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Anti-candidal and anti-virulence efficiency of selected seaweeds against azole resistance *Candida albicans*

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ABSTRACT

Candidiasis in the genital region induces serious infections that can be faced by sexually active women worldwide. Especially, the therapeutic failure of *Candida* species in the past decades has garnered significant research focus. In this study, we investigated the anti microbial activities of six different seaweeds (red, green and brown) were collected from the Gulf of Mannar region using well diffusion and micro dilution technique. An average zone of inhibition obtained were ranged frim 0 to 17 mm. Among the different seaweeds, the methanol extract of *Dictyota bartayresiana* was more susceptible (17.4 ± 0.62 mm) and *Chaetomorpha* sp was least effective (12.29 ± 0.99 mm) against *Candida albicans*. However, the zones of inhibition of methanolic extract of seaweeds were several folds higher than the fluconazole. Micro dilution and time dependent kinetics studies revealed that all the seaweeds have only moderate activity towards *C. albicans* and MFC/MIC show less than 4 for all tested extracts. Conversely, DCM extract of *Turbinaria ornata* showed minimum inhibitory for *Candida* biofilm. The results clearly confirmed that all the extract represent the fungistatic mechanism of inhibition rather than fungicidal except amphotericin B, suggesting that instead of using seaweed extract for killing *C. albicans* better to use to prevent their virulence property.

1. Introduction

The organisms belonging to the genus *Candida* are highly evaluated eukaryotes, commensally associated in the mucosal rich surfaces such as oral cavity, reproductive tract, inner surfaces of the stomach, and sometimes skin surfaces of healthy individuals (Da Silva Dantas et al., 2016; Solis et al., 2018). Although, the role of *Candida* colonisation in healthy individuals not yet to be identified. However primarily *Candida* transformation occurs during child birth from the mother, then the factors influence to demine the colonisation behaviour of *Candida* species (Pappas et al., 2009; Perlroth et al., 2007). Certain factors may attribute to maintain the homeostasis of the *Candida* colonisation (Cole et al., 1996). Likely the layer of the epithelium in the mucosal surfaces acted as the primary defence to prevent the fungal invasion (Salerno Gonçalves et al., 2016; Puel et al., 2012; Struck and Gille, 2013). However, due to the immunodeficiency, defects in host cellular immunity, alteration in the patho-physiology of the sites leads to the development of mucosal infections (De Repentigny et al., 2004). The occurrence of *Candida* colonisation was directly related to host defence, from the mucocutanious candidiasis to invasive candidiasis, a life threading condition (Brown et al., 2012; Pfaller and Diekema, 2007). Growth of *Candida albicans* found in the oral cavity breaks the natural barriers to the oral system thus causing oral lesions/candidiasis. Commonly, the primary risks of oral candidiasis are associated with natural or intended immune compression, improper dental prostheses, HIV etc.

As the commensal organism, *C. albicans* becoming opportunistic pathogen by causes the pathological changes of the vulva and the vaginal mucosal architecture (Pappas et al., 2009; Perlroth et al., 2007). Although, the sign and symptoms may vary according to the individual with respect to age, sexual behaviour, socioeconomic status, clinical complication along with the immunological alterations (Zeng et al., 2018; Fidel and Sobel, 1996; Hellberg et al., 1995). Most frequently curd like white, abnormal vaginal discharge has occurred alongside vaginal itching, reddening and dyspareunia (Yano et al., 2019; Reichman et al., 2017; Sobel, 2016). *C. albicans* shows a unique type of morphological future that participates in the invasion of *Candida* cells to the epithelial barrier. As a consequence, the organism tends respond to the external stimuli that produce fewer diverse features. The yeast to hyphae transition, biofilm formation, white-opaque switching, and production of protease are the important features of developing

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therapeutic failure (Wu et al., 2018). Among them biofilm formation and hyphae transition or filamentation contributing around 80% therapeutic failures developed during the past decades (Privett et al., 2010; Chandra et al., 2001).

