Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. **TITLE OF THE PROJECT**: An assessment of biochemical compounds in Relation with environmental constituents and Phylogenetic relationships and bar-coding of different Seaweeds from Okha coast of Gujarat, India.

2. **NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR** :Dr. Nirmal Kumar J.I. Principal and Professor, Department of Environmental Science & Technology (EST) , Institute of Science & Technology for Advanced Studies & Research (ISTAR) P.O. Box 13, Vallabh Vidyanagar - 388 120 (Gujarat), India.

3. **NAME AND ADDRESS OF THE INSTITUTION**: Institute of Science and Technology for Advanced Studies and Research

4. UGC APPROVAL LETTER NO. AND DATE 42-415/2013 (SR), 7th Nov.2014

5. DATE OF IMPLEMENTATION 13th May 2013

6. TENURE OF THE PROJECT From May, 2013 to March, 2017

7. TOTAL GRANT ALLOCATED 12,30,800/-

8. TOTAL GRANT RECEIVED 11, 68, 547/-

9. FINAL EXPENDITURE 11, 63, 319/-

10. **TITLE OF THE PROJECT** An assessment of biochemical compounds in Relation with environmental constituents and Phylogenetic relationships and bar-coding of different Seaweeds from Okha coast of Gujarat, India.

11. OBJECTIVES OF THE PROJECT

- To characterize bimonthly **biochemical compounds** of selected seaweeds belong to Chlorophyceae, Pheophyceae and Rhodophyceae class: pigments, proteins, amino acids, lipids, phenols, carbohydrates will be estimated for one year.
- To explore the monthly hydro-chemical and geo-chemical properties where seaweeds will be collected to know the interrelation between hydro-geo-chemical properties and biochemical compounds.
- To separate and isolate various **biochemical compounds such** as Fatty Acids, Amino Acids, Alkaloids, and Sterols from seaweeds.
- To explore biocidal activities of seaweeds for antibacterial, antifungal and antioxidant activities on pathological organisms/
- To find out **phylogenic relationships by** DNA **barcoding** among all seaweeds available by using cytochrome c oxidase-I gene

12. WHETHER OBJECTIVES WERE ACHIEVED

(GIVE DETAILS)

1. Seasonal seaweed diversity in relation to hydro-geochemical properties of Bet Dwarka, Okha coast.

Seaweed diversity

Okha coast is rich with diverse group of seaweed species. Presence of suitable substratum both due to coral reefs and other rocks, provide appropriate habitat for most of the algal species. During the study period, seaweeds constituted 121 species enlisted as per the classes (Table 1). The major part of the stranded seaweed is represented by 67 species of Rhodophyta accounting for 55%, followed by 28 species of Chlorophyta contributing 23% and 26 species of Phaeophyta with 22% (Fig. 1). Thus, Rhodophyta shows more prevalence in the seaweeds flora at selected site. Jha *et al*. (2009) observed in their earlier study from Saurashtra coast, more number of Rhodophyta species as compared to Chlorophyta and Phaeophyta.

Table 1.Seasonal variation of identified species belonging to Chlorophyta, Phaeophyta and Rhodophyta.

S. No	Cholorophyta	Pre monsoon (Feb – May)	Monsoon (June– Sept)	Post monsoon (Oct- Jan)
1	*Ulva lactuca* Linnaeus	+	-	+
2	*Entromorpha flexuosa* J.Agardh	+	-	+
3	*Entromorpha prolifera* J.Agardh	-	-	+
4	*Monostroma latissimum* Wiltrock	-	+	-
5	*Ulva fasciata* Delile	+	-	-
6	*Ulva reticulata* Forsskal	+	-	-
7	*Chaetomorpha crassa* Kutzing	+	+	+
8	*Cladophora sp*	-	+	-
9	*Valonia utricularis* C.Agardh	-	-	+
10	*Chamaedoris ausiculata* Borgesen	-	-	+
11	*Bryopsis pennata* Lamouroux	-	-	+
12	*Caulerpa racemosa* J.Agardh	+	-	+
13	*Caulerpa sertularioides* S.Gmelin	+	-	+
14	*Caulerpa veravalensis* Thivy &Charhan	-	-	+
15	*Cladophoropsis javemica* P.silva	-	-	+
16	*Caulerpa taxifolia* C.Agardh	+	-	-
17	*Chlorulerpa microphysa* J. Feldmann	+	+	+
18	*Caulerpa scalpelliformis* C.Agardh	+	+	+
19	*Caulerpa verticillata* J.Agardh	+	+	+
20	*Struvea anastomosans* Harvey	+	-	+
21	*Cladophora glomerate* J.Agardh	+	+	+
22	*Acrosiphonia orientalis* J.Agardh	+	-	+
23	*Udotea indica* A & E. Gepp	+	-	+
24	*Chaetomorpha antennina* Kutzing	+	-	+
25	*Valoniopsis pachynema* Borgesen	+	-	+
26	*Halimeda macroloba* Decaisne	+	-	+
27	*Entromorpha intestinalis* Nees	+	-	+

28	*Caulerpa cylindrial*	+	-	+
Phaeophyta				
1	*Turbinaria ornate* J.agardh	-	+	+
2	*Sargassum cinereum* J.Agardh	-	+	+
3	*Sargassum vulgare* C.Agardh	-	+	+
4	*Sargassum swartzii* J.Agardh	-	-	+
5	*Sargassum johnstonii* Setchell &Gardner	-	-	+
6	*Sargassum tenerrimum* J.G Agardh	-	+	+
7	*Cystoseira indica* Mairh	+	+	+
8	*Sargassum cinctum* J.Agardh	-	+	+
9	*Sargassum plagiophyllum* J.Agardh	-	+	-
10	*Padina boergesenii* Allender &Kraft	+	+	+
11	*Dictyopteris acrostichoides* Bornet	+	+	+
12	*Dictyota dichotoma* Lamouroux	+	+	+
13	*Spatoglossum asperum* J.Agardh	+	-	+
14	*Stoechospermum marginatum* Kutzing	-	-	+
15	*Padina tetrastromaticr* Hauck	-	-	+
16	*Cystoseira trinodis* Mairh	+	-	+
17	*Lobophora variegata* Lamouroux	+	-	+
18	*Iyengaria stellate* Borgesen	+	-	+
19	*Padina boryana* Thivy	+	+	+
20	*Sargassum prismaticum* Chauhan	+	+	+
21	*Cystoseira trinodis* C.Agardh	+	+	+
22	*Dictyopteris australis* Sonder	-	+	+
23	*Dictyota cervicornis* Kutzing	+	+	+
24	*Hydroclathrus clathratus* C.Agardh	+	+	+
25	*Colpomenia sinuosa* Darbes & Solier	+	+	+
26	*Dictyota bartayresiana* Lamouroux	+	+	+
Rhodophyta				
1	*Champia indica* Bogesen	-	-	+
2	*Laurencia sp*	-	+	+
3	*Lausencia obtuse* Lamouroux	-	-	+

4	*Lausencia glandulifera* Kutzing	-	-	+
5	*Botryocladia leptopoda* Kylin	+	-	+
6	*Acanthophora specifera* Borgesen	+	-	+
7	*Odontothulia verovalensis* Krishnamurthy et Vijaya	-	-	+
8	*Rhodymenia sonderi* P.Silva	+	-	+
9	*Coelarthrum muelleri* Borgesen	+	-	-
10	*Cyastexlomium iyenagarii* K.Srinivasan	-	-	+
11	*Hypner valentiae* Montage	-	-	+
12	*Hypnera flagelliformis* Greville	+	-	+
13	*Hypnea valentiae* Montagne	+	-	+
14	*Sarcomema filiforme* Kylin	-	-	+
15	*Solieria robuster* –Kylin	+	-	+
16	*Griffiphsia corallfnoides* C.Agardh	-	-	+
17	*Centrocesae clavulatum* Montayne	+	-	+
18	*Platysiphonia delicata* Cremaeles	+	-	+
19	*Heterosiphonia mulleri* De Toni	-	-	+
20	*Lophocladia lallemandi* Montagne	+	-	-
21	*Chondria dasyphylla* C.Agardh	+	-	-
22	*Wrangalia tanegana* Harvey	+	-	-
23	*Anotrichium tenue* C.Agardh	+	-	-
24	*Laurencia claviformis* Borgesen	+	-	-
25	*Laurencia papillosa* C.Agardh	+	-	-
26	*Gracillaria salicornia* C.Agardh	+	-	-
27	*Gracillaria textori* De toni	+	+	-
28	*Gracillaria corticata* J.Agardh	+	-	-
29	*Scinaia complanata* Collins	+	-	-
30	*Liagora viscida* C.Agardh	+	-	-
31	*Griffithsia corallinoides* Trevisan	-	-	+
32	*Gyrateloupia indica* Borgesen	-	-	+
33	*Gelidium* J.Agardh	-	+	-
34	*Cheilrsporym spectabile* Harvey	+	-	+
35	*Ahnfeltia plicata* Fries	-	-	+
36	*Sarcomema scinaioides* Borgesen	+	-	-

37	*Scinaia hatei* Borgesen	+	+	+
38	*Scinai monoliformis* J.Agardh	+	+	+
39	*Gelidiella acesosa* J. Feldmann	+	+	+
40	*Kappaphycus alvarezii* Doty	+	+	+
41	*Soliera chordialis* J.Agardh	+	+	+
42	*Laurencia pedicularioides* Borgesen	+	+	+
43	*Gracillaria debilis* Borgesen	+	+	+
44	*Stoechospermum marginatum*	+	+	+
45	*Hypnea spinella* Kutzing	+	+	+
46	*Hypnea pannosa* J.Agardh	-	-	+
47	*Tricleocarpa fragilis* Huisman & Townsend	+	-	+
48	*Gastroclonium iyengarii* K. Srinivasan	+	-	+
49	*Dermonema virens* J.Agardh	+	-	+
50	*Champia compressa* Harvey	+	+	+
51	*Gracilaria foliifera* Borgesen	+	+	+
52	*Peyssonnelia obscura* Borgesen	+	-	+
53	*Spyridia hypnoides* Papenfuss	+	+	+
54	*Gracilaria dura* C.Agardh	+	-	+
55	*Amphiroa fragilissima* Lamouroux	+	+	+
56	*Scinaia fascularis* Huisman	+	+	+
57	*Gelidiella acerosa* J. Feldmann	+	+	+
58	*Peyssonnelia obseura* Borgesen	+	+	+
59	*Bostrychia tenella* Lamouroux	+	+	+
60	*Digenea simplex* C.Agardh	+	-	+
61	*Corallina berteroi*	+	-	+
62	*Jania rubens* Lamouroux	+	+	+
63	*Laurencia majuscula* Harvey	+	+	+
64	*Tridecarpa fragilis*	+	-	+
65	*Sebdenia polydactyla*	+	-	+
66	*Cryptonemia undullata* Sonder	+	-	+
67	*Polysiphonia substilissima*	+	-	+

*(+) = presence; (-) = absence

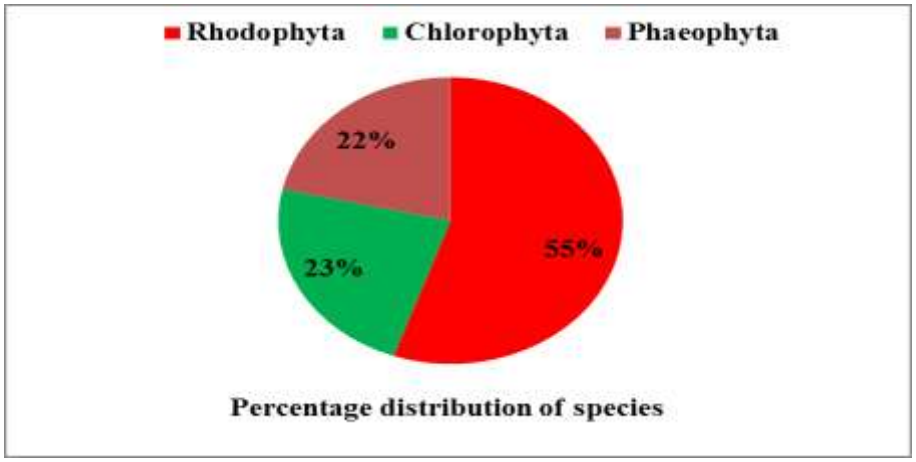


Fig 1 Seaweed species distribution at Bet Dwarka, Okha coast.

Table 2: Total list of seaweeds collected from Bet Dwarka, Okha coast.

Taxonomic groups	Chlorophyta	Phaeophyta	Rhodophyta	Total
Order	7	3	11	21
Families	10	3	23	36
Genera	14	13	47	74
Species	28	26	67	121

Seasonal variation in seaweed diversity and distribution

The seasonal variation in seaweed composition in the present study is correlated with species growth and succession in the natural habitat of Okha coast [Murthy *et al*. (1978)]. The coast is rich in diverse seaweed community and the season for their growth is beginning from November to May. Maximum total number of seaweeds was recorded in post monsoon (101 sp.) season followed by pre monsoon (87 sp.) and monsoon (47 sp.) (Fig.2). The monsoon season is unfavourable for the seaweeds supporting scanty growth of a few species limited to supra littoral zone. However, the dominating growth of Chlorophyta members could be attributed to the simple paranchymatous nature of the fronds. Moreover, the entire algal thallus is exposed to motion, unlike a land plant in which the anchoring roots are beneath the soil and out of the wind. This kind of wave action may hardly be tolerated by the Chlorophyta members with soft thallus. However, the Rhodophyta members, which tend to drift, are mostly of cartilaginous and fragile causing breaking of thallus. *Sargassum* and *Caulerpa* are the two taxa that contributed to maximum stranded seaweed biomass. This could be due to the branched, anatomizing habit of fronds, resulting into extensive spread in the intertidal area as compared to other seaweed species suggested by Thakur *et al*. (2008). Krishnamurthy *et al*. (1967) found that the seaweeds washed ashore during November and December was very less but shortly increased in January.

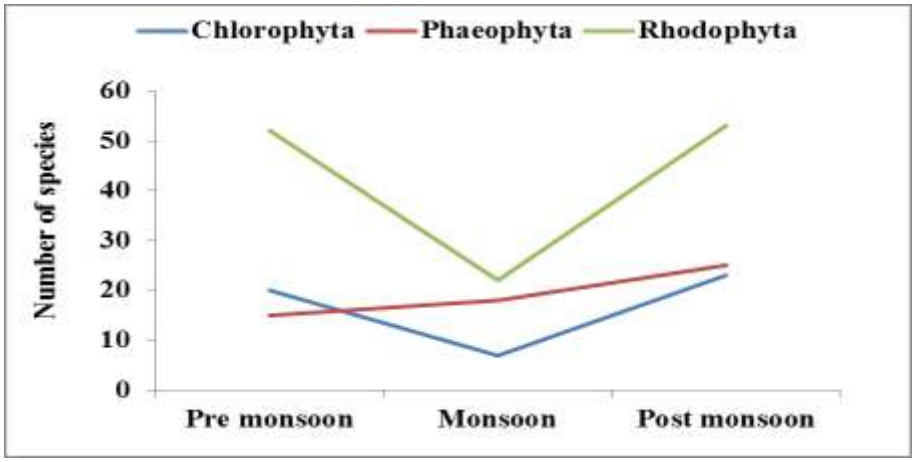


Fig 2.Seaweed seasonal variation Byte Dwarka, Okha coast. Gujarat

Low seaweed population in summer might be due to high temperature, low nutrient level [Nair *et al*. (1986)]. However, both temperature and rainfall does not have any influence on seaweed population. Similar result was also observed by Makwana (2011) in nine different coastal area of Gujarat coast, India. But, Daly and Mathieson (1977) further reported that algae was observed during monsoon might be due to low temperature, low salinity, low desiccation and increased nutrient concentrations in ambient waters. The temporal and spacial changes in ecological patterns are influencing marine biodiversity and community [Hewitt *et al*. (2007); Smale *et al*. (2010)]. In this study, the highest diversity was recorded for Rhodophyta, which is characteristically diverse and abundant in the macroalgae flora of Indian coast (Jha *et al*. (2009). However, total biomass of red algae is lower than those of green and brown algae. This refers to the small filamentous morphology of most Rhodophyta species in the study area. The macroalgal growth and biomass are directly controlled by nutrient availability in water [Pedersen *et al*. (2010); Martínez *et al*. (2012)]. Hence, the result of this study which showed the highest growth of macroalgae in winter may be attributable to increased nutrient availability in this season. *Ulva* species are among the fast-growing algae [Phillips and Hurd (2003)] and showed differences over time, being abundant in winter and early spring then vanishing in summer, which may attribute to the variation in nutrients load in marine water and their seasonal effect at the study area. In terms of vertical distribution, the lower intertidal level showed the highest abundance of macroalgae as it was reported by other studies [Scrosati and Heaven (2007) ; Kang and Kim (2012); Raffo *et al*. (2014)]. On a local scale the upward distribution of organisms is mainly limited by desiccation in the intertidal zones [Nybakken (2001) ; Ingólfsson (2005)]. It appears the red algae are more usually confined to a single zone than the green and brown algae, usually limited to the lowermost zone and only poorly represented at the mid-tide level. However, the species belonging to Chlorophyta and Phaeophyta showed more similarity among different intertidal stages. The differences of photosynthetic pigments of the three main groups of algae and their tolerance to desiccation may be

important in dealing with variation of light intensity and therefore inducing their vertical growth and distribution in the intertidal area [Nybakken (2001)].

