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Bioactive Compound Produced by *Ulva lactuca* and Antifungal Activity against Pathogenic Fungi

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Abstract: *Seaweeds having antifungal activity against different pathogenic fungi (Aspergillus oryzae,Rhizopusartocarpiand Fusariumoxysporum)collected from coastal area of Kunkeshwar, Sindhudurg district of Maharashtra. The main aim of study was to determine antifunal activity of extracts. The ethyl acetate (26.66mm), methanol (18.59mm) and ethanol (18.36mm) extracts demonstrated the highest activity against mycelial growth of Fusarium oxysporum, significantly higher compared to that of Hexane and petroleum ether. Hexaneethanolic extract shows highest activity against Rhizopus artocarpi(15.36mm) and Aspergillus oryzae (11.50mm) respectively. Based on GC-MS analyses compounds with antifungal activity were detected such as 3-Pentatriacontane, 7,9-Di-tert-butyl-1-oxaspiro-(4,5) deca-6,9 diene-2,8-dione, Cyclohexane, 1- (Cyclohexylmethyl)-2 methyl, cis, n- hexadecanoic acid and Cyclohexasiloxane, Dodecamethyl. These compounds had good general antifungal activity and might have potential future agricultural applications.*

Keywords: GC-MS analysis, Antifungal activity, *Ulva,* Seaweeds, *Fusarium*, *Rhizopus*

I. INTRODUCTION

The largest producers of biomass within the marine environment are seaweeds. (Bhadury and Wright, 2004). Seaweeds produces varieties of chemically active metabolites to protect themselves against other settling organisms. These active metabolites, also referred to as biogenic compounds, like halogenated compounds, alcohols, aldehydes, terpenoids are produced by several species of marine macro and microalgae and have antimicrobial, antialgaland antimacrofouling properties which are effective within the prevention of biofouling and produce other likely uses, as in therapeutics (Smit, 2004).

Biologically active compounds which has been isolated in recent years using the seaweed extract was used as novel drugs by the pharmaceuticals industries. Due to rich source of potential protein seaweeds are greatly reported.These bioactive molecules represent a broad range of biological activities such as antibiotic, antimicrobial, antiviral, antitumor and antioxidant (Scheuer, 1990; Tuney*et al*., 2006; Patra *et al*., 2008). Pathogenic fungi are responsible for a substantial loss of plant yield (Sexton and Howlett, 2006). Secondary metabolites extracted from seaweeds are known to possess antifungal properties (Cordeiro *et al*., 2006; Khanzada *et al*., 2007).

This study aimed to see the antifungal activity of Hexane, Petroleum ether, ethyl acetate, methanolic and ethanolic extracts of on strains of fungi*Aspergillus oryzae,Fusariumoxysporum* and *Rhizopus artocarpi*.Qualitative identification of the foremost potential antifungal extract of *Ulva lactuca*, was performed using retention times and mass spectra within the GC/MS analysis.

II. MATERIAL AND METHODS

2.1 Sample Collection

Ulva lactuca was harvested from Kunakeshwar, Sindhudurg district of Maharashtra. After collection, the samples were wash with fresh water to remove associated epiphytes and debris. The cleaned algal materials were then shed dried and ground into fine powder using electric grinder mixer.

Preparation of Extract:

Ten grams of dry algal powder were extracted in 100ml of an organic solvent for 24 hours using an orbital shaker and the extract was filtered through Buchner's funnel using Whatman No.1 filter paper. The filtrate was

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condensed to half of the original volume (50ml) and stored in a glass vial until used (Yuvraj *et al*., 2011). Extraction of algal samples was done using Ethanol, Ethyl acetate and Methanol.

2.2 Test Organism

Asperigillusoryzae, Rhizopus artocarpi and *Fusarium oxysporum*

2.3 Antifungal Assay

Sensitivity of fungal strains to different algal extracts was analyzed using food poisoning method described by Dekker and Glelink (1979).