Marine macro algae are one among the dominated group of flora in the marine ecosystem (Ba-Akdah et al., 2016). To maintain the healthy life style, the macro algae have evaluated to produces several structural and chemical defence mechanisms (Sudatti et al., 2018; Nylund et al., 2013). The secondary metabolites like alkaloids, terpenes, aromatic compounds, phlorotannins and acetogenins (Amsler, 2008; Jormalainen and Honkanen, 2008) were investigated from seaweed for important biomedical applications. Due to the presence of several biologically important substances, macro algae play an attention of biologists and the chemist. They are rich in secondary metabolites such as group of steroids, carbohydrates, peptides, alkaloids etc. to prevent the unwanted growth of epiphytes (Plouguerné et al., 2010; Carvalho et al., 2017; Das et al., 2014). Numerous research evidence are calming that the biological activities of macroalgae due to the presence of various macro and small molecules (Nogueira et al., 2014; Mendes et al., 2013; Namvar et al., 2013; Mtolera and Semesi, 1996). The important biologically active compounds including antibacterial (Amiguet et al., 2011; Glombitza et al., 1975; Amico et al., 1988), antifungal (Arumugam and Rajendran, 2019; Peres et al., 2012; Martinez-Lozano et al., 2000; Bennamara et al., 1999), antiviral (Thompson and Dragar, 2004; Lapshina et al., 2006; Reunov et al., 2009), antimalarial (Chen et al., 2009), anticancer (Vaikundamoorthy et al., 2018; Nishibori et al., 2012; Vizetto-Duarte et al., 2012), anti-inflammatory (Khan et al., 2009; Cumashi et al., 2007), antioxidant (Sullivan et al., 2011; Le Lann et al., 2008) and UV protection.

Generally, algae belonging to the rhodophyta and phaeophyta are most effective against biofilm forming organisms than the green algae (Hellio et al., 2002; Da Gama et al., 2002). Extract of marine algae was the promising source of anti-virulence molecules. Hence the present study was designed exclusively for the anticandidal and the virulence property of different *Candida* sp. and *C. albicans* respectively. In this concern, the following objectives are included (1) to screen the anticandidal property of polar and non polar extract of different seaweeds (2) understating the killing kinetics of seaweed extract towards antibiotic resistance *C. albicans* (3) determine the potential impact of seaweed extract on the biofilm formation and filamentation of *C. albicans*.

2. Materials and methods

2.1. Media, strains and chemicals

YPD (Yeast Extract Peptone Dextrose) agar, agar-agar, YPD broth, methanol, dichloromethane, DMSO (Dimethyl sulphoxide), crystal violet, 96 well microtiter plate, Amphotericin B, PBS pH 7.4 are acquired from Himedia laboratories, Mumbai. The pure cultures of *C. parapsilosis* (450001), *C. krusei* (440002), *C. albicans* (400026), *C. kefyr* (410002) was obtained from National Culture Collection of Pathogenic Fungi, India. All the strains were maintained in glycerol stocks at 4°C until further studied.

2.2. Preparation of seaweed extract

Fresh seaweeds of *Turbinaria ornata, Dictyota bartayresiana, Halimeda tuna, Sargassum white, Chaetomorpha* sp. and *Padina* sp. were collected from Gulf of Mannar, India (Latitude 9° 28′ 12 N; Longitude 79° 18' 44 E) and identified with the help of algalbase (Wendy Guiry and Guiry, 2012). Collected samples were immediately washed with sea water followed by sterile distilled water to remove the epiphytes and other debris. The cleaned seaweeds were shade dried under room temperature and grounded into fine particles. Fifty gram (50 g) of seaweed powder was extracted in 250 ml of different solvent (methanol and dichloromethane) for 24 h on a rotary shaker. The extract was filtered through Whatman no.1 filter paper and condensed using rotary evaporator (to prevent any possible impact of solvents used in the studies). The higher concentration (100 mg/ml) of all seaweed extract was prepared by dissolving the content in 2% of DMSO (non toxic to cells).

2.3. Determination of primary anti-candidal activity

For the preliminary screening, the efficiency of inhibiting candid cells by marine algae extract, the agar well diffusion method was used (Clinical Laboratory Standard Institute M44A, 2009). Sterile YPD agar plates were prepared using 1% yeast extract; 2% peptone: 1% dextrose and 1.7% agar under aseptic condition. The prepared YPD plates were swabbed over with 24 h old *Candida* culture with the cell density of 1×10^6 cells/ml. Followed by, 6 mm in diameter of wells were made and the stock solution 50 µl of different extracts were added. They were strand for 2hrs to diffuse and the plates were incubated at 37°C for 24 h. The diameter of the inhibition zone (mm) was measured and the activity index was calculated (Khan et al., 2018).