Seasonal variation in seawater and sediment chemistry at selected site.

The physiochemical parameter in coastal region mainly affected by water circulation, tidal cycles, waves, morphology and fresh water intrusion [Arhonditsis *et al*. (2000)]. The pH of the seawater at selected site ranged from 7.3 to 8.5, where maximum value was recorded in post monsoon season. The pH is influence the status of ecosystem, the balanced ecosystem range of 5.5 to 8.5 is indicating balanced ecosystem [(Chandrasekhar JS (2003)]. Temperature is one of the important parameter influencing physicochemical characteristics of sea water and biological behaviour of coastal ecosystem [Krishna Kumar *et al*. (2012)]. The temperature variation is one of the influencing factors in the coastal and estuarine system, which may trigger the physicochemical characteristics and also the distribution and abundance of marine flora and fauna [P. Soundarapandian (2009)]. Temperature was reached maximum in pre monsoon period by 30˚C and minimum was noticed in post monsoon with 27.8 ˚C.

Salinity fluctuated from 26.15 to 30.11%, where decreasing salinity in monsoon period due to inflow of rainfall and fresh water influx into the open sea and due to low temperature [Patel (2016)]. The sediment salinity ranged from 0.82 to 0.87% (Fig.7). The salinity act as limiting factor in the distribution of living organism and changes due to evaporation and dilution in the coastal ecosystem [Paramasivam and Kannan (2005)]. Dissolved compounds and ions strongly influenced the electrical conductivity. The increase in conductivity is possibly due to high concentration of the ions in the water. Maximum EC value observed was 57.23mS/cm during pre-monsoon followed by monsoon 52 mS/cm and post monsoon 47.11 mS/cm. The fluctuations in total EC was due to variation in total dissolved solids and salinity. Therefore, conductivity measurements can be used to predict the water quality.

The DO refers to the amount of oxygen dissolved in the sea water and it is particularly important in limnology (aquatic ecology) [Weiss (1970)]. The fate and behaviour of DO is of critical importance to marine organisms in determining the severity of adverse impacts [Best *et al*. (2007)]. Photosynthesis, respiration and nitrification are the main biological process controlling dissolved oxygen concentration by producing and consuming the oxygen [Best *et al*. (2007)]. The study shows the DO varies from 5.65 mg/l to 6.85 mg/l. It is reported that with increasing salinity, the DO level decreased. The DO and temperature are inversely related as temperature influence the dissolve oxygen holding capacity of water [Satheeshkumar and Khan (2012)]. The lower DO content in pre monsoon was due to the significant inverse relationship ($r = -0.841$, $P < 0.05$) between temperature and dissolved oxygen is a natural process because the hot water get easily saturated with oxygen and it reduce the oxygen holding capacity of water. Moreover, observed seasonal variation of dissolved oxygen was mainly due to freshwater influx and ferruginous impact of sediments [Fig. 3] (Appendix-A1& A2). Seawater is more alkaline due

to hardness of water. Calcium and magnesium both play an important role in provoking the toxic effects of various ions and neutralizing the excess acid produced [Raju (2007)]. Maximum calcium and magnesium hardness was recorded during monsoon, whereas minimum was registered in pre-monsoon season. Sodium content was in range of 6808 to 7149.675 mg/L, found higher in pre-monsoon while oppositely in sediment maximum was recorded during monsoon season (Fig.7). The range of calcium and magnesium hardness of sediment was 0.12 to 0.27 mg/g and 0.012 to 0.18, respectively (Fig. 7). The maximum TDS value 38187 mg/l was recorded during monsoon season whereas minimum was recorded in pre-monsoon 33,133 mg/l. TDS consists of inorganic minerals (salts) in ionic (e.g, Na+ , Ca2+, Cl- , HCO3 -), organic material and all soluble substances. Seawater typically has a level of total dissolved solids (TDS) between 33,000–37,000 mg/L (Fig 4).

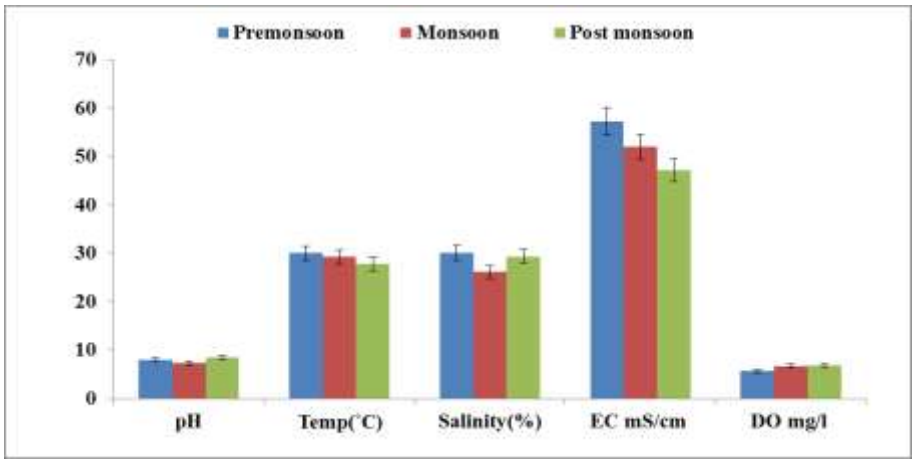


Fig 3 Seasonal variation in pH, temperature, salinity and dissolved oxygen content of seawater.

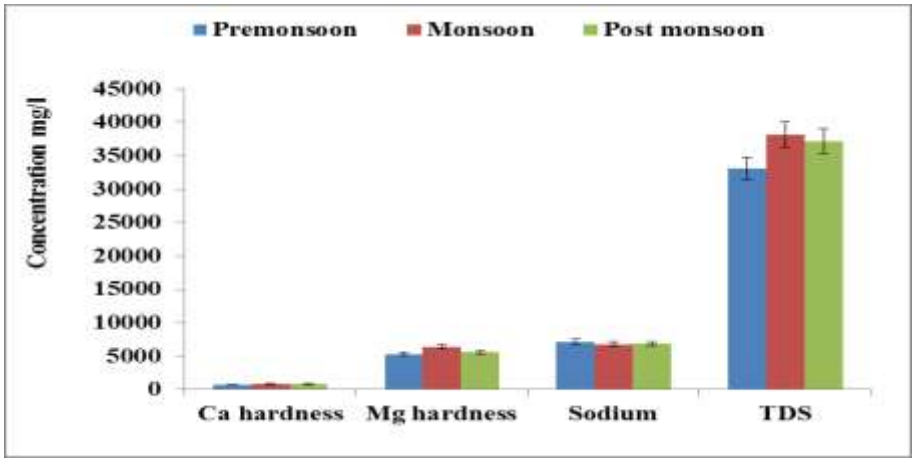


Fig 4 Seasonal variation in Ca-Mg hardness, sodium and TDS content of seawater.

There was not much variation in phosphate concentration, however the nitrate was found maximum during post monsoon season and minimum was registered in monsoon. Nitrate and phosphate recorded in the range from 0.94 to 1.5 mg/l and 2.33 to 4.75 mg/l, respectively. The variations were observed due

to dissimilarity in phytoplankton excretion, oxidation of ammonia, reduction of nitrate and by recycling of nitrogen and bacterial decomposition of planktonic detritus [Asha and Diwakar (2007)]. The high concentration of phosphate was due to the diffusion and migration of phosphorus from the sediment pore water to the overlying water [Faragallah *et al*. (2009)]. The phosphate content of sediment found in the range from 0.027 to 0.13 mg/g, where maximum was recorded in post monsoon season. Similarly nitrate was also found maximum in sediment during post monsoon season with 0.012 mg/g (Fig. 7). Mishra *et al*. (1993a) also reported elevated concentration of phosphate during monsoon season. Potassium was recorded in range of 14.6 to 14.92 mg/l, maximum was found in premonsson season. Sediment analysis of potassium content revealed higher concentration in monsson season (Fig. 7). In coastal waters, nitrogen and phosphorus concentrations of seawater are high in winter and low in summer [Martínez *et al*. (2012)].Nitrates stimulate the growth of plankton and water weeds that provide food for fish. Also, the winter mixing deepening escalated nitrate enrichment into the euphotic zone from deeper water [Al-Qutob *et al*. (2002)], which indicate the maximum nitrate concentrations at the different sites during winter, while the lower concentrations during the other seasons may be due to high booming of phytoplankton observed. Generally, the increase in nitrate content of seawater is followed by and raise in both production and chlorophyll-a level (Fig. 5). Sulfate concentration varied from 261.38 to 326.245 mg/l, where maximum was recorded during post monsoon and minimum in monsoon months. Maximum sedimentary sulphate was recorded during post monsoon season 1.208 mg/g and minimum in pre monsoon period 0.438 mg/g (Fig.7). Bicarbonate and carbonate concentration found in range from 470 to 518.5 and 55 to 81.5 mg/l, respectively (Fig. 7).

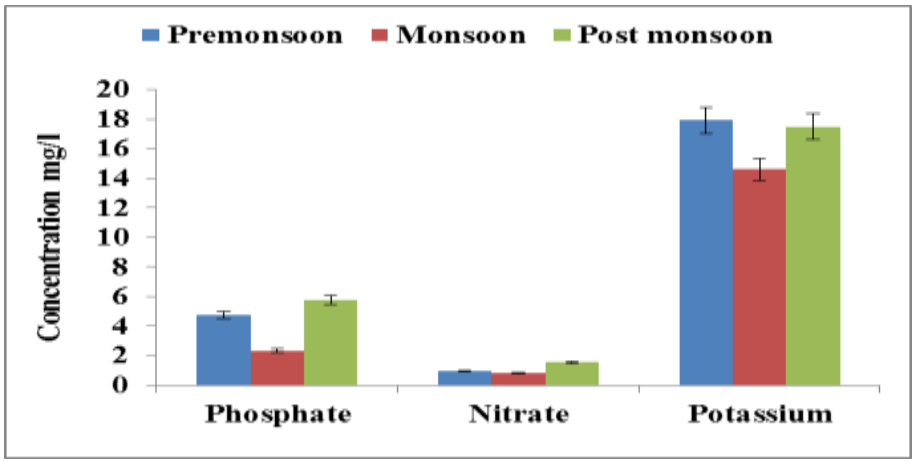


Fig 5 Seasonal variation in phosphate, nitrate and potassium content of seawater.

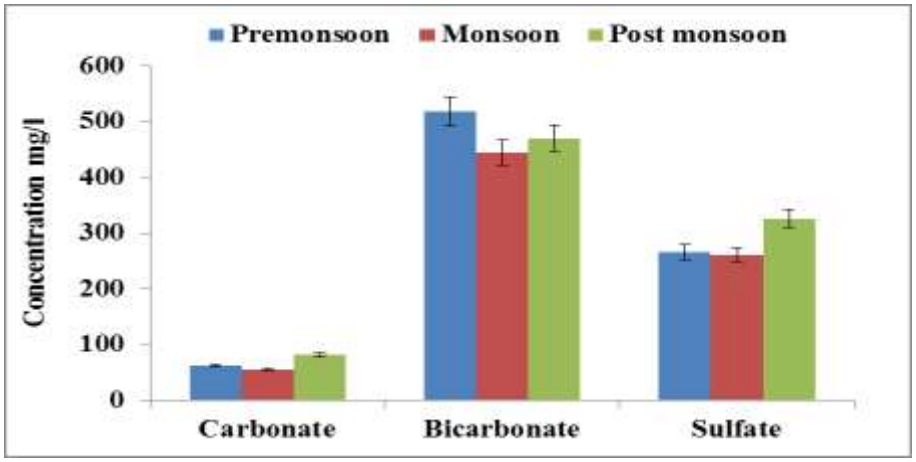


Fig 6 Seasonal variation in carbonate, bicarbonate and sulphate content of seawater

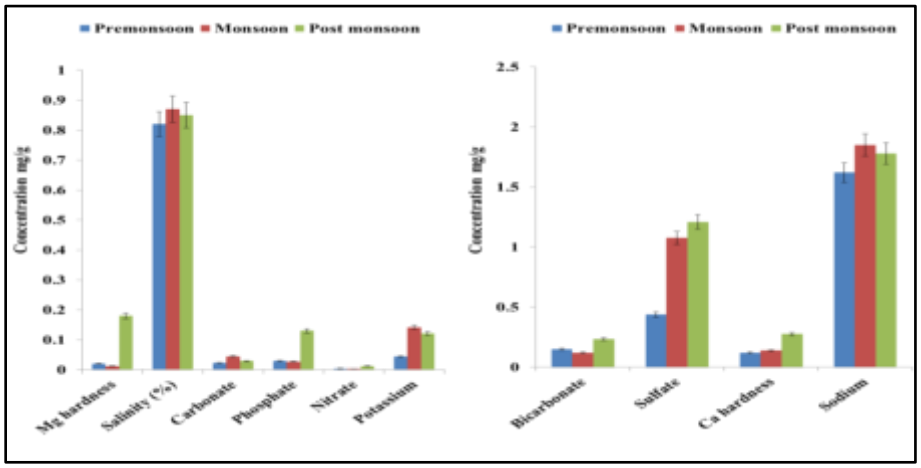


Fig 7 Seasonal variation in geochemical parameters of study area.

2. Selected seaweeds utilized for nutraceutical properties.

Total eighteen species were selected which are belonging to Chlorophyta, Phaeophyta and Rhodophyta (Table 3; Fig 8 -10). The six species from each group was selected on the basis of their huge growth and consistent availability at the selected site round the year.

Table 3.List of eighteen seaweeds selected for detail study.

Sr. No	Name of the species	Order	Family	Class
1	*Caulerpa recemosa*	Ulvales	Ulvaceae	Chlorophyta (Green)
2	*Monostroma latissimum*	Ulvales	Ulvaceae	
3	*Ulva lactuca*	Ulvales	Ulvaceae	

4	*Ulva reticulata*	Ulvales	Ulvaceae	
5	*Struvea anastomosans*	Siphonocladales	Boodleaceae	
6	*Ulva fasciata*	Ulvales	Ulvaceae	
7	*Sargassum tenerrimum*	Fucales	Sargassaceae	Phaeophyta (Brown)
8	*Cystoseira indica*	Fucales	Sargassaceae	
9	*Turbinaria orneta*	Fucales	Sargassaceae	
10	*Dictiyota dichotoma*	Dictyotales	Dictyotaceae	
11	*Iyengaria stellata*	Scytosiphonales	Scytosiphonaceae	
12	*Lobofora variegata*	Dictyotales	Dictyotaceae	
13	*Botrycladia leptopoda*	Rhodymeniales	Rhodomelaceae	Rhodophyta (Red)
14	*Centroceras clavulatum*	Ceramiales	Ceramiaceae	
15	*Halymenia venusta*	Halymeniales	Halymeniaceae	
16	*Hypnea pannosa*	Gigartinales	Cystocloniaceae	
17	*Hypnea spinella*	Gigartinales	Cystocloniaceae	
18	*Dermonema virens*	Nemaliales	Liagoraceae	

Chlorophyta members

Fig 8. Species of Chlorophyta selected for detail study.

1. *Caulerpa racemose*

Caulerpa pale green in colour, 2.5 cm tall closely arranged simple or sparingly split covered with clavate to spherical branchlets; grow in the bunches look like grapes. Mostly found growing on dead corala, sometime rooted in sand and muddy bottom. With moderate abundance, start growing from November to April at lower mid littoral zone , tide pool.

