Screening of Antifungal Activities of Five Algal Crude Extracts

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Abstract:

In the present study, four species of marine algae were collected during different seasons for one year (September 2013 to August 2014). The collected species belonged to Chlorophyta (*Ulva lactuca*), Phaeophyta (*Sargassum denticulatum*, *Hormophysa triquetra*) and Rhodophyta (*Hypnea cornuta*) in addition to one blue- green alga from freshwater (*Spirulina platensis*) was obtained from stock at Hydrobiology Lab, Qanater, Khayria, Qalubia, Egypt. Crude algal extracts were prepared by using different solvents (methanol, ethanol and chloroform) in addition to hot water and cold water extracts. The crude algal extracts were examined for their antifungal efficacy against oral *Candida species* (*Candida albicans, C.tropicalis, C.krusei* and *C.glabrata*) using agar well diffusion method. Results revealed that methanol was the best solvent suited for extraction of bioactive compounds from the tested algae. Chlorophyta (*ulva lactuca*) exhibited the highest antifungal effect followed by Phaeophyta, Rhodophyta and blue green algae. MIC of the most potent algal (*ulva lactuca*) methanol extract was 62.5 mg/ml and MFC was 125mg/ml for the same alga with all the tested oral *Candida species*. (comparison was made between MFC value of *Ulva lactuca* methanol extract with the anti chlorhexidine (0.1mg/ml)(which is a common antimicrobial agents in commercialized oral rinses). by using (PIDG) .The results shown that the *Ulva lactuca* methanol extract better than PIDG of chlorhexidine.

Keywords: Marine algae, Antifungal activity, Solvent extracts, Oral *Candida species*, (MIC) and (MFC).

Introduction:

Algal extracts contain compounds such as carbohydrates, proteins, minerals, oil, fats, polyunsaturated fatty acids as well as bioactive compounds such as antioxidants (polyphenols, tocopherols, vitamin E, vitamin C. mycosporine-like amino acids) , pigments, such as carotenoids (carotene xanthophylls) cholorophylls, and phycobilins (phycocyanin, phycoerythrin), which possess antibacterial, antiviral, antifungal, antioxidative, anti-inflammatory and antitumor properties **(Harun *et al.*, 2014)**. Recently, there is increased interest in naturally produced active compounds as alternatives to synthetic substances. Although these compounds often show lower activity, they are nontoxic and do not leave residues. This implies that there is a

need to develop new and safe products of biological origin, with properties similar to the synthetic, in particular antimicrobial, antifungal, antioxidizing compounds and colorants. These natural compounds are found in algal extracts **(Michalak and Chojnacka 2014).** Oral candidiasis is an opportunistic infection of the oral cavity caused by the overgrowth of *Candida species*, usually of *C. albicans. Candida* species are present as commensal organisms of the oral micro biota in about 20-60% of normal human population (**Aggarual *et al.*, 2018**). The genus *Candida* belongs to yeasts. It is also the most common cause of opportunistic mycoses worldwide. It is a frequent colonizer of human skin and mucous membranes (**Greenberg & Burket's, 2005)**.

Although pharmaceutical industries have produced a number of new antimicrobial drugs in the last few years, resistance to these drugs by microorganisms has increased rapidly (**El bashiti *et al.*, 2011**). While natural products have traditionally been harvested from terrestrial sources such as soil and higher plants, reports show that marine organisms are rich sources of structurally new and biologically active metabolites. Approximately 16,000 marine natural products have been isolated from marine organisms and reported in approximately 6,800 publications. Some of these compounds are unique to marine organisms (**Bhakuni & Rawat, 2005**). According to **Smit, 2004** the discovery of metabolites with biological activity from algae increased substantially in the last three decades. These substances exhibit an appreciable number of distinct biological activities such as antitumor, antiviral, antifungal, insecticidal, cytotoxic, phytotoxic and antiproliferative actions (**Machado *et al.*, 2010**). The majority of these compounds are terpenes and polyphenols (**Blunt *et al.*, 2006**). **Osman *et al.* (2013)** studied the antimicrobial activity of three different macroalgal species belonging to Rhodophyta, Chlorophyta and Phaeophyceae, respectively (*Janiarubens*, *Ulva fasciata* and *Sargassum vulgare*) were collected seasonally in 2007 to 2008 from Abu-Qir bay (Alexandria, Egypt). The different macroalgal species were tested against pathogenic microbes such as *Bacillus subtilis, Staphylococcus aureus* and *Streptococcus aureus*as gram-positive bacteria, *Escherichia coli, Salmonella typhi* and *Klebsiella pneumonia* as gram-negative bacteria and one yeast strain *Candida albicans*. **Shobier *et al.* (2016)** studied the antifungal activity and the chemical constituents of selected macroalgae collected from the Egyptian Mediterranean coast of Alexandria have been investigated against *Fusarium solani*, *Fusarium oxysporum*, *Tricoderma hamatum*, *Aspergillus flavipes* and *Candida albicans*. **Sheikh *et al.* (2018)** evaluated the antimycotic activity of fifteen species of the dominant marine algae were collected during summer 2013 from four selected sites on Red sea coast, Jeddah, Saudi Arabia. The collected species belonged to Chlorophyta, Phaeophyta and Rhodophyta. The crude algal extracts were examined for their antifungal efficacy against *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* and *Candida tropicalis.*

The present study aimed to detect the effect of some algal extracts for four marine macroalgal *Ulva lactuca* (green algae), *Sargassum denticulatum* and *Hormophysa triquetra* (brown algae) and *Hypnea cornuta* (red algae), also one fresh water blue-green alga (*Spirulina platensis*) on the growth of some oral *Candida species* (*Candida albicans*, *Candida tropicalis*, *Candida krousei* and *Candida glabrata*) Also determination of the minimum inhibitory concentration (MIC) , minimum fungicidal concentration (MFC) against the four oral *Candida sp* and the percentage inhibition of diameter growth (PIDG) for the fungal pathogen tested oral *Candida sp*.

Materials and Methods:

Identification of algae:

A-Fresh water alga:

Spirulina platensis **(Nords, Geitler).**

B- Marine algae:

The identification was done according to **Papenfuss (1989)** .*Ulva lactuca* (Linnaeus) (Chlorophyceae), *Sargassum denticulatum* (BØrgesen) (Phaeophyceae) and *Hypnea cornuta* (Rayss and Dor) (Rhodophyceae) and *Hormophysa triquetra* (Kützing) (Phaeophyceae).

Sampling program:

A-Fresh water alga (*Spirulina platensis*)**:**

The tested blue green alga *Spirulina platensis* was obtained from Stock at Hydrobiology Lab, Qanater Khayria, Qalubia, Egypt.

B- Marine algae:

Seaweeds were collected seasonally for one year (September 2013 to August 2014) from the intertidal zone of the Gulf of Suez. Three algal samples: *Ulva lactuca* (Chlorophyceae) during spring , *Sargassum denticulatum* (Phaeophyceae) during autumn and *Hypnea cornuta* (Rhodophyceae) during winter were taken from Site I (Ras El-Adabiya) and one algal sample from Site II (RasSedr) *Hormophysa triquetra* (Phaeophyceae) during summer.

Sampling collection:

Seaweeds, were carefully washed by seawater, collected in plastic bags and kept in an ice –box at 20°C. The frozen seaweeds were lift to thaw and washed with distilled water to get rid of salts then lift to dry in indirect light. After dryness the samples were grinded for further analysis.

Preparation of the algal extracts :

Extraction of the ground algal powder were done using different solvents such as methanol, ethanol and chloroform (separately). Twenty-five grams of each algal powder sample were soaked in 100 ml of each solvent for 24 h (separately) on a rotator shaker at 150rpm at room temperature (25^{o}C: 30^{o}C).The resultant crude extracts were filtered through whatman filter paper no (1).The filtrate was freed from solvent by evaporation under reduced pressure by rotary evaporator Then the obtained residues (crude extracts)were separately suspended in 10%Dimethylsulfoxide (DMSO) to obtain 100mg/ml. Each extract was stored at - 20°C in airtighted glass bottle for the antimicrobial assay **(Cho *et al.*, 2007).**

A- Hot water extract **(Subash *et al.*, 2010)**.

- One hundred gram of powder for each algae was extracted separately with 300 ml of distilled water (90-95°C) for3hr.The coloured syrup was filtered through whatman no.3 paper. then concentrated to 1/4 th of the original volume.
- After that it was cooled and precipitated with 100 ml of ethanol.
- The precipitate was collected by centrifugation for 30 min, dehydrated with diethyl ether and dried at 37°C until diethyl ether free.
- Finally 30 ml ethyl acetate was added to the filter and take the residue.

