Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0502.23**

EVALUATION OF CULTURAL CONDITIONS ON THE GROWTH OF MARINE FUNGI AND THEIR ANTIMICROBIAL ACTIVITY

Neha Keral*, Govindaiah, M. Shivashankar, M.S. Manmohan

Department of Life Science, Jnana Bharathi Campus, Bangalore University, Bengaluru, India.

ABSTRACT: Marine fungi are an ecological group, growing under tremendous pressure for the survival in marine environment. These conditions make them to produce different set of secondary metabolites having different biological activities. The aim of the present study was to isolate marine fungi from marine samples, which were identified morphologically. Their media and salinity concentrations were optimized for the growth and sporulation of the fungal isolates and also different solvent (methanol, ethyl acetate) extracts were screened against human pathogens: *Bacillus cereus* (MTCC 497), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (MTCC 443), *Salmonella typhimurium* (NCIM 2501) and yeast *Candida albicans* (MTCC 227). The marine fungi were identified as *Aspergillus* sp. *Trichoderma* sp. and Unidentified sp. and were selected for the production of secondary metabolites. Four different media and five different artificial sea water (ASW) concentrations were used to optimize the growth and sporulation of marine fungi. Malt extract Agar with 60% ASW proved to be the best media for growth and hence used for fermentation. The broths were extracted with methanol and ethyl acetate separately to get crude extracts. The fungal extracts were screened with Agar Plug method and Agar Well Diffusion method. Methanol extracts showed inhibition against all the pathogens in higher concentration, while ethyl acetate showed at lower concentration. Ethyl acetate extracts of *Aspergillus* sp. (SW-5 and SW- 6) were found to be more potential against the tested pathogens.

KEYWORDS: Marine fungi, Culture media, Artificial Sea Water, *Aspergillus* sp., Antimicrobial activity.

Corresponding Author: Neha Keral*(Ph.D.)

Department of Life Science, Jnana Bharathi Campus,

Bangalore University, Bengaluru, India.

1. INTRODUCTION

Marine environment have been recognized as one of the rich natural producers having varying biological and pharmaceutical importance. A lot of new natural products have been isolated from algae compared to marine fungi which are poorly explored. But the general increased interest in marine and estuarine habitats in the last decades has led to an increase in studies on marine fungi [1]. The searching of new natural products from the marine environment came from the fact that marine plants, animals and microorganisms have adopted themselves to all sorts of extreme environmental conditions and these organisms are constantly under tremendous selection pressure including space, competition, predation, surface fouling and reproduction. Marine fungi are most precisely defined as those that can complete their entire life cycles within the sea, i.e., "those that can grow and sporulate exclusively in a marine or estuarine habitat" [2]. They have unique metabolic and physiological capabilities showing the potential for the production of biologically active metabolites [3]. Marine fungi have proved to be a rich source of new biologically natural products [1], [4], [5]. Because of their particular living conditions, salinity, nutrition, higher pressure, temperature variation, competition with bacteria, viruses and other fungi, they may have developed specific secondary metabolic pathways compared to terrestrial fungi.Although some marine fungal compounds are in preclinical trials, the most promising marine fungal derived analogue in clinical trials is the anticancerous agent plinabulin which is based on the diketopiperazine halamide (also known as phenylhistidin), isolated from the fungus *Aspergillus ustus* [6]. Initially halamide was isolated as a mixture of enantiomers, but the (-) enantiomer alone exhibited a 50-fold increase of *in-vitro* antitumor activity against human cancer cell lines along with inhibition of tubulin polymerization by interacting with the colchicine binding site on tubulin [7], [8]. The difficulties of retrieving a "sustainable, reliable" harvest of marine microorganisms in sufficient quantities of materials, to allow for the study completion and difficulties culturing marine microorganisms *in-vitro* were cited. So, there is a requirement for the optimized procedure for growing marine fungi. This work aims at obtaining optimum physiological conditions for the growth of marine fungi and the production of secondary metabolites. It also consists of screening of marine fungal solvent extracts against some selected human pathogens.

2. MATERIALS AND METHODS

2.1. Study Area and Collection of Samples

Study area was selected based on the extreme coastal conditions faced by the specific area. According to Murali *et al.* the entire Pudducherry coast is segmented into equal lengths of 2.8km (12 segments) and assigned vulnerability ranking from 1 to 4 representing very low, low, high and very high vulnerability respectively [9]. Vulnerability specifies to be a function of the character magnitude, rate of climate variation to which a system is exposed, its sensitivity and its adaptive capacity. The entire coastal extent between Mutialpet and Kirumanpakkan as well as northern part

of Kalapet is designated as the high vulnerability zone, which constitute 50% of the coastline. Considering these adverse conditions, Muthialpet coastline was taken for study of fungi growing under extreme adverse conditions. Specimens such as sediment, water, shell and algae were collected aseptically and transferred in sterile polythene bags and glass bottles from the rocky coastline of Muthialpet, Puducherry during December, 2013.