Czapek Dox Agar medium plates were prepared by mixing one ml algal extract with autoclaved Czapek Dox Agar in a 30ml marked beaker to make final volume of 30ml. The contents were mixed well and poured into a sterile Petri plate. Discs of 8mm of actively growing margins in the plates of eight days old fungal culture were placed inverted on the agar surface of plates at the center. The control was maintained without algal extract. Plates were incubated at 25±2^{0}C in an incubator and linear growth was measured after 72h. Percent inhibition was calculated by using formula-

Percent inhition = (C – T)/C x 100

Where **C** = Diameter of fungus colony in control (mm)

T = diameter of fungus colony in algal extract (mm).

2.4 GCMS Analysis of Seaweeds

Seaweed extracts were analyzed by gas chromatography and mass spectrometry for the quantitative determination of phytochemicals. GC-MS analysis was carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40^{0}C and held for 3 min and the final temperature of the oven was 480^{0}C with rate at 10^{0}C [min.sup.-1]. A two µL sample was injected with splitless mode. Mass spectrum was recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 45 min.

2.5 Statistical Analysis

All experiments were performed in duplicate and replicated a minimum of three times. Results are expressed as means ± SD. The data were subjected to one way analysis of variance (ANOVA) using SPSS 9.0 software and the significant difference was determined at $P < 0.05$ using Duncan multiple range tests.

III. RESULTS

3.1 Antifungal activity of *Ulva lactuca*

Ethanol extract of *U. lactuca* effectively controlled growth of *A. oryzae* (11.50mm) and *F. oxysporum* (18.36mm) whereas hexane extract was effective against *R. artocarpi* (15.36mm) (Table 18). *A. oryzae* was controlled almost by all the solvents except petroleum ether in the present study and all growth zones were comparable to that of standard Percent inhibition of mycelium growth was more than 60 for *A. oryzae* in all the solvents except petroleum ether which was not much effective against other two strains also. Methanolic extract also showed more than 60% arrest of all the fungi while ethanol extracted samples gave promising results for *A. oryzae* (76.60%) and *F. oxysporum* (68.08%) (Fig.1).

Table 1: Effect of *Ulva lactuca* on fungal growth

Solvent	**Growth zone (Diameter in mm)**		
	Aspergillus oryzae	***Rhizopus artocarpi***	***Fusarium oxysporum***
Hexane	13.36 ±0.32^e	**15.36** ±0.32^f	32.46 ±0.50^b
Petroleum ether	32.33 ±0.30^b	26.33 ±0.57^d	28.50 ±0.45^c
Ethyl acetate	15.16 ±0.28^c	40.66 ±0.57^c	26.66 ±0.76^d

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Methanol	12.33 ±0.57[d]	24.86 ±0.80[e]	18.59 ±0.28[f]
Ethanol	**11.50** ±0.50[f]	44.66 ±0.57[b]	**18.36** ±0.32[e]
Control	49.16 ±0.28[a]	65.30 ±0.26[a]	57.53 ±0.50[a]
Streptomycin	11.50± 0.50[f]	12.66± 0.76[g]	13.50± 0.50[g]

Values are mean of three replicates with standard deviation. Different superscript letters within a column indicate significant differences between sample at the level of $P<0.05$