2. *Monostroma latissimum*

Plants usually found in upper mid littoral zone as dark or yellow green patchy bunches, they submerge during high tide, are mainly attached to the rocks exposed to tides. The total size of 2to 3 cm crisped margine of membranes, monochromatic fronds and cells are usually oval shape with 12.5 µm broad with lamina chloroplast. Loosely arranged in groups consist 2 to 4 cells each, tetra, oval or polygonal shaped cells. With scanty abundance, this species found during January to May and August to November.

3. *Ulva lactuca*

Plants bright yellowish-green; grows as large sheets and attached by small disc; leaf-like with undulating wide blades; blades upto 20 cm tall, much broad than long; heart or oval shaped and occasionally with some holes; like waxed paper to touch. It is found in moderately exposed situations on rocks, wood

works or coarse algae, in pools and quiet shallow waters near the low tide mark. It also thrives in brackish water with organic pollution.

4. *Ulva reticulate*

The net like structure of this plant grow mostly in association with other algae. Light to dark green colour, net like structure 10 to 20 cm broad with a number of voids with different shape- oval, circular, oblong or rectangular, membranous. The cells two layer in thickness and dileted arranged cells function as air bladder. Plants grow densely at intertidal pool and water rceiving domestic sewage. Grow from the August to April months.

5. *Struvea anastomosans*

Recorded from warm-temperate and tropical waters worldwide, the thalli of this species tend to occur as scattered, dark green and isolated individuals, although clusters are also encountered. The outline of the frond meshwork is highly irregular, the apical cells of primary laterals neither curving in on the margins nor regularly forming tenacular attachments. The stalks are non-annulate.

6. *Ulva fasciata*

Plant is ribbon shaped dark green in colour attached using small holdfast. Blades 5-100 cm long, are moderately to highly lobbed or cleftede. 2 cells thick blades with cells of some plants taller thanwide. Cells in surface view irregularly square to rectangular, with 1-3 pyrenoids. Reproduction occurring in wide bands along both margins of thin lobes, or large patches on wider lobes. Reproductive areas noticeably light golden in color. Very common and often abundant in most habitats where groundwater or streams enter the ocean. Growing from the month August to April at mid littoral zone and tide pools.

Phaeophyta members

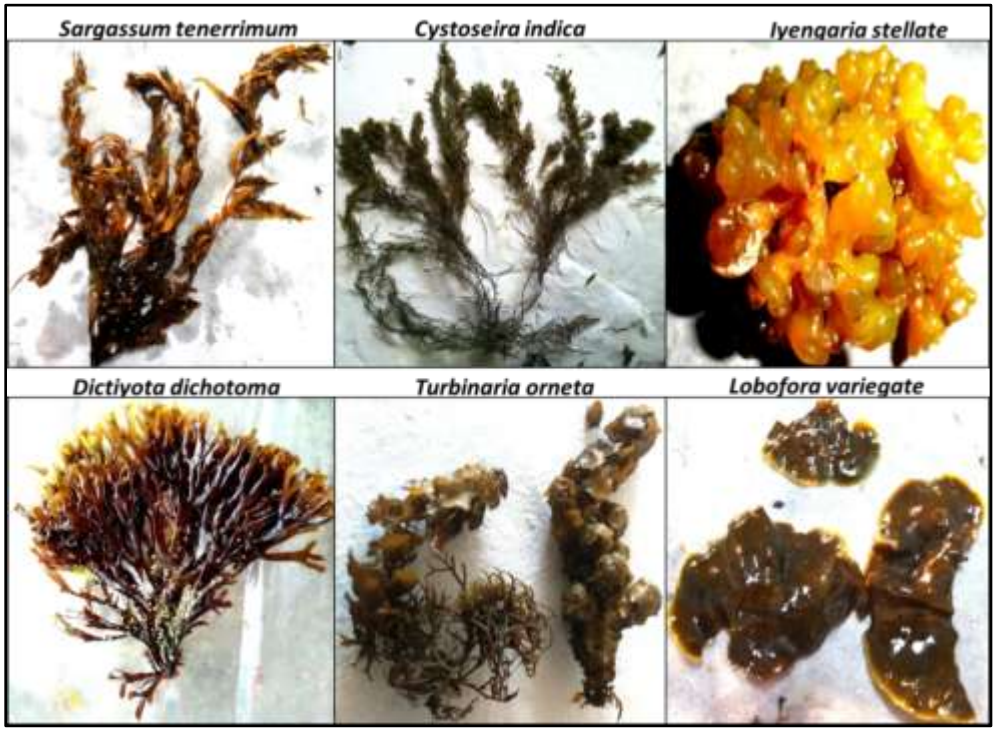


Fig 9.Species of Phaeophyta selected for detail study.

1. *Sargassum tenerrimum*

Plants brown to yellowish brown, pyramidal in shape, densly found in infra littoral zone, sub littoral zone and tidal pools. Small conical disc is holdfast to attached on rocks. Dominantly grow at the habitat and covered other small species. Primary axes terete, alternately branched, cylindrical primarily braches, smooth branchlets; thin leaves 2 to 6 cm long, 0.5 to1.5 cm broad longer and broader below. Racepticals are typical feature of these species,

2. *Cystoseira indica*

Plant dark brown in colour grow dominantly in intertidal rocks and coralliine rocks. 20 to 40 cm in length with terete leafless branched rhizomatous. Luxurious growth of the species found in the intertidal region and remain submerge even during low tide. Alternate spirally arranged branches. Receptacles arranged linear born third or fourth order of branching. Grow from November to April.

3. *Iyengaria stellate*

Plants 5 to 10 cm tall dark brown to yellowish in colour, attached by rhizoids arising from the lower epidermal cells.Smoth solid hollow tubes like structureand spine like projection. Found in intertidal rocks and coralline stones as scattered bundles, dominantly grow from November to April.

4. *Dictyota dichotoma*

Yellowish brown colour plants grow well on intertidal rocks and coralline stones, 12 cm or more in height and ribbon like flat structure, upper part dichotomously forked branches and attached by small disc. Branches scattered both sides of the thallus. Found growing from November to april months.

5. *Turbinaria orneta*

Dark brown colour plants, attached by discoid holdfast on rocks in infra littoral fringe and littoral zone and tide pool. 10 to 50 cm tall irregularly branched, turbinate to obconical, 10- 15 cm broad basal ends. Receptacles racemose, marginal blades obtuse marginal located. Start growing from November to April.

6. *Lobophora variegate*

Dark brown colour plants mostly 10 to 15 cm broad flat. Overlapping in clusters, thalli circular to fan shaped remain submerge during low tide, attached by rhizoids through basal part of the fronds. Growing on intertidal rocks and calcareous stones, dominantly grow from November to April.

Rhodophyta members

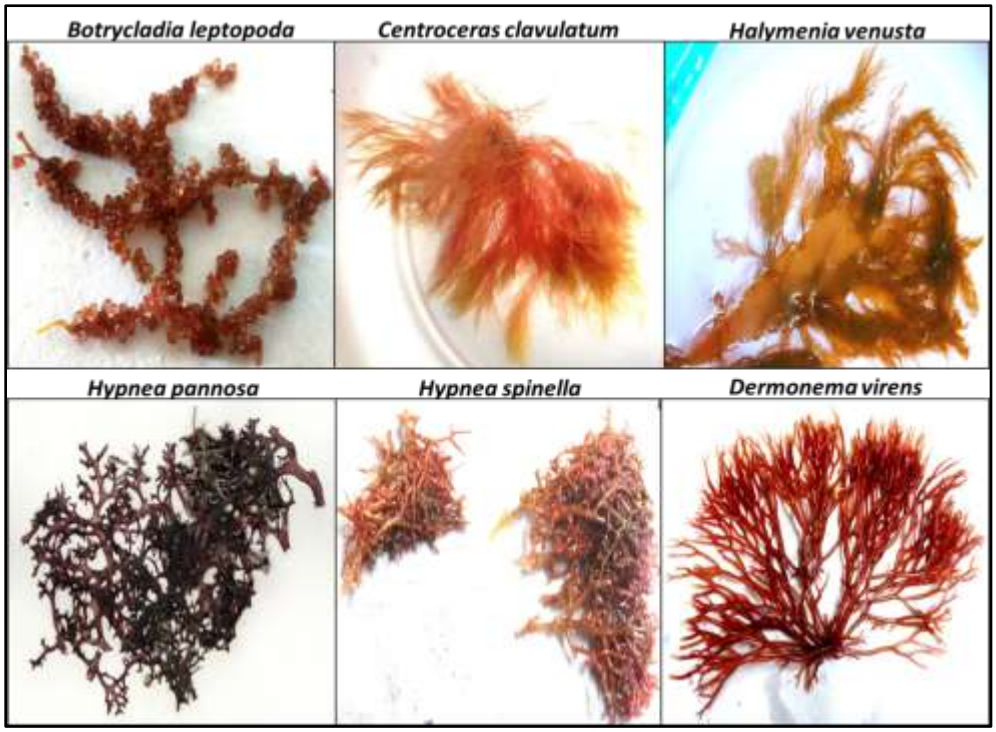


Fig 10. Species of Rhodophyta selected for detail study.

1. *Botrycladia leptopoda*

Red to pinkish red color plants look like red grapes. Maximum 60 cm tall plant attached by discoidal holdfast with cylindrical stem, sparsely branched. Minute stalked vesicles are 1 to 5 mm diameter in size, smooth filled with fluid sub spherical bolls arranged on stem. Growing from November to April, on intertidal rocks, remain submerge during low tide and stay at sub littoral zone.

2. *Centroceras clavulatum*

Dark red colour 5 to 8 cm tall, firm, filamentous and rigid; dichotomously branched filaments. Several spines of 1 or 2 cells with nodes and internodes in younger part, spines are sometimes absent in older portion. Moderately abundant plants growing from November to April on intertidal rocks and calcareous stone in rocks pool and lower mid littoral zone.

3. *Halymenia venusta*

Light rose to red colour plant usually grow upto 30 cm tall with lubricous, large thallus. Plant attached by small basal disc consist fronds with variable shape, divided in many lobes. Margins are densely proliferated up to few millimetres in size. Found moderately growing on intertidal rocks during November to April in lower mid littoral zone and infra littoral fringe.

4. *Hypnea pannosa*

Plants dark pink to purple colour grow up to 10 cm in height, grow as spongy clusters on intertidal rocks. Pyramidal rasomosly arranged from medle part towards the apex. Easily broken small smooth branches and spines. Found growing in lower mid littoral zone and infra littoral fringe and rock pools during November to April months.

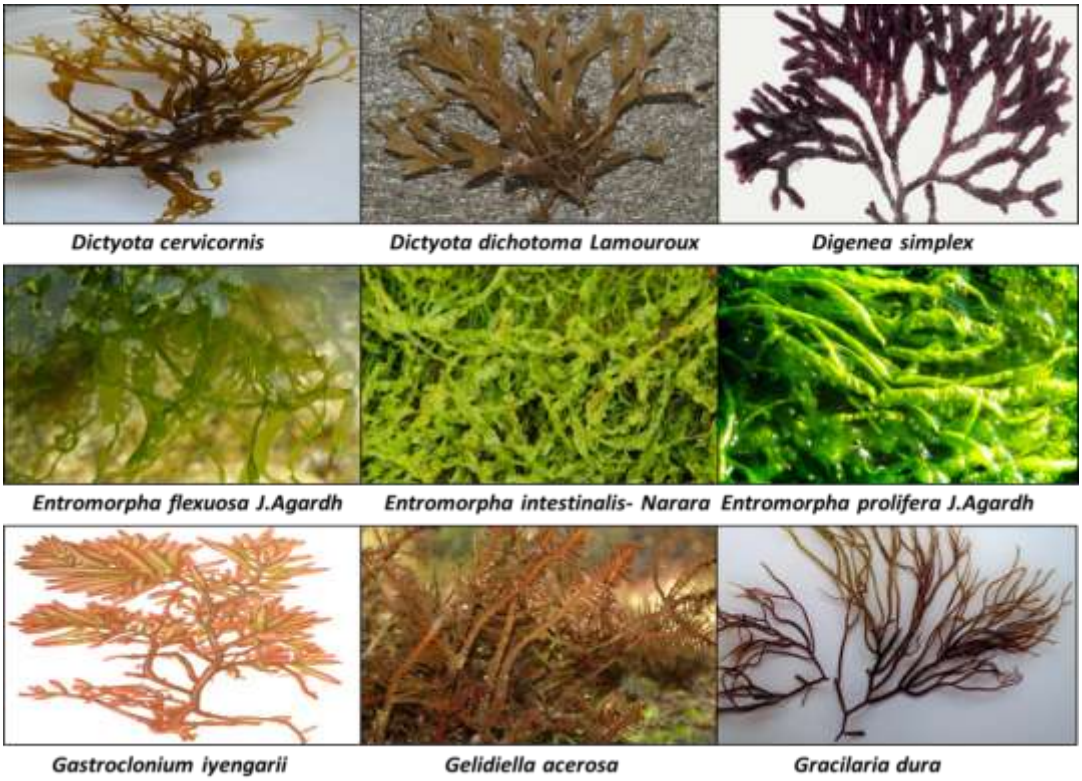
5. *Hypnea spinella* (C.Agardh) Kütz.

Pinkish to dark red plants grow on intertidal rocks attached by basal structure. Grow up to 2.5 cm long as cushion or patches. Short spines alternately arranged and sharply pointed apices. Plant grow with moderate abundance in lower mid littoral zone and infra littoral fringe from July to November.

6. *Dermonema virens*

Dark red colour plants are somewhat gelatinous and un calcified grow up to 10 cm in height. Many axes arising from the basal disc closely branched from base to the tip. Plants with scanty abundance growing on intertidal rocks and coralline stones in mid upper littoral zone, tidal pools from November to April.

List of some seaweed species identified from the Okha coast, Eco-sensitive zone, Western India.



