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Agar Extraction, Physical Properties, FTIR Analysis and Biochemical Composition of Three Edible Species of Red Seaweeds *Gracilaria corticata* (J. Agardh), *Gracilaria dentata* (J. Agardh) and *Gracilariopsis longissima* (S. G. Gmelin) Steentoft, L. M; Irvine and Farnham

Rashida Qari[a]* and Saima Haider[b]

[a]School of Maritime Sciences, Bahria University, Karachi Campus, Karachi, Pakistan

[b]Institute of Marine Sciences, University Campus of Karachi, Pakistan, Pakistan

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Abstract. Three species of red algae *Gracilaria corticata* (J. Agardh), *Gracilaria dentata* (J. Agardh) and *Gracilariopsis longissima* (S.G. Gmelin), Steentoft, L. M; Irvine and Farnham (formerly *Gracilaria verrucosa* (Hudson) were collected from four different sites (Buleji, Hawks Bay, Manora and Paradise Point) of Karachi coast. The *G. corticata* was the dominant species and the highest yield of agar was compared to other studied species. The physical properties such as gel temperature, melting temperature, density, viscosity and gel strength showed large variations. Interestingly, the gel temperature, melting temperature, density, and gel strength had the highest value in *G. corticata* samples collected, while gel viscosity recorded the highest value in *G. dentata* samples. Intensive spectroscopic FTIR analysis was determined in all three species of *G. corticata*, *G. dentata* and *G. longissima*. The bands at 414.7/cm to 3917.2/cm represents stretching and bending vibrations of alcohol O-H, amine N-H, alkane C-H, alkyne C=C, nitriles C=N, carboxyl C=O, nitro aromatic N=O, alkane C-C, nitro methane C-N, aliphatic amines C-N, sulfoxides S=O, alkene C-H alkyl halide C-Cl, C-I groups. The ash content of all studied species (*G. corticata, G. dentata* and *G. longissima*) was in the range of 20-30%, while the carbohydrate content was in the range of 22-24%. The results of this study suggested the utilization of our natural resources present in Karachi coast. This could be achieved by determining the quantity and quality of agar in the edible species of *Gracilaria*/*Gracilariopsis*.

Keywords: agar yield, gel properties, seaweed, FTIR, Karachi coast

Introduction

Agar is a water-soluble gel-forming polysaccharide extracted from agarophyte members of Rhodophyta composed of mainly two sugar residues, D-galactose (51-53%) and 3,6 anhydro-galactose (46%). It is also composed of small proportions of monosaccharides and substituent groups known as disaccharide of hydroxyl groups with sulfate hemiesters and methyl ethers hemiesters and methyl ethers in various combination and more rarely with a cyclic pyruvate ketal as 4,6 O-[(R)-1-carboxyethylidene] acetal $R_2C(OR')^2$ (Cynthia Layse *et al*., 2011; Israel *et al*., 2010; Praiboon *et al*., 2006; Qari and Siddiqui, 1993). *Gracilaria* is the third largest genus in Rhodophyta, with more than 300 species, of which 180 species were accepted taxonomically (Sahu and Sahoo, 2013). The distributions of *Gracilaria* species is cosmopolitan and are commercially important in the production of phyco-colloid agar. The world-wide production of agar was 9600 tonnes valued at US $ 173 million in 2009, while China, Chile and Indonesia were the main producers (Bixler and Porse, 2010).

Gracilaria corticata, *Gracilaria dentata* and *Gracilariopsis longissima* species of *Gracilaria*/*Gracilariopsis* are mainly used for making food grade agar. They are utilized as human food, mostly in salads and soups, as feed for many animals, as potential source for nutrient removal for waste water treatment and as biomass for energy generation (Sahu and Sahoo, 2013). He was evaluated potential growth rates and dry weight yields of numerous species of red seaweeds. The genus *Gracilaria* is considered as the most attractive candidate because of its ability to achieve high yields and produce commercially valuable extracts (Cynthia Layse *et al*., 2011).

Gracilaria species are important for industrial and bio-technological use because they have phycocolloid, the main source of agar α-(1,4)-3,6-anhydro-L-galactose

*Author for correspondence; E-mail: rqari2002@yahoo.com

and ß-(1,3)-D-galactose with little esterification in cell wall (Cynthia *et al*., 2011). These species also produce acrylic acid which acts as an important bioactive metabolites with antibiotic activity. Major lipids such as prostaglandins are abundant in this genus. Variety of lactones are also found in *Gracilaria* from the Pacific Ocean such as, aplysia toxin isolated from *Gracilariopsis longissima* (S.G. Gmelin) Steentoft, L.M. Irvine and Farnham (Cynthia *et al*., 2011). Other constituents are also contained in this genus such as proteins r-phycoerythrin from *Gracilaria longa* and "Gigartinine" (new amino acid) from *Agarophyton chilense* (C. J. Bird, McLachlan, E. C. Oliveira) Gurgel, J. N. Norris, Fredericq (formerly *Gracilaria chilensis*) (Wilcox *et al*., 2001).

Physical and chemical characterization of agar polysaccharides extracted from the Thai and Japanese species of *Gracilaria* have been studied (Praiboon *et al*., 2006). The morphology and agar contents in some important *Gracilaria* species of Indian coasts have also been demonstrated by Sahu and Sahoo (2013). Given the fact that the yield and physical properties of agar such as gelling temperature, gel melting point, gel strength as well as its chemical properties determine its value to the industry (Praiboon *et al*., 2006), studies have been conducted on the properties and seasonal variations of agar yield in *Gracilaria* sp. (Whyte *et al*., 1981). In view of these, this present study was conducted to investigate the quantity and quality of agar in the three edible species of *Gracilaria* found in Karachi coast of Pakistan. Fourier transform infrared spectroscopy was used in this study owing to the fact that this technique is efficient and flexible in use for analyzing phycocolloid extracted from marine plants.