2.4. Determination of minimum inhibitory concentration

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit least visible cell growth in a liquid medium. Briefly, the standardised culture suspension was prepared using overnight grown *Candida* culture. Serially diluted marine algae extract was added to the evenly distributed cell suspension and incubated at 37°C for 24 h. The results were observed after incubation based on the visual appearance of turbidity of the cell suspension. In direction to this, MFC was defined as least concentration to kill the target organism by testing drugs. Each well containing SWE treated cells was streaked on the YPD agar and observed for the viable growth after incubation. MFC and MIC ratio was considered to identify the fungicidal or fungistatic action of sea weed extract (Keepers et al., 2014; Appiah et al., 2017)

2.5. Determination of effects on growth kinetics of the C. albicans

To study the inhibition of specific growth rate of marine algae extracts treated *C. albicans*, the spectrophotometric assay based studies were employed. Briefly, a single colony of *C. albicans* was inoculated in the YPD medium to prepare desired volume of cell suspension. The cell density was adjusted to 1×10^6 cells/ml using sterile 45 ml of YPD broth containing MIC concentration of marine algae extract. A flask containing cell suspension along with the marine algae extract was placed in under incubator for 250 rpm. Amphotericin B served as the positive control and flask without marine algae extract served as growth control. In order to understand the growth behaviour of *Candida* in the presence of marine algae extract, an aliquot of 0.5 ml was collected in the entire test flask. The presence of the viable cells were counted using methylene blue staining and blotted.

2.6. Determination of effect on filamentation of the C. albicans treated with marine algae extract

The cells of *C. albicans* was grown overnight and prepared as described above. For filamentation assay, the prepared cells were incubated in YPD medium containing 1% of human serum at 37°C for 6 h and at every 30mins of incubation, an aliquot of samples were withdrawn and analysed for the present of filamentation. Likely, MIC concentration of different seaweeds extract was added to perform filamentation inhibitory assay as mentioned above. Tube with non exposed cells (extensive filamentation) served as a growth control and MIC concentration of amphotericin B treated cells (no filamentation) served as the positive control. A clean cover slip was placed over the drop and examined under low magnification 100 x for the presence of germ

tubes. After 2hrs of incubation, 20 µl of marine algae extract exposed samples were investigated using compound microscopy (Labomed Lx 300) equipped with the digital camera for photography.

2.7. Determination of biofilm formation and biofilm inhibitory activity

A qualitative method used to evaluate the biofilm formation was done as described by Christensen et al. (1985). The previously prepared cell suspension was added to the sterile test tube contain either marine algae extract, Amphotericin B or without the addition of anything. The tube was incubated at 37°C for 72hrs to grow and mature biofilm. After, the cells contain suspension was discarded without disturbing the biofilm matrix, followed by repeated wash with sterile PBS (Phosphate Buffer Saline) and dried. The dried tubes were stained with 0.1% crystal violet. The excess stain was washed with sterile distilled water and the tubes were then dried in an inverted position for biofilm staining. Biofilm formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation.

A quantitative crystal violet based biofilm assay was performed as described by Pierce et al. (2008), Lee et al. (2013). Briefly, the standardised culture of *C. albicans* with the density of 0.5 was prepared using either RPMI 1640 or PBS and distributed in 96-well polystyrene plates. The plates were incubated for 24 h without agitation at 37°C. To evaluate the biofilm inhibition, the medium was replaced by sterile RPMI 1640 medium containing the minimal inhibitory concentration of test extract and incubated at 37°C for 24 h. Nonadherent cells were removed by repeated washing of each well with sterile PBS then 300 µl of 0.2% crystal violet was used to stain the biofilm matrix. The stain was then extracted using 95% ethanol and the absorbance was measured at 570 nm (OD570).

2.8. Statistical analysis

The dataset was processed using MS-excel program and represented as mean and standard deviation (SD). The statistical significant of positive control (Amphotericin B) and seaweed extracts were compared using One-way ANOVA with the help of SPSS software (16.0 Windows). In all the cases statistical significance was consider at 0.05% levels (95%).

