

Phychemical Studies and Antifungal Activity of *Ulva Rigida* C.A.G SP (Chlorophyta) from Coast of Karachi Pakistan

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Abstract : The green alga *Ulva rigida* c.A,g sp(Chlorophycota) collected from the coast of Karachi, Pakistan, has been allocated for the fatty acid composition. Acid was changed to methyl esters and identified GC - MS. The 3,Saturated, 23 Unsaturated fatty acids was present, The top saturated fatty acids was present n-Hexadecanoate (8.17%) and lowermost saturated fatty acids was n-Docosanoate, (3.14%.) the Unsaturated fatty acid are maximum percentage in Methyl-tricosenote 15.01%, 10 Octadecenoic acid 10.81% , Hexadecanoic acid 7.87%, Heptadecenoate 7.15% and smallest amount n-Heptaecenoic acid 0.03% , 3,8-Dimethyl-27-nonadecanoate ,0.41% ,in which 7 are mononic ,4 are Dioenoic and 5 are trienoic fatty acids were originate . The antifungal activity in A. Niger in Ethanol extract 85.71% and P.funiculosum in Chloroform extract 78% and minimum inhibition activity against A. niger 28.57% in Aqueous extract was observed . and Magnesium (Mg) 3179.58 mg/kg Potassium (K)21248.8 mg/kg Calcium (Ca) 10751, mg/kg and Chromium (Cr) was present in lowest amount 0.65mg/kg and protein in the range 14.34% in Sandpits, Bulaji village 20.6 % and minimum in Hawk's bay 12.46%

Key Words: Alga, Fatty acids, Chloroform extract antifungal activity, Aqueous extract, Sandpits, Bulaji.

Introduction

"Karachi" is a cosmopolitan city of Pakistan and also has a 105 km long coastline located on the northern limits of the Arabian Sea. A number of islands beaches, and the mangrove swamps are the characteristics structures of the coastline. The biggest diversity of Marine flora and fauna is exhibited all over the place the sandy and rocky beaches of cape Monze, Nathiagali, Paka, Paradise, Point, Buleji, Hawks-bay, Sand Pits, and Manora etc. The seaweeds are growing in the attached form along with rocks or found as drift in a huge amount (Shameel & Tanaka 1992). The extracts of seaweeds are useful to have antifungal and antibacterial activities also it resides the plant growth (Abdulsalam 1990, Rizvi & Shameel 2003, Burkholder & Sharma 1969, Chapman 1980, Arasaki & Arasaki 1983, Abbot 1988) The fruit crops and vegetables after gathering were being destroyed by fungal infections. In 1990s, the food safety from mycotoxin infection was under attention of scientist internationally more than previous times. (Shun-Ichi 2005) In developing countries, the assessment of post-harvest decay is 50% from field's transportations, and storage place where as 25% spoilage is reported from industrial countries (Harvey 1978). (Eckert & Ogawa 1985). The seaweeds can be used to combat the different pathological hazards, of fruits and vegetables. Seaweeds can be used as a growth stimulator of fruits and vegetables. (Washington etc. 1999).

1.2 Economical and Medicinal Importance of Seaweeds

1.2.1 Historic Background

The documentation of history of seaweeds was reported from Greek, Chinese and roman empires period . The word "Phycos" is a Greek word meaning seaweeds and Phycology is the study of seaweeds (Vashesta et al 2002). The Chinese were expert in using seaweeds as medicine for the treatment of goiter, tumor, fever, Angioredema, (chest infections) and renal problems and they did mention seaweeds uses in Chinese Pharmacopeias and Media.

CULTURING OF PATHOGENIC (FUNGI)

Potato Dextrose -Agar medium was prepared by using the components:

Potato extracts 4.0g, Glucose 20g, Agar 15g, 1000 ml distilled water, (I Litter) 5.6 ± 0.2 pH. All the contents were mixed and dissolved in distilled water. The solution was autoclaved at 120 °C 15 LB/sq inch pressure for 20 minutes.