Researches on the distribution and morpho-ecological of marine benthic algae along the coast of Balochistan, Pakistan (Shameel *et al*., 2000) as well as biochemical composition and yield of agar from the *Gracilaria corticata* of Karachi coast (Qari and Siddiqui, 1993) have been demonstrated to the best of our knowledge, there is paucity of information on the analysis of agar polysaccharides from FTIR technique in Pakistan. The present study constitute a first approach for utilizing *Gracilaria* red algae species (*Gracilaria corticata*, *Gracilaria dentata* and *Gracilariopsis longissima*) as an industrial source of raw material for agar extraction in Pakistan.

Material and Methods

The monthly fresh samples of *Gracilaria* species (*G. corticata, G. dentata and G. longissima*) were collected (n = 86) at different periods on monthly variation from four different exposed sites of Karachi coast at low tide. During the experimental period, samples collected came from Buleji (n = 33), Hawks Bay (n = 8), Manora (n = 23) and Paradise point (n = 22) *G. corticata* (n = 44) was collected from Buleji, Hawks Bay, Manora and Paradise point, *G. dentata* (n = 21) from Buleji and Manora and *G. longissima* (n = 21) from Buleji and Paradise point at different periods of 2014-2016. All the samples collected were separately placed in pre-labeled plastic bags and brought to the laboratory, carefully cleaned from mud debris and other epiphytes with filtered seawater.

The agar extraction and its physical properties: gelation temperature, melting point, relative density, viscosity and strength were determined by the method of Whyte and Engler (1980). Mohan (2005) method was used for the FTIR analysis of agar samples. The fresh potassium bromide (KBr) crystals (Ft-IR grade) were grinded in mortar using pestle into a fine powder and kept in desiccators. The extracted agar samples were grinded in separate mortar pestle to make an homogenous mixture of sample mixed with KBr powder (1:10). After mixing the powders tablet was made by potassium bromide tablet dye and press at a pressure < 4 ton-ram area. The tablet or disc placed in the sample holder and FTIR spectra were recorded in the range of 4000-400 cm^{-1} on FTIR spectrophotometer (Bruker Vector-22). The carbohydrate content was estimated by using phenol sulfuric acid method (Dubois *et al*., 1956). Ash or total inorganic content was determined by the standard method of AOAC (1990).The total organic content was determined by calculating the difference between the inorganic content and total content.

Results and Discussion

The present data reveals high variability in the agar content in all the three species, sampling sites and collection time (Fig. 1-3). The concentration of agar increased in March and May and start decreasing from June to August (Fig. 1). The high agar concentration was mostly found in winter season. The highest agar content was obtained from Buleji samples as compared to other three experimental sites (Fig. 2) The study reported highest agar extracted from Gracilaria

longissima (13-56%) with mean concentration value of 27.20%. as compared to other two species *G. corticata* and *G. dentata* (Fig. 3).

G. corticata was found throughout the year at Buleji, Manora and Paradise point except during the months of April, August, September and November when samples were not found at Hawks Bay. The agar content of *G. corticata* which varied throughout the years had a range of 14-42% with mean concentration value of 25.09%. Samples collected in Buleji were high in September (42%), while it was also high in Hawks Bay sample (33%) in October (Fig. 4). Samples collected in Manora recorded high value in January and September (28.8 and 28.66%) respectively, while those collected in Paradise Point recorded high value (30%) in January (Fig. 4). *G. dentata* that was collected from major two sites (Buleji and Manora) was not found at Buleji in June and July but was absent only in July at Manora site. The range of agar content in *G. dentata* was 10-40% with mean concentration value of 21.79%. Samples collected in Buleji recorded high values of agar concentration in November (40%), while those collected in Manora recorded high value (30%) in December (Fig. 5). *G. longissima* was also collected from two sites (i.e., Buleji and Paradise point) throughout the year except during the months of July at Buleji and July and September at Paradise Point. The range of agar content in *G. longissima* was 13-56% with mean concentration value of 27.20%. The agar concentration was high in May in both Buleji and Paradise point samples (56 and 30%), respectively (Fig. 6).

The density of agar extract in *G. corticata* was in a range of 0.79-1.71 g/cm^3 in all sample sites. This study recorded high density of agar extract of *G. corticata* in both Buleji and Paradise Point in September (1.71 g/cm^3 and 1.03 g/cm^3), respectively. Samples collected in Hawks Bay recorded high value in October (1.55 g/cm^3) while those sampled in Manora recorded high value (1.04 g/cm^3) in January (Table 1). The density of agar extract in *G. dentata* was in a range of 0.39-1.63 g/cm^3.

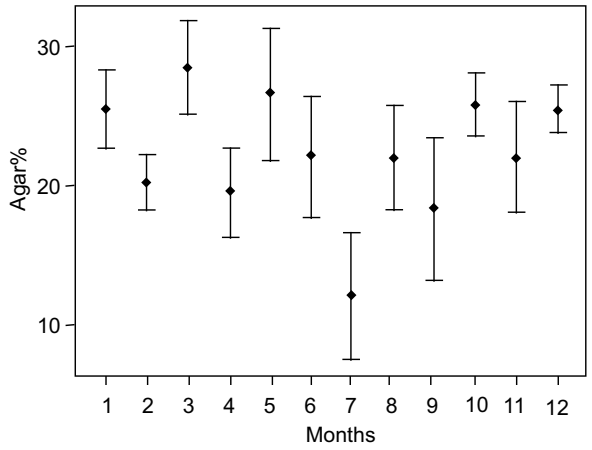

Fig. 1. Monthly variation in agar content (% dry wt.) of *G. corticata, G. dentata* and *G. longissima* collecting from different sites of Karachi coast.

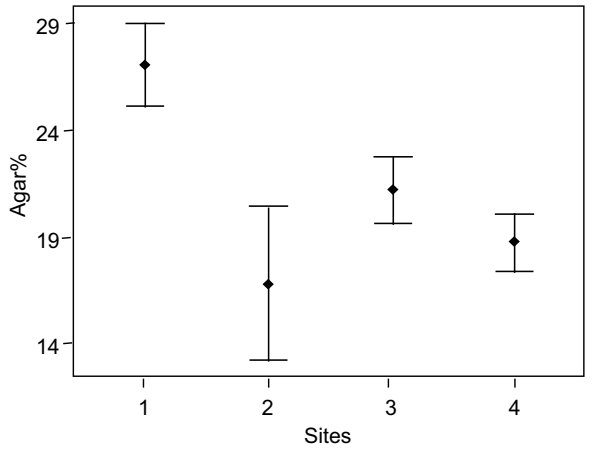

Fig. 2. Variation in agar content (% dry wt.) of *G. corticata, G. dentata* and *G. longissima* collecting from different sites (Buleji, Hawks Bay, Manora and Paradise Point) of Karachi coast.