3. Results and discussion

3.1. Effects of marine algae extract on C. albicans

The results expressed in terms of zone of inhibition and compared with standard antifungal fluconazole. Methanolic extracts of all the tested seaweeds exhibited good inhibition to the tested *Candida* strains rather than DCM extract. An average zone of inhibition of each seaweed was varied from 0 to 17 mm. The methanol extract of *Dictyota bartayresiana* recorded maximum activity against *C. albicans* (17.4 ± 0.62) followed by *Turbinaria ornata* (14.7 ± 0.40) and *Sargassum wightii* (14.4 ± 0.67). *Chaetomorpha* sp. reported least activity as representing 12.29 ± 0.99 mm. Likely, *Dictyota bartayresiana* and *Halimeda tuna* recorded higher zone of inhibition against *C. kefyr* (12.50 ± 0.7; 12.28 ± 1.01), *C. krusei* (11.35 ± 0.91; 12.55 ± 0.77) but it slightly varied for *C. parapsilosis* (*Padina* sp. 13.90 ± 0.14; *Halimeda tuna,* 13.90 ± 2.75). Among the tested marine algae extract, the maximum activity was produced by methanolic extract *Dictyota bartayresiana* and *Halimeda tuna* against *C. albicans* (Table 1). Hence *C. albicans* alone further considers for remaining studies.

Minimum inhibitory concentration (MIC) is defined as the highest dilution or least concentration of the extracts that inhibit the growth of an organism. Determination of the MIC is important in diagnostic laboratories because it helps in confirming the resistance of microorganism to an antimicrobial agent and it monitors the activity of new antimicrobial agents. The MIC and MFC was determined by sub culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of seaweed extract that completely killed the fungi was taken as MFC (Table 2). Micro dilution studies have shown that all the seaweeds shown moderate activity towards *C. albicans* and MFC/MIC was less than 4 for all tested extracts (Table 2). Form this it has confirmed that all the extract represent the fungistatic mechanism of inhibition rather than fungicidal except amphotericin B (Andes, 2003; Ernst et al., 2000).

Table 1
Mean zone of inhibition (mm) representing anti-candidal activity of different seaweeds with reference to standard antifungal.

Organism	Seaweed	Dichloromethane	Methanol
C. parapsilosis	*Turbinaria ornata*	12 .5 ± 0.07	10.6 ± 0.84
	Halimeda tuna	Nil	13.95 ± 2.75
	Chaetomorpha sp.	< 10	11.7 ± 0.42
	Padina sp.	Nil	13.9 ± 0.14
	Sargassum wightii	Nil	12.1 ± 0.14
	Dictyota bartayresiana	Nil	10.5 ± 0.70
C. krusei	*Turbinaria ornata*	13.5 ± 0.70	9.25 ± 1.76
	Halimeda tuna	Nil	12.55 ± 0.77
	Chaetomorpha sp.	Nil	9.45 ± 0.63
	Padina sp.	Nil	10.9 ± 0.14
	Sargassum wightii	< 10	9.1 ± 1.55
	Dictyota bartayresiana	< 10	11.35 ± 0.91
C. albicans	*Turbinaria ornata*	Nil	14.71 ± 0.40
	Halimeda tuna	Nil	13.06 ± 1.32
	Chaetomorpha sp.	12	12.29 ± 0.99
	Padina sp.	10	12.56 ± 0.79
	Sargassum wightii	10	14.47 ± 0.67
	Dictyota bartayresiana	11	17.44 ± 0.62
C. kefyr	*Turbinaria ornata*	9.25 ± 1.06	10.99 ± 0.01
	Halimeda tuna	Nil	12.28 ± 1.01
	Chaetomorpha sp.	Nil	10.06 ± 0.08
	Padina sp.	Nil	9.49 ± 0.69
	Sargassum wightii	Nil	10
	Dictyota bartayresiana	11 ± 1.41	12.5 ± 0.70

Fluconazole shown 0 mm (zone of inhibition) hence it is not mentioned in this table.

Table 2
MIC, MFC and MFC/MIC ration of different solvent extracts of seaweeds against clinical pathogens.

EXTRACTS	SEAWEED	MIC	MFC	MFC/MIC
Methanol	*Turbinaria ornata*	50	100	2
	Halimeda tuna	50	100	2
	Chaetomorpha sp.	25	50	2
	Padina sp.	50	100	2
	Sargassum wightii	25	50	2
	Dictyota bartayresiana	50	100	2
DCM	*Turbinaria ornata*	50	100	2
	Halimeda tuna	50	100	2
	Chaetomorpha sp.	50	100	2
	Padina sp.	50	100	2
	Sargassum wightii	50	100	2
	Dictyota bartayresiana	50	100	2
	Amp B	0.006	0.05	8

3.2. Effects of marine algae extract on growth kinetics of C. albicans

The effects of cellular growth of *C. albicans* in the presence of marine algae extract were studied using time dependent killing assay. The growth of *C. albicans* was limited during its lag phase when the minimal inhibitory concentration of marine algae extract treated. But the growth of organisms was statically increased after entered into the logarithmic