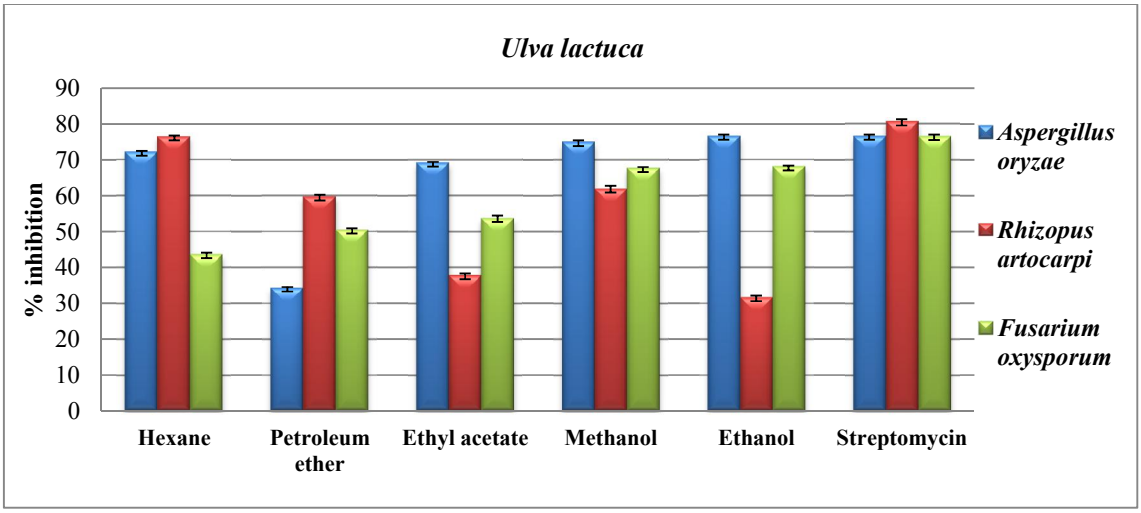

Fig.1 Percent inhibition of fungal growth by *Ulva lactuca*

3.2 Analysis of Volatile Compounds from Hexane, Petroleum ether, Ethyl Acetate, methanol and ethanol Crude Extract Using Gas Chromatography-Mass Spectrometry (GC-MS)

Six compounds were separated in hexane extract of *U. lactuca* (Table 2). The profile displayed one prominent peak of 3- pentatriacontane (89.55%) as the main constituent. Petroleum ether extract had three chemical compounds, out of which 7, 9 di-tert-butyl-1-oxaspiro (4, 5) deca- 6,9- diene had the maximum area (54.36%).

In the ethyl acetate extracted samples of *U. lactuca* six compounds were resolved where cyclohexane, 1-(cyclohexylmethyl)-2- methyl, cis (37.72%) and n-hexadecanoic acid (34%) were prominent ones. Methanol extracted sample had eight compounds n-hexadecanoic acid being the main component (44.90%). Cyclohexasiloxane, dodecamethyl (59.28%) was detected as the major compound in ethanol extract showing presence of seven compounds (Fig. 2).

Table 2: Chemical composition of *Ulva lactuca*

Extracts	RT (min.)	Name of compounds	Molecular formula	Molecular weight	Percent Composition
Hexane	18.539	1,1-Bicyclohexyl, 2-methyl-trans	$C_{13}H_{24}$	180	1.06
	18.817	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	2.35
	20.816	7,9-Di-tert-butyl-1-oxaspiro-(4,5) deca-6,9 diene-2,8-dione	$C_{17}H_{24}O_3$	276	3.74
	20.926	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	1.67
	21.141	1,2 Benzenedicarboxylic acid, butyl-2 ethyl hexyl ester	$C_{20}H_{30}O_4$	334	1.64
	24.500	3-Pentatriacontane	$C_{35}H_{72}$	492	**89.55**
Petroleum	20.811	7,9-Di-tert-butyl-1-oxaspiro-(4,5)	$C_{17}H_{24}O_3$	276	**54.36**

