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Assessment of antibacterial potential of metabolites of marine fungi isolated from coastal region of Mumbai

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ABSTRACT

The current study was carried out to isolate and identify marine fungi from the coastal region of Mumbai and assess their antibacterial potential. *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cladosporium cladosporioides*, *Cladosporium pseudocladosporioides*, *Trichophyton rubrum*, *Penicillium chrysogenum*, *Alternaria alternate*, *Neoscytalidium dimidiatum* and *Aspergillus terreus* were isolated and identified. The metabolite extraction was carried out by broth fermentation and extraction of dry mycelium using organic solvents like chloroform, ethyl acetate, and ethanol. Antibacterial potential of fungal metabolites was assessed by well diffusion method. Different concentrations (2–150 µg/ml) of extracts of broth and dry mycelia were tested against organisms like *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Staphylococcus aureus*. Results revealed that chloroform and ethanolic extracts (2 µg/ml) from *C. cladosporioides* broth fermentation exhibit 100% growth inhibition of test organisms. Mycelium ethanolic extract of *A. fumigatus*, ethyl acetate extract of *C. cladosporioides* and chloroform extract of *C. pseudocladosporioides* exhibited maximum (100%) growth inhibition against all test organisms at 2 µg/ml. The study confirms the antibacterial potential of fungal metabolites and therefore paves a way for further identification of the active principles.

INTRODUCTION

Ever since the existence of humankind, infectious diseases have always been seen as a big threat. However, with the advancement of science and technology and the discovery of penicillin, people were hopeful to combat the threat posed by microorganisms. However, their joy did not last too long due to the emergence of resistance in microorganism against available antibiotics. Development of drug resistance is attributed to the excessive and over use of antibiotics (over the counter availability) (Demain, 1999). The evolution of resistant bacteria is also influenced by mutation and gene exchange between bacteria, therefore leaving most of the current antibiotics obsolete. Since last one and half decade, scientists have turned to natural products for the identification of novel compounds to combat the menace of drug resistance. Microorganisms are seen as the reservoir of natural and novel compounds due to their ability to produce diverse secondary metabolites with their environment (Demain, 1999; Schmidt, 2004). There are a variety of natural resources that can be used to find antibiotics. By studying marine-derived microorganisms, novel antibiotics can be discovered and developed as per several studies, as it is a home to a diverse range of biodiversity (Arumugam *et al*., 2015). The organisms of marine habitat evolve themselves not only to survive in harsh environment but also produce various metabolites which have been found biologically active (Kasanah and Hamann, 2004).

It is now widely acknowledged that marine is home to large and varied micro biomes. In addition to bacteria and archaea, fungi in deep-sea habitats have been extensively investigated (Wang *et al*., 2015). Fungi are known for their wide range of secondary metabolites, which include numerous life-saving medications as well as deadly poisonous mycotoxins. It has been

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found that screening and characterization of metabolites are key concepts in metabolomics (Roy and Banerjee, 2017). Many pharmacologically bioactive compounds have been identified from deep-sea fungi and tested for their anticancer, antifungal, antibacterial, antiviral, and anti-larval properties (Wang *et al*., 2015). Lots of fungi have been isolated from the deep sea or coastal seaside, like *Rhodotorula*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Alternaria*, *Fusarium*, *Engyodontium*, *Sistotrema*, *Schizophyllum*, *Tilletiopsis*, etc. The most dominant species were found to be *Penicillium* and *Aspergillus* (Luo *et al*., 2020).

Novelty of marine-derived compounds and its wide applications have yet to find a way to solutions for many dreadful diseases caused by resistant microorganisms, which are difficult to treat today (Manimegalai *et al*., 2013). Due to a scarcity of knowledge, secondary metabolites of marine fungi are of special interest. Thus, the current study was carried out to isolate and identify marine fungi from the coastal region and to assess their antibacterial potential against several microorganisms.

MATERIALS AND METHODS

Sample collection

Water sample was collected in a sterile glass bottle from the coastal side of Gorai beach, Mumbai, India and stored in refrigerator at 4°C for further study.

Isolation and identification of fungi

Samples were diluted at 10 and 100 folds and spread onto potato dextrose agar (PDA) plate, and incubated for 3–4 days at room temperature. Media was supplemented with streptomycin (30 mcg/ml) for inhibition of bacterial growth. After incubation, hyphae growth was observed on plates, each fungal colony was separated on a new PDA plate for purification. Further identification was done by slide culturing method and lacto-phenol cotton blue staining (Shamly *et al*., 2014). All the experiments were repeated in triplicates.