Gracilaria foliifera

Gracillaria debilis

Gracillaria salicornia C.Agardh

Gracillaria textori De toni

Griffithsia corallinoides Trevisan

Halimeda macroloba

Heterosiphonia mulleri De Toni

Hydroclathrus clathratus

Hypnea pannosa

Hypnea spinella

Hypnea valentiae Montagne

Hypnera flagelliformis Greville

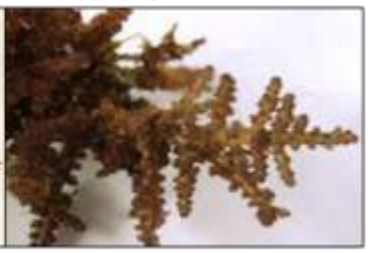
Iyengaria stellate Borgesen

Jania rubens

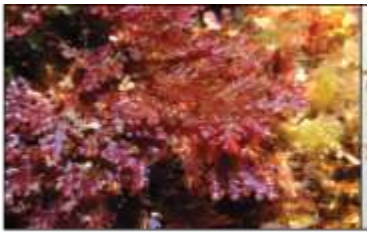
Kappaphycus alvarezii

Laurencia claviformis Borgesen

Laurencia majuscula



Laurencia papillosa C.Agardh



Laurencia sp

Liagora viscida C.Agardh

Lobophora variegata

Lophocladia lallemandi Montagne

Monostroma latissimum Wiltrock

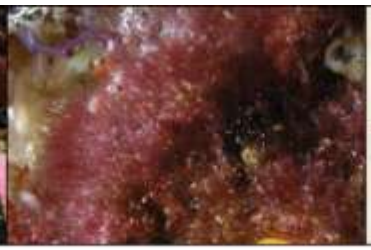
Padina boergesenii Allender &Kraft

Padina boryana

Padina tetrastromaticr Hauck

Peyssonnelia obscura

Peyssonnelia obseura



Platysiphonia delicata Cremaeles

Rhodymenia sonderi P. Silva

Sarcomema filiforme Kylin

Sarcomema scinaioides Borgesen

Sargassum cinctum J.Agardh

Sargassum cinereum J.Agardh

Sargassum johnstonii setchell &Gardner

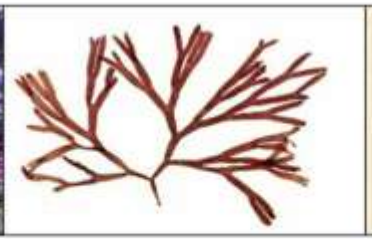
Sargassum plagiophyllum J.Agardh

Sargassum swartzii J.Agardh

Sargassum tenerrimum J.G Agardh

Scinai monoliformis

Scinaia complanata Collins



Scinaia fascularis

Scinaia hatei

Solieria robuster –kylin

Spatoglossum asperum J.Agardh

Spyridia hypnoides

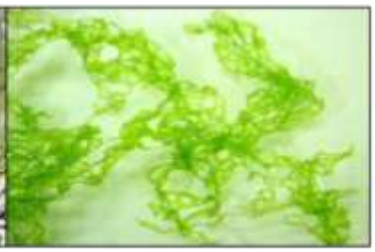
Stoechospermum marginatum Kutzing

Tricleocarpa fragilis

Udotea indica

Ulva fasciata delite

Ulva lactuca Linnaeus



Ulva reticulata forsskal

Valonia utricularis C.Agardh

Valoniopsis pachynema

Wrangalia tanegana Harvey

Ahnfeltia plicata Fries *Acanthophora specifera Borgesen* *Amphiroa fragilissima*

Anotrichium tenue C.Agardh *Bostrychia tenella* *Botryocladia leptopoda kylin*

Bryopsis pennata Lamouroux *Caulerpa racemosa J.Agardh* *Caulerpa scalpelliformis*

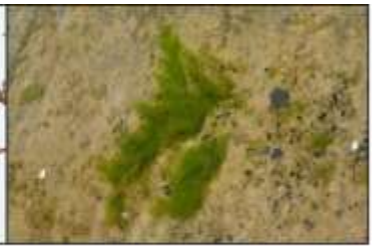
Caulerpa sertularioides S.Gmelin *Caulerpa taxifolia C.Agardh* *Caulerpa veravalensis Thivy &Charhan*

Caulerpa verticillata *Centrocesae clavulatum Montayne* *Chaetomorpha antennina*

Chaetomorpha crassa Kutzing *Champia compressa* *Champia indica bogesen*

Chlorulerpa microphysa

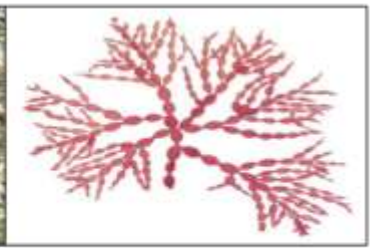
Chondria dasyphylla C.Agardh



Cladophora glomerate

Cladophora sp

Cladophoropsis javemica P.silva



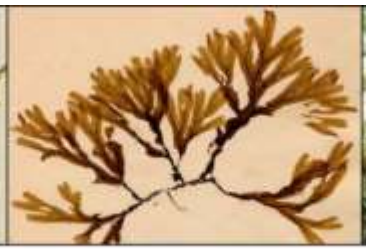
Coelarthrum muelleri Borgesen

Colpomenia sinuosa

Cryptonemia undullata

Cystoseira trinodis

Dictyopteris acrostichoides Bornet

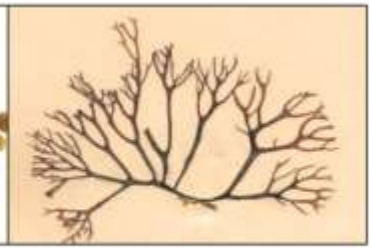


Dictyopteris australis

Dictyota bartayresiana

Gelidiella acesosa

Gelidium J.Agardh

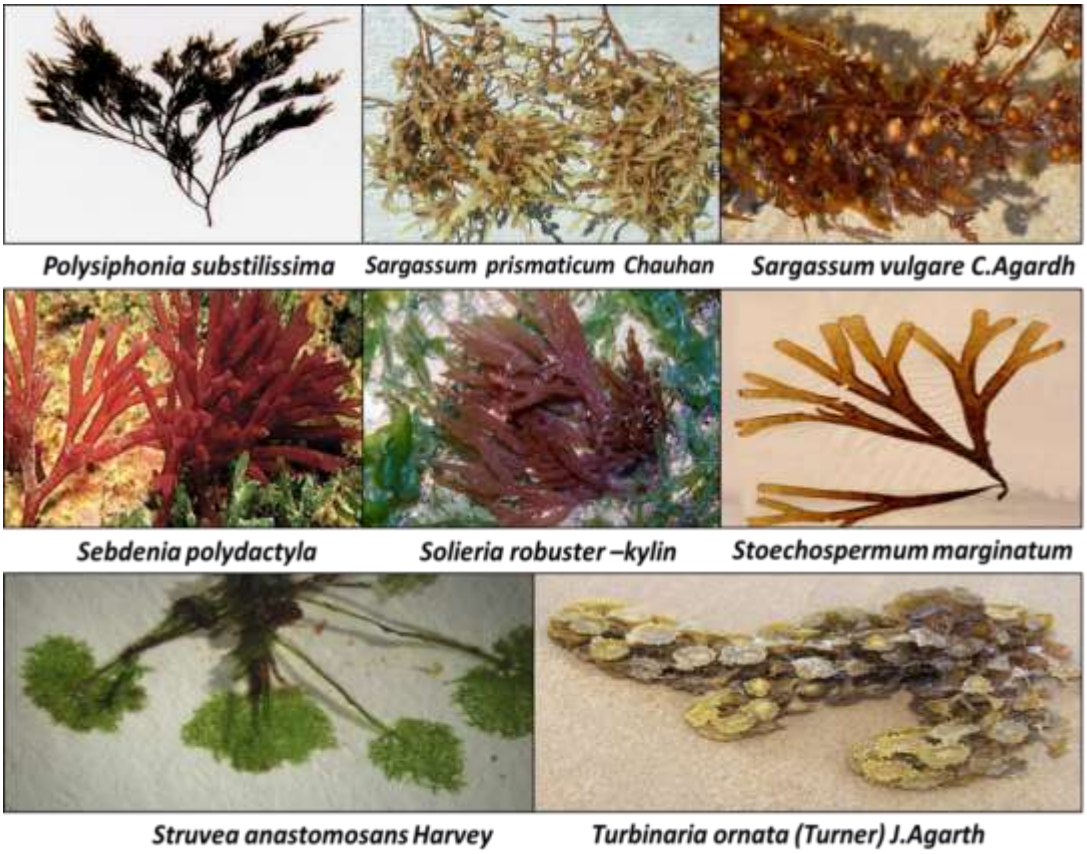


Gracillaria corticata J.Agardh

Hypner valentiae Montage

Lausencia glandulifera Kutzing

Lausencia obtuse lamouroux



3. Exploration of the nutraceutical properties, of selected seaweeds belonging to Chlorophyta, Phaeophyta and Rhodophyta.

The Eighteen seaweed species of three algal groups were analysed for nutraceutical properties: Pigments (total chlorophyll, carotenoids, phycoerythrine, phycocyanin); biochemical composition (total carbohydrate, protein, lipid and amino acids); enzymes activity- (polyphenol oxidase, peroxidase, succinate dehydrogenase); mineral composition and functional group variation of seaweeds; antioxidant activities (DPPH free radical scavenging, H_2O_2 scavenging, reducing power assay, total phenol and flavonoids); antifungal and antibacterial properties of different seaweed extracts; fingerprinting analysis of alkaloids, fatty acids and amino acids in different seaweeds. The detailed descriptions are as under:

Pigments

The pigments are key component of seaweeds involved in photosynthesis and important feature to give them morphological variation. The pigment concentration of eighteen seaweeds was evaluated. The concentration of total chlorophyll of selected species ranged from 0.0106±0.001 to 0.1258±0.01 mg/g; maximum found in *M. latissimum* (0.125±0.01), *S. tenerimum* (0.122±0.02), and *H.*

pannosa(0.106±0.01). The minimum content was observed in *U. lactuca* (0.010±0.001), *U. fasciata* (0.0126± 0.002) and *D. virens* (0.023±0.001) (Fig.11). In the present study maximum chlorophyll content was observed in Chlorophyta species. Similarly Muthuraman and Ranganathan (2004) reported maximum chlorophyll in the green alga *Caulerpa scalpelliformis* among the 12 seaweeds tested which include Phaeophycean and Rhodophycean member also (Fig.11).

The carotenoid concentration fluctuated from 0.075 ±0.002 to 1.863±0.1; the content was greater in *S. tenerimum*(1.863±0.1) followed by *H. venusta* (1.714 ±0.2) and *L. variegata* (1.619 ±0.1). The lower concentration was found in *U. lactuca* (0.075 ±0.002) followed by *U. fasciata* (0.096 ±0.002) and *M. latissimum* (0.12 ±0.002) (Fig.11). Similarly Vimala and Poonghuzhali (2013)have reported maximum concentration from *Sargassum polycystum* (1.71 µg/g) and minimum in *Amphiroa sp* (0.13 µg/g). The highest carotenoid content was recorded in the brown seaweed *D. acrostichoides*, similarly Muthuraman and Ranganathan (2004) have reported maximum carotenoid content in the brown seaweed *S. wightii*.

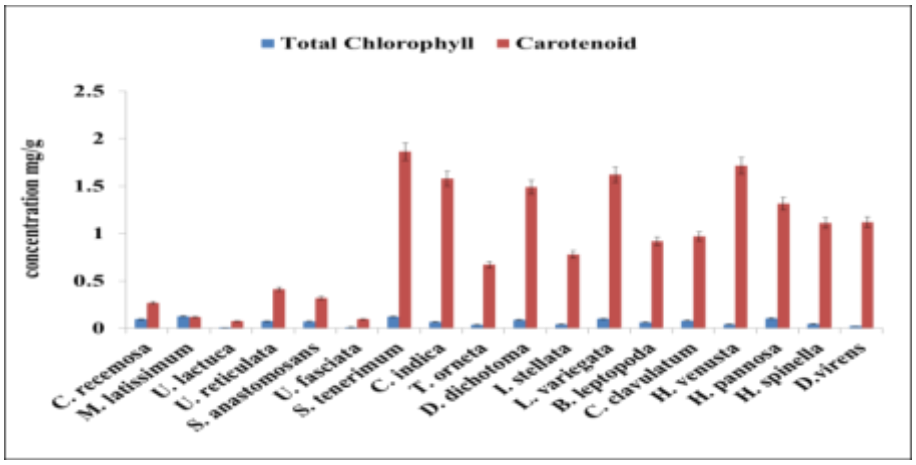


Fig. 11 Total chlorophyll and carotenoid concentration of selected seaweeds

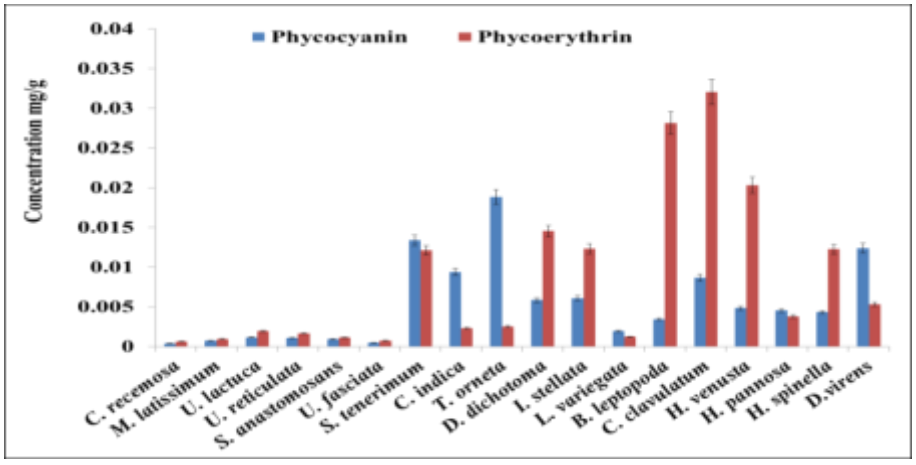


Fig. 12 Phycocyanin and phycoerythrin concentration of selected seaweeds.

The total chlorophyll, concentration found maximum in Chlorophyta species whereas minimum content was observed in Rhodophyta species. JI *et al.* (2009a) recorded maximum chlorophyll from species of Chlorophyta while comparing with species of Rhodophyta and Phaeophyta members. Carotenoid content was greater in Phaeophyta species as compare to Rhodophyta and Chlorophyta. Similarly Vimala and Poonghuzhali (2013)reported that carotenoids content was higher in brown seaweed as compare to green and red species.

Phycocyanin responsible for brown color of Phaeophyta species was found maximum compare to Rhodophyta and Chlorophyta species. Maximum concentration was recorded in T. orneta (0.01883 mg/g) and minimum was registered in *C. recemosa* (0.00039 mg/g). High amount of phycoerythrine in Rhodophyta species gives them red colored appearance was registered lower in Chlorophyta species. Highest content was found in Rhodophyta species C. clavulatum (0.032 mg/g) and lowest was noticed in Chlorophyyta species *C. racemosa* (0.00061 mg/g). Similarly Kumar *et al* (2009) received the trend for studied species according to phycoerythrin content as Rhodophyta > Phaeophyta > Chlorophyta. The results are also corroborated with the findings of Gómez *et al*. (2004) (Fig.12).

Biochemical composition

The biochemical composition of 18 species is presented in Table 4. The carbohydrate content was recorded within the range of 1.21±0.1 to 34.89±0.02% in green seaweeds, 0.50±0.01 to 13.65±0.1% in red and 3.99±0.2 to 21±0.2% brown seaweeds. The maximum concentration was recorded from *U. lactuca* (34.89±0.02%) while, minimum was found in *H. spinella* (0.50±0.01%). The carbohydrate content recorded by Manivannan *et al*. (2009)was comparably lower than currently studied species of *S. tenerimum* (23.55%) and higher than present study of *U.lactuca* (23%). Chakraborty and Bhattacharya (2012)also reported nutritional composition of some seaweed species, with carbohydrates (8.6 ± 0.7 to 42.4 ± 0.7% dw.), protein (32.4 ± 2.5% to 4.3 ± 0.4%), lipid (5.2 ± 0.4% to 0.9 ± 0.3) from the Gulf of Kutch coastline, Gujarat, India. The carbohydrate concentration reported in present investigation for the seaweeds are higher than the contents reported by several authors for daily consumed fruits and vegetables [Schmidt-Hebbel *et al*. (1992)].

The range of protein recorded in green seaweeds was 1.39±0.01 to 20.2±0.1%, brown 0.429±0.7 to 10.23±0.2% and red seaweeds 4.09±0.4 to 18.85±0.01% dry weight. Protein was found higher in species *C. racemosa* (20.2±0.1%) while lower was encountered in *C. indica* (0.429±0.7%). Burtin (2003) reported the protein content ranged from 10 to 30 % red and green seaweeds. Similar range of protein content (40.87±1.43 to 3.33 ± 0.69) was reported by Chakraborty and Santra (2008). Ito and Hori (1989) also reported low protein content (3-15% dw) in brown seaweed and high protein content (10-47% dw) in green and red marine algae. High protein levels and amino acid composition were found in the red seaweed [Fleurence (1999)]. Pillai (1957) reported that protein content did not exceed 12.5% working

on seasonal variation in the biochemical composition of different seaweeds from Mandapam coast. This is in agreement with the result obtained in the present investigation except in the case of *C. clavulatum* (18.85±0.01%), *S. anastomosans* (14.40±0.3%), *U. reticulate* (16.39±0.2%) and *C. racemosa* (20.2±0.1%) and all seaweed protein content fall within the range. The amount of protein of studied species was higher than carrots (1%), lettuce (1.2%) and red pumpkin (0.9%) and lower than Soyabeans (33.8%) reported by Tee *et al*. (1988). The protein content of the seaweeds studied in the present work is higher than that of some terrestrial plants, vegetables, seeds, grains, and eggs [Fleurence (1999); Castro-González *et al*. (1994); Rodríguez-Montesinos and Hernández-Carmona (1991)]. The contribution of biologically good proteins makes these seaweeds suitable for inclusion in both animal (especially marine species) and human diet and foods [Castro-González *et al*. (1994); Jackson *et al*. (1982); Pak and Araya (1996)].