2.2. Isolation of Marine Fungi from Different Samples

The collected samples of water and sand were serially diluted using Artificial Sea Water (ASW) [10] till 10^{-4} and plated on Potato Dextrose Agar (PDA) and Czapek Dox Agar (CDA) prepared in ASW supplemented with 0.1g streptomycin to inhibit bacterial growth by spread plate method [11], [12]. All the plates were incubated at 27 ± 2^0C for 10-15 days or as soon as the colonies appeared prior to spore formation to avoid over estimation due to autoinoculation [11], [13]. The distinct sporulated hyphal colonies on the agar plates were then sub-cultured on sterile CDA and PDA plates, respectively using monohyphal tip method for purification [14]. The algal and shell samples were surface sterilized with 70% ethanol were rinsed with sterile water and pressed onto agar plates to detect any residual fungal spores on their surface. Disinfected algae were cut into pieces and placed on CDA and PDA agar plates containing medium and incubated at 27 ± 2^0C for 10-15 days. Fungal isolates were then transferred onto the fresh medium [15].

2.3. Identification of Fungal Isolates

Identification was achieved by taxonomic processes such as direct comparison of specimens and by the use of cultural and morphological characters like arrangement and attachment of conidia on conidiophores, shape, size and septation of conidia, observed under binocular light microscope (40X) using lactophenol cotton blue stain by taking the marginal colonies of the fungi on clean glass slide. The fungal colonies were identified with the help of keys given by Barghoom and Linder, Johnson and Sparrow, Barnett and Hunter, Kohlmeyer and Kohlmeyer and following the taxonomic arrangement proposed in the 6th edition of Ainsworth and Bisby's Dictionary of Fungi [16], [17], [18], [19], [20].

2.4. Screening for Antimicrobial Activity

The fungal isolates of marine samples were subjected to screening for antimicrobial activity against four human pathogenic bacteria, two Gram positive: *Bacillus cereus* (MTCC 497), and *Staphylococcus aureus* (NCIM 2079); two Gram negative: *Escherichia coli* (MTCC 443),and *Salmonella typhimurium* (NCIM 2501) and a yeast,*Candida albicans* (MTCC 227).

2.4.1. Agar Plug Assay

In agar plug assay, cylindrical pieces were cut out from well grown culture of the fungal isolates. The blocks were placed on petriplates containing media with a fixed amount of test pathogens grown on Luria Bertani (LB) agar for bacteria and Sabouraud Dextrose Agar (SDA) for yeast (10^6 cells / ml). The cultures were kept in refrigerator for 12 hours at $4\text{-}5^0$C for the diffusion of antimicrobial

substance and afterwards they were incubated for 24 hours at 37^0C for bacteria (*S. aureus, S. typhi, B. cereus,* and *E. coli*) and 48 hours at 28^0C for yeast (*C. albicans*). The antimicrobial activity was measured in mm as clear zone of inhibition [21].

2.5. Influence of Culture Media and Artificial Sea Water on Fungal Growth

The isolated fungal species were selected to study the influence of environmental factors on the growth in the laboratory conditions. Effect of abiotic factors viz., culture media, and artificial sea water concentration were tested.

2.5.1. Effect of Culture Media

To find out the effect of culture media on the growth of the marine fungal isolates, four different culture media namely Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA), Malt Extract Agar (MEA), and Artificial Sea Water Agar (ASWA) were used. Autoclaved media were poured in petridishes, upon solidification 5mm fungal disc were transferred aseptically. The plates were incubated at 27 ± 2^0C for 7-15 days. After the incubation period, radial growth of the fungal isolates was recorded in mm [22].

2.5.2. Effect of Artificial Sea Water Concentration

All the ingredients of the media were dissolved in ASW [10] with varying concentration of ASW and sterile deionized water (V/V) respectively to find out obligate and facultative marine fungi. Culture media were prepared with varying percentages of ASW 0%, 20%, 40%, 60%, 80% and 100% respectively. For concentrations that were less than 100% ASW, the other percentage was deionized water. ASW was used instead of natural sea water because the exact ion concentrations are more consistent with ASW and ensures media reproducibility. All the different sea water concentration media were autoclaved and poured into sterilized petridishes, 5mm fungal disc were transferred aseptically onto the agar surface. All the plates were incubated at 27 ± 2^0C for 7-15 days. After incubation period, radial growth (in mm) of the fungal samples was recorded.

