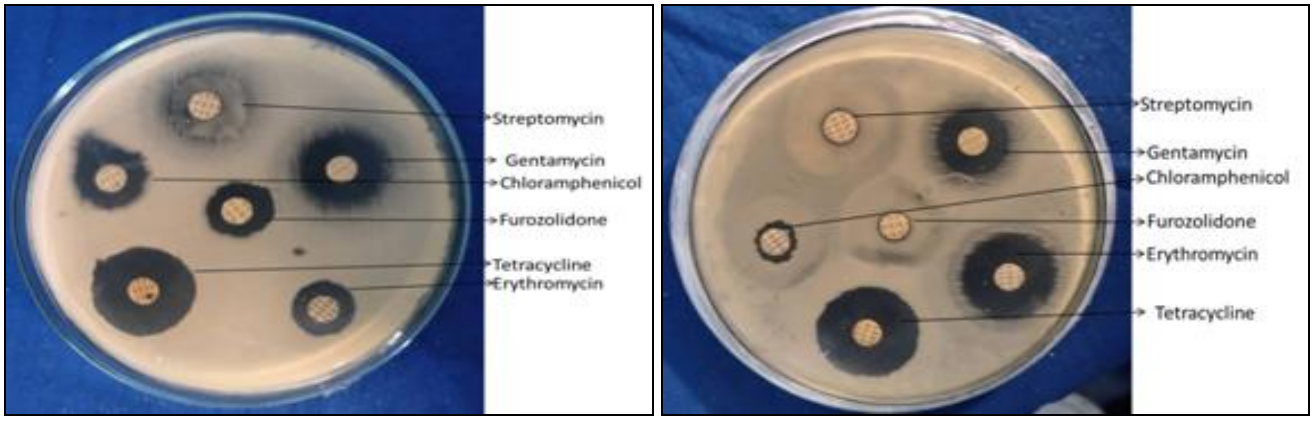


FIG. 4: INHIBITORY ACTIVITY OF THE CRUDE MICROALGAL METHANOLIC EXTRACTS ON *STAPHYLOCOCCUS AUREUS*



VIBRIOHARVEYI *STAPHYLOCOCCUSAUREUS*

FIG. 5: ANTIBIOTIC SENSITIVITY ON PATHOGENIC BACTERIA

TABLE 1: ANTI-BACTERIAL ACTIVITY OF ETHANOLIC EXTRACTS OF FIVE MARINE MICROALGAE

S. no.	Pathogenic Bacteria	Zone of inhibition (mm) of marine microalgae				
		I. galbana	*C. calcitrans*	*T. suecica*	*N. oculata*	*Aphanocapsa* sp.
1	*Vibrio harveyi* (gram -ve)	NA	13±1	20±2	13±1	NA
2	*Staphylococcus aureus* (+ve)	15±1	12±1	13±1	11.5±0.5	NA
3	*Aeromonashydrophila* (-ve)	NA	NA	NA	NA	NA
4	*Escherichia coli* (-ve)	NA	NA	NA	NA	NA

NA-No activity

TABLE 2: ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF FIVE MARINE MICROALGAE

S. no.	Pathogenic Bacteria	Zone of inhibition (mm) of marine microalgae				
		I. galbana	*C. calcitrans*	*T. suecica*	*N. oculata.*	*Aphanocapsas*p.
1	*Vibrio harveyi* (gram -ve)	10±1	12±1	11.5±0.5	NA	NA
2	*Staphylococcus aureus* (+ve)	NA	NA	11±1	NA	NA
3	*Aeromonashydrophila* (-ve)	NA	NA	NA	NA	NA
4	*Escherichia coli* (-ve)	NA	NA	NA	NA	NA

NA-No activity

TABLE 3: BACTERIAL SENSITIVITY WITH COMMERCIALLY AVAILABLE ANTIBIOTICS

S. no.	Antibiotic	Concentration (µg/disc)	Zone of inhibiton (mm)	
			V. harveyi	*S. aureus*
1	Erythromycin	15	14±1	21±1
2	Tetracycline	30	22±1	22±1
3	Furazolidone	50	13±1	NA
4	Gentamycin	10	18±1	16±1
5	Streptomycin	10	17±1	NA
6	Chloramphenicol	30	15±1	10±1