Bioassay

The sterile assay medium at 40-45 °C was poured in to sterile Petri dish and allowed to cool, the strip of highly absorbent paper (12.5 to 10mm diameters) impregnated with required amount (80 to 100 µ.g.) of different extracts such as ethanol, ethyl acetate, Chloroform, Methanol and Aqueous of the sample of designated seaweeds were placed on the surface of solid medium. The test organism was inoculated at the center of plates. The plates were incubated for 72 hours at 30°C for the evaluation of inhibition activity of the fungi. The growth of test fruit fungi compared with the controlled plates the percentage of mycelial inhibition was calculated as follows (Khanzada et al 2007)

$$\% \text{ Mycelial inhibition} = [(dc-dl)/dc] \times 100$$

Dc = colony dia meter in control, dl colony dia meter in treatment.

ANALYSIS OF ELEMENTS FROM SEaweEDS BY ATOMIC ABSORPTION

SPECTOPHOTOMETER (AAS), (Khanzada et al 2007) (Khanzada et al 2013) (Khanzada et al 2014) (Khanzada et al 2015)

Total Protein Analysis:

The protocol of (Khanzada et al 2007) (Khanzada et al 20012) (Khanzada et al 2013) (Khanzada et al 2014) (Khanzada et al 2015)

was used for the determination of total nitrogen, which was calculated using a nitrogen conversion factor of 6.25

TOTAL PROTEIN BY KJELDHAL

The sample was digested in H₂SO₄.con. (30 ml) in the presence of natural catalyst CuSO₄ (1g) and K₂SO₄ (10g), after digestion Sodium hydroxide (NaOH, 33%) were added followed by steam digestion, the distillate was collected in 20 ml boric acid (4%). Then nitrogen contain was determine by titration with HCl (0.01 N). A factor of 6.25 was used to evaluate total protein concentration.

ISOLATION OF FATTY ACIDS.

SEaweED MATERIAL.

Ulva rigida c.A,g sp(Chlorophycota)were collected from Karachi coast and collected materials were washed with water and dried shade at room temperature for 20 days.

EXTRACTION.

The dried seaweeds were chopped into small pieces were dipped two-liter ethanol (EtOH) for about one month at room temperature. The ethanolic extract was filtered and evaporated under, reduced pressure below 40°C using Rotary Evaporator, which yielded dark green gummy residue

SAPONIFICATION.

Quantity of 150 ml. Ethanol (EtOH) and water (H₂O) (1:1, V/V) containing 10% KOH was added to the residue and reaction mixture was refluxing at 100 °C for 6 h. The mixture was concentrated under reduced pressure at rotary evaporator, and thereafter (H₂O) and diethyl ether (Et₂O) were added, and this procedure was repeated their times. The unsaponifiable matter was partitioned and they're by separated. The aqueous alkaline fraction was acidified with 6NHCl (PH 5-6) and then extracted several times with (Et₂O). The total Et₂O fraction was dried over anhydrous Na₂SO₄ and on evaporation of Et₂O residue was obtained.

COLUMN CHROMATOGRAPHY (CC).

The residue containing fatty acids fraction was Chromatographed over silica gel. (70-230mesh Merck) column. The column was first eluted with n-hexane and thereafter Diethyl Ethyl was added in order of increasing polarity. First Fraction was eluted with pure hexane, fraction "A" was eluted from hexane: Chloroform (85:15), fraction "B" from hexane: Diethyl Ethyl (80:20), fraction "C" from hexane: Diethyl Ethyl (75:25), and fraction "D" from hexane: Diethyl Ethyl (70:30)),

ESTERIFICATION

All fractions (A-D) were esterified with diazomethane, 0.5 mg of each fraction was dissolved in MeOH and 0.5 ml of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and was then evaporated. The methylated fatty acid fractions were analyzed first by GC and finally by GC-MS.

IDENTIFICATION.