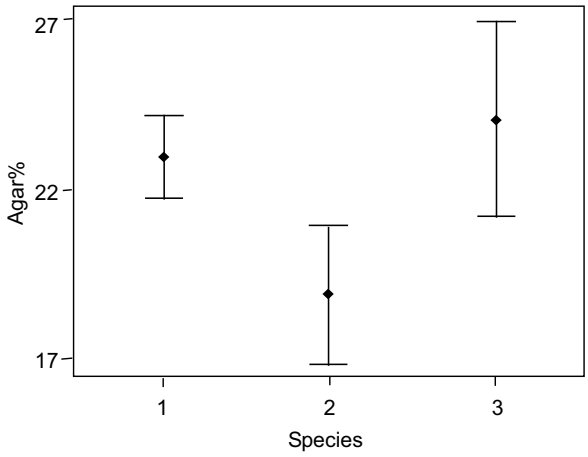

Fig. 3. Variation in agar content (% dry wt.) of *G. corticata, G. dentata* and *G. longissima* collecting from different sites of Karachi coast.

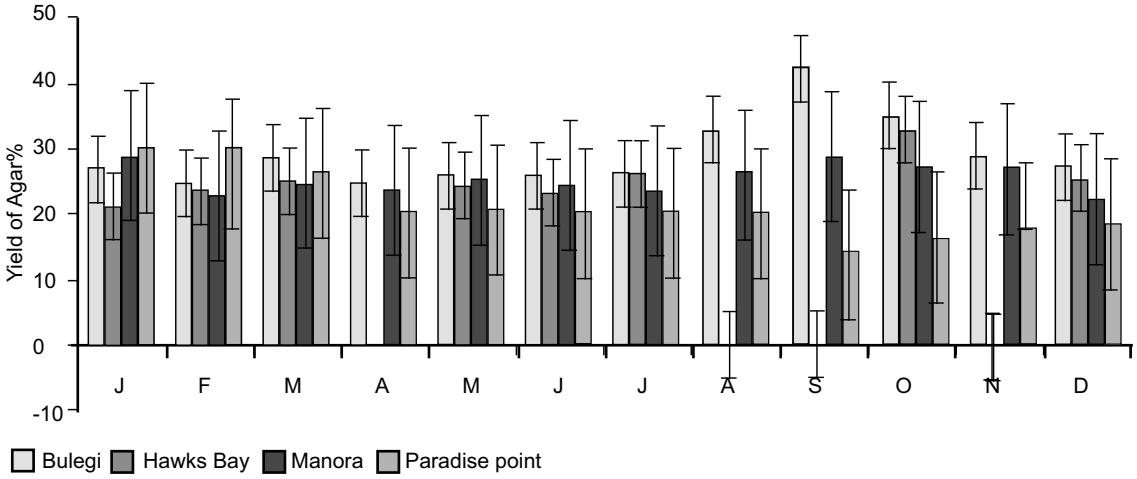

Fig. 4. Seasonal variations in the yield of agar from *G. corticata* collecting from different sites of Karachi coast.

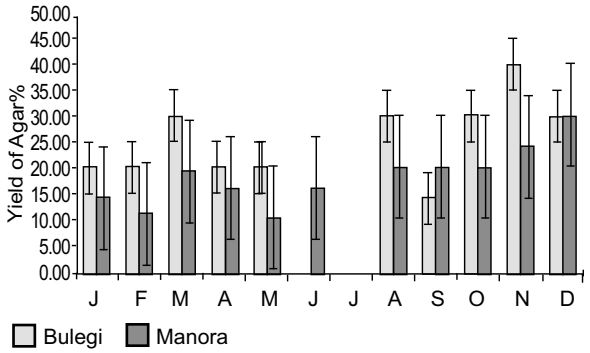

Fig. 5. Seasonal variations in the yield of agar from *G. dentata* collecting from different sites of Karachi coast.

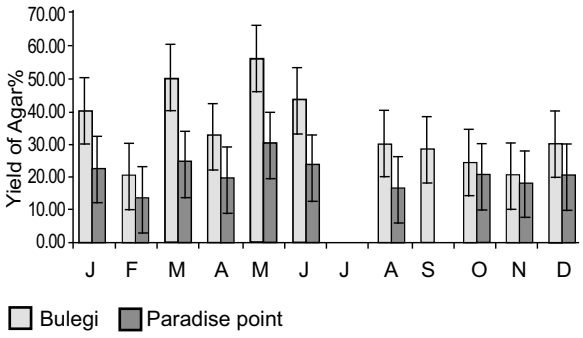

Fig. 6. Seasonal variations in the yield of agar from *G. longissima* collecting from different sites of Karachi coast.