2.6. Production and Extraction of Secondary Metabolites

The fungal isolates exhibiting broad spectrum of antimicrobial activity during screening were subjected for the production of secondary metabolites. The fungal strains were inoculated on petridishes with MEA and incubated at 27 ± 2^0C for 7-10 days using ASW(60%) and then transferred to Erlenmeyer flask containing 200ml Malt Extract Broth made in ASW for 7,14,21 and 28 days in shaker incubator at 27 ± 2^0C. The incubated flasks were filled with 200ml ethyl acetate (EtOAc) and methanol (MeOH) separately and allowed to stand for 24hrs in shaker, then filtered through cheese cloth and whattman filter paper 1. The culture broth was successively extracted with ethyl acetate and methanol thrice (3X200ml per 200ml homogenate) separately. Here, media and solvents were taken in 1:1 (V/V) ratio. The ethyl acetate and methanol extracts were concentrated by rotary evaporator to get the crude extract.

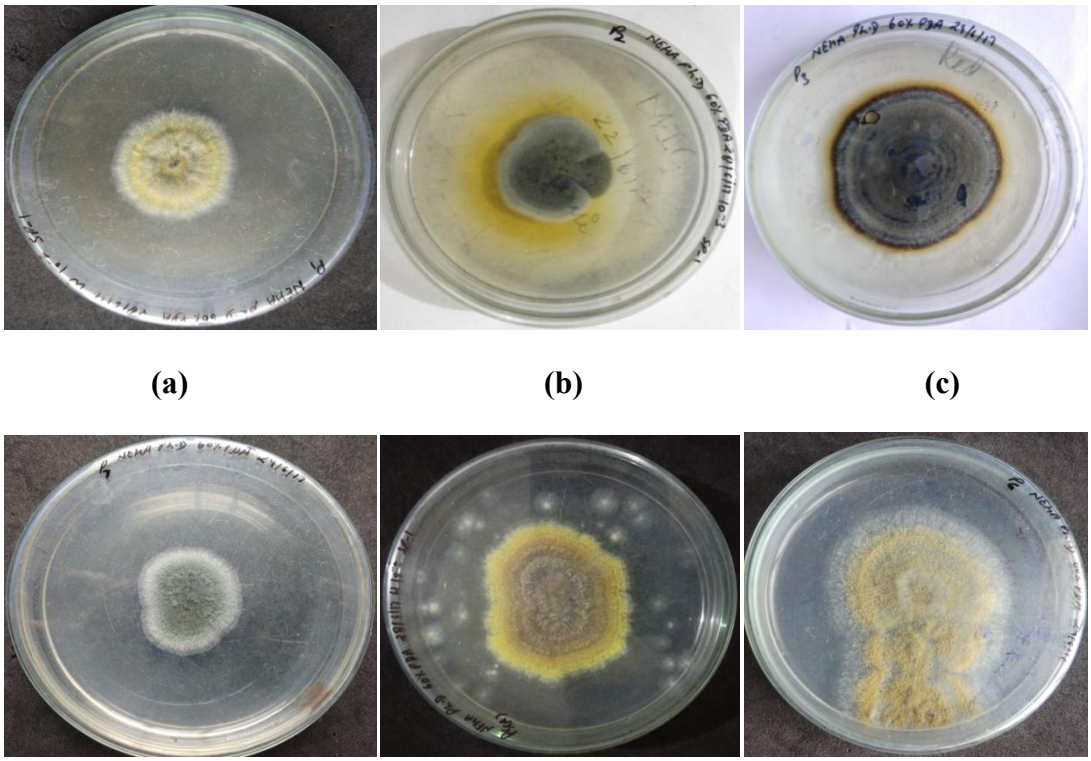
2.7. Antimicrobial Activity of Solvent Extracts by Agar Well Diffusion Method

The extracted secondary metabolites of both ethyl acetate and methanol solvents of different days were dissolved in DMSO (Dimethyl Sulfoxide) at concentrations of 100mg/ml and 10mg/ml, then poured into 5mm diameter well made on petridishes containing media (LB agar for bacteria and SDA for yeast) and inoculated with a fixed amount of test pathogens (inoculums 10^6 cells/ml). Approximately 50µl of the crude extract was poured into the wells, allowed to stand for 2 hours at room temperature and then incubated at 37^0C [23]. Control was set up using DMSO. Zone of inhibition was measured in mm after incubation period of 24 hours for bacteria and 48 hours for yeast.

3. RESULTS AND DISCUSSION

3.1. Isolation and Identification of Marine Fungi

The fungi investigated in this study were isolated from marine samples (water, sand, algae and shells) collected from Muthialpet coastline of Pudducherry. Total of 17 different colonies were obtained, out of which 8 fungal isolates from sea water and 9 from sand. There were no fungal growths in algal and shell plates. Among all the isolates, 6 fungal strains were recurring and were identified based on cultural and morphological characteristics (Fig-1).



