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Biological Control of Some Pathogenic Fungi using Marine Algae Extracts

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ABSTRACT

The present research has been conducted to explore the antifungal potency of the methanol and ethyl acetate crude extracts of seaweeds collected from the red sea, Hurghada, Egypt. The concentration of (50 mg mL^{-1}) was tested against *Alternaria alternata*, *Fusarium oxysporium*, *Alternaria brassicicola*, *Ulocladium botrytis* and *Botryotrichum Piluliferum*. The antifungal activities were expressed as inhibition in dry mass (mg), protein content (μg mL^{-1}) and enzymatic activity of pectinase and cellulase (units mL^{-1}). For comparative study, the biological activity of standard antibiotic Nystatin was also measured. Crude ethyl acetate extract of *Padina gymnospora* and methanolic extract of *codium fragile* exhibited strong activity against most of the tested fungi. All the tested fungi were sensitive to Nestatin, except *F. oxysporium* (the most resistant fungi), where the dry weight recorded from 68 to 69%, protein recorded 69 to 52%, pectinase and cellulase activity 62 to 58% of control. The most sensitive fungi were *U. botrytis* where the dry weight, protein content, pectinase and cellulase activities all were completely inhibited in cellulose and pectin media. While, the most active algae is the ethyl acetate extract of *P. gymnospora* and methanolic extract of *C. fragile*. inhibited pectinase and cellulase enzymes activities for all the tested fungi except *A. brassicicola* and *F. oxysporium*. This report confirms the broad antifungal effect of *C. fragile* using the methanolic extracts rather than ethyl acetate (*P. gymnospora*). Additionally, These Egyptian seaweeds, therefore, considered as a potential source for treating infections caused by the tested plant fungi.

Key words: Antifungal, ethyl acetate extracts, marine algae, methanolic extracts, biological control, pectinase enzyme, cellulase

INTRODUCTION

The orientations for treatment of plant diseases are currently interesting in extraction of natural products as alternatives to synthetic fungicides for their safety and negligible environmental impacts (Brimmer and Boland, 2003). For example, Khalil *et al*. (2005) and Zafar *et al*. (2002) reported some medicinal plants as antifungal activities against plant pathogenic fungi. Osman *et al*. (2011) examined the antifungal activity of some agriculture wastes (rice straw, maize and cotton wastes) against *Rhizoctonia solani* with successful results. Also, seaweeds considered a possible and easy source of antimicrobial compounds due to their variety of secondary metabolites with antifungal activities (Cordeiro *et al*., 2006). Pathogenic fungi are the most organisms responsible for a considerable plant yield losses than other microorganisms (Sexton and Howlett, 2006). Extracted substances from seaweeds confirmed earlier for their antifungal

(Khanzada *et al*., 2007) and antibacterial activities (Salem *et al*., 2011). The crude extracts obtained from herbs essential oils, *Cassia fistula* and *Mesua ferrea* are subjected to broad biological screening for antifungal activity (Sohel and Yeasmin, 2004; Mousavi *et al*., 2009). Nowadays, extraction of new compounds from macro algae confirmed its biocidal activity as antifungal agents (Bhosale *et al*., 2002). Such compounds are extracted from different macroalgae families, like green, brown and red algae (Vallinayagam *et al*., 2009). Tuney *et al*. (2006) recorded positive results for methanol, acetone, diethyl ether and ethanol extracts of *Cystoseira mediterranea* and *Ulva rigida*, for their antifungal activities. According to prior reports, it has been confirmed that most of biologically active compounds can be used as therapeutic agent that are found in seaweeds (Madhusudan *et al*., 2011).

However, the reports on the antifungal activities of seaweed extracts from Egypt are very limited. Hence, this investigation aimed to screen and evaluate the efficiency of organic solvents such as methanol and ethyl acetate extracts from the Egyptian seaweeds as antifungal agents against some plant pathogenic fungi.

MATERIALS AND METHODS

Algae collection and extract preparation: Eight marine algae were collected by hand picking from the red sea in Hurghada, Egypt during June 2009. Algal samples were cleaned from epiphytes, extraneous matter and necrotic were removed. Samples were collected in sterilized polyethylene bags and put in an ice box, then transferred to the laboratory immediately. The collected algae comprise three different families as follows: Phaeophyceae (*Cystoesira myrica, Cystoesira trinodis, Padina gymnospora, Sargassum dentifolium, Sargassum hystrix*); Rhodophyceae (*Actinotrichia fragilis*) and Bryopsidophyaceae (*Codium fragile*). Samples were washed thoroughly with sea water then sterile distilled water, air dried, cut into small pieces and then ground in a tissue grinder (IKA A 10, Germany) until reach fine powder shape.