Gas Chromatography-Mass spectrometry (C-MS)

The fatty acids (methyl ester) fractions were finally analyzed and identified by GC-MS, The analysis was performed on JEOL JMS 600H Agilest 6890N, equipped with 30 m×0.32 ZP-5MS column, stationary phase coating 0.25µm. The column temperature was kept at 70°C for 2 min with increased at the rate of 4°C per min up to 260°C. Injection temperature 250°C, split ratio 1:45, the carrier gas (Nitrogen/Helium) flow rate 1.0 ml/min.

Table.1 Antifungal activity of *Ulva rigida* c.A,g sp(Chlorophycota)against fruit spoiling fungi.

Controlled reading in 72 h at 30 ⁰ C (mm)					
		<i>A. niger</i>	<i>A. flavus</i>	<i>A. ochraceus</i>	<i>P.funiculosum</i>
<i>P.cetricola</i>					
		35mm	40mm	4.2mm	55mm
30mm					
ETHANOL					
Controlled reading	17 mm	22 mm	14 mm	11 mm	18 mm
at 30 ⁰ CAfter 72					
hours (mm).	51	45	66	80	40
Inhibited (%).					

Methanol Controlled reading at 30 ⁰ C After 72 hours (mm). Inhibited (%).	14 mm	11 mm	16 mm	19 mm	18 mm
	60	72	61	65	40
Chloroform Controlled reading at 30 ⁰ C After 72 hours (mm). Inhibited (%).	15 mm	17 mm	14 mm	1 mm	4 mm
	66	57	66	80	53
Ethyl acetate Controlled reading at 30 ⁰ C After 72 hours (mm). Inhibited (%).	12 mm	10 mm	16 mm	12 mm	16 mm
	65	75	61	78	46
Aqueous Controlled reading at 30 ⁰ C After 72 hours (mm). Inhibited (%).	15 mm	13 mm	11 mm	10 mm	18 mm
	57	67	73	81	40

Table 2

S/No	NAME OF ELEMENTS	FORMULA	AMOUNT Mg/ Kg
1	Calcium	Ca	10751.11±11.2
2	Cadmium	Cd	8.68±4.2
3	Chromium	Cr	0.73±0.3
4	Copper	Cu	14.9±6.2
5	Iron	Fe	1653±12.3
6	Lead	Pb	2.39±1.2
7	Potassium	K	21244±22.01
8	Magnesium	Mg	3179.58±12.36
9	Manganese	Mn	13.125±11.2
10	Nickel	Ni	2.96±1.23
11	Zinc	Zn	114.9±15.32

Table.3 Saturated Fatty acids of *Ulva rigida* c.A,g sp(Chlorophycota)analyzed as methyl ester.and (b) Unsaturated Fatty acids of *Ulva rigida* c.A,g sp(Chlorophycota)analyzed as methyl ester.

S.no	SYSTEMATIC NAME	COMMON NAME	MOLECULAR FORMULA	MOL. Wt.	R.R.T	Rel.%ag
(a)Saturated Fatty acids methyl ester.						
1	n-Hexadecanoate	Palmitate	C ₁₇ H ₃₄ O ₂	270	28.8	8.71
2	n-Tridecanoate	Tridecylate	C ₁₄ H ₂₈ O ₂	228	26.13	3.15
3	n-Tetradecanoate	Myristate	C ₁₅ H ₃₀ O ₂	242	24.6	3.46
						15.32
(b)Unsaturated fatty acid methyl ester <i>Codium flabellatum silva</i>						
1.	3,8-Dimethyl-27-nonadecanoate		C ₁₂ H ₂₀ O ₂	196	21.55	0.42
	Tridecatrienoate		C ₁₄ H ₂₂ O ₂	222	19.72	0.53
2.	Methyl-2-Tridecynoate		C ₁₄ H ₂₄ O ₂	224	27.57	0.94
3	Hexadecanoic acid	Pentadecylate	C ₁₆ H ₃₂ O ₂	256	30.37	7.87
4	n-Heptadecanoic acid	Myristate	C ₁₇ H ₃₂ O ₂	208	19.9	0.52
5	Heptadecanoate	Heptadecanoate	C ₁₈ H ₃₆ O ₂	282	33.45	7.14
6	Heptadecadienoate		C ₁₈ H ₃₄ O ₂	280	67.83	1.14
7	Heptadecatrienoate		C ₁₈ H ₃₂ O ₂	278	29.45	2.62
8	10-Octadecenoic acid	Oleate	C ₁₉ H ₃₆ O ₂	296	32.1	10.81
9	n-Octadecanoate	Stearate	C ₁₉ H ₃₈ O ₂	298	32.53	1.89
10	n-Nonadecanoate	Nonadecylate	C ₂₀ H ₄₀ O ₂	312	57.2	1.05
11	n-Heptacosanoate		C ₂₀ H ₅₆ O ₂	424	45.12	0.63
12	Eicosa-8,11,14-	Eicosatrienoate	C ₂₁ H ₃₆ O ₂	320	60.15	1.36