B- Cold water extract **(Subash *et al*.2010)**.

- The same procedure without heating but the 100 gram of powder for each algae(separately) was soaked with 300 ml of distilled water at 4°Cover night.
- The coloured syrup was filtered through whatman no.3 paper. then it was precipitated with 100 ml of ethanol.
- The precipitate was collected by centrifugation for 30 min, dehydrated with diethyl ether and then dried at 37°C until diethyl ether free.
- Finally 30 ml ethyl acetate was added to the filter and take the residue.

Microorganism:

The source of *Candida* isolates:

Oral *Candida* isolates*:*

Candida species used in this study were isolated from oral cavity of diabetic patients from Al-Azhar hospital.

Cultivation of oral *Candida species* and growth condition:

Four oral *Candida species* were employed as test organism which included *Candida albicans, Candida tropicalis, Candida krusei* and *Candida glabrata*.

Sabouraud Dextrose Agar (SDA):

This medium was used for cultivation and growth of *Candida;* it contains (gram/liter)

- 40 Dextrose
- 10 Peptone
- 20 Agar
- 1 Distilled water

The pH of the medium was adjusted at 5.6 ± 0.1 and sterilized by autoclaving at 121ºC for 15 minutes.

The *Candida* strains were cultured on SD agar plates at 37°C for 48 hrs.

All strains were maintained on SD agar and subcultured monthly.

Preparation of inoculation:

Twenty- four hours old cultures of oral *Candida species* were mixed separately with SD broth medium and turbidity was adjusted using spectrometer at wave length of 600 nm in order to obtain absorbance O.D $\simeq$ 0.5, which resulted in final concentration of 10^8 CFU/ml(Colony Forming Unit).

Antifungal susceptibility test:

Antifungal assay :

In vitro the antifungal activity was screened by agar well diffusion method **(Bauer *et al*., 1996).** *Candida species* inoculates(10^8 cells/ml) were spread on SD agar plates and left to dry at room temperature, wells were made on the surface of agar medium with 6 mm cork borer. Each well in plate was filled with 50 µm of algal crude extract using micropipette.

The plates were incubated at 37^{o}C from 24- 48 hrs, At the end of incubation the plates were observed for the zone of inhibition and the diameters of the zones were measured in millimeters **(Karabay-Yavasoglu *et al*., 2007).**

All assays were carried out independently in triplicates and the mean result were calculated, also Fluconazole and dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively.

Determination minimum inhibitory concentrations (MIC):

The minimum inhibitory concentration (MIC) was applied on the crude extracts of *Ulva lactuca* these proved their high effectiveness against some oral *Candida species* by the agar well diffusion method. It was determined by using two fold serial dilution method .

The highest dilution of an extract that still retains an inhibitory effect against the growth isolates of oral *Candida sp.* is known as the MIC **(Misra and Dixit, 1978)**.

The complete protocol of the MIC test is found in the M7-T2 publication of the National Committee for Clinical Laboratory Standards **(2000)**.

Briefly, different crude extracts preparations were subjected to a serial dilution using SD broth medium as a diluents to give final crude extract concentration between 250mg/ml and 31.25 mg/ml. The tubes were inoculated with *Candida* suspension (20 µl/ml broth). Homogenized and incubated at 37^{o}C for 24 h. Following this, the lowest concentration of the crude extract that inhibited the visible growth of oral *Candida sp*. (absence of turbidity) was recorded as the MIC value of the crude extract.

The positive and negative controls were performed using SD broth medium. All MIC tests were performed independently for each *Candida sp*.

Determination of minimum fungicidal concentrations(MFC):

An overnight incubation for the MIC (determin50µl) of each tube which indicate no growth for all respective *Candida sp*, were sub cultured onto SD agar plates. The plates were incubated at 37°C for 24 to 48 h until visible growth was observed. The MFC value was the concentration where no growth or fewer than 3 colonies were obtained to approximately 99 :99.5 killing activity, Clinical and Laboratory Standards Institute **(2002).**

Determination of the Percentage Inhibition of Growth Diameter (PIGD):

The observation for MFC and the percentage inhibition of diameter growth (PIDG) values were determined according to the equation as below:

$$\text{PIDG (\%)} = \frac{\text{Diameter of sample} - \text{Diameter of control}}{\text{Diameter of control}} \times 100$$

Results:

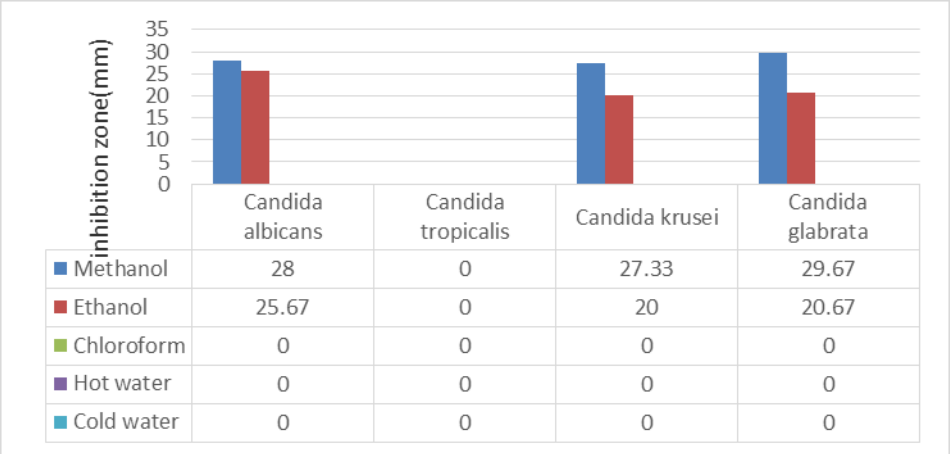
Antifungal activity:

The data in **Figs.1.2 and3** showed that:

i. Methanol extract of *Spirulina platensis* had an effect against *Candida albicans, C. krusei* and *C. glabrata* and the recorded inhibition zones were 28mm, 27.33mm and 29.67mm (respectively), but no effect against *C. tropicalis.*

ii. Ethanol extract of *Spirulina platensis* showed an effect against *Candida albicans, C. krusei* and *C. glabrata* and the recorded inhibition zones were 25.67mm, 20mm and 20.67mm (respectively), but no effect against *C. tropicalis.*

iii. Chloroform, Hot water and Cold water extracts of *Spirulina platensis* showed no effect against any of the tested oral *Candida sp.*

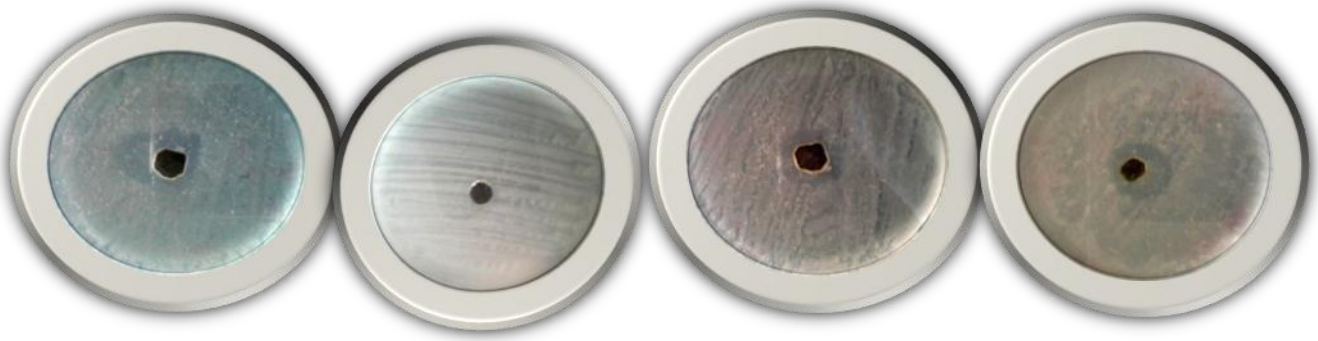


	Candida albicans	Candida tropicalis	Candida krusei	Candida glabrata
Methanol	28	0	27.33	29.67
Ethanol	25.67	0	20	20.67
Chloroform	0	0	0	0
Hot water	0	0	0	0
Cold water	0	0	0	0

Fig. (1) : Antifungal activities of *Spirulina platensis* extracts against oral *Candida sp.*

Candida albicans ***Candida tropicalis*** ***Candida krusei*** ***Candida glabrata***

Fig. (2):Antifungal activities of methanol extract for *Spirulina platensis* concentration (100mg/ml) on oral *Candida* species growth.



Candida all *Candida tropicalis* *Candida krusei* *Candida glabrata*

Fig. (3):Antifungal activities of ethanol extract of *Spirulina platensis* concentration (100mg/ml)on oral *Candida* species growth.