Table 1. Gel density (g/cm^3) of agar extracted from *G. corticata*, *G. dentata* and *G. longissima* collected from different sites of Karachi coast

Species	*G. corticata*				*G. dentata*		*G. longissima*	
sites	Buleji	Hawks bay	Manora	Paradise point	Buleji	Manora	Buleji	Paradise point
J	1.09	1.00	1.04	0.97	0.81	0.50	1.62	0.71
F	0.99	1.10	0.81	0.89	0.81	0.39	0.8	0.42
M	1.15	1.18	0.88	0.84	1.21	0.68	2.02	0.77
A	1.00	0.00	0.84	0.84	0.81	0.57	1.29	0.61
M	1.04	1.14	0.91	0.90	0.81	0.36	2.26	0.97
~~J~~	~~1.04~~	~~1.09~~	~~0.88~~	~~0.87~~	~~0.00~~	~~0.57~~	~~1.74~~	~~0.74~~
J	1.05	1.22	0.86	0.85	0.00	0.00	0.00	0.00
A	1.34	0.00	0.94	0.93	1.22	0.71	1.21	0.51
S	1.71	0.00	1.02	1.03	0.57	0.72	1.14	0.00
O	1.42	1.55	0.98	0.97	1.22	0.72	0.97	0.64
N	1.17	0.00	0.96	0.96	1.63	0.86	0.81	0.58
D	1.11	1.19	0.79	0.79	1.22	1.07	1.21	0.64

High density of agar extract was recorded in Buleji sites in the month of November (1.63 g/cm^3), while high value was also recorded in Manora in December (1.07 g/cm^3). The agar extract of *G. longissima* had density which ranged between 0.42-2.26 g/cm^3 with high values at Buleji and Paradise Point in May (2.26 and 0.97 g/cm^3) respectively (Table 1).

The gel viscosity of *G. corticata* agar extract was in the range of 39-92 cP. In Buleji samples viscosity of agar was high in September (87 cP) and in Hawks Bay samples it was high (92 cP) in October whereas in both Manora and Paradise samples gel viscosity was high in January 80 cP and 83 cP respectively (Table 2). In *G. dentata* sample gel viscosity of agar extract was in the range of 28-83 cP. In both Buleji and Manora samples viscosity was high (83 cP) in November and December respectively (Table 2). The gel viscosity of *G. longissima* agar extracts was in range of 36-116 cP. In both Buleji and Paradise point, it was high in May 116 cP and 83 cP respectively (Table 2).

Gel temperature of *G. corticata* was in the ranged of 24-85 °C. In Buleji, *G. corticata* samples gel temperature was high in September (76 °C), October (85 °C) in Hawks Bay samples collected, and January in samples collected at both Manora and Paradise Point samples (56 and 52 °C) respectively (Table 3). The gel tem-

Table 2. Gel viscosity (cP) of agar extracted from *G. corticata*, *G. dentata* and *G. longissima* collected from different sites of Karachi coast

Species	*G. corticata*				*G. dentata*		*G. longissima*	
sites	Buleji	Hawks bay	Manora	Paradise point	Buleji	Manora	Buleji	Paradise point
J	56	59	80	83	41	39	82	61
F	51	65	63	76	42	31	41	36
M	59	69	68	72	62	53	104	66
A	51	00	65	55	41	44	66	52
M	53	67	70	57	41	28	116	83
J	53	65	67	55	00	44	89	63
J	54	72	66	55	00	00	00	00
A	68	00	72	55	62	55	62	44
S	87	00	79	39	29	55	58	00
O	72	92	75	46	62	55	49	56
N	60	00	74	50	83	66	42	50
D	56	70	61	50	62	83	62	54

Table 3. Gel boiling point (°C) of agar extracted from *G. corticata*, *G. dentata* and *G. longissima* collected from different sites of Karachi coast

Species	*G. corticata*				*G. dentata*		*G. longissima*	
sites	Buleji	Hawks bay	Manora	Paradise point	Buleji	Manora	Buleji	Paradise point
J	49	55	56	52	36	27	73	38
F	44	60	44	47	35	21	35	22
M	52	64	47	45	54	36	91	41
A	45	0	45	34	36	30	58	51
M	47	63	48	35	36	19	102	32
J	47	60	47	34	0	31	78	39
J	47	67	46	34	0	0	0	0
A	60	0	50	34	54	38	54	27
S	76	0	55	24	25	38	50	0
O	64	85	52	28	54	38	43	33
N	52	0	51	31	72	45	36	31
D	44	65	42	32	48	57	48	34

perature for *G. dentata* was in the ranged of 19-72 °C. In Buleji samples collected, gel temperature for *G. dentata* was high in November (72 °C) and December (57 °C) in Manora samples (Table 3). Gel temperature in *G. longissima* agar was in the range of 22-102 °C. It was high in May (102 °C) in Buleji samples collected and April (51 °C) in Paradise Point samples (Table 3).

G. corticata gel melting point was in the ranged of 59-140 °C. In Buleji samples, gel melting point was high in September (133 °C), October (140 °C) in Hawks Bay samples and January in Manora and Paradise Point samples (122 and 127 °C) respectively (Table 4). The *G. dentata* gel melting point was in the ranged of 42-126 °C and recorded high value of (126 °C) in Buleji and Manora samples in November and December (Table 4). Gel melting point in *G. longissima* agar was in the ranged of 54-176 °C. Samples collected in Buleji recorded high value in May (176 °C) and those sampled in Paradise point recorded high value of 127 °C in the month of August (Table 4).

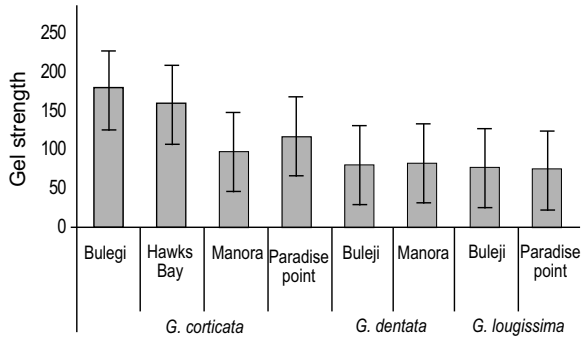

Fig. 7. Gel strength in *G. corticata, G. dentata* and *G. longissima* at different experimental sites of Karachi.

G. corticata samples gel strength was in the ranged of 94-174 g/cm^2. The highest gel strength was found in Buleji samples (174 g/cm^2) as compared to other experimental site samples. This study observed high value of gel strength in *G. dentata* samples collected in Manora (81 g/cm^2) as compared to those collected in Buleji, while gel strength of *G. longissima* in Buleji samples was high (74 g/cm^2) as compared to Paradise Point samples (Fig. 7).

