International Journal of Research and Reviews in Pharmacy and Applied science

www.ijrrpas.com

ANTIFUNGAL ACTIVITY OF CERTAIN SEAWEEDS FROM PUTHUMADAM COAST

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ABSTRACT

Antifungal activity of seaweed extracts in Petroleum ether, benzene, chloroform, Methanol, ethyl acetate, and water extracts was made.Antifungalactivity of five species of Seaweed was explored. This activity was evaluated by Disc diffusion method. The minimum inhibition zone concentrations were also determined for two fungal pathogens namely Pythiumaphanidermatam, Colletotrichumcapsici. All the extracts used in this study were exhibited antifungal activity. The lowest inhibition zone effect was noticed for Sargassumwightii, followed by sargassumillicifolium, andTurbinariaconoides(Brown seaweeds) and the highest inhibiting of zone effect was noted forUlvafasciata (Green seaweed), Gracilariacorticata(red seaweed),Sargassumilicifolium, andTubinariaconoideswas also not growth against Colletotrichumcapsici were completely not inhibition zone in ethyl acetate extract. These results suggest that seaweeds collected fromputhumadam coast in Tamil nadu Gulf of mannarregion is potential significances capacity which makes them interesting for screening of natural products.

Key words: Seaweeds, antifungal activity, solvent extracts, natural substances.

INTRODUCTION

Recent research on marine chemical ecology has clearly shown that many seaweed natural products may also play multiple functions, acting simultaneously as feeding deterrent[1], and defence against pathogens and fouling organism.[2]thereby increasing the adaptive value of their metabolites. Seaweeds produce a large number of secondary metabolites, including terpenes, aromatic compounds, acetogenins, amino acid derived substances, phlorotanin and polyphonic.[3]Seaweeds or marine macroalgae are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and grouped into different classes: green (Chlorophytes), brown (Pheophytes) and red (Rhodophytes) algae.[4] Marine alga was first investigated for the anti-bioactive in 1951 and since then, many works have been carried out on the antibacterial activity of marine plants. Among the green algae, Chaetomorphalinoidesspecies has been extensively studied and is known produce interesting natural products, many of which are broad spectrum feeding deterrents against herbivores or show other biological activities.[5]In particular, interest in polyphenol compounds from marine algae has been growing due to their pharmacological values.[6,7]Since the finding of antibacterial and antifungal activities in many species of marine algae from different part of the world and the isolation of some active compounds from them.[8,9]marine algae have become recognized as potential sources of antibiotic substances.[10]Extracted substances from seaweeds have antibacterial actions and other properties include antifungal activities and growth inhibition of plants.[11,12.13,14] Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.[15]In recent years, there havebeen many reports of macro algae derived compounds that have a broad range ofbiological activities, such as antibacterial, antiviral, antioxidant, anti-inflammatorycytotoxic and antimitotic activities.[16]In the search for these new agents, natural products from plantscontinue to play an important role because of the diversity of their components. Several plant products, including polyphenols, phenolics, terpenoids, and alkaloids have been reported as antimicrobial agents.[17]Seaweeds are known to contain reactive antioxidant molecules, such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites, including carotenoids (α- and βcarotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine) and catechins (e.g., catechin, epigallocatechin, epigallocatechin), gallate, phlorotannins (e.g., phloroglucinol), eckol and tocopherols (α-, χ-, δ-tocopherols) Y. V. Yuan.[18]Brown-algal polyphenols phlorotannins worked as antioxidants, antibacterial and anti-algal compounds.[19,20]

MATERIALS AND METHODS

Algae materials

Four species of marine algae belonging to families such as pheophyceae(sargassumwightii,sargasumilicifolium, Turbinariaconoides), Chlorophyceae(Ulvafasciata) and Rhodophyceae(Gracilariacorticata) were collected in the month of December 2012. The natural habitat in intertidal area of Gulf of Mannarbiosphere, the collection was made during the morning low tide. The collected material was then transported to the laboratory in plastic packet,washed thoroughly in tap water to remove the sand particle and epiphytes. They were dried for 15 days in shade,and mechanically grounded to make powdered form.