Test bacteria

Test organisms such as *Escherichia coli* MTCC 64, *Enterococcus faecalis* MTCC 439, *Enterococcus faecium* MTCC 9728, *Klebsiella pneumonia* MTCC 432, *Bacillus subtilis* 441, and *Staphylococcus aureus* MTCC 96 were obtained from MTCC, Chandigarh, Punjab.

Fermentation and extraction of metabolites

Broth fermentation method and extraction

The fungal strains were cultivated on PDA. After 4–5 days of incubation little block of size 2 cm^2 containing the mycelium was inoculated in 50 ml of potato dextrose broth in a 250 l flask and incubated for 14 days on a rotary shaker at 150 rpm under room temperature (VanderMolen *et al*., 2013).

Solvents (Chloroform and ethanol) in combination and individually were used for extraction of metabolites. 60 ml of ethanol and chloroform (1:1) were used in combination and individually and added in fermentation broth of *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Cladosporium cladosporioides*, *Cladosporium pseudocladosporioides*, *Trichophyton rubrum*, *Penicillium chrysogenum*, *Alternaria alternate, Neoscytalidium dimidiatum* and *Aspergillus terreus*. Flasks were kept on a shaker at 150 rpm for 24 hours and filtered with Whatman paper. Mycelia were separated for further investigation and filtrate was allowed to evaporate (VanderMolen *et al*., 2013). Further, the sticky substance was collected in vials that were weighed earlier and again weighed with metabolite. Then vials were refrigerated at 4°C for further studies.

Dry mycelia extraction

Mycelia from broth fermentation were poured onto a muslin cloth and boiling water was added until the entire agar disappeared. Then mycelia was recovered by inverting the mesh on another cloth, pouring water again to release the mycelium from the mesh and weighing the final cloth. Further cloth was kept with mycelia in a hot air oven at 50°C till it dried out. Then mycelia were collected in a mortar and powdered with the pestle. Weight of powdered mycelia was calculated and stored in a cool place for further study (VanderMolen *et al*., 2013). Extraction was done by using three solvents: chloroform, ethyl acetate, and ethanol. 1 g of each dried mycelia was added to 10 ml of solvent separately and incubated at room temperature for 24 hours. The next day, it was filtered and the filtrate was store in refrigerator at 4°C. Mycelia was again extracted overnight by using more than 10 ml of solvent. That was the second cycle of extraction. Again, this was filtered and both the filtrate were mixed, allowed to evaporate, and the metabolite was collected in weighed vials and refrigerated at 4°C.

Antibacterial activity of crude extract by Well diffusion method

Bacterial cultures *E. coli*, *E. faecalis*, *E. faecium*, *K. pneumonia*, *B. subtilis*, and *S. aureus* were adjusted at 0.5 McFarland standard and spreaded on Mueller Hinton agar plate with a sterile swab moistened with bacterial suspension. Wells of 6 mm diameter were punched onto the agar medium and filled with a concentration that ranged between 2–10 g/ml (2, 4, 6, 8, 10 µg/ml) and 10–150 µg/ml (5, 10, 20, 50, 100, 150 g/ml). 100 µl of metabolite extracted from broth fermentation and dry mycelia was added to wells. Metabolite were allowed to diffuse at room temperature for 2 hours. Plates were incubated in the upright position at 37°C for 24 hours. Wells of positive (streptomycin 30 mcg/ml) and negative control (sterile saline) was also set. After incubation, the diameters of the inhibition zone were measured in millimeter and noted (Balouiri *et al*., 2016).

RESULT

Sample collected site

The sample was collected from the coastal waters of Gorai Beach, Mumbai, India. The pH was found to 5.9 and temperature of the sample was 34°C.

Isolated and identified fungi

Fungal identification was done by using the slide culture method and lacto-phenol cotton blue staining. Few fungal identification books were referred. Total nine fungal isolates were recovered. Thus cultural, morphological, and microscopic examination was done. Identified isolates were *A. fumigatus*, *H. capsulatum*, *C. cladosporioides*, *C. pseudocladosporioides*, *T. rubrum, P. chrysogenum, A. alternate, N. dimidiatum and A. terreus. Isolates* were named alphabetically like A, B, C, D, E, F, G, H, and I as shown in Table 1. Cultural and morphological

Table 1. Cultural and morphological characteristics.