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Fig 1: Marine fungal isolates (a) SW-1, (b) SW-2, (c) SW-3, (d) SW-4,(e) SW-5 (f) SW-6

Thus, all the 6 isolates were further preceded for primary screening of antimicrobial activity. Out of 6, 3 were identified as *Aspergillus* species, one *Trichoderma* species and the rest two were not

showing any sporulation, hence referred as unidentified species. One of the previous studies carried out by Samuel *et al.,* stated that *Aspergillus* and *Penicillium* were an indication for the wealth of fungal biodiversity in the south east coast of Bay of Bengal [12]. Das *et al.* while studying filamentous fungal diversity from the sediments of continental slope of Bay of Bengal reported that *Aspergillus* was found to be the dominant genus [24]. Damare *et al*. also reported *Aspergillus, Penicillium, Cladosporium* and *Fusarium* presence in the deep sea sediment samples [25].

3.2. Preliminary Screening for Antimicrobial Activity

Among all the fungal isolates, 6 recurring marine fungal strains were screened for antimicrobial activity by agar plug method.

Table 1: Preliminary screening for Antimicrobial activity of Marine Fungi against some selected Human Pathogens by Agar Plug Method

Marine fungal cultures			Antimicrobial activity (Zone of Inhibition in mm)				
Sl. No.	**Isolates Code**	**Marine Fungi**	***E. coli***	***S. aureus***	***S. typhi***	***B. cereus***	***C. albicans***
1	SW-1	*Aspergillus* sp.	10.33±0.57^{b}	0±0^{b}	0±0^{d}	11.33±0.57^{b}	8.66±0.57^{b}
2	SW-2	*Unidentified* sp.	10.66±0.57^{b}	10.00±1.00^{a}	0±0^{d}	13.00±1.00^{a}	9.00±1.00^{b}
3	SW-3	*Unidentified* sp.	15.00±1.00^{a}	0±0^{b}	17.66±0.57^{bc}	0±0^{c}	0±0^{c}
4	SW-4	*Trichoderma* sp.	16.00±2.00^{a}	0±0^{b}	21.66±2.08^{a}	0± 0^{c}	0±0^{c}
5	SW-5	*Aspergillus* sp.	10.33±0.57^{b}	0±0^{b}	19.00±1.00^{b}	13.00±1.00^{a}	12.66±0.57^{a}
6	SW-6	*Aspergillus* sp.	11.66±0.57^{b}	0±0^{b}	16.66±1.15^{c}	12.66±0.57^{a}	13.66±0.57^{a}

In each column, zone of inhibition expressed as Mean ± SD (n=3)
The mean values followed by different alphabets differ significantly when subjected to DMRT @ $p \leq 0.05$ among the fungal isolates

All the marine fungal isolates showed potential inhibition against *E. coli.* Only SW-2 suppressed the growth of *S. aureus*. Whereas, *S. typhi* was inhibited by four marine fungal isolate SW-3 to SW-6 while *B. cereus* and *C. albicans* were inhibited by SW-1, SW-2, SW-5 and SW-6. On comparing the antimicrobial activity of all the fungal extracts, four fungal isolates SW-1, SW-2, SW-5 and SW-6 were most promising against all the human pathogens (Table -1 and Fig.- 2) and selected for further studies.