The dried samples (10 g) were extracted in two different solvents: (100 mL of methyl alcohol or ethyl acetate) under stirring condition (50 rpm) for 7 days at room temperature. The solution was filtered through Whatman No. 1 sterile filter paper. The filtrates then were dried using desiccator (Cole- parmer instrument, Chicago). The dried precipitates were dissolved in the above two solvents to give 50 mg mL^{-1} extracts, then stored in airtight bottles in a refrigerator before testing. These crude extracts were screened against common pathogenic fungi as follows:

Fungal source and culture conditions: *Alternaria alternata, Fusarium oxysporium, Alternaria brassicicola, Ulocladium botrytis* and *Botryotrichum Piluliferum* were isolated from infected seeds, identified and subcultured on glucose-Czapek's agar medium (Smith and Dawson, 1944). All the fungal isolates were screened for their abilities to produce extracellular cell wall-degrading enzymes such as pectinase according to Hankin *et al*. (1971) and cellulase according to Eggins and Pugh (1962).

Effect of algal extracts on pectin lyase production: The fungi were grown in Erlenmeyer flasks (100 mL) containing 50 mL of sterilized liquid media, inoculated with a single 6 mm disc cut-out from the margin of a 5 day colony of each fungus grown on glucose-Czapek's agar medium. The flasks were supplemented with 1% of the algal extracts and incubated at 28±1°C for 7 days. Methanol and ethyl acetate was used as a negative control while nystatin used for comparative study.

After 7 days of incubation, the mycelial growth, PL activity and extracellular protein content were determined. The mycelial growth was then filtered through pre-weighted 0.45 μ filter paper using a filter unit. The agar inoculums were removed from the mycelium. The dry weight of the mycelium was left to dry in an oven at 85°C for 24 h. Filtrates were centrifuged at 15.000 g for 15 min at 4°C for assaying of enzymes activities and extracellular of protein content.

PL assay procedures: The PL assay procedures were carried out according to Sherwood (1966) because there is a correlation between virulence of the pathogens and their ability to degrade cellulose and pectin.

Protein content determination: Protein of culture filtrates (as indicators for fungi activities) was determined according to Lowry *et al.* (1951).

Effect of algal extracts on cellulase (exo-1,4-β-glucanase) production: The method described by Nelson (1944) and modified by Naguib (1964) was employed for cellulose determination.

Statistical analysis: The data of all experiments were subjected to analysis by the Least Significant Differences test (L.S.D) using PC-STATE program version 1A, coded by Rao, M.; Blane, K. and Zannenberg, M, University of Georgia.

RESULTS

The effect of marine algal extracts on dry mass, protein and extracellular enzyme functions of different fungi are presented in Table 1 to 6.

The data showed that all the tested fungi were sensitive to nestatin, except *F. oxysporium* where the dry weight recorded 68% of absolute control in pectin medium (Table 1). The most sensitive fungi were *U. botrytis* where the dry weight completely inhibited in both cellulose and pectin media. While, the most active algae, *P. gymnospora* (ethyl acetate extract) where dry weight of all tested fungi was inhibited completely, the exception was *A. brassicicola* (68.6% dry weight compared to control) (Table 1). Methanolic extracts showed much more bioactivity (inhibition of fungal dry weight) than ethyl acetate extracts. For example, the ethyl acetate extracts of *C. fragile*, *A. fragilis* and *C. trinodes* did not show any antifungal activity (indicated as dry weight) against all of the tested fungi (Table 1). *C. fragile* (methanolic extract) also showed high antifungal activity with all tested fungi, except *F. oxysporium* (58% dry weight compared to control) (Table 2).

U. botrytis and *A. brassicicola* are the most sensitive fungi (showed complete inhibition of protein content) when ethyl acetate of and methanolic extracts were employed, respectively (Table 3). While, the most active algae, *P. gymnospora* (ethyl acetate extract) where protein content of all tested fungi was inhibited completely, the exception was *A. brassicicola* (61.6% protein content compared to control) (Table 3). *C. fragile* (methanolic extract) also showed high antifungal activity with all tested fungi, except *F. oxysporium* (69.88%) protein content compared to control (Table 4). Both ethyl acetate of (*C. fragile*, *A. fragilis* and *C. trinodes*) and methanolic extracts of (*S. dentifolium*, *C. myrica* and *C. trinodes*) showed non antifungal activity (based on protein content) for all tested fungi (Table 4).