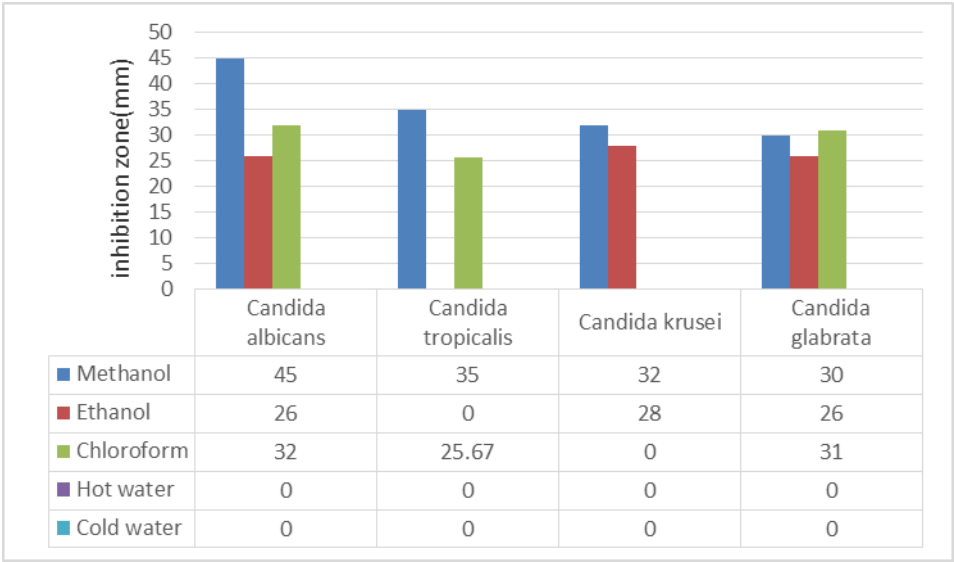
The data in **Figs.4.5.6 and 7** showed that:

i. Methanol extract of *Ulva lactuca* had an effect against all the tested oral *Candida sp*. with inhibition zones of 45,35,32 and 30mm for *Candida albicans*, *C. tropicalis*, *C. krusei* and *C. glabrata* (respectively).

ii. Ethanol extract of *Ulva lactuca* showed an effect against *Candida albicans*, *C. krusei* and *C. glabrata* with inhibition zones of 26mm, 28mm and 26mm (respectively). While it had no effect against *C. tropicalis.*

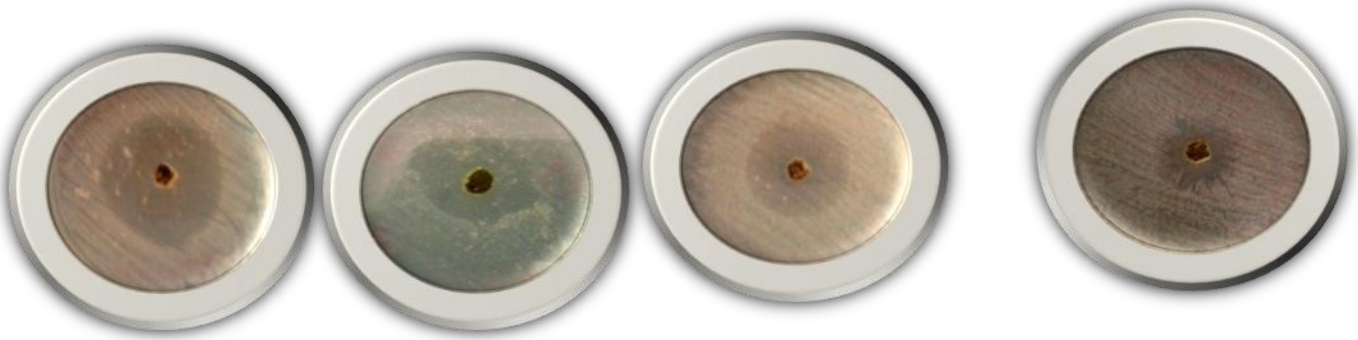
iii. Chloroform extract of *Ulva lactuca* had an effect against *Candida albicans*, *C.tropicalis* and *C. glabrata* with inhibition zones of 32mm, 25.67mm and 31mm (respectively). While it had no effect against *C. krusei.*

iv. Hot water and Cold water extracts of *Ulva lactuca* showed no effect against any of the tested oral *Candida sp.*



	Candida albicans	Candida tropicalis	Candida krusei	Candida glabrata
Methanol	45	35	32	30
Ethanol	26	0	28	26
Chloroform	32	25.67	0	31
Hot water	0	0	0	0
Cold water	0	0	0	0

Fig. (4) : Antifungal activities of *Ulva lactuca* extracts against oral *Candida sp.*

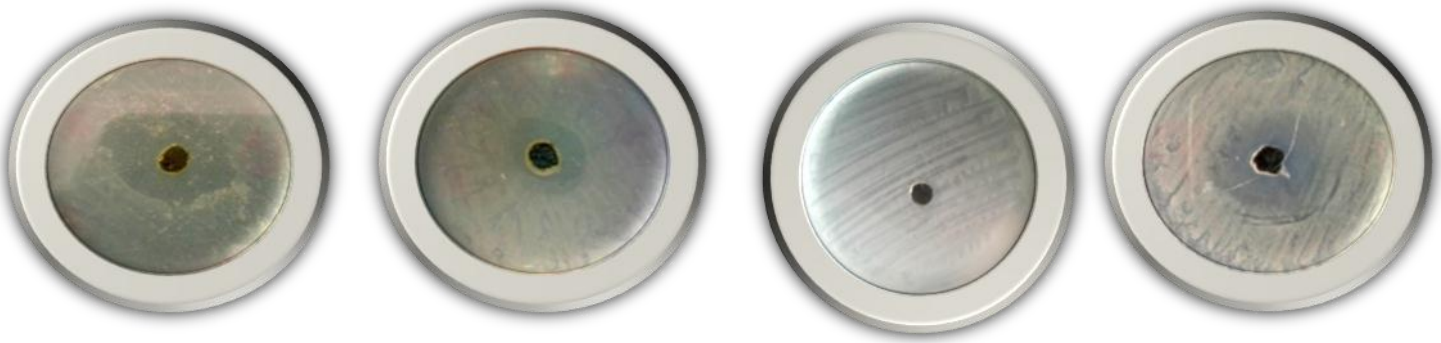


Candida albicans *Candida tropicalis* *Candida krusei* *Candida glabrata*

Fig. (5): Antifungal activities of methanol extract of *Ulva lactuca* concentration (100mg/ml) on oral *Candida* species growth.

Candida albicans *Candida tropicalis* *Candida krusei* *Candida glabrata*

Fig. (6): Antifungal activities of ethanol extract of *Ulva lactuca* concentration (100mg/ml) on oral *Candida* species growth

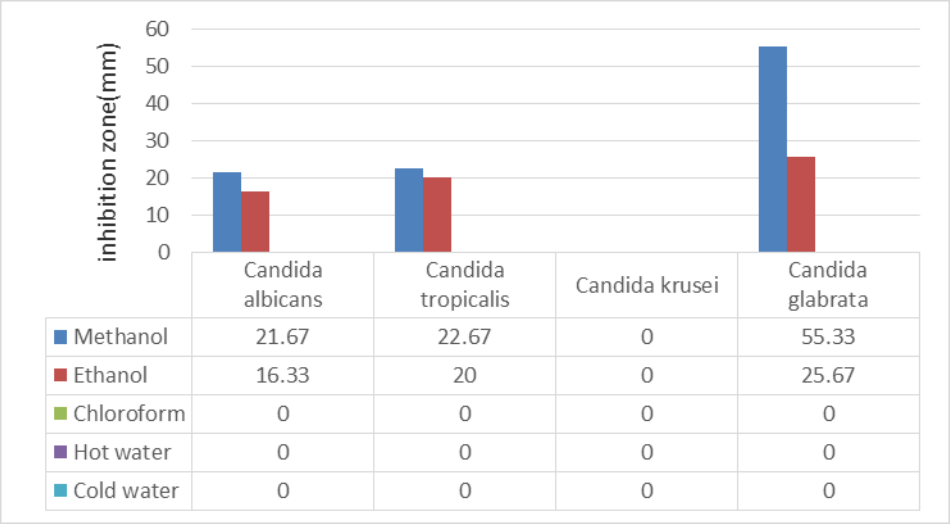


Candida albicans *Candida tropicalis* *Candida krusei* *Candida glabrata*

Fig. (7): Antifungal activities of chloroform extract of *Ulva lactuca* concentration (100mg/ml) on oral *Candida* species growth.