Microorganisms

The two fungal pathogens strains ofmycilia used were pythiumaphanidermatam, colletotrichumcapsici. The microorganisms wereobtained from the collection of Department of Pathology laboratory from Annamalai University in Annamalai nagar.

Preparation of marine macro algal extract

Two methods were used in the extraction phase. In the first one, 10 g of seaweeds powder were macerated for three days in 100 ml sterile distilled water at room temperaturein 25^{o}Candwith mechanically shaker (GEL ROCKER LI-GR-E-100), and then filtered in extract using by what man No 1 paper, and the second extraction was also inPetroleum ether, Benzene, Chloroform, Ethyl Acetate,and Methanolusing bySoxhlet extractor for six hours.

Bioassay

Two pathogens isolates were inoculated into the Potato Dextrose Agar (PDA) plates and incubated at 25^{o}C for 6-7 days to obtain young, actively growing cultures consisting of Pythiumaphanidermatam and Colletotrichumcapsici. Antifungal activity was carried out by theDisc diffusion method.[21]One gram of each extract was dissolved in 10ml of its particular solvent and sameconcentration of 1ml was obtained by using serial dilution method.Disc diffusion were dipped in 1ml of extract of known concentration and left for the solvent evaporation in vacuum chamber withMycelialdiscs, 5 mm diameter, cut from the periphery of the young growing cultures of PythiumaphanidermatamandColletotrichumcapsici, were aseptically transferred to the potato Dextrose (PDA) medium in Petri plates (Muller Hinton). The impregnated disc with extract was placed around the mycelialdisc of Pythiumaphanidermatam and Colletotrichumcapsici. Sterilized disc were placed aroundPythiumaphanidermatamm andColletotrichumcapsiciin control plates.Colony diameter was recorded after the 3rd, 5th, 7th days of each test and compared with respective controls. Three replicates were used for each treatment. Presentinhibition zonewas calculated by the formula.

Total phenolic content

The total phenolic of the extract and fractions were determined with Folin–Ciocalteureagent using the method.[22] and 20 µl of sample were mixed with 300 µl of 2% Na2CO3 and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin-Ciocalteau's phenol reagent were added, and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 765 nm using a spectrophotometer. Phenolic contents are expressed as Gallic acid equivalents per gram (GAE/g) of extract.

Thin layer chromatography

Thin-layer chromatography (TLC) was performed on a silica gel plate. An aliquot of each sample was spotted on the silica gel plate with a developing solvent system ofchloroform/Methanol (10:1, v/v). The spots were visualized by spraying the plates with spraying solution (1% potassium ferric cyanide in water and 1% ferric chloride in water) and the visualized under UV.

RESULTS

Antifungal activity

In the present investigation results was antifungal activity of seaweeds extract in Petroleum ether, benzene, chloroform, Methanol, ethylacetate, and water extracts were given in Table 1-6. Extracts of the five species (Sargassumwightii, Sargassumilicifolium, Turbinariaconoides, Ulvafasciata, Gracilariacorticata) of seaweeds showed antifungal activity against two fungal strains (Pythiumaphanidermatam, andColletotrichumcapsici) were tested in this study. Methanol, ethyl acetate and water algae extract was used as positive control; antifungal activity was observed in lowest growth of inhibition zone. And Petroleum ether, benzene, and chloroformseaweeds extract was used as negative control; antifungal activity was observed in highest growth of inhibition zone. All the seaweed extract in strains growth of inhibition zone compared with control. And

theespeciallySargassumilicifolium, Turbinariaconoidesin Methanol and ethyl acetate extracts was no inhibitionzone withColletotrichumcapsici respectively.

Total phenolic content

The phenolic content was determined using Folin – Ciocalteu reagent and was proposes that Sargassumwightii, sargassumilicifolium, and Tubinariaconoidescontains more of these compounds as Ulvafasciata and Gracilariacorticata although expressed as gallic acid equivalents (GAE) as shown in Table 7. The content of phenolic compounds showed in significant the extraction by Soxhlet showing that extraction method and depends on the polarity of different solvents used.Petroleum ether, benzene, chloroform, Methanol, Ethyl acetate and water extract ofSargassumwightii (6.84mg/GAE 8.96mg), Sargassumilicifolium(6.80mg /GAE 8.96 mg), and Tubinariaconoides(5.92mg/GAE 8.96mg) showed the highest concentration of phenolic compounds. Ulvafasciata (3.36mg/GAE 8.96mg) and Gracilariacorticata (4.30mg/GAE 8.96mg)showed the lowest concentration of phenolic compounds.The results are confirmed by thin layerchromatography (TLC) and a UV spectroscopic studyrecordings of absorptionin maximumabsorbance of phenol is between 270 and 275 nm shown in table 8.