For antifungal assay by pectinase and cellulase enzyme activities (units mL^{-1}), *U. botrytis* with ethyl acetate extracts and *A. brassicicola* with methanolic extracts, were the most sensitive fungi

Table 1: Antifungal assay by dry weight (mg) in pectinase medium

Treatment	*Alternaria brassicicola*	%	*Ulocladium botrytis*	%	*Botryotrichum piluliferum*	%	*Fusarium oxysporium*	%	*Alternaria alternata*	%
Control										
Absolute control	67.78±14.43	100.00	51.98±0.98	100.00	90.60±2.00	100.00	88.68±3.67	100.00	71.40±5.44	100.00
Methyl alcohol	77.38±4.23	114.17	53.65±1.35	103.22	88.42±8.25	101.27	98.25±12.71	110.79	87.08±1.08	121.95**
Ethyl acetate	80.25±5.75	118.41*	56.28±0.03	108.27	90.45±5.15	99.83	83.08±6.40	93.69	79.77±5.27	111.72*
Nystatin	-ve	-ve	-ve	-ve	-ve	-ve	60.65±10.64	68.39**	-ve	-ve
Ethyl acetate extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	90.00±2.00	99.34	21.03±7.84	23.72**	44.55±4.45	62.40**
Caulerpa racemosa	29.96±6.87	44.20**	-ve	-ve	51.51±5.85	56.86**	22.43±4.73	25.30**	32.58±2.53	45.63**
Sargassum dentifolium	32.46±11.90	47.89**	-ve	-ve	66.07±0.00	76.60**	34.07±4.16	38.41**	48.53±0.23	67.96**
Cystoesira myrica	65.98±7.50	97.35	49.00±3.46	94.28	59.33±5.00	76.16**	41.23±8.19	46.49	67.80±2.05	94.96
Padina gymnospora	46.50±8.50	68.61**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Codium fragile	73.05±8.90	107.78	32.53±2.48	62.58	87.10±5.00	96.14	40.17±7.50	45.29*	83.05±3.90	116.32**
Actinotrichia fragilis	73.55±9.75	108.52	26.73±0.23	51.43**	93.40±1.70	103.09	74.40±9.26	83.89*	69.23±5.93	96.95
Cystoesira trinodes	62.75±11.00	92.59	21.36±5.76	41.09**	90.03±1.68	99.38	37.35±5.08	42.11**	74.42±2.08	104.23
Methyl alcohol extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	36.80±0.74	42.09**	22.45±4.24	25.32**	-ve	-ve
Caulerpa racemosa	-ve	-ve	51.90±5.55	99.86	79.15±1.25	87.36*	45.10±3.52	50.86**	-ve	-ve
Sargassum dentifolium	26.32±9.55	38.83**	-ve	-ve	90.95±3.15	109.22	74.63±5.75	84.16**	55.98±2.63	78.40**
Cystoesira myrica	27.89±9.26	41.149**	32.35±0.15	62.24**	85.60±5.00	94.48	37.08±7.43	41.82**	52.30±3.89	73.25**
Padina gymnospora	-ve	-ve	48.27±1.03	92.87	89.00±8.00	98.23	47.70±1.83	53.79**	50.03±7.02	70.08**
Codium fragile	-ve	-ve	-ve	-ve	-ve	-ve	52.57±5.90	59.28**	-ve	-ve
Actinotrichia fragilis	44.43±2.41	65.55**	51.85±5.82	99.76	89.82±3.45	102.82	41.45±5.21	46.74**	-ve	-ve
Cystoesira trinodes	66.10±2.30	97.53	32.34±5.52	62.23**	48.53±4.80	55.52**	31.03±7.38	34.99**	71.60±6.80	100.28
LSD										
5 %	12.14		4.57		11.34		11.21		6.03	
1%	16.19		6.16		15.27		15.10		8.11	

Mean±SD, n = 3. *Significant differences, **Highly significant differences, -ve: Negative results (complete inhibition).