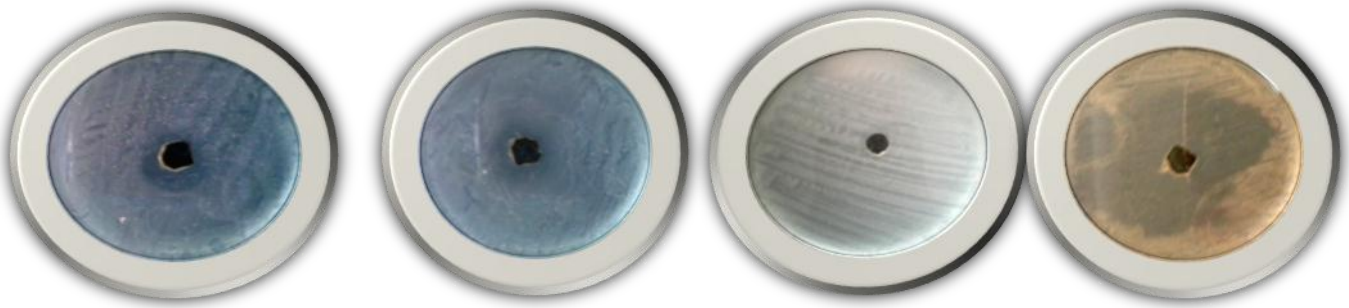
The data in **Figs.8.9 and 10** showed that:

i. Methanol extract of *Sargassum denticulatum* showed an effect against *Candida albicans, C. tropicalis* and *C. glabrata* with recorded inhibition zones of 21.67mm, 22.67mm and 55.33mm (respectively). While it had no effect against *C. krusei.*
ii. Ethanol extract of *Sargassum denticulatum* had an effect against *Candida albicans, C. tropicalis* and *C. glabrata* with recorded inhibition zones of 16.33mm, 20mm and 25.67mm (respectively). While it had no effect on *C. krusei.*
iii. Chloroform, Hot water and Cold water extracts of *Sargassum denticulatum* showed no effect against any of the tested oral *Candida sp.*



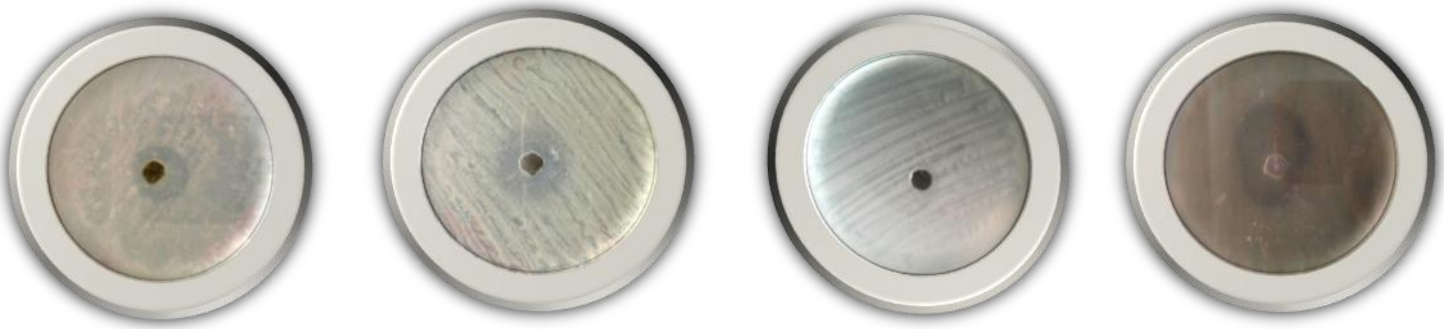
	Candida albicans	Candida tropicalis	Candida krusei	Candida glabrata
Methanol	21.67	22.67	0	55.33
Ethanol	16.33	20	0	25.67
Chloroform	0	0	0	0
Hot water	0	0	0	0
Cold water	0	0	0	0

Fig. (8) : Antifungal activities of *Sargassum denticulatum* extracts against oral *Candida sp.*



Candida albicans ***Candida tropicalis*** ***Candida krusei*** ***Candida glabrata***

Fig. (9): Antifungal activities of methanol extract of *Sargassum denticulatum* concentration (100mg/ml)on oral *Candida* species growth.



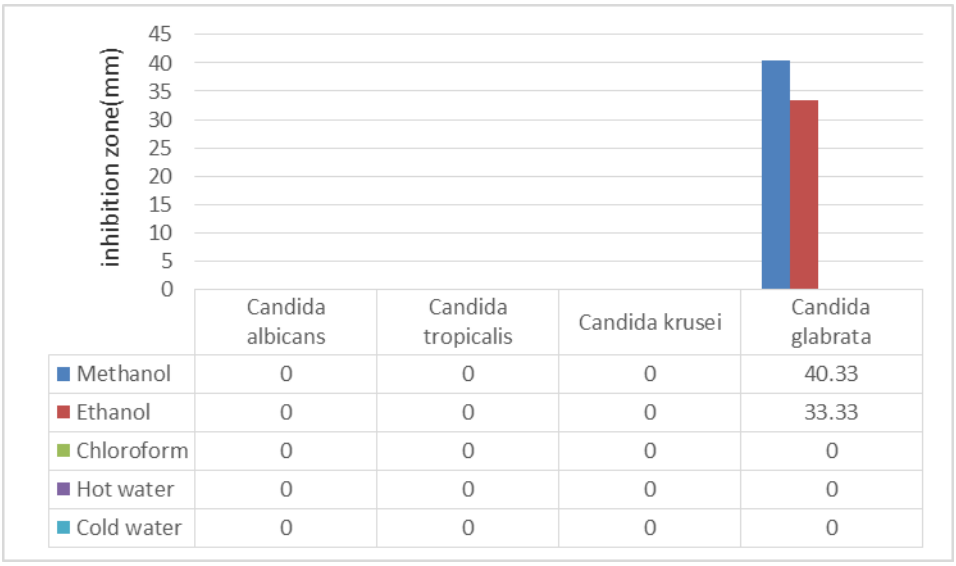
Candida albicans *'andida tropicalis* *Candida krusei* *Candida glabrata*

Fig. (10): Antifungal activities of ethanol extract of *Sargassum denticulatum* concentration (100mg/ml) on oral *Candida* species growth.

The data in **Figs. 11.12.and 13** showed that:

i. Methanol and Ethanol extracts of *Hormophysa triquetra* had an effect against *Candida glabrata* only with inhibition zone of 40.33 and 33.33mm (respectively), but it had no effect against other tested oral *Candida sp*.

ii. Chloroform, Hot water and Cold water extracts of *Hormophysa triquetra* showed no effect against any of the tested oral *Candida sp.*



	Candida albicans	Candida tropicalis	Candida krusei	Candida glabrata
Methanol	0	0	0	40.33
Ethanol	0	0	0	33.33
Chloroform	0	0	0	0
Hot water	0	0	0	0
Cold water	0	0	0	0

Fig. (11) : Antifungal activities of *Hormophysa triquetra* extracts against oral *Candida sp.*

Candida albicans *Candida tropicalis* *Candida krusei* *Candida glabrata*

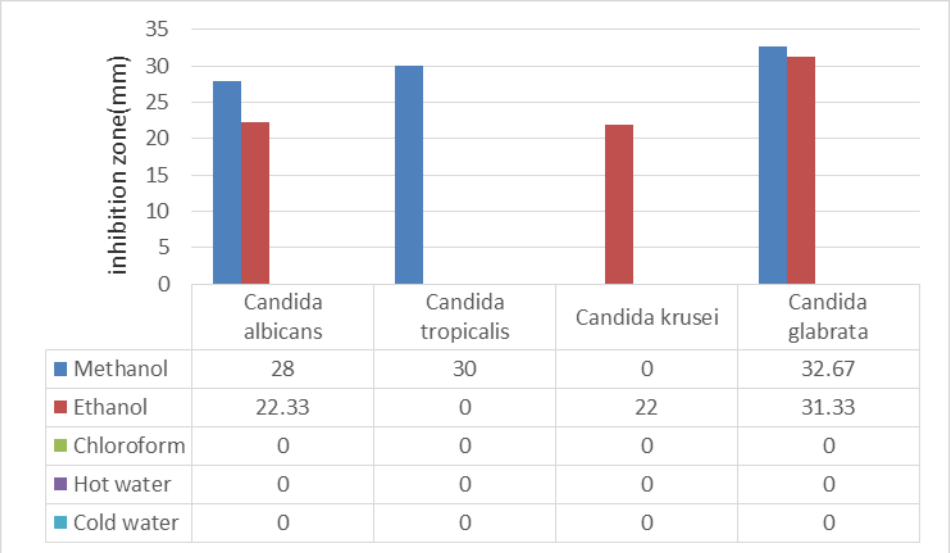
Fig. (12): Antifungal activities of methanol extract of *Hormophysa triqutra* concentration (100mg/ml)on oral *Candida* species growth.