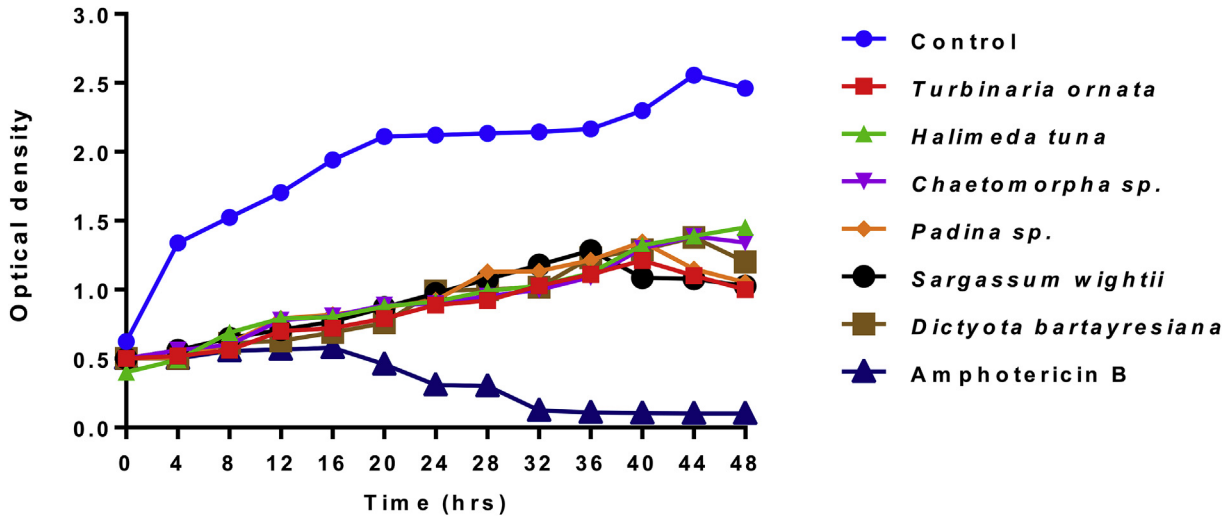

Fig. 1. Alteration of growth kinetics of *C. albicans* treated with methonolic extract of different seaweeds. Cell viability based growth curves of *C. albicans* in presence of minimal inhibitory concentration of marine algae extract (25 and 50 mg/ml), absence drug (growth control), MIC of amphotericin B (0.006 mg/ml; positive control).

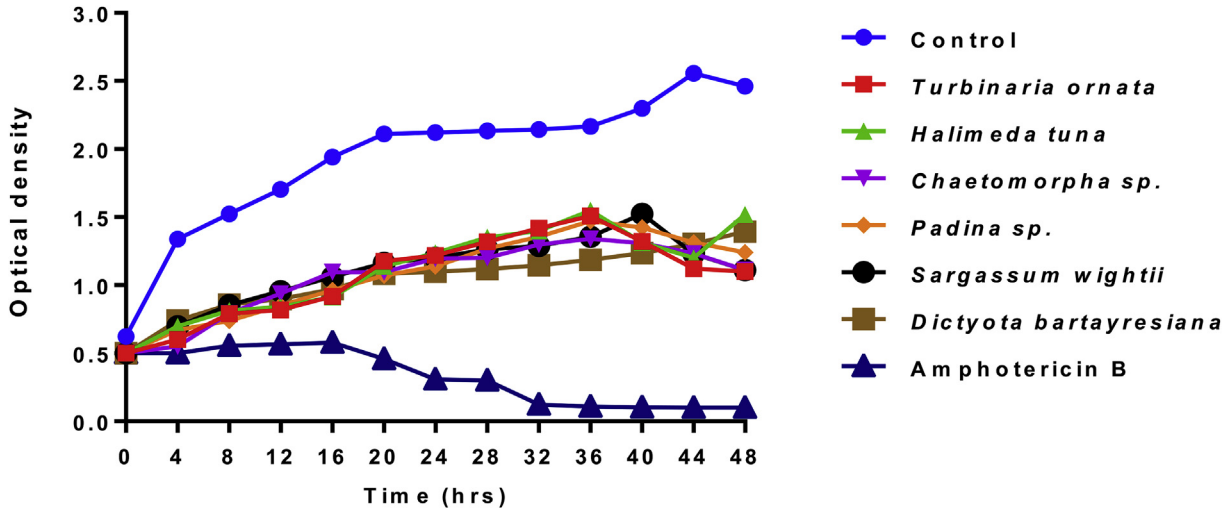

Fig. 2. Alteration of growth kinetics of *C. albicans* treated with dichloromethane (DCM) extract of different seaweeds. Cell viability based growth curves of *C. albicans* in presence of minimal inhibitory concentration of marine algae extract (25 and 50 mg/ml), absence drug (growth control), MIC of amphotericin B (0.006 mg/ml; positive control).