	trienoic acid					
13	Methyl(allcis)-eicosapentaenoate	Methyl eicosapentaenoate	C ₂₁ H ₃₆ O ₂	316	58.17	2.31
14	9-Eicosenoate	Gadoleate	C ₂₁ H ₄₀ O ₂	324	35.5	3.57
15	Eicosadienoate		C ₂₁ H ₃₈ O ₂	322	36.42	2.31
16	Heneicosenote		C ₂₂ H ₄₂ O ₂	338	37.15	3.67
17	11-Docoenoate	Cetoleate	C ₂₃ H ₄₄ O ₂	352	38.73	3.46
18	Methyl-tricosenote		C ₂₄ H ₄₆ O ₂	366	40.27	15.01
19	n-Pentacosenoicacid		C ₂₅ H ₄₈ O ₂	380	41.68	3.46
20	n-Hexacoseoic acid		C ₂₆ H ₅₀ O ₂	394	43.23	2.41
21	Nonacosatrienoicacid		C ₂₉ H ₅₂ O ₂	432	44.15	1.99
23	n-Nnacosanate		C ₃₀ H ₆₀ O ₂	452	69.57	1.05
	TOTAL					84.67
	3,Saturated, 23 Unsaturated, ,Total compounds=26					
	Total %age of Saturated + Unsaturated fatty acid = 99.99					
	(Mol.wt=molecular weight,R.R.T=relative retention time,Rel%age=relative percentage					

Fragmentation of Fatty acids

Spectral data: The GC-mass spectral chromatogram showed the presence of saturated and unsaturated fatty acids methyl esters; the significant ions from the mass spectra of these methyl esters are as follows.

3,8-Dimethyl-27-nonadicneate: 196 (M⁺C₁₂ H₂₀ O₂ 10%) 165(M⁺ 31-8%) 153(M⁺ 43-15%) 139(10%) 125(20%) 111(30%) 97(70%) 83(100%)

Myristate: 242 (M⁺C₁₅H₃₀O₂15%) 211(M⁺31-15%) 199(M⁺43-17%) 157(8%) 143(26%) 129(10%) 101(10%) 87(75%) 73(100%).

Tridecylat : 228(M⁺-C₁₄H₂₈ O₂ 38%) 197(M⁺ -3110%) 185(M⁺ -43-50%) 171(15%) 157(8%) 145(15%) 129(65%) 115(17%) 101(15%) 73(100%).

Methyl-2-Tridecynote: 224 (M⁺C₁₄ H₂₄ O₂ 10%) 193(M⁺- 31-8%) 181(M⁺ -4376%) 167(10%) 153(100%)

Hexadecanate acid: 268(M⁺ C₁₇ H₃₄ O₂ 9%) 237(M⁺ -31,30%) 225(M⁺-43,10%) 211(8%) 197(10%) 183(8%) 169(10%) 155(10%) 141(30%) 127(18%) 113(15%) 99 (65%) 85(60%) 171(70%) 57(100%).