Table 2: Antifungal assay by dry weight (mg) in cellulase medium

Treatment	*Alternaria brassicicola*	%	*Ulocladium botrytis*	%	*Botryotrichum piluliferum*	%	*Fusarium oxysporium*	%	*Alternaria alternata*	%
Control										
Absolute control	127.11±17.03	100.00	90.38±1.44	100.00	85.75±0.61	100.00	149.91±2.71	100.00	124.11±0.01	100.00
Methyl alcohol	120.37±1.45	94.70	80.91±9.35	89.52	90.84±0.04	105.94	133.27±9.74	88.90*	130.51±0.03	105.16
Ethyl acetate	126.63±13.27	99.62	77.12±9.64	85.33*	81.73±9.76	95.31	145.63±4.50	97.14	109.91±0.31	88.56**
Nystatin	-ve	-ve	-ve	-ve	-ve	-ve	104.51±4.57	69.72**	-ve	-ve
Ethyl acetate extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	85.94±0.10	100.22	61.80±9.10	41.23**	50.18±3.85	40.43**
Caulerpa racemosa	90.32±10.98	71.06**	-ve	-ve	62.78±8.00	73.22**	78.48±7.80	52.35**	42.51±5.78	34.26**
Sargassum dentifolium	124.03±4.57	97.58	-ve	-ve	88.20±8.06	102.86	78.84±7.57	52.59**	123.52±10.28	99.53
Cystoesira myrica	105.95±14.03	83.35**	73.41±6.49	81.22**	84.99±4.42	99.11	164.01±15.28	109.40	121.20±10.79	97.66
Padina gymnospora	115.510±1.61	90.87	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Codium fragile	102.43±0.59	80.58**	90.94±7.35	100.62	83.73±10.03	97.65	136.99±6.92	91.38	117.61±6.47	94.77
Actinotrichia fragilis	98.64±3.44	77.60**	90.85±5.01	100.52	56.21±9.10	65.55**	118.24±2.66	78.87**	116.71±8.96	94.04
Cystoesira trinodes	99.77±9.38	78.49**	88.41±7.48	97.82	57.56±5.23	67.13**	81.05±10.17	54.06**	39.81±1.84	32.08**
Methyl alcohol extracts										
Sargassum hystrix	-ve	-ve	81.07±10.67	89.69	46.21±9.64	53.89**	60.25±0.45	40.19**	-ve	-ve
Caulerpa racemosa	-ve	-ve	58.76±12.00	65.01	65.24±7.40	76.09**	72.50±5.84	48.36**	-ve	-ve
Sargassum dentifolium	103.11±1.11	81.12**	-ve	ND	36.59±1.92	42.67**	139.57±4.03	93.11	18.42±0.10	14.84**
Cystoesira myrica	100.45±11.35	79.03**	56.09±12.10	62.06**	91.51±0.67	106.72	80.09±7.65	53.43**	29.12±1.00	23.46**
Padina gymnospora	-ve	-ve	82.08±11.52	90.82	81.68±9.10	95.25	67.06±5.70	44.73**	32.34±0.04	26.06**
Codium fragile	-ve	-ve	-ve	-ve	-ve	-ve	86.92±0.00	57.98**	-ve	-ve
Actinotrichia fragilis	-ve	-ve	62.62±6.96	69.28**	66.16±8.00	77.16**	110.31±6.74	73.58**	-ve	-ve
Cystoesira trinodes	108.65±0.97	85.48**	43.95±9.46	48.63**	85.54±3.85	99.76**	102.57±8.99	68.42**	-ve	-ve
LSD										
5%	12.03		11.62		10.337		13.50		7.43	
1%	16.19		15.66		13.921		18.18		10.00	

Mean±SD, n = 3. *Significant differences, ** Highly significant differences, ND: Not determined, -ve: Negative results (complete inhibition)