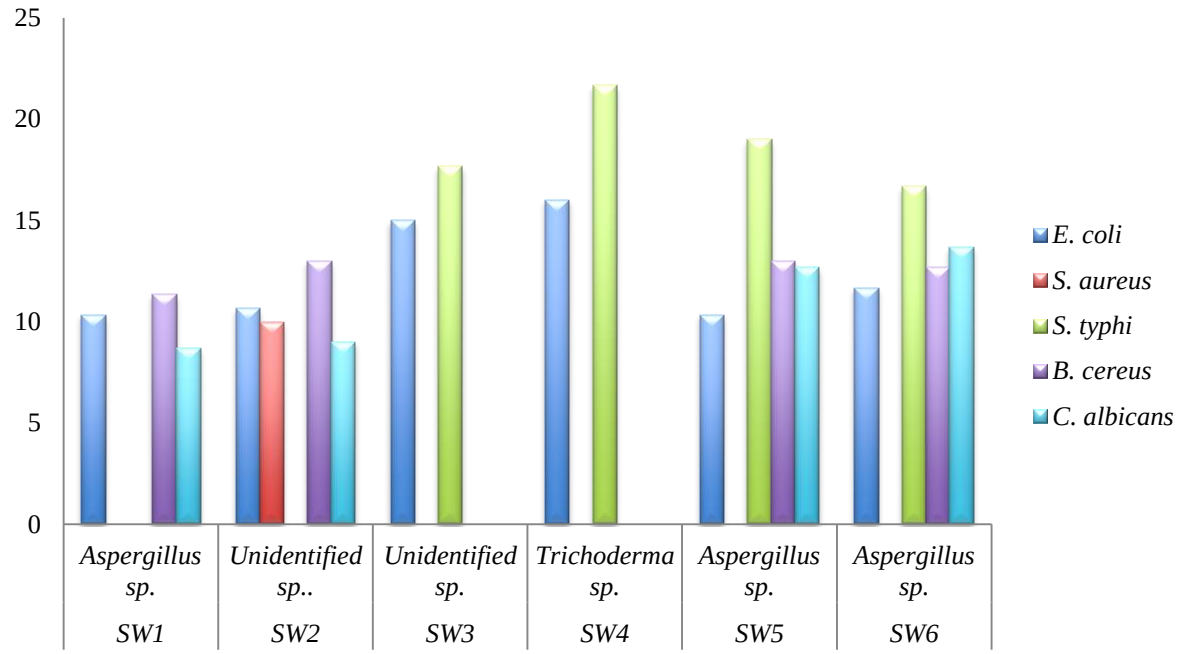


Fig 2: Preliminary screening for Antimicrobial activity of Marine Fungi against some selected Human Pathogens by Agar Plug Method

3.3. Influence of Culture Media and Artificial Sea Water

Saleem stated that marine fungi living in diverse environmental conditions containing high salt levels, typical of the marine situation in association with invertebrates are sensitive to culture media [26]. Based on the primary screening, four fungal isolates were selected. Therefore, an approach was taken to grow marine fungal isolates on different culture media with different ASW concentration. On comparing, different media, it was found out that MEA was the most suitable media for all the four fungal isolates. Considering the ASW concentrations, there was no growth of marine fungal strains in less saline medium. As the salinity increases the rate of growth increases till 60% ASW concentration and gets reduced till it reaches 100% ASW (Table-2). It was found out that there was growth in 100% ASW concentration media but the growth rate was slow and sporulation was late, showing that salinity influences the growth of the fungal strains. There was no growth on Artificial Sea Water Agar which may be due to non – obligate nature of fungal species. Kohlmeyer and Kohlmeyer stated that marine fungi are form of an ecological group and not a taxonomic group [18]. Among these, the obligate marine fungi grow and sporulate exclusively in sea water and their spores are capable of germinating in sea water. On the other hand, facultative marine fungi are more from fresh water or a terrestrial milieu that have undergone physiological adaptations that allow them to grow and possibly also sporulate in the marine environment which is evident in the present study [18].

Table 2: Growth of fungal isolates on different Culture Media using different concentration of Artificial Sea Water (ASW) in mm

Culture Media	Conc. of ASW	Radial Growth in mm			
		SW-1	SW-2	SW-5	SW-6
CDA	20%	0±0	0±0	0±0	0±0
	40%	10.33±0.57	0±0	50.00±1.00	0±0
	60%	48.00±1.00	18.33±0.57	77.00±1.00	25.33±0.57
	80%	37.33±1.15	15.33±0.57	74.00±1.00	12.66±0.57
	100%	06.33±0.57	08.00±1.00	0±0	0±0
PDA	20%	51.66±2.30	13.00±1.00	0±0	0±0
	40%	60.66±2.08	15.66±0.57	0±0	0±0
	60%	81.00±1.00	18.66±0.57	66.00±1.00	24.00±1.00
	80%	34.00±1.00	10.33±0.57	61.00±1.00	12.00±1.00
	100%	44.66±0.57	05.00±1.00	0±0	0±0
MEA	20%	0±0	0±0	0±0	0±0
	40%	40.00±1.00	13.33±0.57	16.33±0.57	54.66±0.57
	60%	78.00±1.00	74.66±0.57	87.00±2.64	42.66±0.57
	80%	74.66±0.57	71.66±0.57	64.00±2.64	0±0
	100%	05.33±0.57	11.66±0.57	02.66±0.57	0±0
ASWA	100%	0±0	0±0	0±0	0±0

In each column, radial growth expressed according to DMRT @ $p \leq 0.05$ as Mean ± SD (n=3)

3.4. Production and Extraction of Secondary Metabolites

The four marine fungal isolates were found to be more favorable antimicrobial agents and hence were selected for the production and extraction of secondary metabolites. The ethyl acetate and methanol extracts were yielded on different fermentation days of 7, 14, 21 and 28 and antimicrobial activities of the extracts were evaluated by using Agar Well Diffusion method.