Isolate name	Media	Colony size (7 days)	Surface texture	Reverse (pigment + color of sulcation)	Color (center)
A	PDA	4 mm	Granular	White, no sulcation	Blue green
B		6 mm	Granular	White, no sulcation	Blue green
C		20 mm	Woolly	White margin, green center, no sulcation	Whitish + little green
D		3 mm	Granular	Black, no sulcation	Dark olive Green
E		5 mm	Granular	Black center, translucent margin, no sulcation	Dark green
F		25 mm	Cottony	Off white, no sulcation	Pure white
G		5 mm	Granular	White, no sulcation	Green (Olive)
H		73 mm	Woolly	White margin, brown, green, cream, dark green zones, no sulcation	Brown, green, cream, dark green, white, black zones
I		69 mm	Cottony/woolly	Off white/yellow pigment, no sulcation	White

Table 1. Cultural and morphological characteristics. (*Continued*)

Isolate name	Zonation	Margin (Color)	Elevation	Growth phase	Shape	Microscope examination
A	White	Ciliate, white	Effuse	Zonate	Myceloid	Hypha conidia and conidiophores, dome-shaped vesicle, closely compacted phialides
B	White	Ciliate, white	Effuse	Zonate	Myceloid	
C	Slight zones with white edge	Fimbriae white	Convex rugose	Filamentous cottony	Filamentous	Mycelial with hyaline hyphae, tuberculated macro conidia spherical, micro conidia
D	-	Ciliate, white	Umbonate	Filamentous zonate	Myceloid	conidia, a distinct hilum, and chains of nine conidia that disarticulate
E	-	Ciliate, White	Raised	Powdery	Myceloid	Conidia, a distinct hilum, and chains of six conidia that disarticulate
F	-	Entire white	Convex papipilate, deep from center	Cottony cloudy	Myceloid	Micro conidia laterally on fertile hyphae
G	White	Ciliate, white	Effuse	Zonate powdery	Myceloid	Filamentous septate hyphae with conidia, phialides
H	Alternate green, brown, cream, white, black zones	Lacerate white	Convex rugose	Filamentous Zonate	Myceloid	Dark brown conidiophore, short beak or no beak
I	-	Ramose white	Convex rugose	Mesh like/ filamentous	Rhizoid	Thick hyphae with septa and long branching polyphialide

characteristics have been mentioned in Table 1, while suspected isolates with growth morphology and microscopic examination have been mentioned in Table 2.

Broth fermentation and extraction

Broth fermentation was carried for all nine isolates named as A, B, C, D, E, F, G, H, and I. Extraction was done in a beaker. Extracted solvent layer in individual and combination form was directly kept for evaporation. Evaporation took around 3–4 days. At the bottom of all beakers, a sticky substance was obtained, which was removed by a sharp spatula and was collected in vials and refrigerated at 4°C. Before collection, vials were weighed. In Table 3, metabolite concentration has been mentioned.

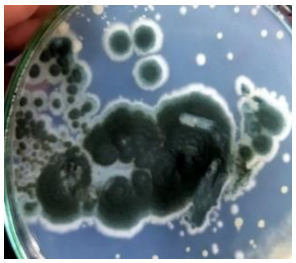
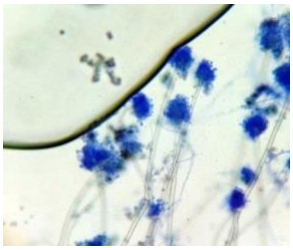
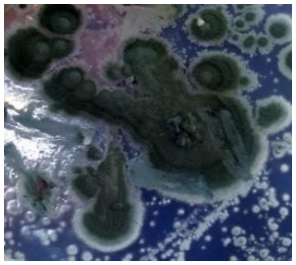
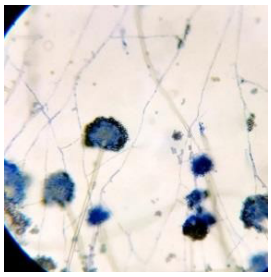
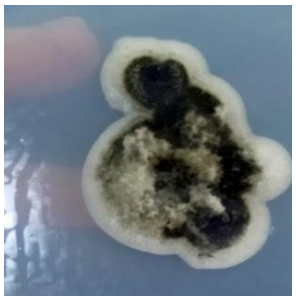
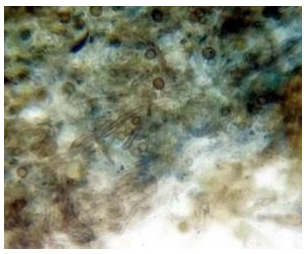
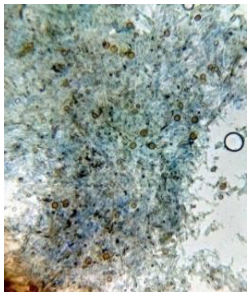
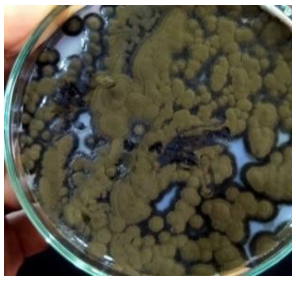
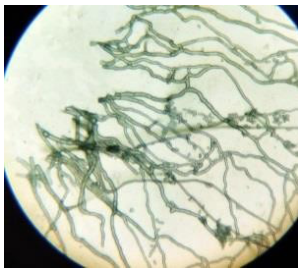
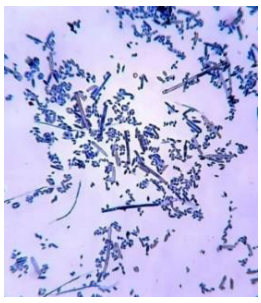
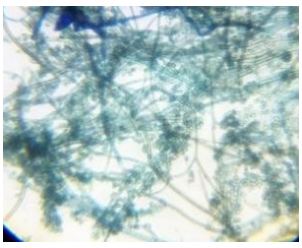
Metabolite extraction from dry mycelial biomass