Table 3: Antifungal assay by protein content (μg mL^{-1}) in pectinase medium

Treament	*Alternaria brassicicola*	%	*Ulocladium botrytis*	%	*Botryotrichum piluliferum*	%	*Fusarium oxysporium*	%	*Alternaria alternata*	%
Control										
Absolute control	39.80±3.48	100.00	104.61±8.85	100.00	56.81±8.24	100.00	63.31±4.16	100.00	42.97±2.32	100.00
Methyl alcohol	37.94±6.73	95.34	98.73±10.21	94.38	59.52±13.55	104.76	73.90±1.58	116.74	43.32±3.36	100.81
Ethyl acetate	42.58±2.09	107.00	104.22±9.09	99.63	56.97±5.40	100.27	61.69±5.38	97.44	41.93±2.17	97.57
Nystatin	-ve	-ve	-ve	-ve	-ve	-ve	44.13±4.02	69.71	-ve	-ve
Ethyl acetate extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	53.80±2.69	94.69	29.59±2.56	46.74	29.21±0.97	67.97**
Caulerpa racemosa	23.25±0.35	58.42**	-ve	-ve	40.26±6.00	70.87**	27.20±3.57	42.96	28.97±2.92	67.43**
Sargassum dentifolium	23.25±1.04	58.42**	-ve	-ve	36.94±3.60	65.02	28.66±1.34	45.28	31.33±3.07	72.92**
Cystoesira myrica	39.15±0.58	98.35	100.66±6.27	96.23	35.16±2.46	61.89**	30.83±2.59	48.70	43.74±1.77	101.80
Padina gymnospora	24.53±0.46	61.63**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Codium fragile	38.27±0.35	96.17	55.73±9.98	53.28**	51.32±5.36	90.34	40.88±6.63	64.58	42.20±0.67	98.20
Actinotrichia fragilis	39.35±3.71	98.87	62.23±3.65	59.49**	57.12±11.80	100.55	46.92±5.24	74.10	44.48±2.09	103.51
Cystoesira trinodes	37.97±2.32	95.39	72.13±6.34	68.95**	55.65±2.71	97.96	34.93±6.21	55.17	32.14±0.67	74.80
Methyl alcohol extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	34.23±4.20	60.25**	26.73±1.16	42.22	-ve	-ve
Caulerpa racemosa	-ve	-ve	102.13±10.24	97.63	52.48±1.16	92.38	41.97±1.17	66.29	-ve	-ve
Sargassum dentifolium	24.72±0.70	62.11**	-ve	-ve	59.13±8.04	104.08	50.63±6.76	79.97	34.70±1.54	80.74**
Cystoesira myrica	21.36±0.35	53.68**	37.02±11.30	35.39**	53.10±2.44	93.47	27.81±0.81	43.93	33.61±4.42	78.22**
Padina gymnospora	-ve	-ve	98.65±8.14	94.31	56.58±12.94	99.59	43.59±3.64	68.85	25.69±0.35	59.78**
Codium fragile	-ve	-ve	-ve	-ve	-ve	-ve	40.81±9.93	64.45	-ve	-ve
Actinotrichia fragilis	29.98±1.84	75.32*	95.94±2.10	91.72	35.93±3.16	63.25	40.57±3.17	64.09	-ve	-ve
Cystoesira trinodes	37.05±1.67	93.08	61.69±10.36	58.97**	39.49±7.48	69.51*	32.840±3.77	51.87	42.89±1.49	99.82
LSD										
5%	4.86		9.75		8.14		7.34		3.18	
1%	6.55		13.14		10.96		9.88		4.29	

Mean±SD, n = 3.*Significant differences, **Highly significant differences, -ve: Negative results (complete inhibition)

Table 4: Antifungal assay by protein content (μg mL^{-1}) in cellulase medium

Treatment	*Alternaria brassicicola*	%	*Ulocladium botrytis*	%	*Botryotrichum piluliferum*	%	*Fusarium oxysporium*	%	*Alternaria alternata*	%
Control										
Absolute control	41.35±3.50	100.00	96.87±8.84	100.00	59.13±0.05	100.00	86.51±4.16	100.00	49.93±0.54	100.00
Methyl alcohol	40.65±6.73	98.32	91.77±10.21	94.73	70.35±0.23	118.96	89.37±13.95	103.31	45.64±0.76	91.40
Ethyl acetate	39.49±2.08	95.51	96.49±9.09	99.60	65.48±0.17	110.72	77.93±11.78	90.08	51.21±0.38	102.56
Nystatin	-ve	-ve	-ve	-ve	-ve	-ve	45.68±3.54	52.80**	-ve	-ve
Ethyl acetate extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	69.26±0.20	117.13	42.58±4.83	49.23**	29.98±0.42	60.04**
Caulerpa racemosa	36.82±0.35	89.06*	-ve	-ve	47.22±0.68	79.86**	50.32±0.94	58.16**	37.48±0.09	75.06**
Sargassum dentifolium	38.22±1.04	92.43	-ve	-ve	60.14±0.17	101.70	57.59±3.02	66.57**	46.84±0.17	93.81**
Cystoesira myrica	45.18±0.58	109.26*	92.93±6.27	95.93	58.36±0.08	98.69**	77.23±2.59	89.27	45.79±0.21	91.71**
Padina gymnospora	41.12±0.46	99.44	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Codium fragile	42.16±0.35	101.96	78.93±9.98	81.48**	54.96±0.24	92.94	83.65±7.31	96.69	46.41±0.52	92.95
Actinotrichia fragilis	27.43±3.71	66.33**	85.43±3.65	88.19*	39.88±0.58	67.44	64.70±13.67	74.79**	48.81±0.18	97.75
Cystoesira trinodes	34.85±2.32	84.29**	88.37±6.34	91.22	45.91±0.28	77.64	45.29±5.03	52.35**	47.38±0.61	94.89
Methyl alcohol extracts										
Sargassum hystrix	-ve	-ve	80.32±10.24	82.92**	39.96±0.14	67.57**	62.46±7.65	72.20**	-ve	-ve
Caulerpa racemosa	-ve	-ve	-ve	-ve	43.20±0.08	73.06	55.89±9.12	64.60**	-ve	-ve
Sargassum dentifolium	35.55±0.70	85.97**	-ve	-ve	43.67±0.30	73.85	73.83±6.76	85.34**	26.19±0.29	52.45**
Cystoesira myrica	30.79±0.35	74.47**	52.48±11.30	54.18**	60.84±0.32	102.88	51.01±0.81	58.97**	28.12±0.08	56.32**
Padina gymnospora	-ve	-ve	98.65±8.14	101.84	56.58±0.26	95.69	47.15±3.25	54.50**	25.69±0.32	51.45**
Codium fragile	-ve	-ve	-ve	-ve	-ve	-ve	60.45±2.81	69.88**	-ve	-ve
Actinotrichia fragilis	29.98±1.84	72.51**	41.04±2.09	42.36**	52.17±0.35	88.23	67.64±7.82	78.19**	-ve	-ve
Cystoesira trinodes	36.24±1.67	87.66**	56.27±10.36	58.09**	55.11±0.23	93.20*	58.36±8.47	67.46**	-ve	-ve
LSD										
5%	3.53		10.98		0.46		11.84		0.55	
1%	4.75		14.79		0.63		15.94		0.73	