phase. Also, growth pattern was comparably similar to the growth control, although significantly lesser to the positive control amphotericin B (Figs. 1 and 2). In contrast, at 50 mg/ml of DCM extract of all the tested seaweeds the growth of *Candida* has slightly varied as compared to unexposed cells. However, the unexposed cells shown the viability of around 95% as directly proportional to the SWE treated cells (Figs. 1 and 2).

3.3. Marine algae extract attest the filamentation of C. albicans

Elongated daughter cells from the round mother cells without constriction at their origin are referred to as true germ tubes while constricted hyphae as pseudohyphae. In YPD, the standard strains of *C. albicans* formed true germ tubes after 1 h at 37°C. The results of filamentation inhibition by different seaweed extract were presented in Table 3. Out of 12 different extracts tested, all of them inhibiting the formation of germ tubes after 1 h at 37°C while the untreated cell shows the extended induction of filamentation during the incubation. The rate and size of the filament was increased rapidly until it reaches 3-4 h of incubation, after that no viable increase in the size of filamentation was observed even after 24hrs. It showed that amphotericin B exhibits 100% of inhibition while control cells showed 100% filamentation after 2 h of incubation (Table 3; Fig. 3). As a continuation of primary

Table 3
The potential antibiofilm and filamentation activity exhibits by marine algae.

Extracts	Seaweed	Biofilm	Filamentation
Methanol	*Turbinaria ornata*	No disturbance	No filamentation
	Halimeda tuna	Disturbance	No filamentation
	Chaetomorpha sp.	Disturbance	No filamentation
	Padina sp.	Disturbance	No filamentation
	Sargassum wightii	Disturbance	No filamentation
	Dictyota bartayresiana	Disturbance	No filamentation
DCM	*Turbinaria ornata*	No disturbance	No filamentation
	Halimeda tuna	No disturbance	No filamentation
	Chaetomorpha sp.	Disturbance	No filamentation
	Padina sp.	Disturbance	No filamentation
	Sargassum wightii	No disturbance	No filamentation
	Dictyota bartayresiana	No disturbance	No filamentation
	Amp B (μg/mL)	Disturbance	No filamentation

