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Screening of potential seaweeds against *Fusarium species* isolated from fruits and vegetables in Baluchistan, Pakistan

Asnad[1*], Tanver Abbass[2]

[1]*Department of Biochemistry, Marine Science, Lasbela University of Agriculture, Water and Marine Science Baluchistan, Pakistan*

[2]*Department of Microbiology, University of Karachi, Pakistan*

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Abstract

The aim of the present study was to screen antifungal activity of the water and ethanol extracts of eight seaweed species *Sargassam swartzii*, *Dictyota dichotoma*, *Padina tetrastromatica* ,*Sargassum tennerrimum* ,*Halimda tuna* , *Jania capillacea, Scinaia shameelii* and *Coelarthum muelleri* against fungi(*Fusarium solani* and *F. oxysporum*) isolated from deteriorated fruits and vegetables. The water extract of *Padina tetrastromatica* and ethanolic extract of *Padina tetrastromatica* and *Sargassum tennerrimum* showed activity against *Fusarium solani* and *F. oxysporum* by well diffusion and disc diffusion method respectively .The potential seaweeds further fractioned into hexane, ethyl acetate and methanol fractions respectively and checked their activity against pathogens, Hexane and methanol fractions showed activity against isolated pathogens. Maximum inhibition was noticed with the methanol fraction as compare to hexane fraction for both potential seaweeds.

* **Corresponding Author:** Asnad ✉ drasnadkhan@gmail.com

Introduction

Spoilage fungi were caused pathogenicity in local and imported fruit and vegetables by secreting degrading enzymes (Rashad *et al.*, 2011). The estimated post-harvested spoilage of fruits is about 20-25% in developed countries (Oelofse *et al.*, 2006). Fungi were caused toxigenicity in fruits (Stinson *et al.*, 1981). *F. oxysporum* was produced high concentration of xylenase *(*Simoes *et al.*, 2006) and another study showed that F. oxysporum was produced the highest pectolytic enzymes (Bahkali *et al.* 1997**).**Fungi are the most important and prevalent pathogens which caused destructive and economically important losses in most fresh fruits and vegetables **(**Sommer, 1985). Seaweeds are considered as source of bioactive compounds which have broad spectrum of biological activities. Compounds have been detected in green, brown and red algae with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities (Newman *et al.*, 2003). Seaweeds have secondary metabolites with antiviral, antibacterial and antifungal activities (Caccamese *et al.***,** 1980; Del Val AG *et al.*, 2001; Perry NB *et al.*, 1991). Many seaweed were screened for their antimicrobial activity (Reichelt & Borowitzka, 1984). Extracted substances from marine algae have antibacterial and antifungal activities (Abdussalam, 1990; Scheuer & P.J .,1990; Rizvi, 2003; Burkholder, 1969; Chapman *et al.*, 1980; Arasaki *et al.*, 1983; Abbot t& I.A.,1988).

Although Pakistan's seaweed natural resources are unlimited but these resources need to be explored for their potential activity against pathogens and develop herbal fungicide against fruit pathogens and reduce the uses of hazard chemicals. The aim of the present study was to screen antifungal activity of the water and ethanol extracts of eight seaweed species represented by four Phaeophyta (*Sargassam swartzii, Dictyota dichotoma, Padina tetrastromatica* and *Sargassum tennerrimum*), one Chlorophyta (*Halimda tuna*) and three Rhodophyta (*Jania capillacea, Scinaia shameelii* and *Coelarthum muelleri*) against fungi (*Fusarium solani* and *F. oxysporum*) isolated from deteriorated fruits and vegetables.

Materials and methods

Collection of seaweeds

The seaweeds *Sargassam swartzii, Dictyota dichotoma, Padina tetrastromatica, Sargassum tennerrimum, Halimda tuna, Jania capillacea, Scinaia shameeli*i and Coelarthum *muelleri* were collected from Sandspit and Buleji coastal areas of Karachi, Pakistan in Jan 2012 to Jun 2013 and remove sand particles and epiphytes by washing the seaweeds with fresh water followed by distilled water and finally dried under shade for 20 days then used for extraction.

Extractions

Ethanol 100% solvent is used for extraction of dried seaweed at (1:3 *w/v*) ratio, after 20 days, the solution was filtered using Wattman No. 42 paper and concentrated by rotary evaporator and then checked the fungicidal activity of crude extract against pathogens. The potential crude extract was further fractioned into hexane, ethyl acetate and methanol fraction and aqueous fractions were obtained by using separating funnel and then checked the fungicidal activity of fractions against pathogens for the best fractions screening

Fruit and Vegetables materials collection

Various types of deteriorated fruits and vegetables were purchased from Uthal, Vinder and Bela market, Baluchistan, Pakistan.