The lipid content in green seaweeds ranged from 1.2±0.2 to 4.8± 0.2%, brown from 1±0.05 to 4.2±0.3%, red seaweeds 1.2±0.2 to 2.6±0.2% dry weight which found relatively low in seaweeds (1-5% dry weight) in the present study which is also in corroboration with the findings of Burtin (2003) and Polat and Ozogul (2008). The lipid was ranged from 4.8 to 1% dry weight where maximum was registered in *C. racemosa* (4.8±0.2) and minimum in *I. stellata* (1± 0.05). The lipid contents of some studied species are comparable to those observed by Kumari *et al*. (2010). In this study lipid content of *U. lactuca* is lower than that of previous study reported by Chakraborty and Bhattacharya (2012). The lipid content of studied species was higher compared to that of several vegetables reported by Tee *et al*. (1988) which have less than 1.0% lipid content except for soyabean. The total lipid content of these seaweeds is similar to that of cereals (rice and rye) and legumes (common beans, chick-pea and broad bean), which is less than 2%, and more than carrots, spinach and tomatoes ranging from 0.2 to 0.8% [Muñoz de Chávez *et al*. (1996); Herbreteau *et al*. (1997); Schmidt-Hebbel *et al*. (1992)].

Ash of the selected seaweeds was recorded higher amount in red species *C. clavulatum* (50± 0.5) and minimum found in *C.indica* (20± 0.2). Ash content in green seaweeds ranged from 20±0.5 to 44±0.5 %, brown 20±0.2 to 40±0.2% and red seaweeds 38±0.3 to 50±0.5%. JI *et al*. (2009b) recorded ash content of *U. lactuca* (34.8±0.20) and *C. racemosa* (36.4±0.14) which is higher than present studied of same species. Rupérez (2002) reported that the ash contents of the seaweeds were much greater than those of terrestrial plants other than spinach and few vegetables. The average mean concentration of ash was higher in red species followed by brown and red species. In general, the brown seaweeds have higher ash contents than other seaweed types [Rupérez (2002)]. In contrast to these findings, the present study reveals the higher concentration of ash in red seaweeds than green and brown. The ash value of

seaweeds in the present study was higher than some vegetables such as Swiss chard, spinach and some algae [Castro-González *et al*. (1994); Pak and Araya (1996)].

Total free amino acids of the selected green seaweeds ranged from 3.36±1.2 to 135.36±2.5 mg/g, brown species 23.76±0.23 to 364.8±8.67mg/g andred seaweeds23.52±0.12 to 58.08±5.2 mg/g. The amino acid content was encountered greater in. *D. dichotoma* (364.8±8.67 mg/g) and lower was registered in green species *U. reticulata* (3.36±1.2). Chakraborty and Santra (2008)reported the free amino acid content of *U. lactuca* (161.68± 3.11mg/g) collected from Sunderban is higher than the present findings. Chakraborty and Bhattacharya (2012)recorded amino acid content of *C. racemosa* (96.5± 3.9) is comparable with the same species in present study.

Table 4. Biochemical composition of selected seaweeds (Data ± SE, n=3)

	Carbohydrate%	**Protein %**	**Lipid %**	**Ash %**	**Amino acids mg/g**
Caulerpa recemosa	8.8± 0.2	20.2±0.1	4.8± 0.2	32±0.1	84.48±1.2
Monostroma latissimum	6.22±0.1	1.39±0.01	1.2±0.4	40±0.4	11.76±0.6
Ulva lactuca	34.89±0.02	12.72±0.2	1.7±0.2	26±0.2	135.36±2.5
Ulva reticulata	9.87±0.3	16.39±0.2	1.2±0.2	20±0.5	3.36±1.2
Struvea anastomosans	10.05±0.2	14.40±0.3	2.9±0.1	44±0.5	82.32±3.2
Ulva fasciata	1.21±0.1	3.69±0.5	2.3±0.3	24±0.3	38.4±0.98
Sargassum tenerrimum	21±0.2	5.33±0.5	2.5±0.1	30±0.1	30±1.21
Cystoseira indica	3.99±0.2	0.429±0.7	1.8±0.1	20±0.2	28.8±0.56
Turbinaria orneta	6.99 ±0.2	1.77±0.3	1.1±0.1	32±0.2	23.76±0.23
Dictyota dichotoma	5.51 ±0.04	4.96 ±0.2	4.2±0.3	38±0.2	364.8±8.67
Iyengaria stellata	20.47±0.6	10.23±0.2	1±0.05	35±0.4	181.2±5.3
Lobofora variegata	4.2±0.3	2.31±0.1	2.5±0.5	40±0.2	37.92±2.1
Botrycladia leptopoda	13.65±0.1	6.078±0.02	2.6±0.2	40±0.2	29.28±0.3
Centroceras clavulatum	7.82±0.02	18.85±0.01	1.5±0.2	50±0.5	32.64±0.4
Halymenia venusta	5.26±0.2	4.09±0.4	1.3±0.02	46±0.7	50.4±3.24
Hypnea pannosa	4.99±0.3	10.32±0.2	1.6±0.1	46±0.6	35.28±1.3

Hypnea *spinella*	0.50±0.01	10.82±0.1	1.2±0.2	48±0.6	23.52±0.12
Dermonema virens	8.19±0.1	5.62±0.3	1.8±0.3	38±0.3	58.08±5.2

Minerals composition

The elements like Sodium (Na), Calcium (Ca), Potassium (K) and Magnesium (Mg) are present in significant amounts in the seaweeds [Nisizawa (2002)]. The sodium concentration was found maximum in red seaweed species *D. virens* 15.8% while minimum amount were recorded in green seaweed species *U. fasciata* 0.5%. Potassium concentration was recorded the highest in *D. dichotoma* 24.7% and lower in *H. spinella* 0.2% (Fig 13). In the present study brown seaweeds showed lower average Na/K value (0.22) than red (2.72) and green (1.24) seaweeds. It was observed that seaweeds living in ocean contain predominantly Na and their salts. In the present study K content in the brown seaweeds is much greater, compared to green and red seaweed species. Seaweeds accumulate more K and their salts than Na El-Said and El-Sikaily (2013). However, potassium is an essential element for the growth and metabolic activities of plants and seaweeds [Sivakumar and Arunkumar (2009)]. The K/Na balance is important to control hypertension and suffer from excessive excretion of potassium [Barzilay *et al*. (2006); Zillich *et al*. (2006)]. Sodium and potassium in the present data are strongly correlated ($p < 0.05$) with green and red seaweed as they might play an important role in the electrolyte balance [Krishnaiah *et al*. (2008)] but negatively correlated with brown algae due to low Na and high K concentration.

Magnesium, being the important constituents of the chlorophyll molecules found higher in green seaweed species *U. fasciata* 12.8% whereas lower concentration encountered in brown seaweeds *T. orneta* 0.5%. Magnesium known as a calcium regulator and hypomagnesemia is one of the causes of hypocalcemia [Torres and Cannata (2003)]. Calcium recorded maximum in *H. spinella* 31.6% and minimum in *H. venusta* 0.5% which is required for the formation of bones and teeth structure, for blood clotting, and also controls nerves and muscles functions. Red, green, and brown seaweeds give Ca/Mg averages of 3.95, 0.48, and 7.82, respectively. Thus, brown seaweeds supply better calcium sources than the red and greens ones. In contrast the finding of El-Said and El-Sikaily (2013) shows higher concentration of Calcium in green seaweeds than red and brown seaweeds. Accordingly, the high significant correlation between calcium and magnesium ($p < 0.05$) might be reason for the substitution of calcium by magnesium in calcite seaweed's component. Chloride content was higher in *T. orneta* 17.2% and lower in *H. spinella* 0.6%. Iron recorded higher in *C. clavulatum* 5.6 %, while minimum found in *U. lactuca* 0.6% (Fig 14). A high variation was also found in species belonging to the same genera, which suggests that there are factors related to the environment or age of species influencing the Fe concentration [Farías *et al*. (2002)]. The minerals concentration differ with different species even in

controlled condition [(Struck *et al*. (1997); Phaneuf *et al*. (1999); Rupérez (2002); Van Netten *et al*. (2000);Farías *et al*. (2002)].

The aluminium concentration was the highest in red seaweed species especially in *H. pannosa* 6.6%, whereas the lowest found in *T. orneta* and *U. lactuca* 0.1%. Silicon (Si) is one of the most predominant elements, taking part in healing plants in response to environmental stresses [Sahebi *et al*. (2015)]. Silicon was recorded maximum in *H. pannosa* 13.9%, while minimum in *U. fasciata* 0.19%. Magnesium, aluminium and silicon were not detected in *H. venusta* species of red seaweeds. Highest phosphorus content was detected in red seaweed *D. virens* 0.7%, the lowest was found in *M. latissimum* 0.07%. Maximum sulfur/sulphur content encountered in *U. fasciata* 13% and minimum in *I. stellata* 0.1% (Fig 15). During the study, heavy metals (Pb, Cr, Ni, Co, Hg, As, Cd, Cu, Ag etc) were found below detectable levels from the seaweeds which shows non-toxic nature of seaweeds.

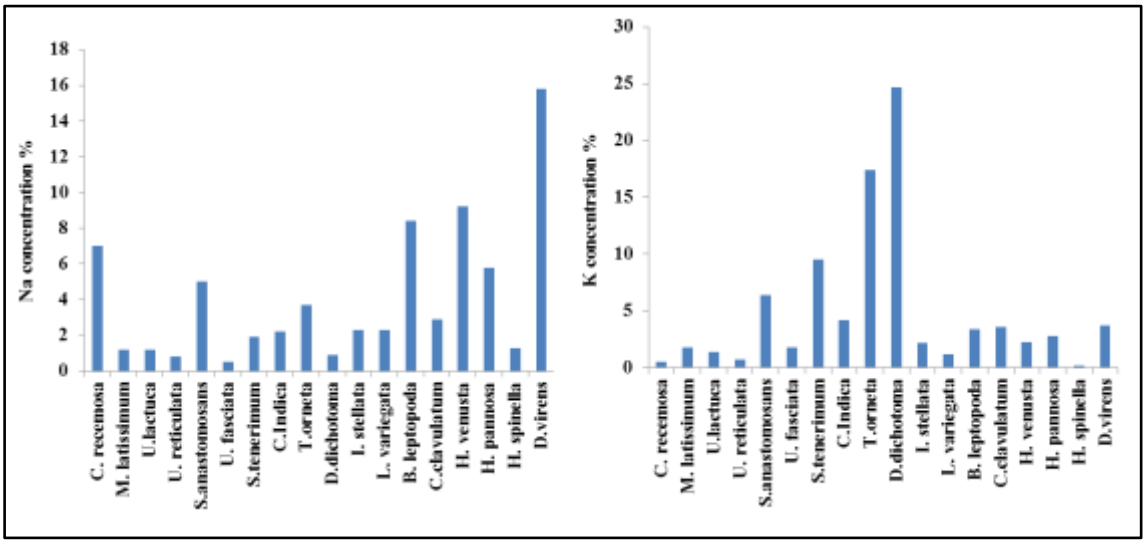


Fig 13.Na and K content of selected seaweeds.

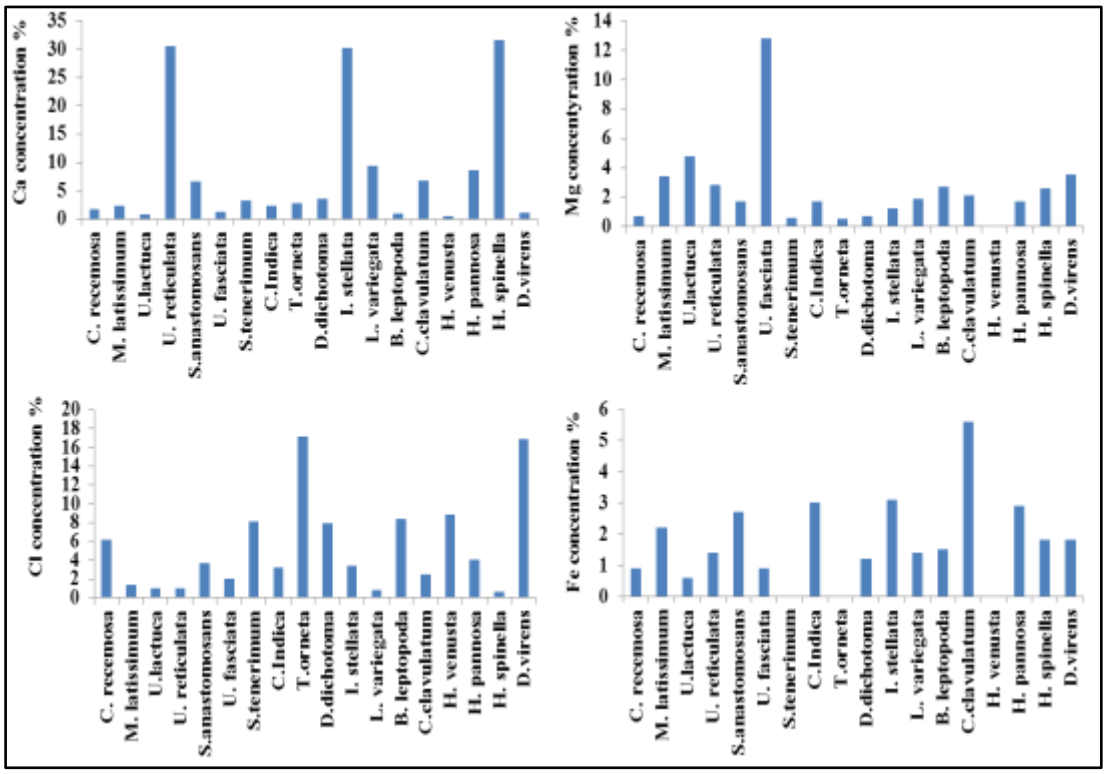


Fig 14.Ca, Mg, Cl and Fe content of selected seaweeds.

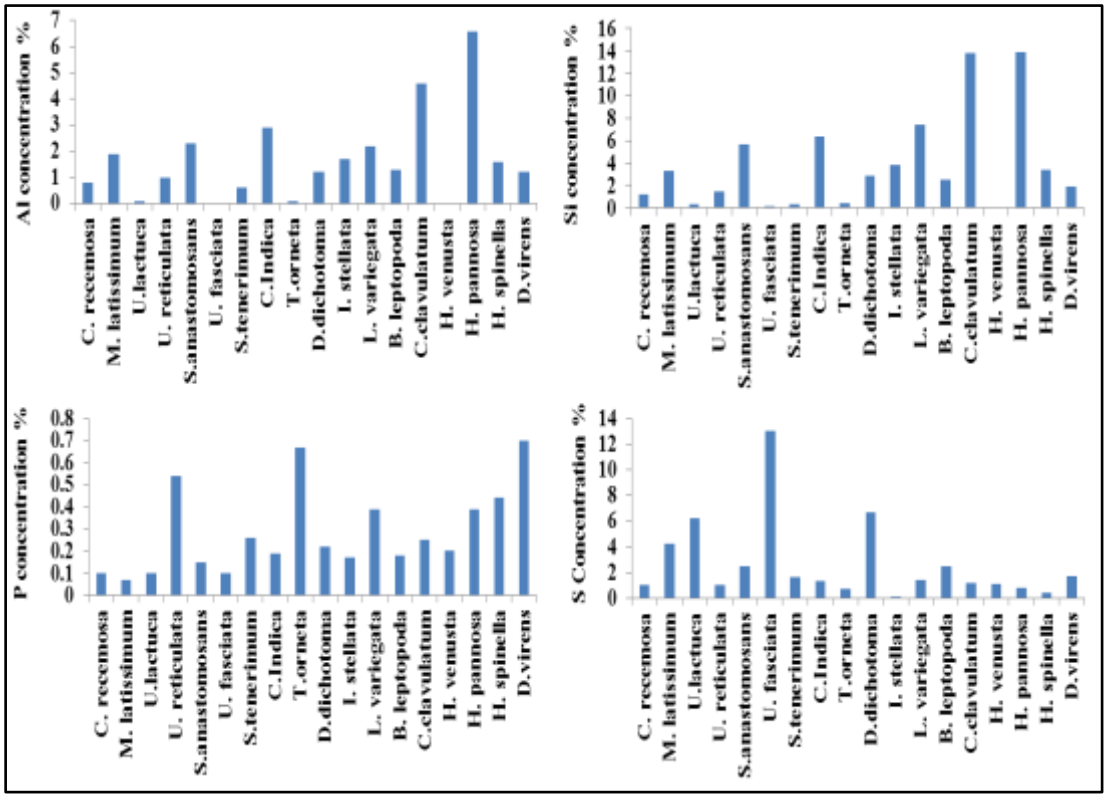


Fig 15.Al, Si, P and S content of selected seaweeds.