Mycelia were collected from a broth fermentation process. It was dried in a hot air oven and powdered by a mortar and pestle. Further extraction of mycelial powder was carried out by using three different solvents like chloroform, ethyl acetate, and ethanol. Extraction was done twice; the filtrate was collected and evaporated. The sticky substance at the bottom of the plate was collected with a spatula and transferred to weighed vials and refrigerated at 4°C. The weight of the extracted metabolite has been mentioned in Table 4.

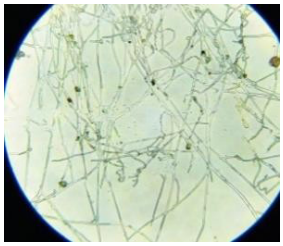
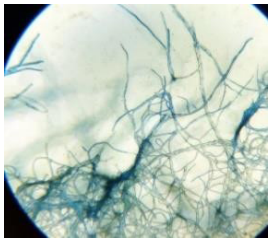
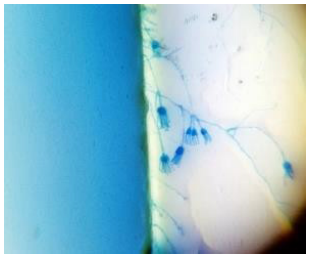
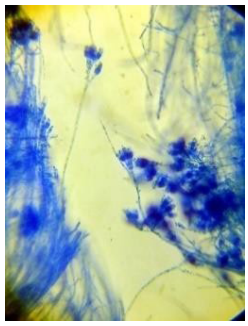
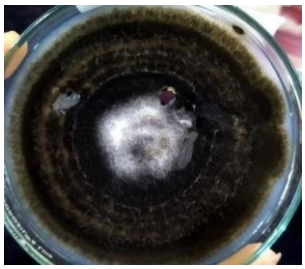
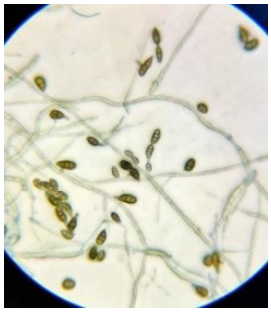
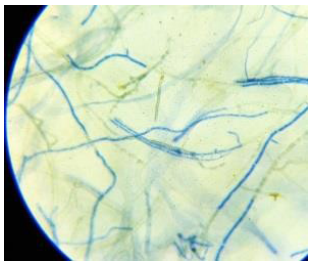
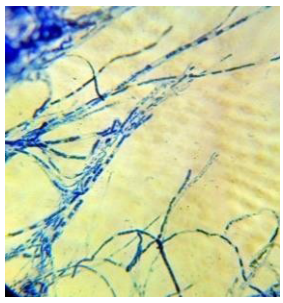
Antibacterial activity of metabolites extracted by broth fermentation and dry mycelia

Antibacterial activity for both types of metabolites collected from broth fermentation and dry mycelia was done by well diffusion method against *E. coli*, *E. faecalis*, *E. faecium*, *K. pneumonia*, *B. subtilis*, and *S. aureus*. The activity was performed in a range of 10–150 and 2–10 µg/ml. A range of 10–150 µg/ml showed complete inhibition of all the cultures for some extracts which was tested further. So the next range was reduced to 2–10

Table 2. Fungal Isolation and Microscopy.

Isolate	Growth on plate	Microsopic examination		Identified fungi
A				*A. terreus*
B				*A. fumigatus*
C				*H. capsulatum*
D				*C. cladosporioides*
E				*C. pseudocladosporioides*

Continued

Isolate	Growth on plate	Microsopic examination		Identified fungi
F				*T. rubrum*
G				*P. chrysogenum*
H				*A. alternate*
I				*N.dimidiatum*

µg/ml. In terms of few metabolites from broth extraction, inhibition was observed against all selected bacteria, with the only difference in metabolite concentration. Inhibition zone was measured in millimeter. Inhibitory activity by broth fermented extract is shown in Table 5 and Figure 1 and for dry mycelia extracted metabolites it is shown in Table 6 and Figure 2.