Isolation of fruit and vegetables spoilage fungi

Sterilized sharp razor is used for cutting the diseased tissues with some healthy tissues then transferred into PDA plates which contain antibiotic such as penicillin (100000 units/L) and streptomycin (0.2 g/L). Plates were incubated for 5 days at 28°C under 12 hours light and dark conditions (Naureen *et al.*, 2009).

Identification of fungi

The pure isolated fungi were identified by Dr.Tanveer Abbas microbiologist Department of Microbiology, University of Karachi, Pakistan

In vitro antifungal activity

For the ethanol extract we used disc diffusion method in which we used respective solvent dilution of 200 mg/mL of each extract and Sterilized thick filter paper discs which were impregnated at 1-4mg/disc for each extract .After dried transferred the discs at periphery of the Petri dishes containing Czepak's Dox agar (pH 7.2). The culture of *Fusarium solani* and *F. oxysporum* 5mm discs were placed at the center of the dish. Respective solvents impregnated disc were used as negative control and with fluconazole used as positive control (Ara J., 1998). For water extract, we used well diffusion method (Perez *et al.,* 1990). Three times replicated each treatment and incubated the plates at 28 °C and observations were daily recorded.

Result and discussion

The value of fresh vegetables and fruits is increasing day by day so it is important to control post-harvest diseases (Eckert *et al.*, 1967). Fungi cause significant loss of 10-30% (Agrios & G.N., 2005). It is reported that A. niger caused spoilage in sweet orange (Bali *et al.*, 2008). *F. oxysporum* is produced pectin degrading enzymes (Ten have *et al.,* 2001; De las Heras *et al.,* 2003; Li R *et al.,* 2004). *Fusarium spp are also produced* cell wall degrading enzymes while *Aspergillus spp.* are produced several toxic metabolites, such as malformins, naphthopyrones **(**Frisvad *et al.*, 1991) and they can produce Ochratoxins (OTA), a mycotoxin which is a very important toxin for human and animal health (Peraica *et al.,* 1999).

Table 1. Deteriorated fruits and vegetables collected from Lasbela , Balochistan.

No	Fungi with host	Scientific name
a	*Fusarium oxysporum*	
1	Turnip	*Brassica rapa* var. *rapa*
2	Round gourd	*Praecitrullus fistulosus* Stocks) Pangalo
3	Banana	*Musa acuminata*
4	Grape	*Vitis vinifera* L.
5	Apple	*Malus pumila* Mill.
6	Common bean	*Phaseolus vulgaris* L.
b	*Fusarium solani*	
1	Bell pepper	*Capsicum annum* L.
2	Taro	*Colocasia esculenta* (L.) Schott
3	Pumpkin	*Cucurbita moschata* L.
4	Bottle gourd	*Lagenaria siceraria* (Molina) Standl
5	Tomato	*Lycopersicon esculentum* Mill.

Table 2. In vitro growth inhibition of *Fusarium solani* and *Fusarium oxysporum* by water extracts of seaweed.

No	Seaweeds	*Fusarium solani* (mm)	*Fusarium oxysporum* (mm)
1	Control (Distilled water)	0	0
2	Standard (Fluconazole) 10 ug/well	18	20
	Phaeophyta		
1	*Sargassam swartzii*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
2	*Dictyota dichotoma*		
	2mg	0	0
	3mg	0	0
	4mg	0	0

3	*Padina tetrastromatica*		
	2mg	0	0
	3mg	4	6
	4mg	8	9
4	*Sargassum tennerrimum*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
	Chlorophyta		
5	*Halimda tuna*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
	Rhodophyta		
6	*Jania capillacea*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
7	*Scinaia shameelii*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
8	*Coelarthum muelleri*		
	2mg	0	0
	3mg	0	0
	4mg	0	0

Table 3. In vitro growth inhibition of *Fusarium solani* and *Fusarium oxysporum* by ethanol extracts of seaweed.

No	Seaweeds	Fusarium solani (mm)	*Fusarium oxysporum* (mm)
1	Control Ethanol	0	0
2	Standard Fluconazol 10 ug/disc	18	20
	Phaeophyta		
1	*Sargassam swartzii*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
2	*Dictyota dichotoma*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
3	*Padina tetrastromatica*		
	2mg		
	3mg	11	9
	4mg	13	15
4	*Sargassum tennerrimum*		
	2mg		
	3mg	9	7
	4mg	14	12
	Chlorophyta		
5	*Halimda tuna*		
	2mg	0	0
	3mg	0	0

	4mg	0	0
	Rhodophyta		
6	*Jania capillacea*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
7	*Scinaia shameelii*		
	2mg	0	0
	3mg	0	0
	4mg	0	0
8	*Coelarthum muelleri*		
	2mg	0	0
	3mg	0	0
	4mg	0	0

Table 4. In vitro growth inhibitions of *Fusarium solani* and *Fusarium oxysporum* by the fractions of ethanol extracts of seaweed.