3.5. Antimicrobial Activity of Crude Extract by Agar Well Diffusion Method

The extracts of both methanol and ethyl acetate solvents procured from marine fungal isolates exhibited a broad spectrum of antimicrobial activity against human pathogens and the zone of inhibition were measured and tabulated in Table-3 and Table-4. The methanol extracts of all the four fungal isolates were sticky in nature, while ethyl acetate extracts were in powder form. Both the extracts exhibited antimicrobial activity.Among the methanol extracts represented in Table-3, the highest activity recorded from marine fungal isolate SW-1 was against *C. albicans* on its 14th days being 40.00mm while the least activity was against *S. aureus* on its 14th day being 11.00mm. SW-2 exhibited highest inhibition against *C. albicans* (35.66mm) on its 7th fermentation day and least

against *S. aureus* (14.00mm) on its 28th day. Both SW-5 and SW-6 had highest inhibition against *C. albicans* 35.66mm and 31.00mm respectively on its 21st fermentation day while least against *S. aureus* 12.66mm on 14th day and 11.33mm on 7th day respectively.

Table 3: Antimicrobial activity of Methanol extracts of Fungal isolates on different fermentation period by Well Diffusion Method

Sl. No.	Fermentation Period (days)	Antimicrobial activity (Zone of Inhibition in mm)				
		E. coli	***S. aureus***	***S. typhi***	***B. cereus***	***C. albicans***
(a) SW1 *Aspergillus* sp.						
1	7	22.66±0.57	11.33±1.15	23.00±1.00	23.66±1.15	35.33±0.57
2	14	21.66±1.52	11.00±1.00	20.66±0.57	20.00±1.00	40.00±1.00
3	21	21.66±0.57	12.33±0.57	21.33±1.15	20.33±1.15	30.66±1.52
4	28	20.33±1.52	12.66±0.57	23.33±1.52	16.33±1.15	29.00±1.00
(b) SW2 *Unidentified* sp.						
5	7	26.66±1.52	16.66±0.57	24.66±0.57	24.33±0.57	35.66±1.15
6	14	21.00±1.00	14.33±0.57	19.66±0.57	17.33±1.52	30.66±0.57
7	21	24.66±1.15	19.00±1.00	26.33±0.57	29.00±1.00	34.33±1.15
8	28	21.33±1.52	14.00±1.00	20.00±1.00	21.33±1.15	31.00±1.00
(c) SW5 *Aspergillus* sp.						
9	7	22.00±1.00	16.66±1.15	22.33±1.15	21.00±2.00	28.66±1.52
10	14	19.66±1.15	12.66±0.57	24.66±0.57	26.00±1.00	28.66±0.57
11	21	22.66±0.57	25.66±0.57	29.66±0.57	27.66±1.15	35.66±1.15
12	28	23.33±1.15	13.33±1.52	23.66±0.57	21.00±1.00	26.66±0.57
(d) SW 6 *Aspergillus* sp.						
13	7	23.66±0.57	11.33±1.52	22.33±0.57	23.00±1.00	28.00±1.00
14	14	23.33±1.15	12.33±1.15	23.00±2.00	25.00±1.00	30.66±0.57
15	21	25.66±0.57	13.00±1.00	26.66±0.57	23.00±1.00	31.00±1.00
16	28	22.66±0.57	27.00±1.00	32.33±0.57	26.00±1.00	24.00±1.00

In each column, zone of inhibition expressed according to DMRT @ $p \leq 0.05$ as Mean ± SD (n=3)

Among the ethyl acetate extracts, SW-1 recorded highest antimicrobial activity against *S. typhi* with 25.33mm inhibition zone on its 7th day while least against *S. aureus* (15.33mm) on 14th day. SW-2 exhibited highest inhibition against *S. aureus* (33.33mm) on its 7th day, while SW-5 and SW-6 exhibited highest activity against *S. typhi* (26.66mm) and *E. coli* (32.00mm) respectively on 21st day of fermentation. It was observed that the overall antimicrobial activity of methanol and ethyl acetate extracts decreased with time for strain SW-1 and SW-2, being highest on 7th day. While

for SW-5 and SW-6, the activity was highest on 21st day (Table-4). Li *et al*. reported presence of two new dihydroisocoumarin derivatives, aspergillumarins A and B, extracted from the marine-derived fungus *Aspergillus*sp., isolated from mangrove tree *Bruguiera gymnorrhiza* collected from the South China Sea. Both the compounds showed minimal antibacterial activity against *S. aureus* and *B. cereus* at the concentration of 50µg/ml [27].The results were compared to antimicrobial studies of secondary metabolites of marine fungi which have been reviewed and well documented by Debbab *et al.*[28].