DISCUSSION

Antimicrobial resistance among clinical microorganisms makes it difficult to treat infectious disorders. Since the continuing study of terrestrial sources generated known microorganisms and metabolites in the past, scientists and academics throughout the world have been searching for novel antimicrobials by varying microbial sources. A review of current literature suggests that microorganisms originated from the sea are potential source of bioactive compounds (Rajasekar *et al*., 2012). Marine fungus is one of the most significant and abundant sources of novel natural compounds for the pharmaceutical and medical sectors as per several studies (Arumugam and Ponnusami, 2017; Aslam *et al*., 2018). Secondary metabolites are formed and released in response to nutrient deprivation, competition, or any other type of metabolic

stress that limits marine fungal growth. Antibiotics, anticancer, and co-stimulatory chemicals are examples of secondary metabolites that can be generated by a variety of metabolic processes (Shabana *et al*., 2021).

As part of this study, a few of the sea-derived fungi were screened, isolated, and metabolite extraction was done to study their antimicrobial metabolite capacity. As the marine environment is a much-diversified habitat, the isolation of fungi from such an environment can be a challenging task. Here, the major focus in the initial studies was nutrition for the isolates. As many studies have majorly focused on synthetic media for the desired growth of isolates, here we have also used a synthetic medium that is PDA because a nutritionally rich medium can help in the isolation of maximum isolates from a sample (Kossuga *et al*., 2012). Fungal isolates found to be *A. fumigatus*, *H. capsulatum*, *C. cladosporioides*, *C. pseudocladosporioides*, *T. rubrum*, *P. chrysogenum, A. alternate, N. dimidiatum* and *A. terreus*. Many studies have isolated fungi from sea samples and all fungi are found to be common. Some studies also says that due to the mixing of water from different sources, some terrestrial and soil-based fungi can enter the marine habitat (Preedanon *et al*., 2016; Shabana *et al*., 2021). As the study was focused on metabolite extraction and antimicrobial properties, both were done, and antimicrobial activity was detected in nearly half of the isolates. It includes fungi named *A. fumigatus*, *C. cladosporioides*, *C. pseudocladosporioides*, and *P. chrysogenum.* These isolates have shown antibacterial activity in previous studies also with differences in concentration of metabolites (Christophersen *et al*., 1998; Wang *et al*., 2015).

Metabolite extraction can be done in different ways. Here it has been carried out by the broth fermentation method, along with dry mycelium extraction, which was also done to check the metabolite content of the biomass. Extraction was done by using solvents like chloroform, ethyl acetate, and ethanol with varied polarities. In most of the fungal metabolite studies, extraction was done only in relation to broth fermentation (Rajasekar *et al*., 2012; Song *et al*., 2019), a few studies had focus on mycelium extraction (Synytsya *et al*., 2017; Wong Chin *et al*., 2021). Both the methods are different concerning protocols as well as results. As we can see in the study, broth fermentation has given a good yield, but when we compare it with dry mycelium extraction, even this has given an almost equal yield. Even though dry mycelium extraction was done using fungal biomass from broth fermentation, an equal yield in less biomass was obtained. So it can be said that for further studies we can just rely on the dry mycelial process. So, for any metabolite related studies, a huge bank of fungal mycelial biomass can be made and used for metabolite studies as the requirement for biomass is less compared to the synthetic broth requirement. For comparison purposes, solvents with varying degrees of increasing polarity were used in both extraction processes. In the broth fermentation process, chloroform has given the highest metabolite yields compared to ethanol. While in the dry mycelial extraction process, ethanol has given the highest yield compared to chloroform. The reason behind these differences will be understood only after the characterization of metabolites, as it will

Table 3. Weight of extracted metabolite from Broth fermentation.

Metabolites	In mg	Metabolites	In mg
AC	32.9	FE	98.67
BC	104.8	GE	100.00
CC	126.4	HE	68.3
DC	126.3	IE	120.00
EC	110.8	A mix	63.90
FC	98.83	B mix	33.00
GC	80.00	C mix	87.69
HC	55.68	D mix	34.56
IC	86.46	E mix	112.2
AE	57.2	F mix	139.0
BE	62.6	G mix	31.1
CE	11.6	H mix	135.0
DE	71.4	I mix	23.2
EE	39.50		

A–I = isolates; C = chloroform; E = ethanol; mix = mixture of chloroform + ethanol.

Table 4. Weight of extracted metabolite from dry mycelium biomass.