The results of FTIR analysis of agar extracted from *G. corticata* showed different peak values at 3872.8. 3820.7, 3890.2, 3840.0, 3789.9, 3396.4 and 2925.8 for functional groups alcohols, amines and alkanes (O-H, N-H, C-H), 2117.7 for alkynes and nitriles (C≡C, C≡N), 1645.2 and 1542.9, carbonyl amide, nitro methane and aromatic (C=O, N=O), 1419.5 for alkane (C-C), 1323.1 for alkane and nitro methane (C-N, N=O), 1251.7 aliphatic amines (C-N), 1055.0 for sulfoxides (S=O), 862.1 for alkene (C=H), 769.5 and 584.4 for alkyl halide (C-Cl), 432.0 alkyl halide (C-I) (Fig. 8 and Table 5).

G. dentata agar extract results showed different peak values at 3832.3, 3917.2, 3766.7, 3691.5, 3409.9, 2920.0, 2850.6, 2673.2 for same functional groups alcohols, amines and alkanes (O-H, N-H, C-H), 2059.8 for alkynes and nitriles (C≡C, C≡N), 1633.6, 1595 and 1575.7 for carbonyl amide, nitro and aromatic (C=O, N=O), 1465.8

Table 4. Gel melting point (°C) of agar extracted from *G. corticata*, *G. dentata* and *G. longissima* collected from different sites of Karachi coast

Species	*G. corticata*				*G. dentata*		*G. longissima*	
sites	Buleji	Hawks bay	Manora	Paradise point	Buleji	Manora	Buleji	Paradise point
J	85	90	122	127	63	59	127	93
F	77	99	96	116	62	46	62	54
M	90	106	104	110	94	80	158	101
A	78	0	99	85	63	67	101	80
M	81	103	106	87	63	42	176	68
~~J~~	~~81~~	~~99~~	~~103~~	~~85~~	~~0~~	~~67~~	~~133~~	~~97~~
J	82	110	100	85	0	0	0	0
A	104	0	110	85	94	84	94	127
S	133	0	121	59	44	84	88	0
O	111	140	114	70	95	84	76	84
N	91	0	113	76	126	101	63	76
D	86	107	93	78	94	126	94	84

and 1436.9 for alkane (C-C), 1251.7 for alkane and nitro methane (C-N, N=O), 1039.6 and 933.5 for sulfoxides (S=O), 875.6 and 721.3 for alkene (C=H), 671.2 and 532.3 for alkyl halide (C-Cl), 468.7 and 437.8 (C-I) (Fig. 9 and Table 6).

The agar extracted from *G. longissima* showed also different peak values at 3793.7, 3384.8 and 2925.8 for functional groups alcohols, amines and alkanes (O-H, N-H, C-H), 2113.8 for alkynes and nitriles (C≡C, C≡N), 1639.4 and 1546.8 for carbonyl amide, nitro and aromatic

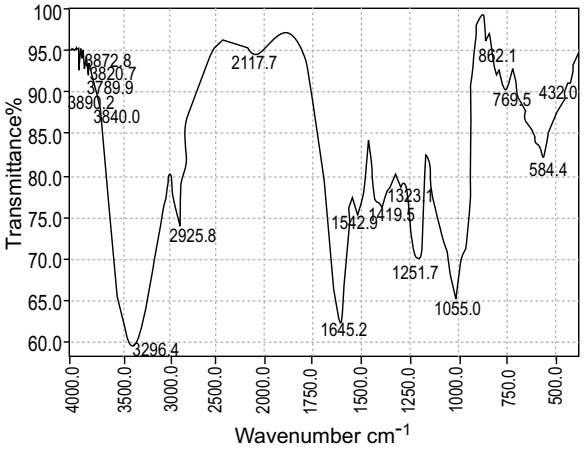

Fig. 8. FTIR spectrum of agar in species of *G. corticata.*

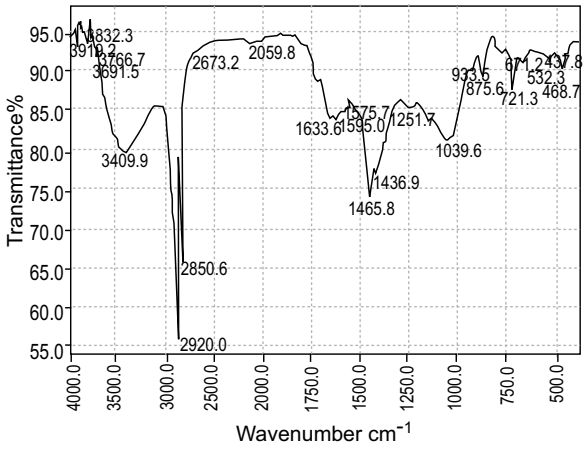

Fig. 9. FTIR spectrum of agar in species of *G. dentata.*

Table 5. FTIR absorption frequency/(cm), intensity estimation and functional groups of agar extracted from *G. corticata*

IR Frequency	Bond/(cm)	Functional groups	Intensity estimation	Type of vibration	Sample IR frequency/(cm)	References
4000-2500	O-H, N-H, C-H	Alcohol, Amine, Alkane	Strong, Sharp	Stretch, Free	3872.8, 3820.7, 3890.2, 3789.9, 3840.0, 3396.4 2925.8	(Faust, 1997), (Radhika and Mohaideen, 2015), (Fernando *et al*., 2017)
2500 – 2000	C≡C, C≡N	Alkyne, Nitriles	Medium	Stretch	2117.7	(Faust, 1997)
2000-1500	C=O, N=O,C=C	Cabonyl amide, Nitro methane, Aromatics	Very weak, Medium, Strong	Stretch	1645.2 1542.9	(Faust, 1997), (Pavia *et al*.,2009), (Radhika and Mohaideen, 2015)
1500 -1400	C-C	Alkane	Medium to weak	Stretch	1419.5	(Radhika and Mohaideen, 2015)
1400 – 1300	C-N, N=O	Alkane, Nitro methane	Medium	Bending	1323.1	(Pavia *et al*., 2009), (Radhika and Mohaideen, 2015)
1300 – 1200	C-N	Aliphatic amines	Strong	Stretch	1251.7	(Faust, 1997), (Pavia *et al*., 2009), (Radhika and Mohaideen, 2015)
1200 – 1000	S=O	Sulfoxides	Strong	Stretch	1055.0	(Pavia *et al*., 2009)
1000 – 700	C-H	Alkene	Weak	Bending	862.1	(Pavia *et al*., 2009)
700 -500	C-Cl	Alkyl halide	Strong	Stretch	769.5 584.4	(Faust, 1997), (Pavia *et al*., 2009)
200 – 500	C-I	Alkyl halide	Strong	Stretch	432.0 2009)	(Faust, 1997), (Pavia *et al*.,