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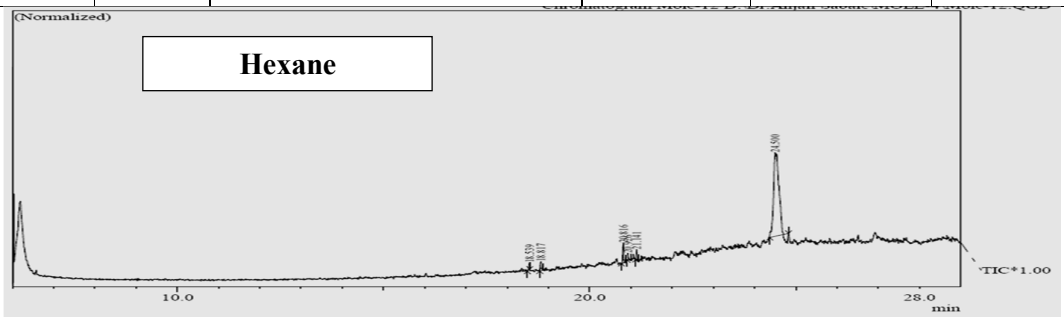
ether		deca-6,9 diene-2,8-dione			
	21.251	Hexadecanoic acid, ethyl ester	$C_{18}H_{38}O_2$	284	21.00
	22.989	Ethyl oleate	$C_{20}H_{38}O_2$	310	24.64
Ethyl acetate	13.506	2-Napthalene methanol, decahydro-alpha	$C_{15}H_{26}O$	222	2.66
	13.907	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	5.48
	13.992	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	3.24
	14.363	Cyclohexane, 1-(Cyclohexylmethyl)-2 methyl, cis	$C_{14}H_{26}$	194	**37.72**
	16.736	n- hexadecanoic acid	$C_{16}H_{32}O_2$	256	**34.00**
	17.336	Cyclic octaatomicsulphur	S_8	256	16.91
Methanol	14.871	Cyclohexanone, 3-(3-3-dimethylbutyl)	$C_{12}H_{22}O$	182	5.87
	16.148	1,4-Eicosadiene	$C_{20}H_{38}$	278	13.64
	16.386	E-10-Methyl-11- tetra96+ Decen-1-01 acetate	$C_{17}H_{32}O_2$	268	3.52
	16.579	3,7,11,15-Tetramethyl- 2-hexadecen-1-01	$C_{20}H_{40}O$	296	6.29
	16.893	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.30
	17.254	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	**44.90**
	18.760	Acetic acid, 3,7,11,15-tetramethyl- hexadecyl ester	$C_{22}H_{44}O_2$	340	12.25
	23.176	1,2- Benzenedicarboxylic acid, diodooctyl ester	$C_{24}H_{38}O_4$	390	11.23
Ethanol	6.611	Ethane,1,1,1-triethoxy	$C_8H_{18}O_3$	162	2.85
	6.832	Phenol	C_6H_6O	94	0.96
	11.296	Cyclohexasiloxane, Dodecamethyl	$C_{12}H_{36}O_6Si_6$	444	**59.28**
	14.182	3-isopropyl-1,1,1,7,7,7-hexamethyl-3,5,5- tris (trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	576	25.12
	15.702	Pentanoic acid,2,2,4 trimethyl 3-carboxyisopropyl, isobutyl ester	$C_{16}H_{30}O_4$	286	3.44
	16.392	Cyclooctasiloxane, hexadecamethyl	$C_{16}H_{48}O_8Si_8$	592	5.23
	19.675	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.11