Metabolites ethanol	In mg	Metabolites ethyl acetate	In mg	Metabolites chloroform	In mg
AE	128.3	AEA	164.7	AC	107.3
BE	50.3	BEA	71.2	BC	24.3
CE	46.9	CEA	37.2	CC	14.4
DE	87.5	DEA	87.9	DC	39.9
EE	142.7	EEA	121.6	EC	68.3
FE	8.3	FEA	38.1	FC	26.3
GE	59.1	GEA	40.6	GC	22.0
HE	44.7	HEA	26	HC	12.2
IE	27.1	IEA	24.9	IC	14.4

AE–IE = ethanol; AEA–IEA = ethyl acetate; AC–IC = chloroform.

Table 5. Inhibitory activity of metabolites extracted by broth fermentation.

Isolates with respectiv e solvent initials	Zone of inhibition in mm						
	Concentration in µg/ml	*E. coli*	*E. faecalis*	*E. faecium*	*K. pneumonia*	*B. subtilis*	*S. aureus*
AC	2	TBTC	-	-	TBTC	TBTC	TBTC
	4	TBTC	-	16 mm	TBTC	TBTC	TBTC
	6	TBTC	TBTC	27 mm	TBTC	TBTC	TBTC
	8	TBTC	TBTC	TBTC	TBTC	TBTC	TBTC
	10	TBTC	TBTC	TBTC	TBTC	TBTC	TBTC
AE	2	-	-	14 mm	-	-	TBTC
	4	8 mm	TBTC	14 mm	-	-	TBTC
	6	10 mm	TBTC	21 mm	-	13 mm	TBTC
	8	22 mm	TBTC	27 mm	TBTC	20 mm	TBTC
	10	36 mm	TBTC	40 mm	TBTC	32 mm	TBTC
BC	2	TBTC	-	-	TBTC	TBTC	TBTC
	4	TBTC	-	16 mm	TBTC	TBTC	TBTC
	6	TBTC	TBTC	27 mm	TBTC	TBTC	TBTC
	8	TBTC	TBTC	TBTC	TBTC	TBTC	TBTC
	10	TBTC	TBTC	TBTC	TBTC	TBTC	TBTC
BE	2	-	-	14 mm	-	-	TBTC
	4	8 mm	TBTC	14 mm	-	-	TBTC
	6	10 mm	TBTC	21 mm	-	13 mm	TBTC
	8	22 mm	TBTC	27 mm	TBTC	20 mm	TBTC
	10	36 mm	TBTC	40 mm	TBTC	32 mm	TBTC
DC	2	15 mm	TBTC	19 mm	-	16 mm	-
	4	15 mm	TBTC	20 mm	16 mm	17 mm	TBTC
	6	16 mm	TBTC	29 mm	21 mm	20 mm	TBTC
	8	25 mm	TBTC	29 mm	30 mm	26 mm	TBTC
	10	30 mm	TBTC	30 mm	36 mm	30 mm	TBTC
DE	2	TBTC	16 mm	28 mm	TBTC	-	TBTC
	4	TBTC	20 mm	TBTC	TBTC	15 mm	TBTC
	6	TBTC	22 mm	TBTC	TBTC	20 mm	TBTC
	8	TBTC	28 mm	TBTC	TBTC	27 mm	TBTC
	10	TBTC	32 mm	TBTC	TBTC	28 mm	TBTC
E mix	2	-	-	TBTC	-	-	TBTC
	4	-	15 mm	TBTC	TBTC	TBTC	TBTC
	6	TBTC	15 mm	TBTC	TBTC	TBTC	TBTC
	8	TBTC	27 mm	TBTC	TBTC	TBTC	TBTC
	10	TBTC	32 mm	TBTC	TBTC	TBTC	TBTC
G mix	2	-	-	-	-	-	-
	4	-	15 mm	-	-	-	-
	6	18 mm	30 mm	11 mm	28 mm	30 mm	15 mm
	8	23 mm	TBTC	30 mm	TBTC	TBTC	27 mm
	10	34 mm	TBTC	42 mm	TBTC	TBTC	32 mm

AC, BC, DC = chloroform extract; AE, BE, DE = ethanol extract; E mix, G mix = mixture of chloroform and ethanol; TBTC = zone too big to count means complete inhibition; - = stands for no inhibition.