Table 6. FTIR absorption frequency/(cm), intensity estimation and functional group of agar extracted from *G. dentata*

IR Frequency	Bond/(cm)	Functional groups	Intensity estimation	Type of vibration	Sample IR frequency/(cm)	References
4000-2500	O-H, N-H, C-H	Alcohol, Amine, Alkane	Strong , Sharp	Stretch,Free	3832.3, 3917.2, 3766.7, 3691.5, 3409.9, 2920.0, 2850.6, 2673.2	(Faust, 1997), (Radhika and Mohaideen, 2015), (Fernando *et al*., 2017)
2500 – 2000	C≡C, C≡N	Alkyne, Nitriles	Medium	Stretch	2059.8	(Faust, 1997)
2000-1500	C=O, N=O	Cabonyl amide, Nitro, Aromatic	Very weak, medium, Strong	Stretch	1633.6, 1595, 1575.7	(Faust, 1997), (Pavia *et al*.,2009), (Radhika and Mohaideen, 2015)
1500 -1400	C-C	Alkane	Medium to weak	Stretch	1465.8, 1436.9	(Radhika and Mohaideen, 2015)
1400 – 1300	C-N, N=O	Alkane, Nitro methane	Medium	Bending	1251.7	(Pavia *et al*., 2009), (Radhika and Mohaideen, 2015)
1200 – 1000	S=O	Sulfoxides	Strong	Stretch	1039.6, 933.5	(Pavia *et al*., 2009)
1000 – 700	C-H	Alkene	Weak	Bending	875.6, 721.3	(Pavia *et al*., 2009)
700 -500	C-Cl	Alkyl halide	Strong	Stretch	671.2, 532.3	(Faust, 1997), (Pavia *et al*., 2009)
200 – 500	C-I	Alkyl halide	Strong	Stretch	468.7, 437.8	(Faust, 1997), (Pavia *et al*., 2009)

(C=O, N=O), 1423.4 for alkane (C-C), 1382.9 for alkane and nitro methane (C-N, N=O), 1261.4 aliphatic amines (C-N), 1074.3 for sulfoxides (S=O), 933.5 and 850.5 for alkene (C=H), 603.7 for alkyl halide (C-Cl), 488 and 414.7 for (C-I) (Fig. 10 and Table 7).

The carbohydrate contents in all three species of *Gracilaria* are uniform and ranged from 22-24%.

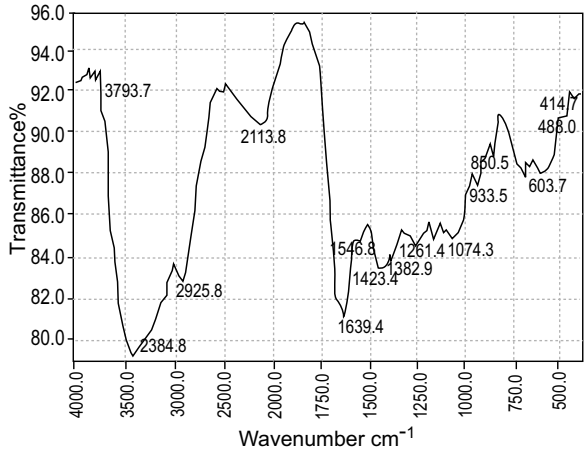

Fig. 10. FTIR spectrum of agar in species of *G. longissima*.

Mostly the carbohydrate contents in *G. corticata*, *G. dentata* and *G. longissima* were 22% except in Buleji and Hawks Bay samples of *G. corticata* carbohydrate that was 24 and 23% respectively (Fig. 11). The ash contents in all three species of *Gracilaria* were 20% except in Buleji coast sample of *G. dentata* that was 30% (Fig. 12).

The results of two way analysis of variance (ANOVA) of agar of three species *G. corticata*, *G. dentata* and

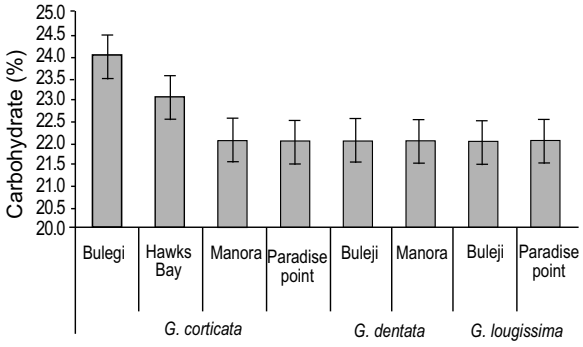

Fig. 11. Carbohydrate content in species of *Gracilaria* collecting from different sites of Karachi coast.

Table 7. FTIR absorption frequency/(cm), intensity estimation and functional group of agar extracted from *G. longissima*