Candida albicans *Candida tropicalis* *Candida krusei* *Candida glabrata*

Fig. (13): Antifungal activities of ethanol extract of *Hormophysa triqutra* concentration (100mg/ml) on oral *Candida* species growth.

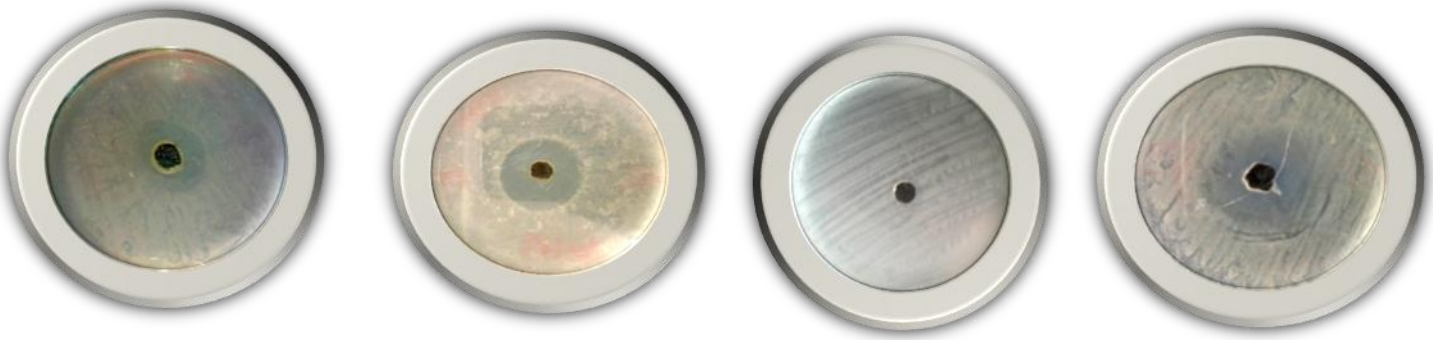
The data in **Figs. 14. 15 and 16** showed that:

i. Methanol extract of *Hypnea cornuta* showed an effect against *Candida albicans, C. tropicalis* and *C. glabrata* with inhibition zones of 28mm, 30mm and 32.67mm (respectively). While it had no effect against *C. krusei.*
ii. Ethanol extract of *Hypnea cornuta* showed an effect against *Candida albicans, C. krusei* and *C.glabrata* with inhibition zones of 22.33mm, 22mm and 31.33mm (respectively). While it had no effect on *C. tropicalis.*
iii. Chloroform, Hot water and Cold water extracts of *Hypnea cornuta* showed no effect against any of the tested oral*Candida sp.*



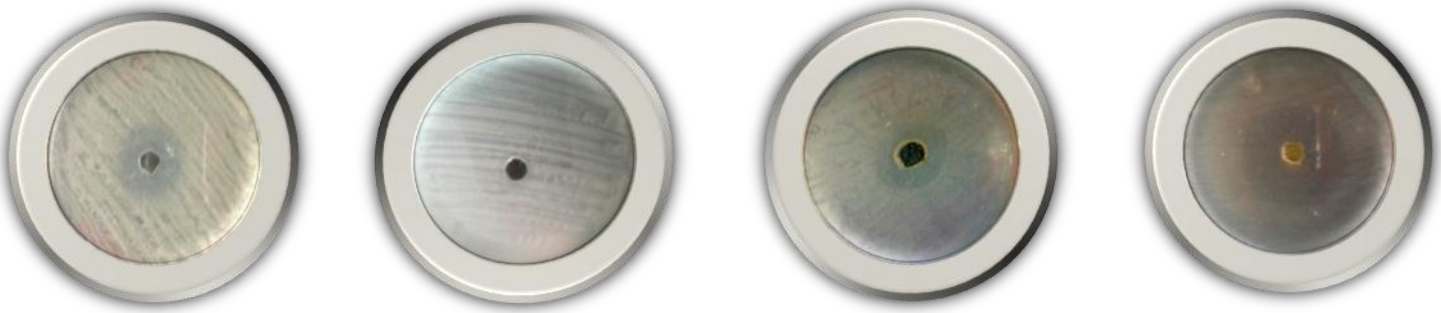
	Candida albicans	Candida tropicalis	Candida krusei	Candida glabrata
Methanol	28	30	0	32.67
Ethanol	22.33	0	22	31.33
Chloroform	0	0	0	0
Hot water	0	0	0	0
Cold water	0	0	0	0

Fig. (14) : Antifungal activities of *Hypnea cornuta* extracts against oral *Candida sp.*



Candida albicans ***Candida tropicali*** ***Candida krusei*** ***Candida glabrata***

Fig. (15): Antifungal activities of methanol extract of *Hypnea cornuta* concentration (100mg/ml) on oral *Candida* species growth



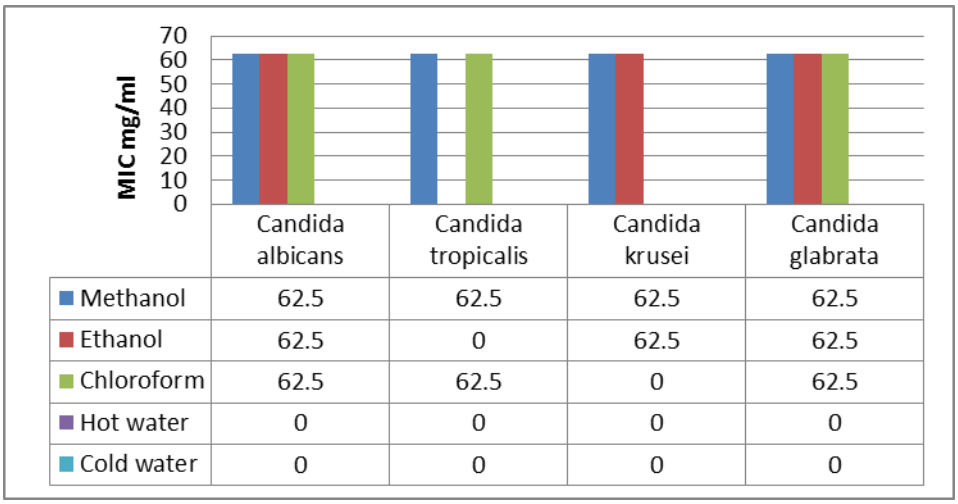
Candida albicans ***Candida tropicalis*** ***Candida krusei*** ***Candida glabrata***

Fig. (16): Antifungal activities of ethanol extract of *Hypnea cornuta* concentration (100mg/ml) on oral *Candida* species growth

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC):

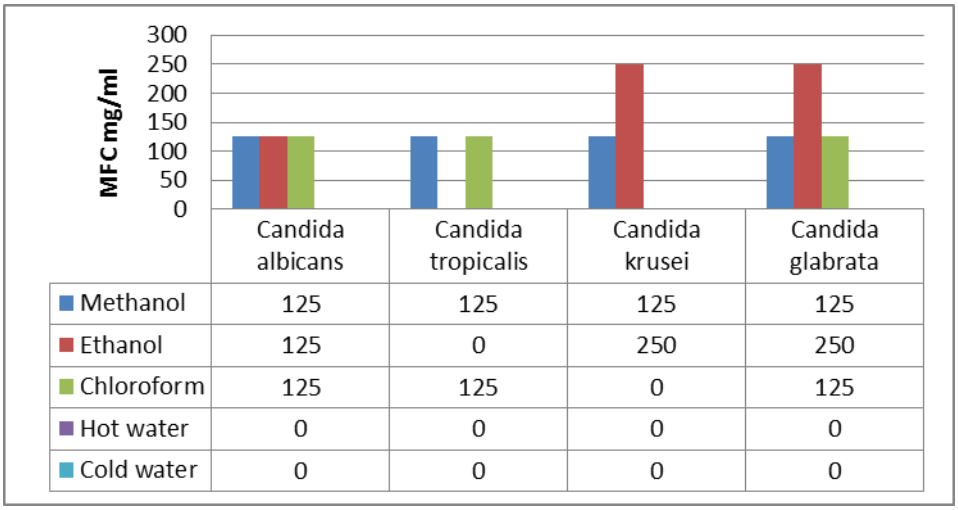
As shown in **Fig.17** The MIC value of *Ulva lactuca* methanol extract was 62.5mg/ml against all the tested oral *Candida species*, whereas the MIC value of ethanol extract was 62.5mg/ml against *Candida albicans, C. krusei* and *C. glabrata* , but it had no activity against *C. tropicalis*. As well as the MIC value of chloroform extract was 62.5 mg/ml against *Candida albicans, C. tropicalis* and *C. glabrata* , but it had no activity against *C. krusei*. The extract by hot water and cold water had no activity against all the tested oral *Candida sp.*