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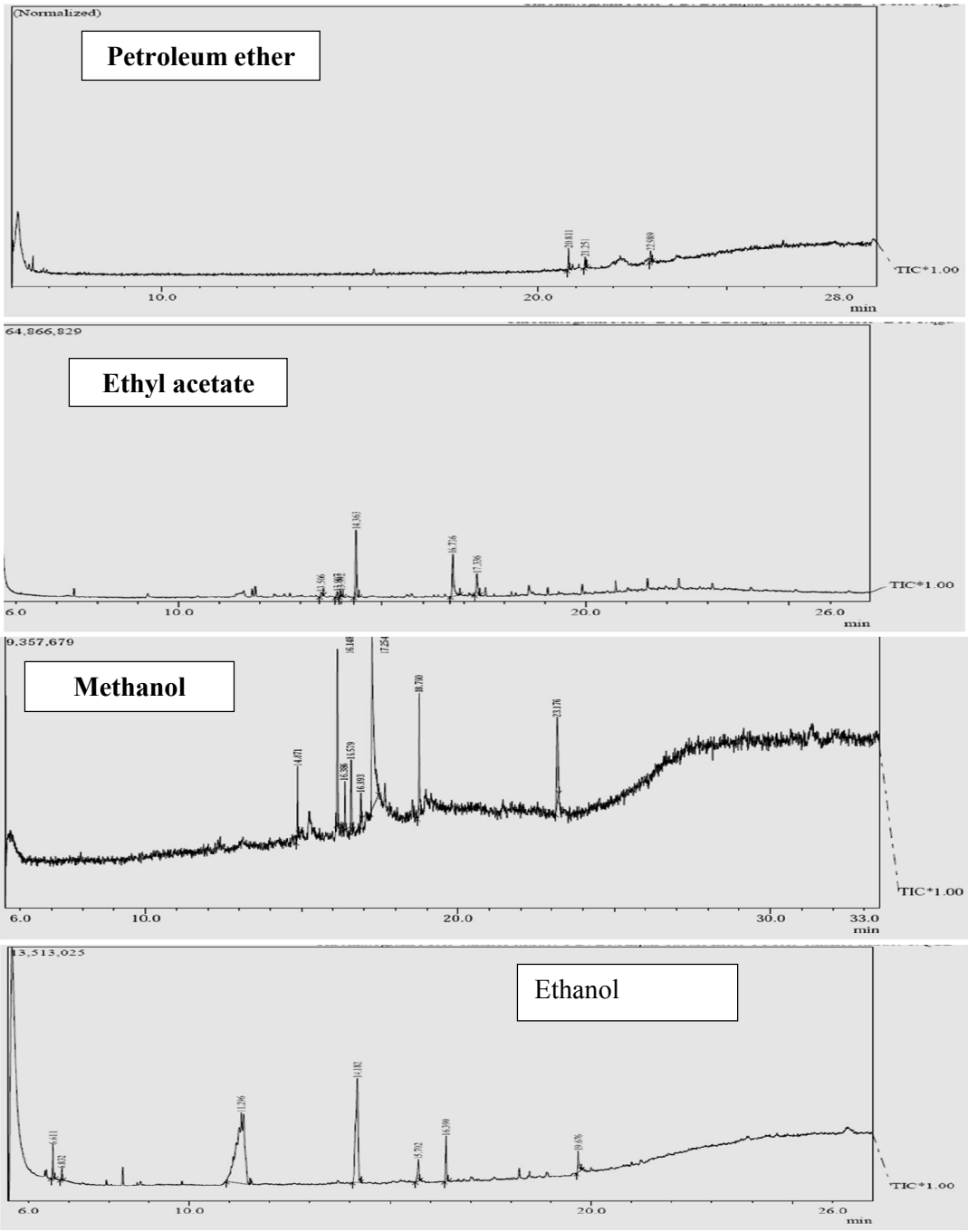

Fig. 2 GC-MS profiles of Ulva lactuca

IV. DISCUSSION

*Aspergillus oryzae*apperared to be the most sensitive fungus in the present collection, as its growth was remarkably inhibited by the extracts of all the three seaweeds. The growth of *F. oxysporum* was moderately inhibited by green seaweeds while *R. artocarpi* was acted upon selectively and was a resistant pathogen in the present lot.

Several workers have reported antifungal activity of ethanol, methanol, hexane and ethyl acetate extracts of *Ulva fasciata* and *Chaetomorphaantennina* against a variety of pathogenic fungi (Febles*et al*., 1995; Ali *et al*., 2000).

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Antifungal activities of *Ulva lactuca* and *U. rigida* in methanol extract have been reported (Barreto *et al*., 1997; Saidani*et al*., 2012).

The fatty acid composition of green seaweeds reported in the present study agrees with the general observation made by Pohl and Zurheide (1979) that marine macroscopic chlorophyta primarily synthesize C_{16} and C_{18} fatty acids and C_{20} and C_{22} fatty acids are usually less abundant.

In conclusion, the findings of the current study showed that *Ulva lactuca*had significant antifungal activity against *Aspergillus oryzae*. Hexane crude extract showed the highest inhibition on the mycelial growth of *A. oryzae* and *R. artocarpi*. GC-MS analysis showed that all solvent crude extract contained macrolides compounds, 3-Pentatriacontane, 7,9-Di-tert-butyl-1-oxaspiro-(4,5) deca-6,9 diene-2,8-dione, Cyclohexane, 1- (Cyclohexylmethyl)-2 methyl, cis, n- hexadecanoic acid and Cyclohexasiloxane, Dodecamethyl. These results indicate that *Ulva lactuca* had the potential to be developed as a biocontrol agent to control different plant pathogenic fungi.

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