1	*Sargassum tennerrimum*	*Fusarium solani*	*Fusarium oxysporum*
a	n-Haxane		
	2mg	0	0
	3mg	3	5
	4mg	7	9
b	Ethyl-acetate		
	2mg	0	0
	3mg	0	0
	4mg	0	0
c	Methanol		
	2mg	0	0
	3mg	5	7
	4mg	11	13
2	*Padina tetrastromatica*		
a	n-Haxane		
	2mg	0	0
	3mg	9	8
	4mg	12	10
b	Ethyl-acetate		
	2mg	0	0
	3mg	0	0
	4mg	0	0
c	Methanol		
	2mg	0	0
	3mg	10	9
	4mg	14	13

Seaweeds provide a rich source of structurally diverse secondary metabolites. These are mainly terpenes ,acetogenins, alkaloids and polyphenolics, with many of these compounds being halogenated (Watson SB. &Cruz-Rivera E**.,** 2003**)**.The functions of these secondary metabolites are defense against herbivores, fouling organisms and pathogens. Lipid-soluble extracts and organic solvent substances of seaweeds were determined for antimicrobial activity (Mahasneh *et al.*, 1995; Sukatar *et al.,* 2006). Seaweed extracts have been shown to induce resistance to frost, fungal and insects attack .In the present study, deteriorated fruits and vegetables were purchased from Uthal, Vinder and Bela market, Baluchistan, Pakistan such as Turnip, Round gourd, Banana, Grape, Apple and Common bean. Fungi were isolated from these fruits and vegetables and slides were prepared for identification. *F. oxysporum* was isolated from six

fruits and vegetables (Turnip, Round gourd, banana, grape, Apple and Common bean) and *Fusarium solani* from five fruits and vegetables(Bell pepper, Taro, Pumpkin, Bottle gourd and Tomato) table-1.Antifungal activity of ethanol and water extract of eight seaweed species represented by four Phaeophyta (*Sargassam swartzii, Dictyota dichotoma*, *Padina tetrastromatica* and *Sargassum tennerrimum*), one Chlorophyta (*Halimda tuna*) and three Rhodophyta (*Jania capillace*a, *Scinaia shameelii* and *Coelarthum muelleri*) were studied against fungi (*Fusarium solani and F. oxys*porum) isolated from deteriorated fruits and vegetables. The results of antifungal activity against tested pathogens were tabulated in the Table 2 & 3. The water extract of *Padina tetrastromatica* (3mg/well and 4mg/well) showed promising antifungal activity against all the test organisms compared with the standard fluconazole table 2. The ethanol extracts of *Padina tetrastromatica and Sargassum tennerrimum* at (3mg/disc and 4mg/disc) showed the significant antifungal activity against all the test organisms compared with the standard fluconazole. *Padina tetrastromatica* showed best results as compare to *Sargassum tennerrimum* at 3mg/disc and 4mg/disc against isolated pathogens that is (11mm and 13mm)for *Fusarium solani* and (9mm and 15 mm) for *F. oxysporum* while the *Sargassum tennerrimum* showed antifungal activity for *Fusarium solani* is(9mm and 14mm) and for *F. oxysporum* is (7mm and 12mm) at 3mg/disc and 4mg/disc respectively table 3. The crude extract of potential seaweeds (*Sargassum tennerrimum* and *Padina tetrastromatica*)are further fractionated into n-Haxane, Ethyl acetate and methanol fractions respectively. In *Sargassum tennerrimum* Haxane and methanol fractions showed activity against isolated pathogens and ethyl acetate did not show activity against pathogens. Maximum inhibition was noticed with the methanol fraction (5mm and 11mm) for *Fusarium solani* and (7mm and 13mm) for *F. oxysporum* at 3mg/disc and 4mg/disc respectively as compare to hexane fraction which is (3mm and 7mm) for *Fusarium solani* and (5mm and 9mm) for *F. oxysporum* at 3mg/disc and 4mg/disc respectively table 4.In *Padina tetrastromatica* Haxane and methanol fractions also showed activity against isolated pathogens and ethyl acetate did not show activity against pathogens. Maximum inhibition was noticed with the methanol fraction (10mm and 14mm) for *Fusarium solani* and (9mm and 13mm) for *F. oxysporum* at 3mg/disc and 4mg/disc respectively as compare to hexane fraction which is (9mm and 12mm) for *Fusarium solani* and (8mm and 10mm) for *F. oxysporum* at 3mg/disc and 4mg/disc respectively table 4. From the above preliminary studies on the antifungal activities, the crude extracts of *Padina tetrastromatica* and *Sargassum tennerrimum* showed promising activity against *Fusarium solani* and *F. oxysporum* shows potential use of these marine seaweeds against a range of microbial populations. The research work can further be extended to isolate secondary metabolites that attributes to the antifungal activity and also develop herbal fungicide from these seaweed to reduce the economical loss and uses of chemicals hazards

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