The cluster analysis of studied seaweed species were analysed for biochemical composition like Carbohydrates, Protein, Lipid, Ash content to know chemical variation among the species. The seaweed species belong to the same group has variation in their chemical composition and this was observed that

some of the species were more closed to species of another group (Fig 16). A dendrogram was obtained by Ward hierarchical clustering, using squared Euclidean distance for 18 seaweed species (Fig 17). Four different clusters were obtained, whereby all the species of different groups were grouped together according to similarity in their biochemical composition, except *U. lactuca*, which formed a separate group. This indicates the biochemical composition of present study was not fulfil to separate the species belongs to same class and same group because of non-significant differences among the species.

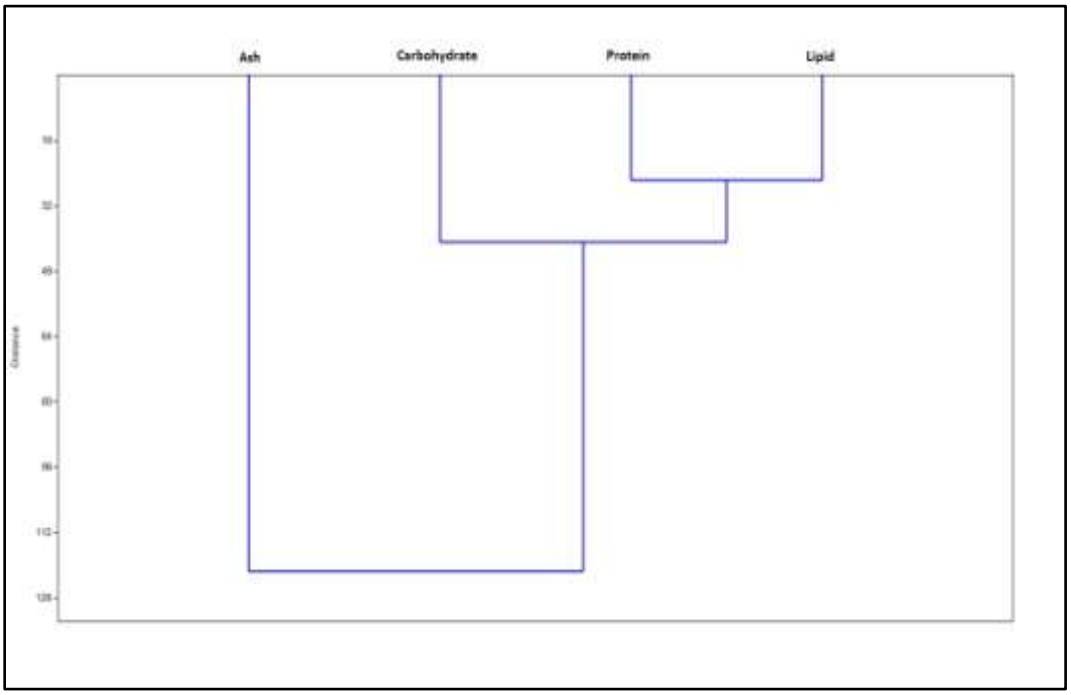


Fig 16.Cluster analysis of biochemical content of eighteen seaweed species.

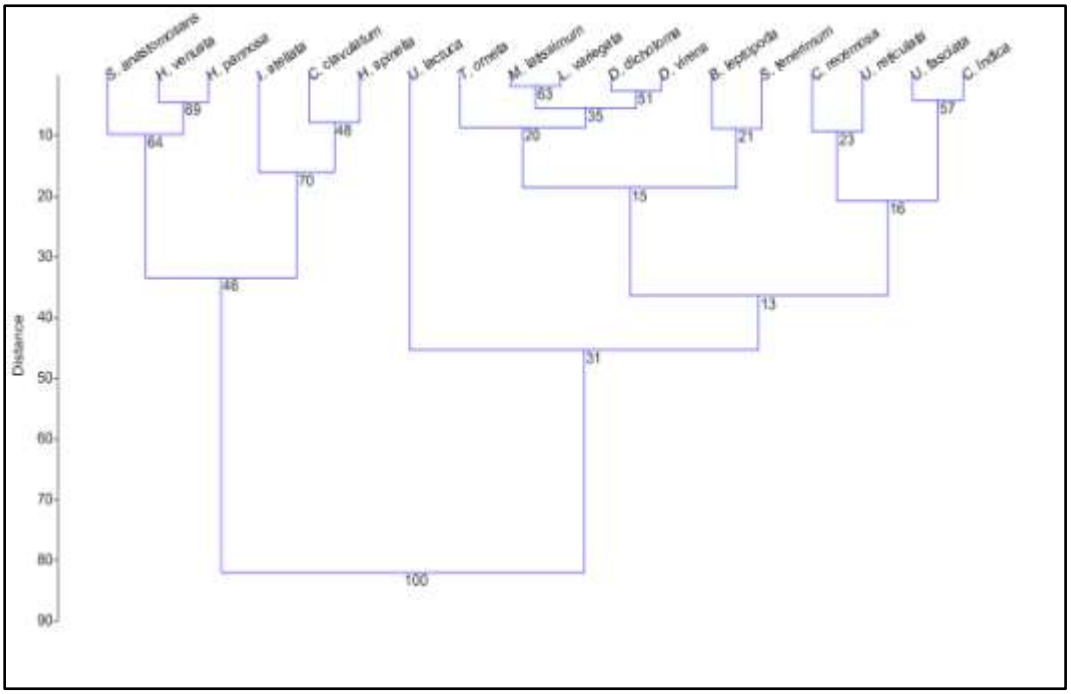


Fig 17.Dendrogram for hierarchical cluster analysis of eighteen seaweed species.

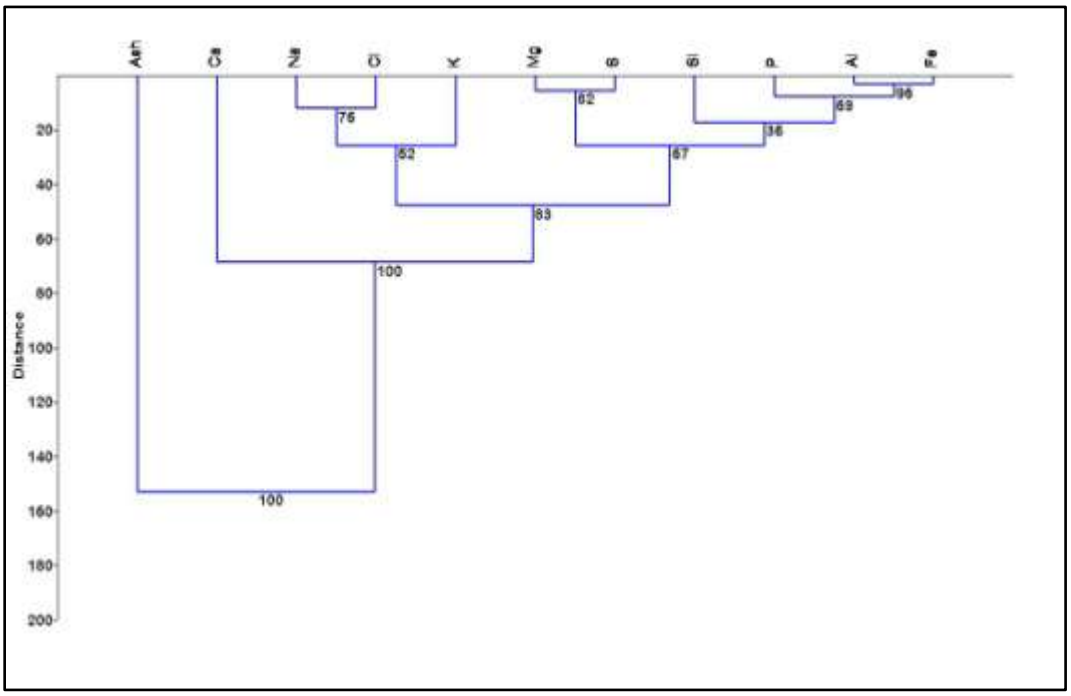


Fig 18.Cluster analysis of Ash and minerals content of selected seaweed species.

The correlation between biochemical compositions of species indicates that carbohydrate was positively correlated with protein only by the value of 0.25 while protein shows positive correlation with lipid and ash content of all the species with 0.20 and 0.15 respectively. The lipid content negatively correlated with carbohydrate (-0.04) and ash (-0.16) content, similarly ash content negatively correlated with carbohydrate (-0.12) and lipid (-0.16). This is confirmed by clustered analysis of biochemical composition where ash content showed negative correlation with other compounds (Fig 16). The cluster analysis indicates ash content positively correlate with minerals contents (Fig 18). It is known that higher levels of ash are associated with higher amounts of mineral elements [Rodrigues *et al*. (2015)]. The biochemical compounds of all the species have shown non-significant difference at P>0.05.

Principal Components Analysis (PCA)

Principal Components Analysis (PCA) was performed on the nutrient composition (biochemical and minerals) to assess the relationship between species belonging to the three different classes. In the scores plot, species clustered according to their nutrient composition (Fig 19), suggesting that each species has a distinct biochemical profile and supporting earlier evidence that lipid content may be a biochemical marker for each taxonomic group [Kumari *et al*. (2010)]. Dawczynski *et al*. (2007) also mention that seaweeds can be classified as red, brown or green algae depending on their nutrient and chemical composition. The fig shows clear difference between the species belonging to different classes. The placement of Chlorophyta and Phaeophyta in the right quadrants indicate less variation in the biochemical composition between them while Rhodophyta in the right quadrants, specify the more variation in comparison with o two groups mainly variation in the ash content.

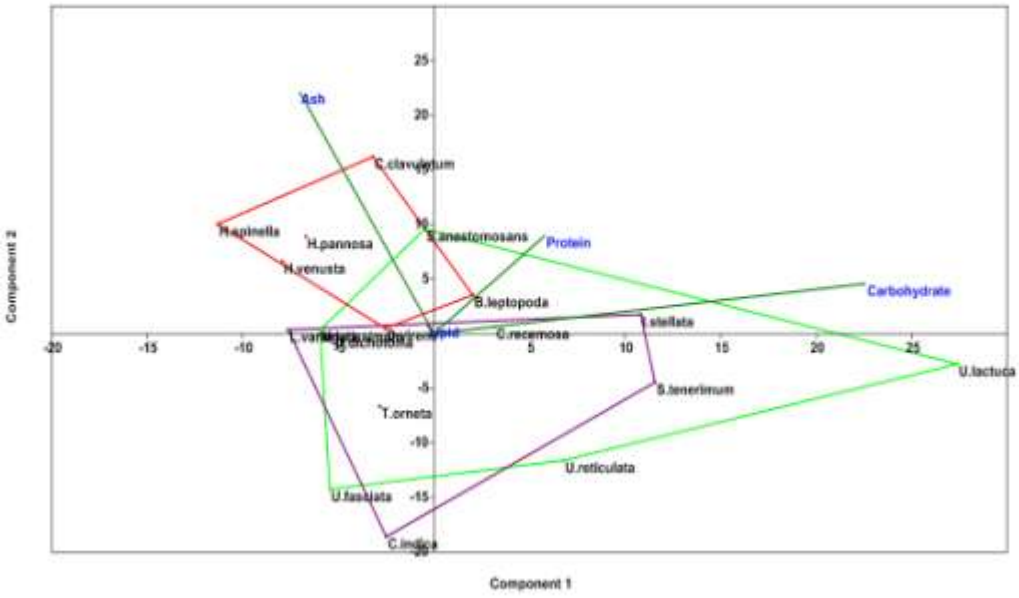


Fig 19. PCA plot of the selected seaweeds biochemical profiles

Enzyme activity

The activity of different enzymes like Succinate dehydrogenase, Polyphenoloxidase and Peroxidase of selected seaweeds has been shown in Fig 20. Succinate dehydrogenase is mitochondrial iron containing enzyme that involved in many metabolic processes mainly, respiration and energy production in the cell.

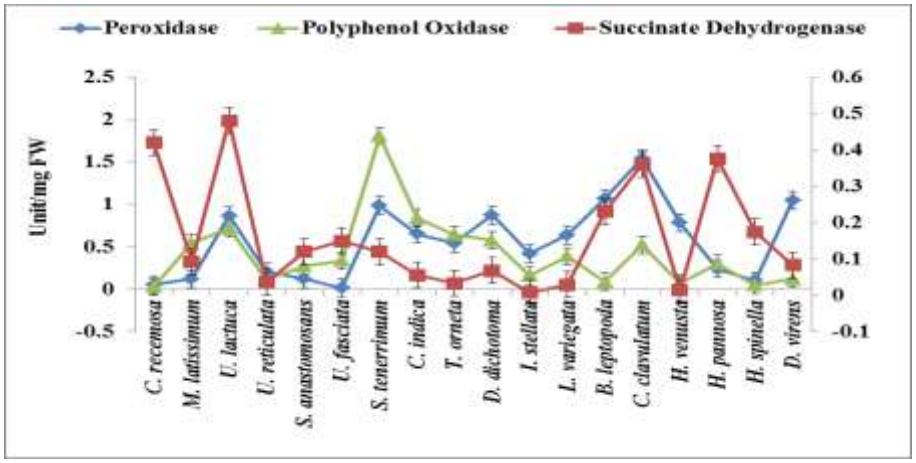


Fig 20. Succinate dehydrogenase Peroxidase, and Polyphenol Oxidase activity of selected seaweeds.

The maximum activity was observed in Chlorophyta members compared to Phaeophyta and Rhodophyta. The enzyme activity ranged from 0.008 to 0.481 unit/mg FW, where highest activity was recorded in *U.lactuca* and minimum was found in *I. stellata*. Peroxidases are group of enzymes containing heme cofactor in their active sites involved in plant defense mechanism. The enzyme activity recorded in the ranged from 0.0165 to 1.542 unit/mg FW. The maximum activity was registered in *C.clavulatum* followed by *B.leptopoda* and *D.virens* while minimum was encountered in *U.*

*fasciata*and*C. recemosa*. The enzyme polyphenol oxidases have properties to catalyze different phenolic compounds from monophenols to ortho-diphenols and vice versa, they are also known as copper enzymes. Polyphenol oxidase activity was found higher in Phaeophyta followed by Chlorophyta and Rhodophyta. The enzyme activity ranged from 0.0034 to1.807 unit/mg FW. The significant values were obtained from *S.tenerrimum*, *C.indica* and *T.orneta* whereas lowest was observed in *C. racemosa, H. spinella* and *H.venusta*.

Variation in different enzymes activity of seaweeds was explored by Kumar *et al*. (2010) from the Okha coast. Polyphenol oxidase activity of *U.lactuca* and *C.racemosa* is comparable with the results obtained by Kumar *et al*. (2010) whereas succinate dehydrogenase and peroxidase activity were highly fluctuated compared to the studied species. Peroxidase activity has been studied by many researcher in several species of plant, vegetables and soyabeans [Nagaraja *et al*. (2009) ; Sakharov (2004); Bania and Mahanta (2012)] but few attempts were made to study enzymatic variation in seaweeds. Many researchers reported the occurrence of various antioxidants, polysaccharides, dietary fibers, minerals, proteins, amino acids, vitamins, polyphenols and carotenoids in seaweeds [Burtin (2003)].

Antioxidants compounds produce by seaweeds protect them from environmental stresses [Lesser (2006)]. In the intertidal zones of rocky beaches around the world, marine algae are exposed to constantly fluctuating and extreme abiotic conditions in, for example, temperature, salinity, pH, and toxic metal pollution, induce production of reactive oxygen species [Davison and Pearson (1996), Mittler (2002), Pinto *et al*. (2003)]. Several endogenous defence mechanisms, including enzymatic and non-enzymatic, act in the cells to provide protection against oxidative damage. Important enzymes that scavenge reactive oxygen species (ROS) include polyphenol oxidase, peroxidase and catalase [Noctor and Foyer (1998), Ahmad *et al*. (2012)] and nonenzymatic metabolites like ascorbic acid [Khan and Ashraf (2008)], salicylic acid [Gautam and Singh (2009)], proline and quercitol [Rached-Kanouni and Alatou (2013)] and low concentration of H_2O_2 [Wahid *et al*. (2007)] that quench these oxygen radicals and protect membranes from injurious effects of ROS [Foyer and Noctor (2003)].