Also as represented in **Fig.18** The MFC value of *Ulva lactuca* methanol extract was 125 mg/ml against all the tested oral *Candida species*, whereas the MFC value of ethanol extract was 125 mg/ml against *Candida albicans* and was 250 mg/ml against *C. krusei* and *C. glabrata* (independently), but it had no activity against *C. tropicalis*. As well as the MFC value of chloroform extract was 125 mg/ml against *Candida albicans, C. tropicalis* and *C. glabrata* (independently), but it had no activity against *C. krusei*. The MFC value of extracts by hot water and cold water had no activity against all the tested oral *Candida species.*



	Candida albicans	Candida tropicalis	Candida krusei	Candida glabrata
Methanol	62.5	62.5	62.5	62.5
Ethanol	62.5	0	62.5	62.5
Chloroform	62.5	62.5	0	62.5
Hot water	0	0	0	0
Cold water	0	0	0	0

Fig. (17): Minimum Inhibition Concentration (MIC) mg/ml of *Ulva lactuca* extracts against the tested oral *Candida species*.



	Candida albicans	Candida tropicalis	Candida krusei	Candida glabrata
Methanol	125	125	125	125
Ethanol	125	0	250	250
Chloroform	125	125	0	125
Hot water	0	0	0	0
Cold water	0	0	0	0

Fig. (18): Minimum Fungicidal Concentration (MFC) mg/ml of *Ulva lactuca* extracts against the tested oral *Candida species*.

Determination of the percentage inhibition of diameter growth (PIDG):

Table (1): show the determination of the percentage inhibition of diameter growth (PIDG) for the tested oral *Candida species*. It revealed that the *Ulva lactuca* extract exhibited a higher inhibition ability compared to chlorhexidine(a positive control used in this study) which is a common antimicrobial agents in commercialized oral rinses.

Table (1): The percentage inhibition of diameter growth (PIDG):

Isolates	Diameter inhibition zone (mm)				PIDG(%)
	Ulva lactuca extract		Chlorhexidine		
	MFC	DIZ	MFC	DIZ	
Candida albicans	125mg/ml	16.37mm	0.1mg/ml	11.33mm	44.48%
Candida tropicalis	125mg/ml	15.33mm	0.1mg/ml	13.33mm	15%
Candida krusei	125mg/ml	15mm	0.1mg/ml	12.33mm	21.65%
Candida glabrata	125mg/ml	16.33mm	0.1mg/ml	14mm	16.64%

Discussion:

Usage of commercial antibiotics for human disease treatment produces undesirable side effects. Cell extracts and active constituents of various algae may be potential bioactive compounds of interest in the pharmaceutical industry ***(*Ely *et al.*, 2004*).***

In the present study the results showed that the methanol extracts of the five tested algae *(Spirulina platensis, Ulva lactuca, Sargassum denticulatum, Hormophysa triquetra* and *Hypnea cornuta)* had the highest antifungal activity against the selected human pathogenic fungi (*Candida albicans, C.tropicalis, C.krusei* and *C.glabrata*) where as the highest values of inhibition zones for *Sargassum denticulatum* and *Hormophysa triquetra* methanol extracts against *C.glabrata* were of 55.33 and 40.33mm (respectively).

These results were in agreement with that obtained by **El-Sheekh *et al.* (2015)**who found that the methanol extracts have the strongest inhibition against the tested microorganisms with inhibition activity percentage of 42.3%, followed by ethanol extracts with inhibition activity percentage of 33.5%. However ethyl acetate extracts have 14% inhibition activity, whereas chloroform showed the lowest inhibition activity percentage of 10.3% for all algae extracts against all tested fungi. Therefore, methanol was the best solvent for extractions. As well as, the results were in agreement with the results obtained by **Pandian *et al.* (2011)** who found that the tested petroleum-ether, chloroform and methanol of *Acanthaphora spicifera* in vitro for its antifungal activity against *Candida albicans,*

Microsporum gypseum, Aspergillus niger, respectively by disc diffusion techniques. The methanol extract of *Acanthaphora spicifera* showed higher antibacterial and antifungal activity compared to the other two extracts. Also, the results were in harmony with the results obtained by **Prabha *et al.* (2013)** who found that the methanolic extract showed higher antimicrobial activity compared to ethanol and acetone. The methanol extract of *Kappaphycus dvarezii*, the zone of inhibition was 6mm against *Micrococcus leutues*, *Escherichia Coli* and *Aspergillus flavus* and 5mm against *Staphylococcus aureus, Aspergillus fumigates* and *Candida albicans*.

In addition, the results were cording with the results obtained by **Sekar and Kolanjinathan(2015)** who found that the crude methanolic extract of *Padina gymnospora* showed maximum mean zone of inhibition against *Candida albicans* (15±09 mm) followed by *Turbinaria conoides* (15±09mm), *Sargassum wightii* (15±0.5mm), *Canlerpa racemosa* (12±0.8mm) and *Acanthophora spicifera* (12±0.6mm) at 300 mg/nl. The crude hexane extract of marine macroalgae showed minimum zone of inhibition against *Candida albicans* when compared to the other solvent extracts.

Methanol is a good solvent for extraction and it is frequently used in biology because of its high polarity . it is capable of extracting both lipophilic and hydrophilic molecules or substances. The other advantage is that it be removed easily at room temperature because it is highly volatile **Fiedler *et al*.(2005)**.

In the present study the results showed that the green algae (*Ulva lactuca*) methanol extract was the most active against all the tested oral *Candida species* (*Candida albicans, C.tropicalis, C.krusei* and *C.glaborata*) with inhibition zones of 45,35,32 and 30mm (respectively). Followed by Phaeophyta, Rhodophyta and blue green algae, where they had similar effects against the tested oral *Candida species*.

These results were in agreement with that obtained by **Osman *et al.* (2010)** who found that the most active seaweeds was *Ulva fasciata* (Chlorophyceae) against all tested microorganisms (*Bacillus subtitis, Staphylococcus aureus*and *streptococcus aureus* as gram-positive bacteria, and *(Escherichia coli, Salmonella typhi* and *Klepsiella pneumonia* as gram-negative bacteria) and one yeast strain *Candida albicans.* Followed by Rhodophyta and phosophyta. Also, the present results were in agreement with the results obtained by **Kandhasamy and Arunachalam (2008)** who reported that the Chlorophyceae showed high antimicrobial activity than other of the tested algae (Rhedophyceae and Phaeophycaea).

As well as, the present results were in agreement with the results obtained by **Sheikh *et al.* (2018)** who found that the Chlorophyta exhibited the highest antimycetic effect against *Candida albican, C.tropicalis, Aspergillus flavus, A. funmigatus* and *A. niger* followed by Rhodophyta and Phaeophyta.

The reason for the convergence of these results were the similarity of the environmental conditions to which algae have been exposed.

In the present study the results showed that the *Ulva lactuca* methanol extract was active against all the tested oral *Candida species* with the MIC and MFC of 62.5 and 125 mg/ml, Whereas the *Ulva lactuca* ethanol extract was active against *Candida albicans, C.krusei* and *C.glabrata* with the MIC and MFC of 62.5 and 125-250 mg/ml (respectively). Also *Ulva lactuca* chloroform extract was active against *Candida albicans, C.tropicalis* and

C.gabrata with the MIC and MFC of 62.5 and 125 mg/ml. In general the values of MFC were higher than the corresponding values of MIC.

These results were in agreement with that obtained by **Saleh andAl-Mariri (2018)** who reported that the methanolic *Sargassum vulgare* extract was the strongest by showing the lowest MIC value of 0.11 and 0.133 mg/ml^{-1} against *Aspergillus nigher* and *Candida albicans*, (respectively) and the lowest MFC value of 1.67 mg/ml^{-1} for the broth fungal strains. Also, the results were in agreement with the results obtained by **Saleh and Al-Mariri (2017)** who reported the inhibitory effect of *Ulva lactuca* (Chlorophyta), *Dilophusspiralis* (Phaeophyta) and *Jania rubens* (Rhodophyta) marine seaweeds against 2 fungal (*Candida albicans* and *Aspergillus niger* straine using aqueous and six organic extracts. The previous investigation showed that the lowest MIC value was recorded to be 0.106 mg/ml^{-1} with *Ulva lactuca* methanolic extract against broth fungal strains and with acetone and hexane against *Candida albicans* moreover, the lowest MFC value (0.266 mg/ml^{-1}) was observed with *Dilophus spiralis* chloroform against broth fungal strains.