IR Frequency	Bond/(cm)	Functional groups	Intensity estimation	Type of vibration	Sample IR frequency/(cm)	References
4000-2500	O-H, N-H, C-H	Alcohol, Amine, Alkane	Strong , Sharp	Stretch,Free	3793.7, 3384.8, 2925.8	(Faust, 1997), (Radhika and Mohaideen, 2015), (Fernando *et al*., 2017)
2500 – 2000	C≡C, C≡N	Alkyne, Nitriles	Medium	Stretch	2113.8	(Faust, 1997)
2000-1500	C=O, N=O	Cabonyl Amide, Nitro, Aromatic	Very weak, medium, Strong	Stretch	1639.4, 1546.8	(Faust, 1997), (Pavia *et al*., 2009), (Radhika and Mohaideen, 2015)
1500 -1400	C-C	Alkane	Medium to Weak	Stretch	1423.4	(Radhika and Mohaideen, 2015)
1400 – 1300	C-N, N=O	Alkane, Nitro methane	Medium	Bending	1382.9	(Pavia *et al*., 2009), (Radhika and Mohaideen, 2015
1300 – 1200	C-N	Aliphatic amines	Strong	Stretch	1261.4	(Faust, 1997), (Pavia *et al*., 2009), (Radhika and Mohaideen, 2015
1200 – 1000	S=O	Sulfoxides	Strong	Stretch	1074.3	(Pavia *et al*., 2009)
1000 – 700	C-H	Alkene	Weak	Bending	933.5, 850.5	(Pavia *et al*., 2009)
700 -500	C-Cl	Alkyl halide	Strong	Stretch	603.7	(Faust, 1997), (Pavia *et al*., 2009)
200 – 500	C-I	Alkyl halide	Strong	Stretch	488, 414.7	(Faust, 1997), (Pavia *et al*., 2009)

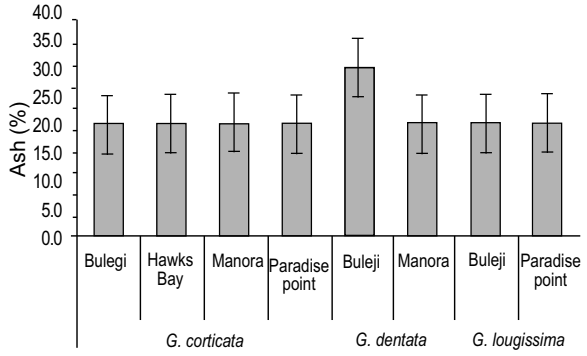

Fig. 12. Ash content in species of *Gracilaria* collecting from different sites of Karachi coast.

Table 8. Analysis of variance (ANOVA) for agar in *G. corticata*, *G. dentata* and *G. longissima* at four different sites of Karachi coast

	Analysis of variance					
Source	DF	Seq SS	Adj SS	Adj/MS	F	P
Month	11	1760.30	1760.30	160.03	1.89*	0.052
Sites	3	1508.77	1862.30	620.77	7.35**	0.000
Species	2	727.11	727.11	363.56	4.31*	0.017
Error	79	6671.40	6671.40	84.45		
Total	95	10667.57				

* = significant at P < 0.05 and ** = significant at P < 0.001.

G. longissima showed that there were highly significant variations observed between sites (P < 0.001), months (P < 0.05) and species (P < 0.05). The differences in species, sites and months in present results reveal that agar content was different in all three studied species at different sites in different times (Table 8). The data for agar concentrations of the three experimental species used in this study were analyzed to determine the relationship in between agar of the three species of Gracilaria at different sites found. Positive significant correlations were found in agar of *G. corticata* collected from Buleji and Manora (r2 = 0.653), *G. dentata* collected from Buleji and Manora (r2 -= 0.688) and *G. longissima* collected from Buleji and Paradise Point (r2 = 0.782). The insignificant correlation was found in between agar, carbohydrate and ash content.

The present study demonstrated that the yield of agar varied seasonally. The results for yield of agar extracted from the three species of *Gracilaria* used in this present study were mostly similar to the values reported by Buriyo and Kivaisi (2003). The yield of agar, gelation temperature and gel melting temperature of agar solution of samples used in this study were similar when compared to that of Praiboon *et al*. (2006). Present study showed greater concentration of agar for *G. corticata* (14-42%), *G. dentata* (10-40%) and *G. longissima* (13-56%) when compared with the previous study from Karachi coast (Qari and Siddiqui, 1993) and Philippine coast for the same genus species (Barros, 2013). The gel relative density (0.79-1.71 g/cm^3) and gel melting temperature (59-140 °C) of *G. corticata* agar in the present study were quite similar to the values (1.08-1.1 g/cm^3 and 91.1-92.5 °C, respectively) reported by Qari and Siddiqui (1993) for the same species, whereas gel viscosity values were lower (39-92 cP) and gel temperature (24-85 °C) and gel strength (94-174 g/cm^2) values were higher than previous study (Qari and Siddiqui, 1993) and two other Philippine species of same genus *Gracilaria* by (Barros, 2013).

The unusually high gelling temperature observed in this study was partly due to the very viscous solution, which resisted the sinking of glass beads used in the gelling temperature determination (Barros, 2013). High viscosity and melting temperature values indicate a high molecular weight polymer (Whyte and Englar, 1981). The yield of agar of *G. longissima* sample collected from Buleji was high (20-56%) as compared to *G. longissima* sample collected from Paradise Point. The agar content of *G. longissima* in this present study was similar to the values reported in previous study of Sahu and Sahoo (2013) for the same species. The yield of agar was high during winter season. This may be due to high biomass in winter because in this period high nutrient availability and low temperature and light intensity (Qari, 2017). The relative density of *G. longissima* agar solution was almost similar as compared to the results of Qari and Siddiqui (1993).

FTIR is a valuable tool for measuring many chemical constituents in plants and seaweeds to reveal some qualitative aspects regarding the organic compounds (Lammers *et al*., 2009). The results of FTIR analysis of agar extract showed strong and sharp absorption peaks in the 2673.2-3872.8/cm region (Alcohol, amine, alkane), 1055.0 -1251.7 (Aliphatic amines, Sulfoxides) and 432.0-769.5 (Alkyl halide) region in all agar samples (Fernando *et al*., 2017; Radhika and Mohaideen, 2015; Pavia *et al*., 2009; Faust, 1997). The absorbance peak of weak band at 1639.4/cm represent C=C stretching vibration, indicative of the lignin (Kubo and Kadla, 2005; Chatjigakis, 1998). The absorbance peak at 1423.4/cm representing bending vibration of C-H group indicated the presence of amino acids (Kannan, 2014). The absorbance peak of medium band observed at 2113.8/cm representing the stretching vibration of C≡C group, whereas the absorbance peak at 1382.9/cm representing bending vibration of C-H group indicating the presence of amino acids (Kannan, 2014). The carboxyl and hydroxyl functional groups are mainly found in polysaccharides and are primary constituent of seaweeds found in all studied samples, and can be used in medicine (Kannan, 2014). The other chemical groups which are characteristic of present agar samples are alkyne, nitriles, carbonyl amide, nitro, aromatic alkane, alkane nitro methane and alkene (Radhika and Mohaideen, 2015; Pavia *et al*., 2009; Faust, 1997). The types of vibration were mostly stretch free, stretch and binding.