Functional group variation

FT-IR analysis shows different functional groups present in studied species (Table 5). IR patterns of each species revealed clear difference in their frequencies and variation in the functional groups. Chlorophyta species is having different frequencies and functional groups but there were some common frequency recorded in all the six species, at 3435.79, 2925.19, 2856.55, 1632.99, 1384.67, 1316.12, 665.25, 779.83, 520.56 cm^{-1} which indicate the presence of amino acids, aliphatic compounds, ester, pectin, lignin, alkenes, sulfates, fatty acids. The frequencies at 471.51 and 465.78 cm^{-1} shows weak S-S Stretching, disulfides bond were only recorded in *C. racemosa* and *S. anastmosans* species, respectivelywhereas species of Ulvacae family, *U. lactuca, U reticullata, U. fasciata* and *M. latissimum*

shows the similar type of pattern in IR frequency. *U. lactuca*, shows the frequency at 3428.90, 2959.01, 2925.19, 2856.55, 1631.88, 1421.18, 1384.67, 1314.83, 1112.64, 851.59, 779.69, 665.64, 620.04, 522.60 cm-^{1} which represent polysaccharides, amino acids, aliphatic compounds, pectin, cutin, alkanes, carbohydrates, glucose, fatty acids, phosphates compounds. Frequencies at 3430.93, 2959.01, 2925.34, 2856.55, 1629.51, 1458.49, 1413.53, 1384.57, 1315.32, 1264.36, 1104.62, 984.41, 855.68, 779.99, 621.42, 522.60 cm^{-1} shows presence of amino acids, aliphatic compounds, ester, pectin, cutin, lignin, carbohydrates, polysaccharides, glucose, fatty acids, sulfates and phosphates compounds in *U. fasciata*. Presence of compounds amino acids, aliphatic compounds, ester, pectin, cutin, lignin, cellulose, carbohydrates, starch and polysaccharides, glucose, fatty acids, sulfates, phosphates, disulfides at the frequencies 3435.79, 2923.26, 2856.55, 1632.99, 1428.15, 1384.03, 1335.88, 1316.12, 1249.83, 1164.24, 1111.11, 1059.59, 1035.50, 869.98, 827.07, 779.83, 665.25, 618.64, 557.34, 520.56 cm-1.

M. latissimum shows the frequencies at 3436.31, 2925.06, 2852.45, 1629.83, 1456.44, 1415.58, 1384.67, 1315.03, 1107.48, 1035.50, 857.72, 779.89, 619.41, 520.56 cm-1 indicate presence of amino acids, aliphatic compounds, ester, pectin, cutin, lignin, carbohydrates, starch and polysaccharides, glucose, fatty acids, sulfates, phosphates.*S. anastomosans* and *C. racemosa* shows the same range of IR frequences except 1259.60, 1166.28, 908.81 cm^{-1} which is only present in *C. racemosa* indicate the presence of lignin, cellulose and polysaccharides compounds (Fig. 21&22).

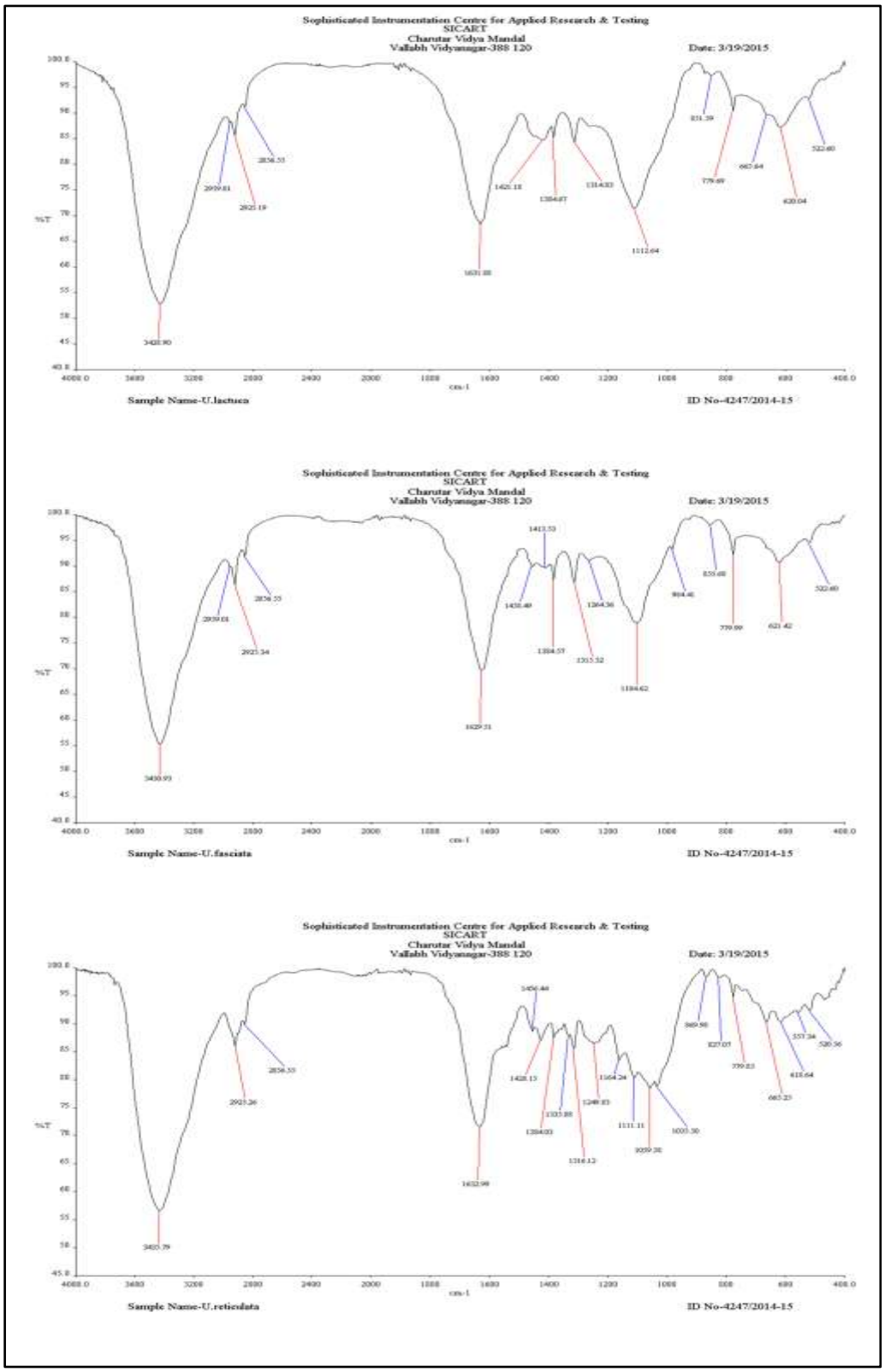


Fig 21. Functional groups analysis of *U.lactuca*, *U.fasciata* and *U.reticulata*.

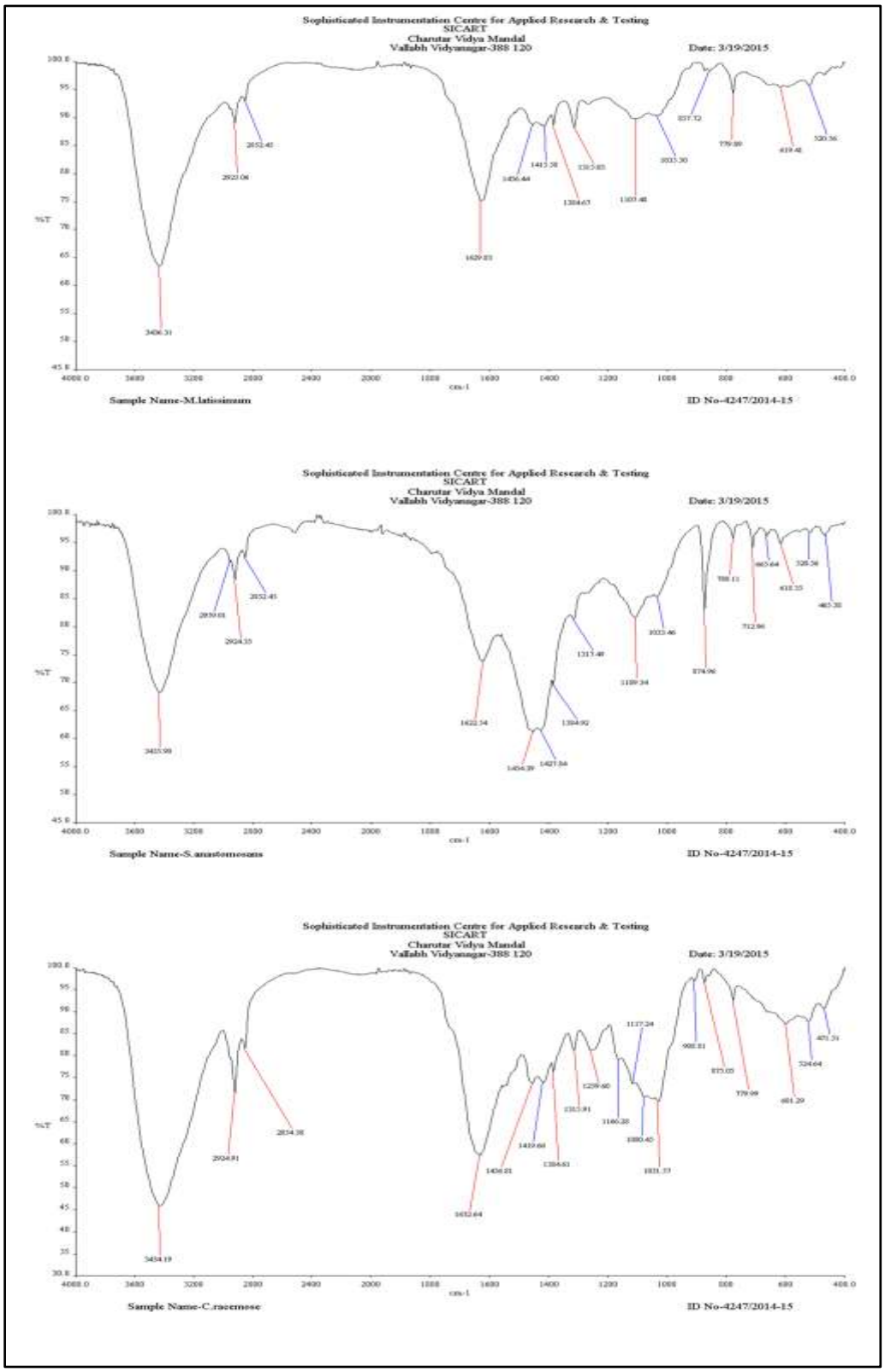


Fig 22. Functional groups analysis of *M. latissimum*, *S. anastomosans* and *C. racemosa*.

There are some common frequencies recorded from almost all the Phaeophyta species are 1035.50, 1384.92, 2854.48, 2924.59, 3435.79 cm^{-1} which represent functional groups; S=O Stretching (sulfonides), S=O Stretching (Sulfonamides), C-H Symmetry stretching (aliphatic), CH3 and CH2

stretching, OH Stretching indicating presence of starch and polysaccharides, lignin, aliphatic compounds and amino acids. *D. dichotoma*, *I. stelleta* and *L. variegata* were recorded with few IR frequencies at 523.61, 779.90, 1315.34 and 1630.12 cm^{-1} specify the functional group, iodo components P-O stretch, out of plane N-H wagging, S=O stretching (sulfone), C=O stretching indicate presence of phosphates, fatty acids, alkanes, ester and pectin compounds. *C. indica* shows very unique pattern of IR frequency compare to other species except 1114.74 and 1466.96 cm^{-1} which was also detected in *D. dichotoma* and *I. stelleta*, respectively. IR frequencies at 802.55 and 1101.91 were only recorded from the S. *tenerrimum*, represents the out of plane C-H bending and Si-O functional groups for glucose, galactose. *D. dichotoma* shows exclusive frequencies at 471.51, 618.36, 931.28 and1040.16 cm^{-1} indicates occurrence of S-S Stretching, C=S Stretching (sulfides), =C-H bend, S=O Stretching (sulfonides) functional groups for disulfides, sulfates, polysaccharides, Starch and polysaccharides. 1268.45 and 2963.11 frequencies were only recorded from L. variegate while 810.72, 855.68, 1057, 1421.81, 1532.05, 3430.63 were encountered only in from *T. orneta* . *I. stellata* also showed different frequencies at 657.47, 696.29, 712.89, 798.46, 1031.72, 1086.59, 1264.36, 1468.93, 2959.01 cm^{-1}were not detected in any other Phaeophyta members (Fig. 23&24; Table5).

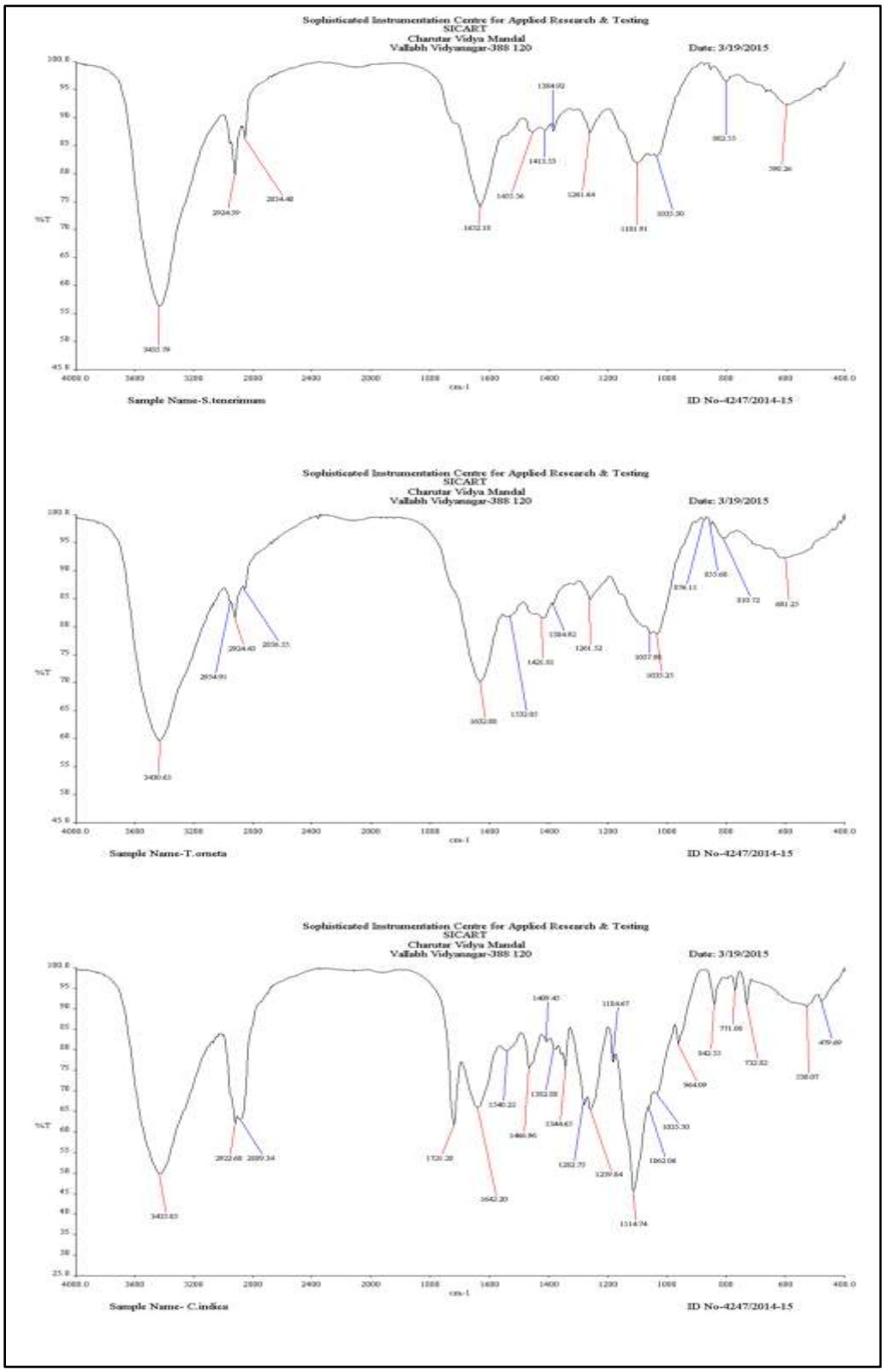


Fig 23. Functional groups analysis of *S. tenerrimum*, *T. orneta* and *C. indica*.