DISCUSSION

Antibacterial and antifungal activity of extract prepared from the rhizome to Mediterraneanseagrassposidonia, also noted antimicrobial activity of marine algae from Brazilian northern.[23] Coast of recorded the antimicrobial activity ofblue-green and green algae,[24]the studied seven algae for its antimicrobial activity against Aspergillusflavus, Aspergillusniger and Alternariabrasica.The results highlighted the strongly in vitro antifungal activity of the tested algal extracts, although in recent years, most of the compounds of marine algae were reported as antibacterial in human medicine. It is expected that the antifungal activity found by us to be done in the presence of bioactive molecules, as phenolic compounds (phlorotannins, terpenes, and alkaloids), polysaccharides or fatty acids, many of these structures being identified as antimicrobials[25].Since most available antifungal agents possess only fungistatic activities, the search for novel antifungal agents with fungicidal properties may provide an alternative treatment for dermatomycoses.[26]Using organic solvents which are able to extract a large quantity of lipophilic compounds (glycolipid, phenolic-terpenoidsds, unsaturated-fatty acids and hydroxylated unsaturated-fatty acids), the higher antifungal activity found in Methanol extracts, compared to water extracts (data not shown) could be explained)[27,28]Biochemical analysis are currently undertaken to determine the structure and nature of these compounds.The results showed that the phenolic content of the crude extract of SargassumVulgarewas higher than that of the crude extract of PadinaPavonica, which proposes that SargassumVulgarecontains more of these compounds as PadinaPavonicaalthough the fraction Ethyl acetate of the latter showed a greater level which is consistent with the literature[29]. It is known that ionone has a deterring action against some arthropods, and that it possesses antibacterial and antifungal activity[30]Marine organisms have several active chemicals such as antioxidant and antimicrobial compounds. In this research, the antioxidant and antimicrobial activity of brown algae from the Aegean Sea were investigated.[31]In this present investigation the tested Petroleum ether,benzene, chloroform, Methanoland water macroalgal extracts exerted antifungal activity against two plant fungal species (Pythiumaphanidermatam and colletotrichumcapsici). Fungal mycelial growth was strongly inhibited zone ofMethanol and Ethyl acetate extracts. And the high amount of phenolic compound in brown macro algal species, these results is confirmed by thin layer chromatography (TLC) and a UV spectroscopic study recordings of absorption.

ACKNOWLEDGMENTS

The Author thanks the authorities of Annamalai University and the Head of Department of Botany, Annamalai University and The Professor and Head, Department of Pathology, Faculty of agriculture, Annamalai University, Annamalainagarfor providing necessary facilities and encouragement to carry out this research work.

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Seaweeds	Phythiumaphanidermatam			Colletotrichumcapsici		
	IIIrd days (%)	Vth days(%)	VIIth days(%)	IIIrd days(%)	Vth days(%)	VIIth days(%)
Sargassumwightii	10	28	36	15	-4	-13
Sargassumilicifolium	20	20	-33	40	16	6.6
Turbinariaconoides	25	-4	-16	30	20	13
Ulvafasciata	16	-4	-13	25	16	6.6
Gracilariacorticata	40	12	-6.6	20	8	-6.6

Each value is presented as mean ± standard deviation (n = 3)

Table-1: The antifungal activity of seaweeds in petroleum ether extracts.

Seaweeds	Phythiumaphanidermatam			Colletotrichumcapsici		
	IIIrd days (%)	Vth days(%)	VIIth days(%)	IIIrd days(%)	Vth days(%)	VIIth days(%)
Sargassumwightii	15	-4	-13	-5	-8	-20
Sargassumilicifolium	10	12	-20	10	-16	-30
Turbinariaconoides	5	-20	-26	10	-20	-20
Ulvafasciata	-5	-28	-33	-20	-28	-26
Gracilariacorticata	-15	-36	-40	-30	-40	-33

Each value is presented as mean ± standard deviation (n = 3)

Table-2: The antifungal activity of seaweeds in Benzene extracts.