The results of extraction of all three studied species *G. corticata, G. dentata* and *G. longissima* suggested that these edible species are good source of agar that could be used commercially. The functional group alcohols, amines, alkanes, alkynes, nitriles, carbonyl amide, nitro methane, aliphatic amines, sulfoxides, alkene and alkyl halide observed in the experimental seaweeds used in this study as demonstrated in the FTIR analysis indicates that all the experimental seaweeds used in this study could be used medicinally in the treatment of various human diseases. It is therefore suggested that these edible species of seaweeds should be cultivated so as to protect the blue economy or marine resources.

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Conflict of Interest. The authors declare no conflict of interest.

References

A.O.A.C. 1990. *Official Methods of Analysis of Association of Official Analytical Chemist*. 15th eds., 684 pp., Association Official Analytical Chemists. Arlington, Virginia, USA.

Barros, F.C.N., da Silva, D.C., Sombra, V.G., Maciel, J.C., Feitosa, J.P.A., Freitas, A.L.P., de Paula, R.C.M. 2013. Structural characterization of polysaccharide obtained from red Seaweed Gracilaria caudata (J Agardh). *Carbohydrate Polymers*, **92:** 598-603.

Bixler, H.J., Porse, H. 2010. A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology*, **23:** 321-335.

Buriyo, A.S., Kivaisi, A.K. 2003. Standing stock, agar yield and properties of *Gracilaria salicornia* harvested along the Tanzanian coast, western Indian Ocean. *Journal of Marine Sciences*, **2:** 171-178.

Cynthia Layse F. De Almeida, Heloina De S. Falcão, Gedson R. De M. Lima, Camila De A. Montenegro, Narlize S. Lira, Petrônio F. De Athayde-Filho, Luis C. Rodrigues, Maria de Fátima V. De Souza, José M. Barbosa-Filho, Leônia M. Batista. 2011. Bioactivities from Marine Algae of the Genus *Gracilaria*. *International Journal of Molecular Sciences*, **12:** 4550-4573.

Dubois, M., Giles, K.A., Hamilton, J.K., Rebors P.A., Smith, F. 1956. Calorimetric method for determination of sugars and related substances. *Analytical Chemistry*, **28:** 350-356.

Faust, B. 1997. Modern chemical techniques: an essential reference for students and teachers. *The Royal Society of Chemistry, Unilever*, **5:** 127-128.

Israel, A., Einav, R., Seckbach, J., 2010. *Seaweeds and their Role in Globally Changing Environments*. 479 pp., Springer Dordrecht Heidelberg London, New York, USA.

Kannan, S. 2014. FT-IR and EDS analysis of the seaweeds *Sargassum wightii* (brown algae) and *Gracilaria corticata* (red algae). *International Journal of Current Microbiological Applied Sciences*, **3:** 341-351.

Kubo, S., Kadla, J.F. 2005. Hydrogen bonding in lignin: a Fourier transform infrared model compound study. *Biomacromolecules*, **6:** 2815 -2821.

Lammers, K., Keil, G.A., Dighton, J. 2009. FTIR study of the changes in carbohydrate chemistry of three New Jersey Pine Barrens leaf litters during simulated control burning. *Soil Biology and Biochemistry*, **41:** 340-347.

Mohan, J. 2005. *Organic Spectroscopy Principles and Applications*, 2nd eds., 548 pp., Narosa Publishing House, Daryagani, Delhi, India.

Pavia, D.L., Lampman, G.M., Kriz, G.S. 2009. *Introduction to Spectroscopy*, 4th edition, 511 pp., Brooks/Cole, Cengage Learning.

Praiboon, J., Chirapart, A., Akakabe, Y., Bhumibhaman, O., Kajiwara, T. 2006. Physical and chemical characterization of agar polysaccharides extracted from the Thai and Japanese species of *Gracilaria*. *Science Asia*, **1:** 11-17.

Qari, R. 2017. An assessment of seaweeds diversity and distribution at the beach of Nathia gali, Karachi, Pakistan. *Journal of Marine Science Research Development*, **7:** 228. doi: 10.4172/2155-9910.1000228

Qari, R., Siddiqui, S.A. 1993. Biochemical composition and yield of agar from the seaweed *Gracilaria corticata* of Karachi coast (Pakistan). *Marine Research*, **2:** 77-81.

Radhika, D., Mohaideen, A. 2015. Fourier transform infrared analysis of *Ulva lactuca* and *Gracilaria corticata* and their effect on antibacterial activity. *Asian Journal of Pharmaceutical Clinical Research*, **8:** 209-212.

Sahu, N., Sahoo, D. 2013. Study of morphology and agar contents in some important *Gracilaria* species of Indian coasts. *AJPS*, **4:** 52-59.

Shameel, M., Khan, S.H., Afaq, H.S. 2000. Biodiversity of marine benthic algae along the coast of Balochistan, Pakistan. *Pakistan Journal of Marine Biology*, **6:** 69-100.

Whyte, J.N.C., Englar, J.R. 1980. Chemical composition and quality of agars in the morphotypes of *Gracilaria* from British Columbia. *Botanica Marina*, **23:** 277-283.

Whyte, J.N.C., Englar, J.R., Saunders, R.G., Lindsay, J.C. 1981. Seasonal variations in the biomass, quantity and quality of agar from the reproductive and vegetative stages of *Gracilaria verrucosa* type. *Botanica Marina*, **24:** 493-501.

Wilcox, S.J., Bloor, S.J., Hemmingson, J.A., Furneaux, R.H., Nelson, W.A. 2001. The presence of gigartinine in a New Zealand *Gracilaria* species. *Journal of Applied Phycology*, **13:** 409-413.