Mean±SD, n = 3. *Significant differences, **Highly significant differences, -ve: Negative results (complete inhibition)

Table 5: Antifungal assay by pectinase enzyme activity (units mL^{-1}) in liquid medium

Treament	*Alternaria brassicicola*	%	*Ulocladium botrytis*	%	*Botryotrichum piluliferum*	%	*Fusarium oxysporium*	%	*Alternaria alternata*	%
Control										
Absolute control	5.20±0.73	100.00	3.00±0.47	100.00	2.66±0.33	100.00	4.73±1.00	100.00	5.039±0.38	100.00
Methyl alcohol	5.26±0.53	101.28	3.33±0.27	111.11	2.73±0.13	102.50	4.90±0.37	103.52	5.26±0.07	104.41
Ethyl acetate	5.08±0.77	97.86	2.83±0.43	94.44	2.50±0.10	93.75	4.43±0.43	93.66	4.71±0.39	93.39
Nystatin	-ve	-ve	-ve	-ve	-ve	-ve	2.98±0.79	62.91**	-ve	-ve
Ethyl acetate extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	2.53±0.07	95.00	1.68±0.29	35.45**	3.00±0.88	59.47**
Caulerpa racemosa	1.50±0.57	28.85**	-ve	-ve	1.43±0.68	53.75**	2.10±0.17	44.37**	1.78±0.48	35.24**
Sargassum dentifolium	2.76±0.17	53.21**	-ve	-ve	1.87±0.33	70.00	1.99±0.25	42.02**	2.95±0.33	58.59**
Cystoesira myrica	4.82±0.58	92.74	3.08±0.25	102.59	1.63±0.03	61.25**	2.76±0.10	58.45**	4.37±0.37	86.78*
Padina gymnospora	3.56±0.36	68.59**	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Codium fragile	2.15±0.45	41.45**	2.17±0.15	72.22**	2.46±1.60	92.50	2.49±0.21	52.72**	4.80±0.13	95.15
Actinotrichia fragilis	4.98±0.83	95.81	1.85±0.26	61.85**	2.54±0.18	95.42	3.84±0.23	81.22*	4.55±0.51	90.31
Cystoesira trinodes	4.93±0.35	94.87	1.40±0.13	46.67**	2.38±0.20	89.17	3.26±0.37	69.01**	4.64±0.30	92.07
Methyl alcohol extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	1.63±0.70	61.25**	1.80±0.20	38.03**	-ve	-ve
Caulerpa racemosa	-ve	-ve	2.78±0.38	92.59	2.50±0.20	93.75	1.77±0.23	37.32**	-ve	-ve
Sargassum dentifolium	1.20±0.07	23.08**	-ve	-ve	2.69±0.28	100.83	3.93±0.13	83.10	1.88±0.71	37.23**
Cystoesira myrica	2.10±0.10	40.39**	2.26±0.07	75.56**	2.61±0.36	97.92	2.36±0.43	50.00**	2.86±0.29	56.83**
Padina gymnospora	-ve	-ve	2.78±0.33	92.59	2.51±0.10	94.17	1.70±0.50	35.92**	3.33±0.27	66.08**
Codium fragile	-ve	-ve	-ve	-ve	-ve	-ve	1.83±0.03	38.73**	-ve	-ve
Actinotrichia fragilis	3.03±0.23	58.33**	2.84±0.23	94.82	2.45±0.20	92.08	2.32±0.41	49.06**	-ve	-ve
Cystoesira trinodes	5.01±0.35	96.37	2.36±0.23	78.89**	1.77±1.10	66.25*	2.01±0.53	42.49**	4.57±0.31	90.75
LSD										
5%	0.69		0.38		0.86		0.85		0.61	
1%	0.93		0.51		1.16		1.15		0.82	