Moreover, **Kim *et al.* (2014)** recorded antifungal activity of ethyl acetate edible brown seaweed *Eisenia bicyclis* extract against *Candida species*. The previous investigation showed that MIC/MFC values ranged between 4-32/16-64 mg/ml^{-1} against *Candida albicans*, overall, ethyl acetate. Soluble extract was the most potent with MIC/MFC values ranged between 4-8/16 mg/ml^{-1} against the tested strain.

In the present study the results showed that the percentage inhibition of diameter growth (PIDG) for the tested oral *Candida species* (*Candida albicans, C.tropicalis, C.krusei* and *C.glabrata*). It revealed that the *Ulva lactuca* extract exhibited a higher inhibition ability compared with chlorhexidine (a positive control used in this study) which is a common antimicrobial agents in commercialized oral rinses. Based on the percentage, *Candida albicans* was highly affected (44.48%) followed by *C.krusei* (21.65%), *C.glabrata* (16.64%) and *C.tropicalis* (15%). Since the present study was the first to conducte this PIDG test on algae extracts.

So the present results will be compared with that obtained by **Himratul-Aznita *et al.* (2011)** and **Nordin *et al.*, (2013)** who used the PIDG test on higher plants.

These results were in agreement with that obtained by **Himratul-Aznita *et al.* (2011)** who found that the percentage inhibition of diameter growth (PIDG) of *Piper betle* crude aqueous extract against oral *candida species* (*Candida albicans, C.tropicalis, C.krusei, C.lusitaniae, C.dubliniensis, C.glabrata* and *C.parapsilosis*), was exhibited a higher inhibition ability compared to chlorhexidine, were the PIDG values for *Candida albicans, C.tropicalis, C.lusitaniae, C.dubliniensis* and *C.glabrata* has shown that the aqueous extract of *Piper betle* outstrips the positive control used, that was 0.12% w/v chlorhexidine with PIDG values of more than 50% at *Piper betle* concentration of 25mg/ml. In contrast, PIDG for *C. krusei* and *C.parapsilosis* Shown that at 25mg/ml concentration of *Piper betle* extract has little influence on growth inhibition compared to chlorhexidine. Thus, the results obtained have shown the potential use of *Piper betle* extract as antifungal agent and thus significantly contribute to its antifungal development. as well as, the results were in agreement with that obtained by **Nordin *et al.*, (2013)** who found that the determination PIDG for *Brucea javanica* extract compared with chlorhexidine. Based on the percentage, *Candida glabrata* and *C. dubliniensis* and followed by *C. lusitaniae* were highly affected by

the extract of *Brucea javanica* which outstrips the chlorhexidine (positive control). The PIDG for *C. albicans* however outstrips the positive control at high concentration of 200 mg/ml, suggesting that the effectiveness of the extract on each *Candida* cell was dose-dependent. The other *Candida species* were less affected by the control and this explains the effectiveness of CHX as a reference in many clinical trials.

The reason for the convergence of these results were the algal extract contain compounds such as carbohydrates, proteins, minerals, oil, fats, polyunsaturated fatty acids as well as bioactive compounds such as antioxidants (polyphenols, tocopherols, vitamin E, vitamin C. mycosporine-like amino acids) and pigments, such as carotenoids (carotene xanthophylls) cholorophylls, and phycobilins (phycocyanin, phycoerythrin), which possess antibacterial, antiviral, antifungal, antioxidative, anti-inflammatoryand antitumor properties.

Conclusion:

The results proved the promising antifungal potency of the used solvent extracts for some algae Chlorophyta (*Ulva lactuca*), Phaeophyta (*Sargassum denticulatum*, *Hormophysa triquetra*) and Rhodophyta (*Hypnea cornuta*) from North-east of the Gulf of-Suez and the Red Sea coast, Egypt.in addition to one blue- green alga from freshwater (*Spirulina platensis*) was obtained from stock at Hydrobiology Lab, Qanater, Khayria, Qalubia, Egypt. It has been suggested that the active antifungal compounds in seaweeds were found to be interesting. Thus exploration of such biological agents might be a probable resource of an array of biologically active compounds and the present results will ensure a starting point for exploiting natural bioactive substances presents in the extracts of marine algae. Such compounds may serve as leads in the development of new pharmaceuticals. Consequently, our future research direction is toward isolation, purification and identification of the bioactive ingredients to understand their bio prospects.

References:

Aggarual, N.; Bhateja, S.; Arora, G. and Yasmin, T. (2018). Candidiasis. The most common fungal infection of oral cavity. Biomedical Journal of Scientitic and Technical Research, (1-5).

Bauer, A.W.M.; Kirby, M.; Sherris, C. and Truck, M. (1996). Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology; 45(4): 493-496.

Bellinger, E.G. and Sigee, D.C.(2015). Freash water algae identification, enumeration and use as bioindicators. ISBN. 978-1-118-91716-9 (hard book)

Bhakuni, D.S. and Rawat, D.S. (2005). Bioactive marine natural products. New Delki, p. 103-124.

Blunt, J.W.; Carroll, A.R.; Copp, B.R.; Davis, R.A.; Keyzers, R.A. and Prinsep, M.R. (2006). Marine natural products. Natural Product Report, Londres, B. 23, p. 26-78.

Cho, S.k.; Kang, S.E.; Cho, J.; Kim, A.; Park, S.; Hong, Y. and Ahn, D. (2007). The antioxidant properties of brown seaweed *(Sargassumsiliquastrum)* extracts. J. Med. Food. 10(3): 479-485.

Clinical and Laboratory Standards Institute (2002). Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard-second edition. Wayne : Clinical and Laboratory Standards Institute, P. 51.

El-bashiti, T.R.; Elmanama, A.A. and Masad, A.A. (2011). The antibacterial and synergistic effects of some Palestinian plant extracts on *Escherichia coli* and *Staphylococcus aureus*. Functional plant science and Biotechnology, (57-62).

El-Sheekh, M.M.; El-Shafay, S.M. and El-Ballat, E.M. (2015). Production and characterization of antifungal active substance from some marine and freshwater algae. International Journal of Environmental Science and Engineering (IJESE), 6:85-92.

Ely, R.; Supriya, T. and Naik, C.G. (2004). Antimicrobial activity of marine organisms collected of the coast of south East India. Journal of experimental marine biology and ecology, 309(1), 121-127.

Fiedler, E.G.; Grossmann, D.; Burkhard, K.G.; Weiss, C. and Witte. (2005). "Methanol". Ullmann's Encyclopedia of Industrial Chemistry. Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley VCH. doi:10.1002/ 14356007.a16_465. ISBN 978-3527306732

Greenberg, M.S. and Burker's, G.M. (2005). Oral Medicine Red and White Lesions of the oral cavity.Delhi, India, BC. Decker Inc, Elsevier, pp (85-125).

Harun, R.; Yip, J.W.S.; Thiruven Kadam, S.; Ghani, W.A.K. (2014). Algal biomass conversion to bioethanol. A step. By step assessment Biotechniol, 9, 73-86.

Himratal-Aznita, W.H.; Mohd-Al-Faisal, N. and Fathilah, A.R. (2011). Determination of the percentage inhibition of diameter growth (PIDG) of *Piper betle* crude aqueous extract against oral *Candida species.* Journal of Medicinal Plants Research, 5(6) : 878-884.

Kandhasamy, M. and Arunachalam, K.D. (2008). Evaluation of in vitro antibacterial property of sea weeds of southwest coast of India. African Journal of Biotechnology, 7(12): 1959-1961.

Karabay-Yavasoglu N.U, Sukatar A, Ozdemir G and Horzum Z (2007). Antimicrobial Activity of Volatile Components and Various Extracts of the Red Alga *Janiarubens. Phytother. Res*., **21:** 153-156.

Kim, K.H.; Eom, S.H.; Kim, H.J.; Lee, D.S. and Nshimlyumukiza, O. (2014). Antifungal and synergistic effects of an ethyl acetate extract of the edible brown seaweed Elseniabiculis against Candida species. Fish Aquat. Sci., 17:209-214.

Machado, F.L.D.S.; Kaiser, C.R.; Costa, S.S.; Gestinari, L.M. and Soares, A.R. (2010). Atividade biologica de metabolites Secundarios de algas marinhas do genero laurenica. Brazilian Journal of pharmacognosy, (3) 441-452.

Michalalk, I. and Chojnacka, K. (2014). Algal extracts: technology and advances. Engineering in life sciences, 14, 581-591.