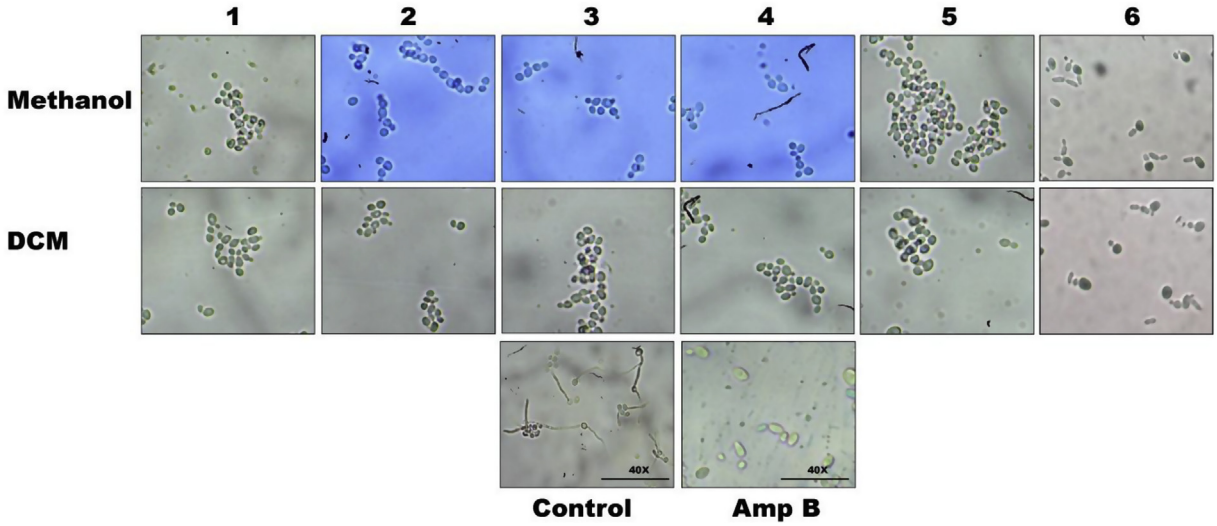

Fig. 3. The anti-filamentation effects of different seaweed extract (1. *Turbinaria ornata*, 2. *Halimeda tuna*, 3. *Chaetomorpha* sp., 4. *Padina* sp., 5. *Sargassum wightii*, 6. *Dictyota bartayresiana; Amp B* – Amphotericin B) treated *C. albicans* cells shown distinguished morphological variation after 2hrs. Exclusively control samples presenting with fully extended filaments with the positive sign of virulence property of the test isolate.

screening, MIC concentration of all the seaweeds blocks the yeast to filament transition.

3.4. Effects of marine algae extract on the structural architecture of candida biofilm matrix

Before performing the biofilm inhibition assay, *C. albicans* was tested for the ability to produce biofilm using the crystal violet staining method. It has confirmed that the isolate used in the study have the ability to produce biofilm after inhibition. To overcome the current antifungal therapy, metabolically active *Candida* cells confers to form mat like structure which can directly attributed to the reduction of susceptibility. Therefore, our investigations further depth into anti-biofilm activity against drug resistance *Candida* biofilm matrix. A standard dose of different seaweeds along with positive control was exposed to 24hrs grown *Candida* matrix and biofilm inhibition was quantified using crystal violet assay.

Biofilm of *C. albicans* was found to be susceptible to DCM extract of *Turbinaria ornata, Halimeda tuna, Sargassum wightii, Dictyota bartayresiana* at MIC values of 50 mg/mL. The cells treated with marine algae extract reduces the biofilm matrix of *Candida*, the results are shown in Fig. 4. When considering, *Turbinaria ornata* methanolic extract has less potency to inhibition of *Candida* biofilm. At least concentration 25 mg/ml of methanolic extract of *Sargassum wightii* and *Chaetomorpha* sp completely dissociates the matured biofilm *C. albicans*.

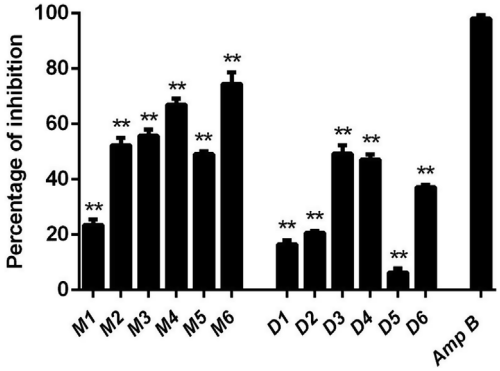

Fig. 4. Significant variation in the percentage of biofilm formation after 24hrs exposed to different seaweed extracts. The prefix of X axis indicates the solvent used to prepare the extract (M – methanol; D – DCM; 1. *Turbinaria ornata*, 2. *Halimeda tuna*, 3. *Chaetomorpha* sp., 4. *Padina* sp., 5. *Sargassum wightii*, 6. *Dictyota bartayresiana; Amp B* – Amphotericin B; ** = $p < 0.001$).

The quantitative assessment of candida cellular matrix has shown considerable variations during the exposure to differential extracts of marine algae (Fig. 4). However, the biofilm activity was diverse with respect to species of seaweeds and nature of solvents. The percentage of viable biofilm matrix of *C. albicans* was dramatically reduced as compared to the growth control (100%), but it was lesser inhibition of Amphotericin B (98.21 ± 1.18) (Fig. 4). Maximum inhibition 74.55 ± 4.11% of biofilm was acquired by methanolic extract of *Dictyota bartayresiana* with the statistical significant ($F = 91.09$; $p < 0.001$) followed by *Padina sp.* 67.08 ± 2.12 ($F = 49.53$; $p < 0.001$), *Chaetomorpha sp.* 55.78 ± 2.27 ($F = 21.13$; $p < 0.001$). Also, all the tested extract showed significant reduction in candida biofilm matrix but the methanolic extract has more activity than the DCM extracts of the tested seaweeds.