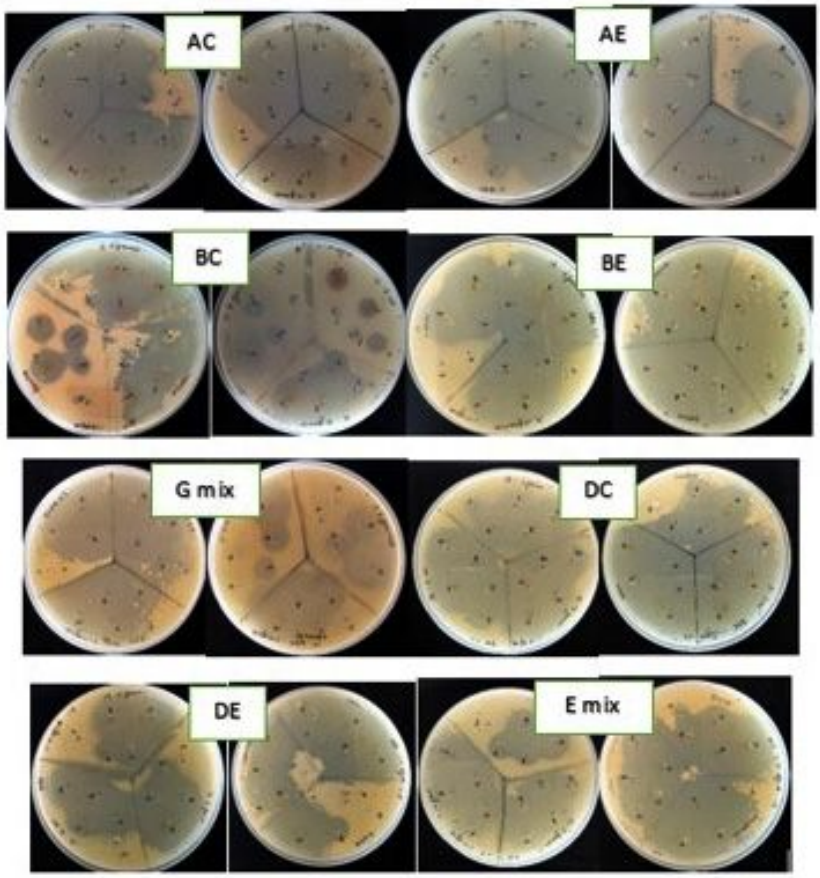


Figure 1. Well diffusion method by broth fermented metabolites.

reveal the exact components present in a metabolite and its nature and chemical properties. This will help in comparing the solvent and extraction techniques.

Despite the absence of extensive metabolic analysis, the results could provide insight into the metabolic activity of fungi under a variety of solvents and extraction techniques.

Both types of the extracts were tested against two Gram- negative *E. coli*, *K. pneumonia*, and four Gram-positive microorganisms, *E. faecalis*, *E. faecium*, *Bacillus subtilis*, and *S. aureus*. Both Gram-negative and positive samples were selected to check the broad-spectrum activity of metabolites. The range selected was between 10–150 and 2–10 µg/ml. The range of 10–150 µg/ml was found to be quite higher as it completely inhibited all the microbes, so the range was further reduced. Major activity was seen against *S. aureus* by some of the extracts. In one study, *A. fumigatus was* found to inhibit *S. aureus* at concentrations of 15.63, 1.95, and 3.90 µg/ml (Hussein *et al*., 2022). In current study, inhibition by *A. fumigatus* metabolite of all solvents was even at 2 µg/ml, so at further reduced concentration might also show inhibition. Here, affectivity is found to be greater. Similarly, *P. chrysogenum* metabolite extract showed maximum inhibition of up to 6 µg/ml against all microbes. One study shows inhibition of *S. aureus* by *P. chrysogenum* metabolite extract between ranges of 31.25 and 1,000 µg/ml (Visamsetti *et al*., 2016). Also, one study showed inhibition of *Bacillus cereus* ATCC 11778 and *Streptococcus faecalis* ATCC 19433 at minimum inhibitory concentration (MIC) values of 32 and 64 µg/ml (Trinh *et al*., 2018).

Compare to this study, inhibition against *S. faecalis* was found to be in the range 4–10 µg/ml and inhibition against *B. cereus* was found to be in the range of 2–10 µg/ml. Regarding *C. cladosporioides* and *C. pseudocladosporioides* inhibitory activities, they were tested against other bacteria like *Xanthomonas campestris*, (Silber *et al*., 2014) *E. coli*, (Li *et al*., 2017) or any other phytobacterial diseases. Here we found its activity against all the selected test organisms, so it can be further studied against human diseases. As per one study, mycelium extract shows more inhibition than broth fermented extract (Synytsya *et al*., 2017) but here in our study, it showed equal inhibitory activity against all microbes. Not much difference was seen.

As antibacterial activity of both extracts was performed here, if we try to compare antibacterial metabolites from both methods, metabolites extracted from broth fermentation have shown the highest inhibitory activity against microbes as compared to metabolites from the mycelial extraction method.

Table 6. Inhibitory activity of metabolites extracted by dry mycelia.