Seaweeds	Phythiumaphanidermatam			Colletotrichumcapsici		
	IIIrd days (%)	Vth days(%)	VIIth days(%)	IIIrd days(%)	Vth days(%)	VIIth days(%)
Sargassumwightii	10	12	-16	5	-8	-10
Sargassumilicifolium	15	20	-23	15	-16	-20
Turbinariaconoides	20	24	-26	10	-28	-23
Ulvafasciata	20	28	-33	20	-28	-33
Gracilariacorticata	10	12	16	5	-4	-6.6

Each value is presented as mean ± standard deviation (n = 3)

Table-3: The antifungal activity of seaweeds in Chloroform extracts.

Seaweeds	Phythiumaphanidermatam			Colletotrichumcapsici		
	IIIrd days (%)	Vth days(%)	VIIth days(%)	IIIrd days(%)	Vth days(%)	VIIth days(%)
Sargassumwightii	20	4	16	45	4	16
Sargassumilicifolium	30	8	26	-	-	-
Turbinariaconoides	25	8	6.6	10	20	16
Ulvafasciata	35	4	10	10	40	33
Gracilariacorticata	36	6	10	12	40	32

Each value is presented as mean ± standard deviation (n = 3)

Table-4: The antifungal activity of seaweeds in Methanol extracts.

Seaweeds	Phythiumaphanidermatam			Colletotrichumcapsici		
	IIIrd days (%)	Vth days(%)	VIIth days(%)	IIIrd days(%)	Vth days(%)	VIIth days(%)
Sargassumwightii	25	8	6.6	25	40	16
Sargassumilicifolium	35	16	6.6	10	20	6.6
Turbinariaconoides	40	20	6.6	-	-	-
Ulvafasciata	30	4	10	35	40	6.6
Gracilariacorticata	28	6	12	32	38	8

Each value is presented as mean ± standard deviation (n = 3)

Table-5: The antifungal activity of seaweeds in Ethyl acetate extracts.

Seaweeds	Phythiumaphanidermatam			Colletotrichumcapsici		
	IIIrd days (%)	Vth days(%)	VIIth days(%)	IIIrd days(%)	Vth days(%)	VIIth days(%)
Sargassumwightii	35	16	6.6	50	36	26
Sargassumilicifolium	40	24	6.6	60	40	33
Turbinariaconoides	45	32	23	45	32	23
Ulvafasciata	30	8	3.3	30	8	3.3
Gracilariacorticata	32	15	6.6	35	35	6.6

Each value is presented as mean ± standard deviation (n = 3)

Table-6: The antifungal activity of seaweeds in water extracts

Seaweeds	Petroleum ether extract	Benzene extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Water extract
Sargassumwightii	6.08	5.42	5.74	5.70	6.84	5.90
Sargassumillicifolium	5.98	5.37	5.69	5.65	6.80	5.82
Turbinariaconoides	5.90	5.35	5.67	5.54	5.93	5.78
Ulvafasciata	3.86	3.45	3.48	3.36	3.77	3.73
Gracilariacorticata	4.96	4.46	4.68	4.53	4.30	4.67

Table-7: Total phenolic content of seaweeds extract with different solvent in soxhlet.

Seaweeds	Petroleum ether extract (Rf v)	Benzene extract (Rf v)	Chloroform extract (Rf v)	Methanol (extract (Rf v)	Ethyl acetate extract (Rf v)	Water extract (Rf v)
Sargassumwightii	8.46	8.00	8.15	7.53	7.07	7.38
Sargassumillicifolium	8.00	5.83	6.66	7.00	5.83	6.33
Turbinariaconoides	7.63	6.00	6.90	7.27	6.36	6.54
Ulvafasciata	7.27	5.63	6.18	5.81	6.00	5.45
Gracilariacorticata	6.90	5.45	5.81	6.18	6.72	6.54

Rf v = Relative frequency value.

Table-8: Rf Values of Spots Separated on TLC Plate from different fractions