Table 4: Antimicrobial activity of Ethyl Acetate extracts of Fungal isolates on different fermentation period by Well Diffusion Method

Sl. No.	Fermentation Period (days)	Antimicrobial activity (Zone of Inhibition in mm)				
		E. coli	***S. aureus***	***S. typhi***	***B. cereus***	***C. albicans***
(a) SW1 *Aspergillus* sp						
1	7	24.33±1.15	21.33±1.15	25.33±1.15	24.33±0.57	27.33±1.52
2	14	22.30±3.05	15.33±0.57	21.66±2.08	17.00±1.00	20.66±0.57
3	21	22.66±0.57	16.00±1.00	21.66±0.57	20.33±2.08	22.33±0.57
4	28	20.66±0.57	18.33±0.57	24.00±1.00	18.33±1.52	21.66±0.57
(b) SW2 *Unidentified* sp.						
5	7	28.66±1.52	33.33±0.57	28.00±1.00	25.00±1.00	28.33±0.57
6	14	22.66±0.57	22.00±1.00	24.33±0.57	21.66±0.57	24.00±1.00
7	21	24.00±1.00	16.66±0.57	23.00±1.00	19.66±0.57	24.66±0.57
8	28	21.00±1.00	26.66±0.57	25.66±0.57	23.00±1.00	27.33±0.57
(c) SW5 *Aspergillus* sp						
9	7	22.66±0.57	20.33±0.57	24.00±1.00	23.00±1.00	20.00±1.00
10	14	20.33±0.57	18.66±0.57	24.00±1.00	18.66±1.52	23.00±1.73
11	21	24.66±0.57	25.33±0.57	26.66±1.52	25.66±0.57	26.33±1.15
12	28	25.66±0.57	21.66±1.52	25.00±1.73	25.33±0.57	27.33±1.52
(d) SW 6 *Aspergillus* sp						
13	7	24.66±0.57	31.33±1.52	26.33±0.57	27.00±1.00	22.66±0.57
14	14	25.00±1.00	31.66±0.57	26.00±1.00	29.33±0.57	24.66±0.57
15	21	32.00±2.00	26.66±0.57	27.00±1.00	26.00±1.00	28.00±1.73
16	28	21.00±1.00	26.66±0.57	21.66±0.57	24.00±1.00	22.33±1.15

In each column, zone of inhibition expressed according to DMRT @ $p \leq 0.05$ as Mean ± SD (n=3)

4. CONCLUSION

In the present study, marine fungi were isolated from the marine samples. Different culture media and salinity were used to optimize the growth of the fungal isolates. The optimized media was observed to be MEA with 60% ASW concentration in the media. The isolated fungal strains were identified as of *Aspergillus* sp., *Trichoderma* sp., and two were unidentified as these strains did not sporulate in laboratory conditions. Preliminary antimicrobial screening of six fungal isolates was done using Agar Plug method. Among the six fungal strains, four showing promising antimicrobial activity were kept for fermentation on different days (7, 14, 21 and 28) for obtaining methanol and ethyl acetate extracts. Both the extracts were then tested against human pathogens for their antimicrobial activity. All the extracts showed different range of antimicrobial activity. On comparing both the solvent extracts, ethyl acetate extracts were more promising than methanol extracts. SW-5 and SW-6 showed most inhibiting effect against all the pathogens proving to be the most promising marine fungi to be further used for drug discovery. The above results were comparable to the research work previously done by several researchers which confirm that the *Aspergillus* sp of marine fungi possesses a significant antimicrobial activity, which was confirmed in our study.

CONFLICT OF INTEREST

Authors have no conflicts of interest.

REFERENCES

1. Liberra K, Lindequist U. Marine Fungi- A prolific resource of biologically active natural products. Pharmazie. 1995; 50: 583-588.
2. Kohlmeyer, J, Spatafora, J.W., Volkmann-Kohlmeyer, B. Lulworthiales, a new order of marine Ascomycota. Mycologia. 2000; 92(3): 453-458.
3. Pietra F. Secondary metabolites from marine microorganisms – bacteria, protozoa, algae and fungi- achievements and prospective. Nat. Prod. Rep. 1997; 14: 453-64.
4. Cuomo, V., I. Palomba, A. Perretti, A. Guerriero, M. D' Ambrosio and F. Pietra. Antimicrobial activities of marine fungi. J. Mar. Biotechnol. 1995; 2: 199-204.
5. Jensen R. Paul and Fenical William. Marine microorganisms and drug discovery: Current status and Future potential. Drugs from the Sea. 2000: 6-29.
6. Kanoh K., Kohno S., Asari T., Harada T., Katada J., Muramatsu M., Kawashima H., Sekiya H.and Uno I. (-)- Phenylahistin: A new mammalian cell cycle inhibitor produced by *Aspergillus ustus*. Bioorg. Med. Chem. Lett.1997; 7: 2847-2852.
7. Kanoh K., Kohno S., Katada J., Hayashi Y., Muramatsu M. and Uno I. Antitumor activity of phenylahistin *in-vitro* and *in-vivo*. Biosci. Biotechnol.Biochem.1999b; 63: 1130-1133.
8. Kanoh K., Kohno S., Katada J., Takahashi J. and Uno I. (-)- Phenylahistin arrests cells in mitosis by inhibiting tubulin polymerization. J. Antibiot.1999c; 52: 134-141.