4. Discussion

The antimicrobial activity of the marine algae was varied with respect to season, geography, habitat, growth stage and nature of solvents etc. (Manivannan et al., 2011). Non polar solvents like benzene, diethyl ether, acetone and chloroform to be the better choice for extracting antimicrobial principle from the seaweeds (Martinez-Nadal et al 1966; Rosell and Srivastava, 1987; Hellio et al., 2004; Mendes et al., 2013). Dichloromethane extract of marine algae was rich in fatty acid as well as some steroids showing 20% of in vitro antifouling activity against 35 isolates with lesser cytotoxic to sea urchin larvae and mouse fibroblasts (Hellio et al., 2004). As a consequence of the Hellio et al., (2004), the methanolic extract of all the seaweeds were effective than the DCM extract. It was directly correlated with Parekh et al. reported that alcoholic extract of Indian seaweeds for antibacterial activity (Parekh et al., 1984; Nogueira et al., 2014)

In general, seaweeds are more effective against Gram positive bacteria than Gram negative and some yeast belonging to *Candida* genus (Mendes et al., 2013; Mtolera and Semesi, 1996). The influence of seaweed mediated activity may be attributed due the presence of thick cell wall in gram negative and yeast (Salem et al., 2011). But the activity was several times lesser than the commercial antibiotics (Nogueira et al., 2014; Mendes et al., 2013; Mtolera and Semesi, 1996). Clinically insignificant results were observed by test *Padina sanctae crucis* methanolic extract against several *Candida* species including

Candida tropicalis and *Candida kruzei* and the MIC results were > 1024μg/ml (Nogueira et al., 2014). In similar to this the present study has reported that the MIC values fall between 25 and 50 mg/ml. It was 100 folds greater than positive control amphotericin B. Methanolic extract of *Padina boergesenii*, *Sargassum wightii*, *Turbinaria conoides* extracts shown the broad spectrum of antibacterial activity (Kumar et al., 2008; Kandhasamy and Arunachalam, 2008). The lipophilic compound of *Gracilaria corticata, Stocheospermum marginatum* and *Acrosiphonia orientalis* are highly active against Gram negative bacteria (Shanmughapriya et al., 2008).

Filamentation from yeast cells are more complex and important mechanism of the physiology of *C. albicans* responded by several signalling pathway. There are several environmental factors stimulates the filamentation in *C. albicans* including human serum, bovine serum albumin etc (Kadosh and Lopez-Ribot, 2013; Sudbery, 2011; Sudbery et al., 2004). Although, the importance of filamentation in *C. albicans* was studied well (Kadosh and Lopez-Ribot, 2013). However, the studies discussing marine macro algae mediated arrest of *Candida* filamentation was limited. Therefore, we further examined the filamentation inhibitory ability of algae extracts against filamentation positive *Candida* strain. In addition to the response of inducing filamentation, the selected seaweed extracts showing promising effects on filamentation under invitro condition. In our study, we evaluated germ tube production at 37°C after 1 h of incubation in serum-free YEPD medium as done by (Kim et al., 2002). Results we have found by two extracts, methanol and dichloromethane have more potency to arrest key virulence factor of the test organism.

In addition to the filamentation, biofilm formation is one among the most important task to characterize pathogenicity of the test organism. The quantitative biofilm inhibition was varied from 6.38% to 74.55% for different seaweed extract. However, the present results were entirely different from the study reported by Culioli et al. (2008). A polysaccharides fucoidan at concentration 250μg mL^{-1} completely inhibits the biofilm formation in *S. mutans* and *S. sobrinus*. (Jun et al., 2018). Also, meroditerpenoids of *Halidrys siliquosa* produces significant antibiofilm activity against the bio fouling organisms at < 2.5 μg/mL concentration (Culioli et al., 2008). Biofilm formations on the medical aids are the serious concern towards the therapeutic failure because the strong biofilm matrix was resistance to the commercially available antibiotics and some natural products.

5. Conclusion

In the present work we are first reporting the anti-candidal activity of six different seaweeds. Although seaweeds shown comparatively lesser activity than the commercial antifungal Amphotericin B it has several folds higher than the fluconazole. Particularly, alcoholic extracts of brown algae exhibits maximum inhibition against *C. albicans* and followed by other microorganism. The results confirm that the bioactive principle might be due to polar in nature. Furthermore, it is clear that all the tested extracts significantly illustrate the anti-filamentation activity and antibiofilm activity against virulence *C. albicans*. Therefore, it is great necessary to analyse their mechanistic approaches for better understanding. Although this is the suitable time to uncover the phyto-skeleton for the drug resistance candidiasis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101195.

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