NA-No activity

DISCUSSION: The chrysophyte *Isochrysis galbana* has been reported to have a wide range and degree of inhibitory activity against pathogens such as *P. aeruginosa*, *K. Pneumonia*, *Proteus vulgaris*, *P. fluorescens*, *S. typhi*and *B. subtilis* [15, 28-32]. In the present study ethanolic extract of *I. galbana* also showed high inhibition (IZ-15mm) against *S. aureus*, whereas methanolic extract showed moderate inhibition (IZ-10mm) against *V. harveyi*. *Chaetoceros launderi*, tested against fungi and significant activity was observed against all the dermatophytes [33]. Selvendran & Michael Babu [34] reported *Chaetoceros calcitrans* is best controller of all the shrimp bacterial pathogens. George *et al.* [35] reported methanolic extract of *Chaetoceros* showed moderate activity against *V. harveyi* & *S. aureus*, but no activity with ethanolic extract. In the present study, *C. calcitrans* showed only moderate activity against shrimp bacterial pathogens like *V. harveyi* & *S. aureus*. The present study showed that the microalgal extracts obtained from *Tetraselmis suecica* claimed to be the best inhibitor against the growth of *V. harveyi* and *S. aureus*. This study is in concurrence with the one where *T. suecica* showed

activity against a number of pathogenic *Vibrio* species including *V. alginolyticus, V. parahaemolyticus* and *V. vulnificus* [36]. Similarly, *Tetraselmis* showed inhibitory activity against soil bacteria [15], *V. harveyi* [37] and *E. coli* & *S. aureus* [28, 30]. Over 132 marine microalgae when screened against six strains of bacteria revealed that methanolic and hexane extracts were more effective against *S. aureus* and *Streptococcus faecalis* and less effective with regard to *Bacillus subtilis* [38]. Ethanolic extract of *Noctiluca scintillans* showed antibacterial activity against *E. coli* while extract in acetone inhibited the growth of *S. faecalis* [39]. In the present study ethanolic extracts of *I. galbana, C. calcitrans, T. suecica* and *N. Oculata* showed the best results which are in concurrence with the report that the growth of bacterial pathogens *i.e. Psuedomonas vulgaris (*26.9%), *Shigella* sp. (26.3%) and *Salmonella typhi* (22.6%) [32].

Ochromonas sp., *Prymnesium parvum,* and a number of blue-green algae produce toxins that may have potential pharmaceutical applications [16]and *Oscillatoria* sp. showed activity against *S. aureus* & *E. coli* [40], but in the present study, both ethanolic & methanolic extracts of *Aphanocapsa* sp. showed no activity against all four bacterial pathogens. The temperature in incubation, pH of the culture medium, incubation period, medium constituents, and light intensity are the important factors influencing antimicrobial agent production [41].

In the present study, streptomycin showed no inhibition activity against *S. aureus*, whereas ethanolic extract of *N. oculata* has shown moderate to high inhibition against both *S. aureus* and *V. harveyi*. Surendhiran *et al.* [42] tested *N. oculata* FAME against different microbial strains; among them *E. coli* was found to be more sensitive with an inhibition zone of 27 mm than other microorganisms such as *B. subtilis* (16 mm) and *S. aureus* (17 mm). Hassi *et al.* [30] also mentioned ethanolic extract of *N. gaditana* showed positive against *E. coli*. But in the present study *N. oculata* were did not show any activity against *E. coli*. The strains of *V. harveyi* were sensitive to the antibiotics tested except Ampicillin [43]. He also reported that chloramphenicol showed the highest zone of inhibitions *i.e.,* 31, 25, 26 mm, against *V. harveyi* strains of PSU 2015, AAHRC1, and AAHRC2, respectively. In the present study, all the tested antibiotics showed inhibition against *V. harveyi* and *S. aureus*, but furazolidone and streptomycin showed no activity against *S. aureus.* Tetracycline showed the highest zones of inhibition (22 mm) against *V. harveyi* and *S. aureus.* In the present study, almost all microalgal extracts have shown greater or equal inhibitory activity against V. harveyi and S. aureus.

CONCLUSION: Since, marine microalgae seem to be a potential source for antibacterial compounds and especially show inhibitory activity against the bacterial pathogens of fish and shrimp more like commercial antibiotics. Further explorations towards this venture are needed for healthy and sustainable aquaculture.

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