Palmitate: 270(M⁺ C₁₇ H₃₂O₂ 40%) 239(M⁺ -31,30%) 225(M⁺-43,10%) 211(8%) 197(10%) 183(8%) 169(10%) 155(10%) 141(30%) 127(18%) 113(15%) 99 (65%) 85 (60%) 171(70%) 57(100%).

Heptadectrienote: 278 (M⁺C₁₈H₃₂O₂6%) 247(M⁺-31,10%) 235(M⁺-43,11%) 221(10%) 207(8%) 151(100%).

Tetradecanate: 256($M^+C_{16}H_{32}O_2$ 40%) 225($M^+-31,10\%$) 213($M^+-43,30\%$) 199(10%) 185(18%) 171(20%) 157(19%) 143(12%) 129(55%) 115(20%) 101(15%) 87(30%) 73(100%).

Oleate: 296($M^+C_{19}H_{36}O_2$ 10%) 265($M^+-31,50\%$) 253($M^+-43,8\%$) 225(30%) 141(15%) 127(14%) 113(15%) 99(65%) 85(58%) 71(78%) 57(100%) .

Stearate: 298 ($M^+ C_{19} H_{38} O_2$ 23%) 267($M^+ -31,10\%$) 255($M^+ - 43,20\%$) 241(7%) 227(8%) 213(9%) 199(15%)185(9%) 143(30%) 129(18%) 115(8%) 101(10%) 87(78%) 73(100%)

Heptadecenoate: 282($M^+C_{18}H_{38}O_2$ 10%) 251($M^+-31,9\%$) 239($M^+-43,10\%$) 225 (17%) 183(10%) 169(16%) 155(18%) 127(14%) 113(50%) 99(90%) 85(97%) 71(100%)

Octadecadienoate: 294($M^+ C_{19} H_{34} O_2$ 7%) 263($M^+ -31,8\%$) 251($M^+-43,10\%$) 223 (8%) 195(10%) 167(9%) 153 (10%) 139(13%) 125 (18%) 111 (40%) 97 (72%) 83 (60%) 69 (72%) 55 (100%).

Gadoleate: 324($M^+C_{21}H_{40}O_2$ 7%) 293($M^+31,6\%$) 281($M^+43,7\%$) 225(7%)197(8%) 183(8%) 169(10%) 141(13%) 127(13%) 113(15%) 99(27%) 85(65%) 71(78%) 57(100%).

Eicosadienoate: 322($M^+C_{20}H_{36}O_2$ 7%) 291($M^+-31,6\%$) 297($M^+-43,8\%$) 171(8%) 115(20%) 87 (100%).

Heneicosenote: 338($M^+ C_{22} H_{44} O_2$ 8%)307($M^+ -31,6\%$) 295($M^+ - 43,7\%$) 267 (7%) 253(8%) 239(7%) 225(8%) 211(9%) 197(8%) 183 (10%) 85 (72%) 71(100%).

Cetoleate: 352($M^+C_{23}H_{44}O_2$ 7%) 321($M^+-31,8\%$) 309($M^+-43,7\%$) 295(8%) 225(8%) 197(10%) 183(11%) 155(12%) 141(13%) 127(15%) 113(20%) 99(30%) 85(75%) 71(100%).

Methyl-tricosenote: 366($M^+C_{24}H_{46}O_2$ 8%), 335($M^+-31,7\%$) 323($M^+43,6\%$) 309(9%) 281(8%) 253(10%) 239(11%) 225(10%) 211(10%) 197(11%) 183 13%) 169(14%) 155(17%) 141(18%) 127 (20%) 113(23%) 99(30%) 85(75%) 71(90%) 57(100%)

n-Pentacosenoic acid: 380($M^+ C_{25}H_{48}O_2$ 6%) 349($M^+ -31,8\%$) 337($M^+ - 43,7\%$) 253 (9%) 211(8%) 169(10%) 155 11%) 141(15%) 127(17%) 113(19%) 99(22%) 85(70%)71(83%) 57(100%)

n-Hexacoseoic acid: 394 ($M^+ C_{19} H_{34} O_2$ 9%) 363 ($M^+ -31,7\%$) 351($M^+ - 43,8\%$) 295(10%) 253(7%) 239(9%) 197(10%) 183(9%) 169(12%) 155(12%) 141(13%)127 (15%) 113(19%) 99(25%) 85(55%) 71(75%) 57(100%).