Mean±SD, n = 3, *Significant differences, **Highly significant differences, -ve: Negative results (complete inhibition)

Table 6: Antifungal assay by cellulase enzyme activity (units mL^{-1}) in liquid medium

Treatment	*Alternaria brassicicola*	%	*Ulocladium botrytis*	%	*Botryotrichum piluliferum*	%	*Fusarium oxysporium*	%	*Alternaria alternata*	%
Control										
Absolute control	2.99±0.23	100.00	5.25±0.17	100.00	2.91±0.05	100.00	6.03±0.24	100.00	3.39±0.54	100.00
Methyl alcohol	2.77±0.32	92.86	4.85±0.09	92.39	2.77±0.23	95.41	6.59±0.37	109.29	3.57±0.76	105.51
Ethyl acetate	3.28±0.28	109.82	5.01±0.54	95.43	2.67±0.17	91.74	5.68±0.35	94.25	3.63±0.37	107.09
Nystatin	-ve	-ve	-ve	-ve	-ve	-ve	3.55±0.09	58.85**	-ve	-ve
Ethyl acetate extracts										
Sargassum hystrix	-ve	-ve	-ve	-ve	2.61±0.20	89.91	2.53±0.32	42.04**	1.84±0.42	54.33**
Caulerpa racemosa	2.13±0.47	71.43**	-ve	-ve	1.81±0.68	62.39**	1.73±0.05	28.76**	2.29±0.09	67.72**
Sargasum dentifolium	2.37±0.44	79.46*	-ve	-ve	2.69±0.17	92.66	2.75±0.05	45.58**	3.17±0.17	93.70
Cystoesira myrica	2.69±0.32	90.18	4.45±0.24	84.77**	2.64±0.08	90.83	5.63±0.18	93.36	3.12±0.21	92.13
Padina gymnospora	3.25±0.17	108.93	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Codium fragile	2.96±0.16	99.11	4.61±0.32	87.82*	2.69±0.24	92.66	5.39±0.38	89.38*	3.28±0.52	96.85
Actinotrichia fragilis	2.85±0.24	95.54	4.51±0.41	85.79**	2.43±0.58	83.49	5.28±0.83	87.61*	3.23±0.18	95.28
Cystoesira trinodes	2.85±0.32	95.54	3.71±0.90	70.56**	1.68±0.28	57.80**	2.43±0.18	40.27**	3.07±0.61	90.55
Methyl alcohol extracts										
Sargassum hystrix	-ve	-ve	3.79±0.51	72.08**	2.16±0.14	74.31**	1.81±0.26	30.09**	-ve	-ve
Caulerpa racemosa	-ve	-ve	-ve	-ve	1.92±0.08	66.06**	2.00±0.40	33.19**	-ve	-ve
Sargassum dentifolium	3.36±0.81	112.50	-ve	-ve	1.55±0.30	53.21**	5.25±0.20	87.17**	1.12±0.29	33.07**
Cystoesira myrica	2.16±0.21	72.32**	2.59±0.24	49.24**	2.93±0.32	100.92	2.19±0.24	36.28**	1.28±0.08	37.80**
Padina gymnospora	-ve	-ve	4.21±0.28	80.20**	3.07±0.26	105.51	2.53±0.24	42.04**	1.31±0.32	38.58**
Codium fragile	-ve	-ve	-ve	-ve	-ve	-ve	2.59±0.46	42.92**	-ve	-ve
Actinotrichia fragilis	2.05±0.32	68.75**	0.93±0.38	17.767**	2.00±0.35	68.81**	4.16±0.00	69.03**	-ve	-ve
Cystoesira trinodes	2.35±0.36	78.57*	3.09±0.33	58.88**	2.61±0.23	89.91	3.71±0.61	61.504**	-ve	-ve
LSD										
5%	0.52		0.54		0.46		0.57		0.55	
1%	0.69		0.73		0.62		0.76		0.73	

Mean±SD, n = 3. *Significant differences, **Highly significant differences, -ve: Negative results (complete inhibition)

(did not show any enzymes function activity) (Table 5). *F. oxysporium* is the most resistance fungus, whereas the fungicide nystatin did not inhibit its pectinase and cellulase activities (growth percentage was 62.9% as compared to control) (Table 5). On the other hand, the most active algae were, *P. gymnospora* (ethyl acetate extract) where pectinase and cellulase enzymes activity was inhibited completely all the tested fungi, the exception was *A. brassicicola* (68.5%) as compared to control (Table 5).