9. R. Mani Murali, M. Ankita, S. Amrita and P. Vethamony, Coastal vulnerability assessment of Puducherry coast, India, using the analytical hierarchical process. Nat. Hazards Earth Syst. Sci. 2013; 13: 3291-3311.
10. Lyman, J., Fleming H.R. Composition of Sea water. J. Marine *Res.*1940;3: 134-146.
11. Raghukumar, C., Saeelu, G., Loka Bharati, P.A. Nair, S., Mohandass, C. Microbial biomass and organic nutrients in the deep sea sediments of the central Indian Ocean Basin. Mar. Georesour. Geotechnol. 2001; 19: 1-6.
12. Raghukumar, C., Raghukumar, S., Saeelu, G., Gupta, S.M., Nath, B. N., Rao, B. R. Buried in time: culturable fungi in deep sea sediment core from the Chagos Trench, Indian Ocean. Deep-sea Res. 2004; I 51: 1759-1768.
13. Samuel, P., Prince, L., and Prabakaran, P. Assessment of mycological diversity of marine sediment of south east coast of Tamil Nadu, India. Euro. J. Exp. Bio. 2011;1(3): 130-138.
14. Ainsworth, M.A. Isolation techniques for basidiomycetes. UNESCO/ WFCC Committee.1993; 11.
15. Klemke, C., Kehraus, S., Wright, A. D. and Koenig, G. M. New secondary metabolites from the marine endophytic fungus *Apiospora montagnei*. J. Nat. Prod. 2004; 67: 158-1063.
16. Barghoorn E.S., and Linder D.H. Marine Fungi: Their Taxonomy and Biology. *Farlowia.*1944; 3: 395-467.
17. Johnson, T.W., and Sparrow, F.K. Fungi in Oceans and Estuaries. J. Cramer, Weinheim, Germany. 1961: 668.
18. Barnett, H.L., and Hunter, B.B. Illustrated genera of imperfect fungi, 3rd ed. *Burgess Publishing* Co., Minneapolis, Minnesota. 1972: 241.
19. Kohlmeyer, J., and Kohlmeyer, E. Marine Mycology - The Higher Fungi. Academic Press; *London, UK.* 1979.
20. Anisworth, G.C. Ainsworth and Bisby's Dictionary of Fungi, 6th ed., Commonwealth Mycological Institute, 1971, Kew, Surrey, England.
21. Ramesha, A and Srinivas, C. Antimicrobial activity and phytochemical analysis of crude extracts of endophytic fungi isolated from *Plumeria acuminata* L. and *Plumeria obtusifolia* L. Euro. J. Exp. Bio. 2014;4(2): 35-43.
22. Miriam H. Kossuga, Stelamar Romminger, Camila Xavier, Marilia C. Milanetto, Milene Z. do Valle, Eli F. Pimenta,*et al.* Evaluating methods for the isolation of marine- derived fungal strains and production of bioactive secondary metabolites. Brazilian Journal of Pharmacognosy. 2012; 22(2): 257-267.
23. Igbinosa O.O, Igbinosa E.O. and Aiyegoro O.A. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). African Journal of Pharmacy and Pharmacology. 2009; 3(2): 058-062.

24. Das Surajit, Lyla P.S., Khan Ajmal Syed. Filamentous fungal population and species diversity from the continental slope of Bay of Bengal, India. Acta Oecologica. 2009; 35(2): 269-279.
25. Damare. S., Raghukumar C, Raghukumar S. Fungi in deep sea sediments of the Central Indian Basin. Deep-Sea Res. 2006; 1(53): 14-27.
26. Muhammad Saleem, Muhammad Shaiq Ali, Shafqat Hussain, Abdul Jabbar, Muhammad Ashrafand, Yong Sup Lee. Marine natural products of fungal origin**.** Nat. Prod. Rep. 2007;24: 1142–1152.
27. Shangde Li, Meiyan Wei, Guangying Chen and Yongcheng Lin. Two new dihydroisocoumarine fronthe endophytic fungus *Aspergillus* sp. collected from the South China Sea. Chemistry of Natural Compounds. 2012; 48 (3): 371-373.
28. Abdessamad Debbab, Amal H. Aly, Wen H. Lin and Peter Proksch. Bioactive Compounds from Marine Bacteria and Fungi. Microbial Biotechnology. 2010;3(5): 544–563.