n-Heptacosanoate: 424 ($M^+ C_{20} H_{28} O_2$ 7%) 393 ($M^+ -31,7\%$) 381($M^+ - 43,7\%$) 241(10%) 213(9%) 185(8%) 171(10%) 157(15%) 143(13%) 129(19%) 115(30%) 87 (78%) 59(100%).

Nonacosatrienoic acid: 432($M^+ C_{29} H_{52} O_2$ 8%) 401($M^+ -31,7\%$) 389($M^+ - 43,7\%$) 221(8%) 193(9%) 179(7%) 165(10%) 151(12%) 137(15%) 123(19%) 95(30%) 81 (75%) 67(86%) 53(100%).

Nonadecylate: 312($M^+C_{20}H_{40}O_2$ 10%) 281($M^+-31,30\%$) 269($M^+-43,10\%$) 255(10%) 241(25%) 213(15%) 199(10%) 185(9%) 157(10%) 143(25%) 115(40%) 87(75%) 73(78%) 59(100%).

Eicosatrienoate: 320($M^+C_{21}H_{36}O_2$ 20%) 289($M^+-31,10\%$) 277($M^+43,12\%$) 263(8%) 221(30%) 207(10%) 193(11%) 165(9%) 137(8%) 123(9%) 109(12%) 95(19%) 81(16%) 67(25%) 53(100%).

Heptadecadienoate: 280($M^+_{18}H_{34}O_2$,35%) 249($M^+-31,20\%$) 206($M^+43,15\%$) 193(18%) 178 19%) 164(13%) 150(18%) 136(26%) 122(40%) 94(90%) 80(97%) 66 (100%).

RESULTS AND DISCUSSION.

The top saturated fatty acids were present n-Hex decanoate (8.15%) and lowermost saturated fatty acids was n-Docosanoate, (3.14%.) the Unsaturated fatty acid are maximum percentage in Methyl-tricosenote 15.01%, 10 Octadecenoic acid 10.81%, Hexadecanoic acid 7.87%, Heptadecenoate 7.15% and smallest amount n-Heptaecenoic acid 0.03%, 3,8-Dimethyl-27-nonadicneate ,0.41%, in which 7 are mononic ,4 are Dieonoic and 5 are trienoic fatty acids were originate. The antifungal activity in *A. Niger* in Ethanol extract 85.71% and *P.funiculosum* in Chloroform extract 78% and minimum inhibition activity against *A. niger* 28.57% in Aqueous extract was observed . and Magnesium (Mg) 3179.58 mg/kg Potassium (K) 21248.8 mg/kg Calcium (Ca) 10751, mg/kg and Chromium (Cr) was present in lowest amount 0.65mg/kg and protein in the range 14.34% in Sandpits, Bulaji village 20.6 % and minimum in Hawk's bay 12.46%