C. fragile (methanolic extract) also showed high antifungal activity with all tested fungi, except *F. oxysporium* (42.9% compared to control) of cellulase enzyme activities (Table 6). Both ethyl acetate of (*C. fragile*, *A. fragilis* and *C. trinodes*) and methanolic extracts of *C. myrica* showed non antifungal activity (based on pectinase and cellulase enzymes activities) for all tested fungi (Table 6).

DISCUSSION

Biological natural products isolated earlier from seaweeds are recommended as potential biocidal and pharmaceutical agents (Rangaiah *et al*., 2010). Broad ranges of data are now available for the *in vitro* anti-fungal activities of different seaweeds families (Tuney *et al*., 2006). The results obtained recorded the higher antifungal activity for ethyl acetate extracts of *P. gymnospora* and methanolic extracts of *C. fragile*. Then, the methanolic and ethyl acetate extracts of *S. hystrix* as indicated by inhibition in dry weight, protein content, pectinase and cellulase enzymes activities. The phenolic compound released from dried crudes of seaweed extracts may be answerable for their antimicrobial properties. This was confirmed earlier by Cox *et al*. (2010) who found that phenolic compounds responsible for the antifungal activities of seaweeds. Other scientists have also reported that phenolic compounds from different plant sources could inhibit various food-borne pathogens (Plaza *et al*., 2009; Osman *et al*., 2011). This may be due to the impact of these antifungal compounds on spore germination (El-Mehalawy, 2003). The above results may also be due to the effect of these antifungal on cell wall shifting its permeability (Wen-Bao *et al*., 2000) or the antifungal suppressed the early stages of mycelial growth (El-Mehalawy, 2003).

In pectin and cellulose medium, there was a linear relationship between protein amount released to the medium and fungal enzymes activities. Obviously, some algal extracts was able to inhibit the activity of pectinase only while cellulase enzyme exhibit some activity for the same fungus. For example, methanolic extract of *S. hystrix* was able to inhibit pectinase enzyme but had no effect on cellulase enzyme of U. botrytis (Table 5, 6). The explanation of this case was confirmed earlier by Karthikaidevi *et al*. (2009). This may explain the specific target (receptors) of the antimicrobial compounds of these algal extracts against one enzyme. This result could be related to the presence of bioactive metabolites present in this species of algae which are not soluble in one solvent but they can be soluble in the other.

It is worth mentioning that, the efficiency of extraction of antifungal natural products from marine macroalgae was higher with methanol as confirmed earlier with the obtained results. Manilal *et al*. (2009) tested several solvents and found that methanol was determined to be the best solvent for isolation of bioactive secondary metabolites from dried red algae followed by ethyl acetate and dichloromethane. These results indicated that extraction method had definite effects on the isolation of bioactive principles. The effectiveness of extraction methods reported by many authors showed that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate (Paul and Puglisi, 2004). Also, this ensures the importance of selection the appropriate solvent and extraction method to be used. Some extracts seemed to be specific in

their activity against some pathogens, which ensures the presence of specific bioactive compounds against certain targets.

Pectinases functions were employed for its important role for fungal virulence and help in the infection process (Jones *et al*., 1972). Since, they corrupt pectin subunits of the middle lamella and of primary cell wall, support the colonization of plant tissues (Favaron *et al*., 1994). Consequently, it supplying nutrients for the fungus through the early stages of infection (Deo and Shastri, 2003). The results obtained showed that pectinase activity significantly decreased when *P. gymnospora* and *C. fragile* extracts were employed. A wide range of results of in vitro anti-fungal activities of extracts of green algae, diatoms and dinoflagellates have been reported to affect fungal enzymes activities (Tuney *et al*., 2006). Another important explanation provided by Cordeiro *et al*. (2006), who approved that chitinasses and β-1,3-glucanase (that can affect pectinase function) are truly recognized as natural antifungal proteins widely found in plants and seaweeds.

The results also reported that crude *P. gymnospora* and *C. fragile* extracts (50 mg mL^{-1}) within pectin and cellulose media showed the same effectiveness compared to fungicide nystatin. And among all the seaweeds, *P. gymnospora* is considered as the ideal antifungal macroalga tested.

CONCLUSION

From cellulase and pectinase tests, we can conclude that, maximum antifungal activities was recorded in Bryopsidophyaceae (*C. fragilis*) and Phaeophyaceae (*P. gymnospora*) which was the most effective seaweeds and minimum antifungal activities were recorded for *C. myrica* a member of the Phaeophyaceae.

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