Isolates with respectiv e solvent initials	Zone of inhibition in mm						
	Concentration in µg/ml	*E. coli*	*E. faecalis*	*E. faecium*	*K. pneumonia*	*B. subtilis*	*S. aureus*
AC	2	-	-	-	TBTC	-	TBTC
	4	TBTC	-	16 mm	TBTC	-	TBTC
	6	TBTC	TBTC	27 mm	TBTC	-	TBTC
	8	TBTC	TBTC	TBTC	TBTC	TBTC	TBTC
	10	TBTC	TBTC	TBTC	TBTC	TBTC	TBTC
AEA	2	-	-	TBTC	-	-	TBTC
	4	-	-	TBTC	-	-	TBTC
	6	-	27 mm	TBTC	-	TBTC	TBTC
	8	39 mm	28 mm	TBTC	16 mm	TBTC	TBTC
	10	36 mm	28 mm	TBTC	18 mm	TBTC	TBTC
AE	2	TBTC	TBTC	16 mm	TBTC	-	TBTC
	4	TBTC	TBTC	20 mm	TBTC	-	TBTC
	6	TBTC	TBTC	22 mm	TBTC	TBTC	TBTC
	8	TBTC	TBTC	30 mm	TBTC	TBTC	TBTC
	10	TBTC	TBTC	36 mm	TBTC	TBTC	TBTC
BC	2	15 mm	-	16 mm	-	-	-
	4	-	-	20 mm	-	8 mm	-
	6	-	7 mm	26 mm	TBTC	19 mm	5 mm
	8	14 mm	7 mm	28 mm	TBTC	21 mm	17 mm
	10	18 mm	21 mm	30 mm	TBTC	25 mm	26 mm
BEA	2	-	-	TBTC	-	-	TBTC
	4	-	-	TBTC	-	-	TBTC
	6	-	27 mm	TBTC	-	TBTC	TBTC
	8	39 mm	28 mm	TBTC	16 mm	TBTC	TBTC
	10	36 mm	28 mm	TBTC	18 mm	TBTC	TBTC
BE	2	TBTC	TBTC	16 mm	TBTC	-	TBTC
	4	TBTC	TBTC	20 mm	TBTC	-	TBTC
	6	TBTC	TBTC	22 mm	TBTC	TBTC	TBTC

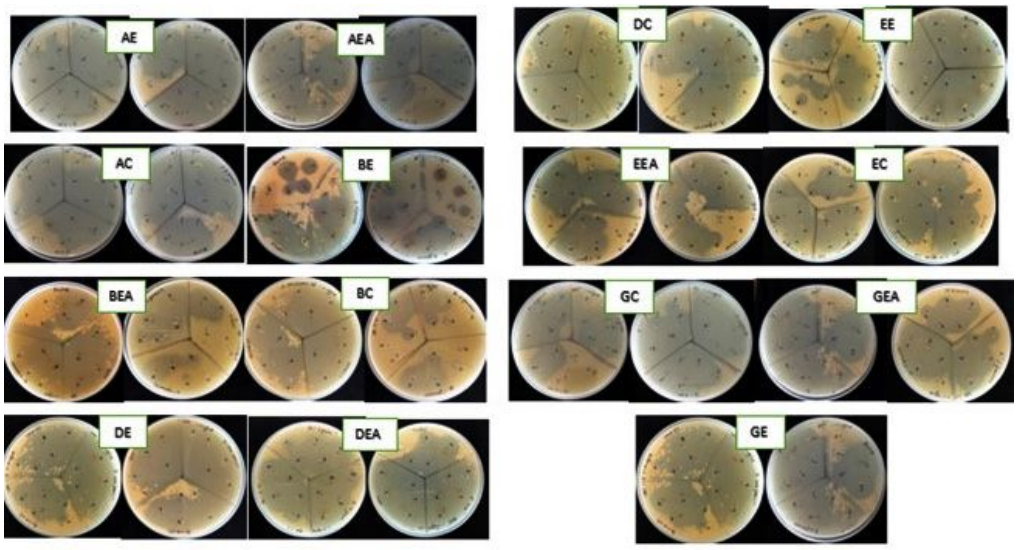


Figure 2. Well diffusion method by mycelial extracted metabolites.

By analyzing the results, it was found that diversified fungi that are present in the marine environment can produce metabolites. Further study on MIC needs to be done for specific concentration. As a result, future research should take these findings into account and investigate them using more diverse methods and criteria. Further studies can be performed by exploring more marine samples and different isolates.

CONCLUSION

The study revealed the antibacterial potential of fungal metabolites against both Gram-positive and Gram-negative bacteria. The marine fungal metabolites could lead to the development of natural and novel drugs and help to combat drug resistance menace. Further, metabolomics needs to be carried out to characterize the bioactive compounds.

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LIST OF ABBREVIATIONS

ATCC, American Type Culture Collection; MIC, Minimum Inhibitory Concentration; MTCC, Microbial Type Culture Collection and Gene Bank; PDA, Potato Dextrose Agar; rpm, rotation per minute.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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