The maximum inhibition activity was observed in Aqueous extract against test organism *P. funiculosum* 81% in ethanol extract *P. funiculosum* 80% and in Chloroform extract The inhibition activity was observed against *P. funiculosum* 80%. The moderate inhibition activity was detected in *P. funiculosum* 78% and *Aspergillus flavus* 75% in Ethyl acetate extract and *Aspergillus flavus* 72% in Methanol extract and *Aspergillus ochraceus* 73% in Aqueous extract the lowest inhibition activity was recorded in ethanol extract *Aspergillus flavus* 45% inhibition activity against *P. cetricola* 40% in Methanol extract minutest inhibition activity against *P. cetricola* 40% was seen. Aqueous extract lowest inhibition activity against *P. cetricola* 40% was noticed. In Ethyl acetate extract smallest inhibition against *P. cetricola* 46 %. If we compare with the pervious reported data then The maximum inhibition activity was observed against *P. funiculosum* 81% in chloroform extract. The maximum inhibition activity was observed against test fungi, *A. ochraceus* 80% in ethyl acetate extract: The maximum inhibition activity was observed against test organism *P. funiculosum* 78% and methanol extract: showed the 73% in ethanol extract against the *A. ochraceus* and minimum antifungal activity showed the minimum inhibition against *A nigier* 20% Ethyl acetate extract As cited in literature, the eighty two macroalgae 18 chlorophyceae, 25 pheophyceae and ecorded was 25% (Sandpits), in *Codium laevigatum* from sites of (Sandpits), coast of Karachi comparing this data with total protein contents on dry weight basis shown 32% (Sandpits), 29% (Manora), 25% (Buleji and Paradise-Point) and 29.45% from Hawks Bay sites in *S. robusta* of Karachi coast. (Khanzada, *et al.*, 2007) which is quite comparable with the problem contains results of *Codium laevigatum*. (Khanzada *et al* 2014) Studies on the antimicrobial and antifungal activities of 50 species of Marine benthic algae antimicrobial activity of certain seaweeds against the 4-gram positive and 4-gram negative bacteria and 4 fugal species. Whereas 24 species showed the activity and others where less inhibition are found in Methanolic extract. 22 algal species inhibited the growth of gram-positive bacteria. *Stoechospermum maculatum* and *Stoechospermum marginatum* were the most active algae. These algae were collected from Karachi Coast. (Usamngani *et al* (1986)

The different locations of Karachi coast was analyzed for the composition of Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn elements The present research work analysis the 11 different metals which are The amount of Mg was the highest among them (Mg) Magnesium 3179.58 mg/kg, Potassium (K) 21244 mg/kg, (Ca), Calcium 10751.11 mg/kg, (Fe) Iron 1653 mg/kg,, (Zn) Zinc 114.9 mg/kg, , (Cu), Copper 14.9 mg/kg, , (Mn), Manganese 13.125 mg/kg, , (Cd), Cadmium 6.68 mg/kg, (Cr), Chromium 0.73 mg/kg, (Ni), Nickel 2.96 mg/kg, ,(Pb) Lead 2.39 mg/kg, .(Table 1) if we compare with the pervious reported data then the Ca is present in highest as 80750 ppm and lowest as 6880 ppm (Rizvi and Shameel, 2001), The concentration of Mg, K, Fe, Zn was higher than other elements and the amount of Cd, Ni, Pb and Cr was minimum from 1 to 7 ppm, whereas Cu and Mn was 11 to 20 ppm and Zn was 122 to 411 ppm as cited in *S. robusta* from the

Karachi coast.(Khanzada *et al.*, 2007) Karachi from different places of the coast of Ca, Cd, Cr, Cu, Fe, K, Mg, MN, Ni, Pb and Zn were analyzed for the formation of the elements. the amount of iron (Fe) was the highest 2694.6 mg / kg potassium (K) 12007 mg / kg, calcium (Ca) 19710.3, Mg / kg of magnesium (Mg) 497.2, Mg / kg, Zinc (Zn) 337 Mg / kg, manganese (Mn) 22.67, Mg / kg, copper (Cu) 15.22, nickel (Ni) 8.8 Mg / kg cadmium (CD) 5.0 Mg / kg of lead (Pb) 2.11, Mg / kg of chromium (Cr) 1.9 Mg / kg, the highest among them the amount of iron. 2694.6 Mg / kg, calculated as the maximum amount Codium lavigateum recovery perspective Buleji 15961.0 Mg minimum amount of seaweed samples were collected from different samples from Manora said. Fe, K, CA, Mg, MN and the concentration of copper, 1 to 8 Mg / kg was less than other elements and Cd, Ni, Pb and million while the amount of Cu and Mn from 11 to 15 Mg / Kg Codium lavigateum coast of Karachi Zn 337 Mg /Kg was 290. (Khanzada et al. 2014).

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