Misra, S.B. and Dixit, S.N. (1978). Antifungal properties of leaf extract of *Ranunculus sceleratus* L. Experientia, 34: 1442-1443.

NCCLS. (2002). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard 3rd ed. NCCLS document M100-S12. Wayne, PA, National Committee for Clinical Laboratory Standards.

Nordin, M.A.; Wa Haran, W.H. and Abdul Razak, F. (2013). Antifungal susceptibility of growth inhibitory response of oral *Candida species* to *Brucea javanica* Linn. Extract. BMC complement Altern Med, (131:342).

Osman, M.E.; Abushady, A.M. and Elshobary, M.E. (2010). In vitro screening of antimicrobial activity of extracts of some macroalgae collectivity from Abu-Qir bay Alexandria, Egypt. African Journal of Biotechnology, 9(12): 7203-7208.

Pandian, P.; Selvamuthukumar, S.; Manavalan, R. and Parthasarthy, V. (2011). Screening of antibacterial and antifungal activities of red marine algae *Acanthaphora spicifera* (Rhodophyceae), Biomed scientitic Research, 3: (444-448).

Papenfuss, G.F. (1989): A history, catalogue and bibliography of Red Sea benthic algae. Israel Journal of Botany. 17,1-2.

Prabha, V.; Prakash, D.J. and Sudha, P.N. (2013). Analysis of bioactive compounds and antimicrobial activity of marine algae *Kappaphycus alvarezii* using three solvents extracts. International Journal of Pharmaceutical sciences and research, 4(1) : 306-310.

Saleh, B. and Al-Marriri, A. (2018). Antifungal activity of crude seaweed extracts collected from latakia coast, Syria, Journal of fisheries and Aquatic Science., 13:49-55.

Saleh, B. and Al-Mariri, A. (2017). Antimicrobial activity of the marine algal extracts against selected pathogens. J. Agric. Sci. Technol., 19:1067-1077.

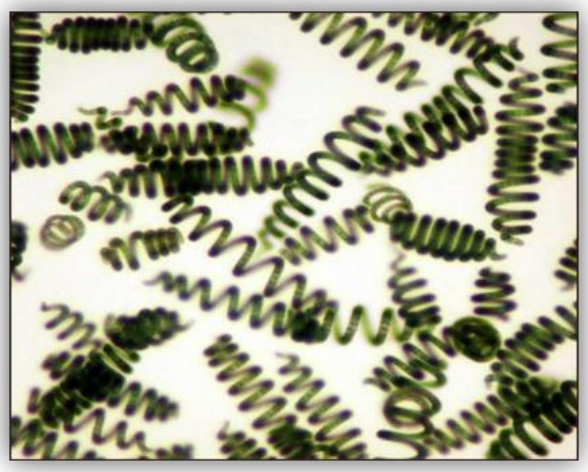
Sekar, D. and Kolanjinathan, K. (2015). Screening of antifungal activity of selected macroalgae against *Candida albicans* and *Candida glabrata.* Indo-Asian Journal of Multidisciplinary Research, (325-319).

Sheikh, H.; El-Naggar, A. and Al-Sobahi, D. (2018). Evaluation of antimycotic activity of extracts of marine algae collected from Red Sea coast, Jaddah, Saudi Arabia. Journal of Biosciences and Medicines, 6, 51-68.

Shobier, A.H.; Abdel Ghani, S.A. and Barakat, K.M. (2016). GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. Egyptian Journal of Aquatic Research, 42, 289-299.

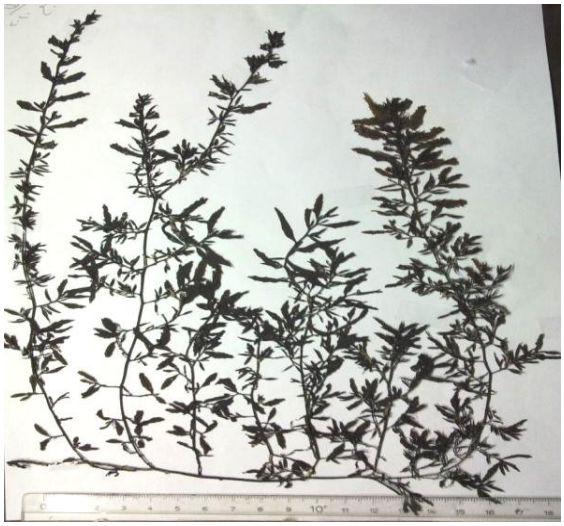
Smit, A.J. (2004). Medicinal and pharmaceutical uses of seaweed natural products : A review. Journal of Applied Phycology, Dordrecht, V. 16, p. 245-262.

Subash, A.; Hanumantha, R.B.R. and Adoor, G.S. (2010). In vitro antioxidant and in vivo anti inflammatory potential of crude polysaccharide from *tubinaria ornate* (Marine Brown Alga), *food ChemToxicol*,48(1),187-192.



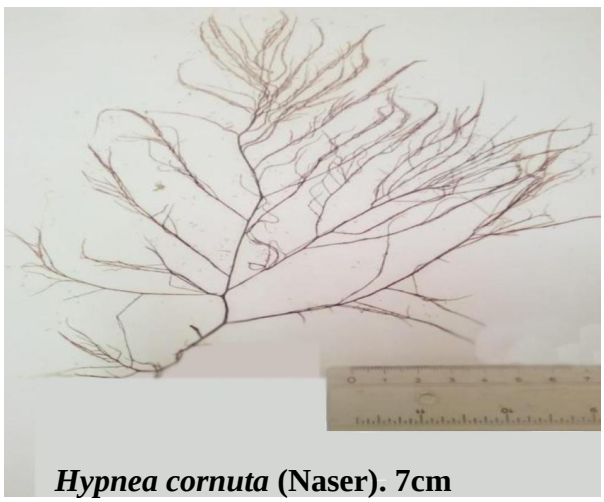
Spirulina platensis **(Nords) .40X**

Ulva lactuca **(Linnaeus). 2cm**



Sargassum denticulatum **(BØrgesen). 10 cm**

Hormophysa triquetra **(Kützing). 2cm**



Hypnea cornuta **(Naser). 7cm**

الملخص باللغة العربية

فحص الانشطة المضادة للفطريات لخمسة مستخلصات خام من الطحالب

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٤ باحث في المعهد القومي لعلوم البحار والمصايد.

في هذه الدراسة تم جمع أربعة أنواع من الطحالب البحرية خلال مواسم مختلفة لمدة عام واحد (سبتمبر ٢٠١٣م إلى أغسطس ٢٠١٤م). تم الحصول على الأنواع التي تم جمعها والتي تنتمي إلى Chlorophyta (*Ulva lactuca*), Phaeophyta (*Sargassum denticulatum*, *Hormophysa triquetra*) and Rhodophyta (*Hypnea cornuta*)

بالإضافة إلى طحلب Blue green algae من المياه العذبة *(Spirulina platensis)*. تم الحصول عليه من مختبر القليوبية مصر. تم تحضير مستخلصات الطحالب الخام باستخدام مذيبات مختلفة (الميثانول والإيثانول والكلوروفورم) بالإضافة إلى طريقة الاستخلاص بالماء الساخن والماء البارد. تم فحص مستخلصات الطحالب الخام لفعاليتها المضادة للفطريات ضد أنواع المبيضات الفموية (*Candida albicans, Candida tropicalis, Candida krusei* and *Candida glabrata*) باستخدام طريقة انتشار الآغار. أظهرت النتائج أن الميثانول هو أفضل مذيب مناسب لاستخراج المركبات النشطة حيوياً من الطحالب المختبرة. عرضت Chlorophyta (*Ulva lactuca*) أعلى تأثير مضاد للفطريات يليه *Phaeophyta* و*Rhodophyta* وطحلب Blue green. كان أدنى تركيز مثبط (MIC) لمستخلصات الطحالب الأكثر فاعلية (*Ulva lactuca*) مستخلص الميثانول ٦٢.٥ ملغ/مل وكان الحد الأدنى لتركيز مبيدات الفطريات(MFC) ١٢٥ ملجم / مل لنفس الطحلب مع جميع أنواع المبيضات الفموية التي تم اختبارها. تم إجراء مقارنة بين قيمة MFC لمستخلص الميثانول أولفا لاكتوكا مع مضادات الكلورهيكسيدين (٠.١) ملجم / مل (وهو مضاد تجاري شائع للميكروبات لشطف الفم) باستخدام طريقة النسبة المئوية لتثبيط نمو القطر (PIDG) ضد أنواع المبيضات الفموية المختبرة. أوضحت النتائج أن مستخلص ميثانول أولفا لاكتوكا كان الأفضل بالنسبة ل PIDG من الكلورهيكسيدين (مضاد حيوي).