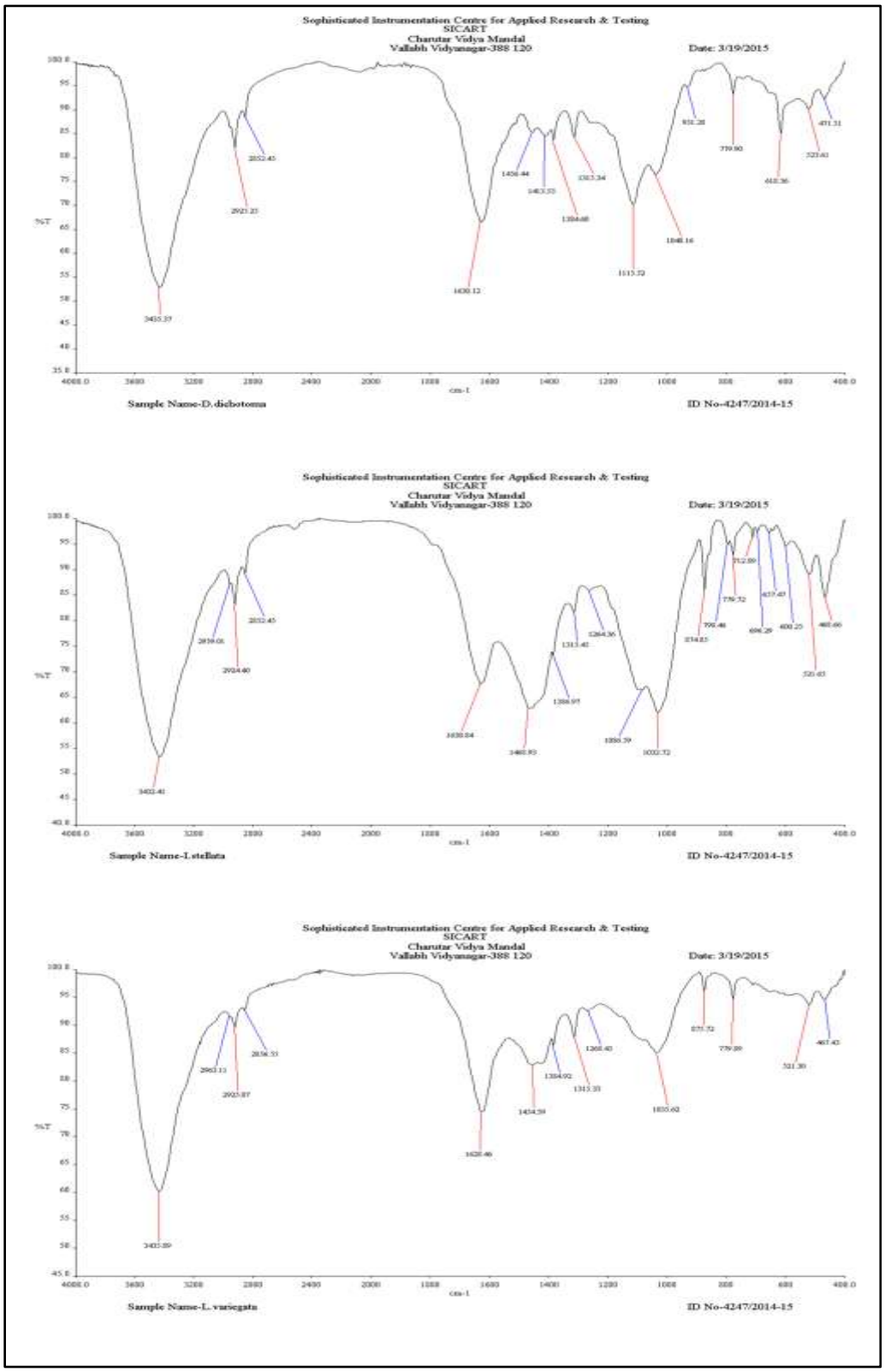


Fig 24. Functional groups analysis of *D. dichotoma*, *I. stellate* and *L. variegata*.

In Rhodophyta species common frequencies at 468.79, 523.85, 779.25, 1315.45, 1384.92, 2852.45, 2925.04, 2959.01, 3435.21 cm^{-1} indicates presence of S-S Stretching, iodo components P-O stretch, out of plane N-H wagging, S=O Stretching (sulfone), S=O Stretching (Sulfonamides), C-H Symmetry

stretching (aliphatic), N-H Stretching, CH3 and CH2 stretching, O-H stretching functional groups indicate disulfides, phosphates, fatty acids, alkanes, lignin , aliphatic compounds and amino acids compounds. *B. leptopoda* were recorded with frequencies at 657.47, 1102.54, 1192.84, 1422.18, 1642.08 cm^{-1} indicates presence of sulphate, cellulose, carbohydrates, cutin, ester and pectin. Frequencies at 1352.45, 1425.79, 1624.36, 1795.65 cm^{-1} were only found in *C. clavulatum* shows presence of alkanes, cutin, ester and pectin compounds. *D. virens* showed IR pattern at 604.04, 898.54, 1042.84, 1253.53, 1322.71, 1417.62 cm^{-1} revealed C=S Stretching (sulfides), Out of plane C-H bending , S=O Stretching (sulfonides) , C-O Stretching (phenols), S=O Stretching (sulfone), O-H bending functional groups indicated sulphate, polysaccharides, lignin, alkanes and cutine compounds. 584.15, 620.68, 1027.18, 1257.95, 1413.53, 2963.11 cm^{-1} frequencies were noted from *H. vanusta* indicates presence of phosphates, sulfates, starch, lignin, cutin, aliphatic compounds. 651.34 and 1896.70 frequencies were unique to the *H. pannosa* species whereas 796.42, 861.33, 1734.35 and 1789.52 cm^{-1} were only found in *H. spinella*. Both Hypnea species recorded with some common frequencies at 874.91, 1031.96, 1469.67, 1629.83 cm^{-1} shows glucose, galactose, starch, cutin, ester, pectin and functional groups such as Out of plane C-H bending, S=O Stretching (sulfonides), C- O Stretching, C=O Stretching and N=O asymmetric (Fig. 25&26; Table 5)

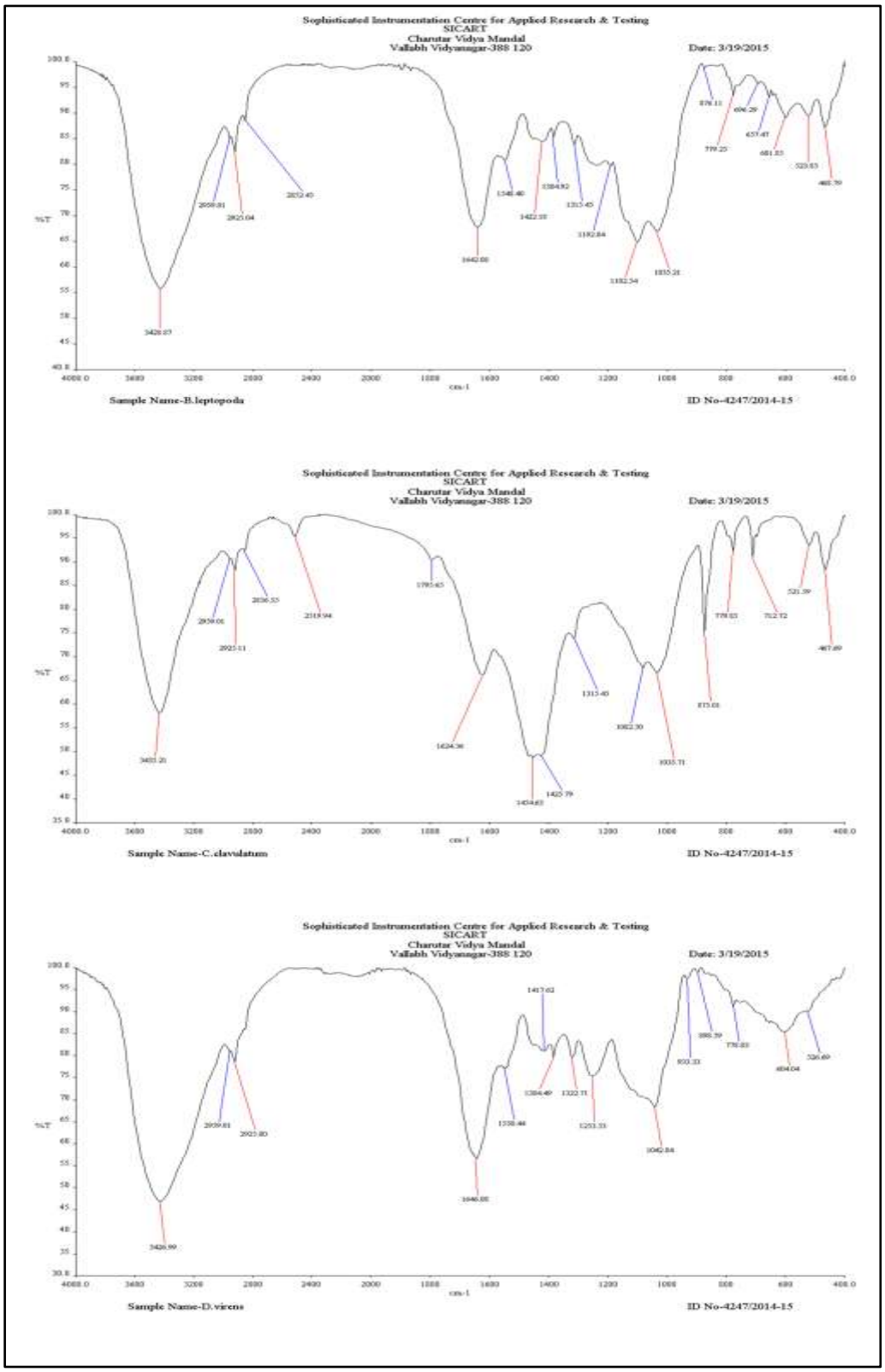


Fig 25. Functional groups analysis of *B. leptoptoda*, *C. clavulatum* and *D. virens*.

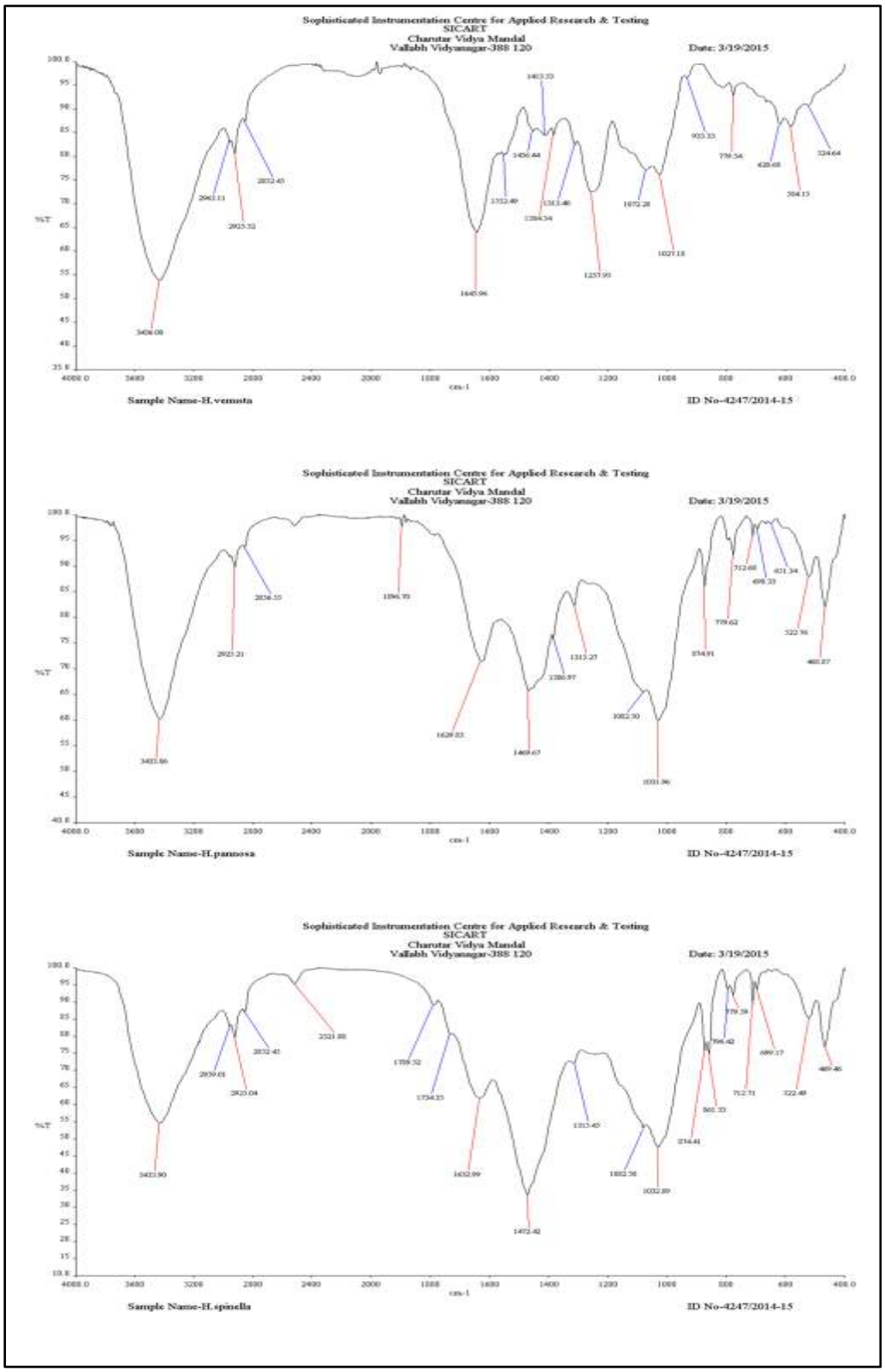


Fig 26. Functional groups analysis of *H. venusta*, *H. pannosa* and *H. spinella*.

Table 5 FT-IR frequency, intensity estimation and functional group of studied species (Kannan 2014).

Frequency cm-^{1}	Intensity estimation	Functional Groups	Compound
3435.79	S	O-H Stretching	Amino acids
2959, 2925	W	N-H Stretching CH3 and CH2 stretching	Aliphatic Compounds
2856,2852,2854	W	C-H Symmetry stretching (aliphatic)	Aliphatic Compounds
1632, 1631,1622	S	C=O Stretching, N=O asymmetric Stretching (Nitrate)	Ester, Pectin
1458, 1456, 1454, 1428, 1415,1413	W	C- O Stretching O-H bending	Cutin
1384	W	S=O Stretching (Sulfonamides)	Lignin
1316	W	S=O Stretching (sulfone)	Alkanes
1264, 1259, 1249	W	C-C-O,S=O Stretching C-O Stretching (phenols)	Lignin
1166, 1117,1112,1107, 1101	W	C-F Stretching Si-O	Cellulose Carbohydrates
1080,1035, 1040	M, S	C-F Stretching S=O Stretching (sulfonides)	Cellulose Starch and polysaccharides
984,908, 931		=C-H bend	polysaccharides
875,869, 857, 851, 827	W	Out of plane C-H bending	Glucose, Galactose
780, 712	W	Out of plane N-H Wagging	Fatty acids
665, 621, 601	W	C-S Stretching C=S Stretching (sulfides)	Sulfates
557,524,522,520	W	C-Cl stretch alkyl halides Brominated and Iodo Components P-O Stretch	Phosphates
471, 465	W	S-S Stretching	Disulfides

[S-strong, W-weak, M- medium]

FT-IR is a valuable tool for measuring many chemical constituents in plants [Lammers *et al*. (2009)] and seaweeds and it is used to reveal some qualitative aspects regarding the organic compounds. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. The use of FTIR analysis in plant biology is quiet limited compared to its applications in other areas. The FTIR analysis in the present study revealed the important present chemical compounds in the seaweeds. The strong absorption bands at 3435 and 1632 cm-1 in all the species C-H, O-H and NH stretching vibrations, characteristic of the presence of amino acids [Rao *et al*. (1964)]. CH3 and CH2 groups [Lammers *et al*. (2009) and Bellamy (1975)] indicative of the chlorophyll groups at 2925 and 2856 cm-1.The peak around 1622-1653 cm-1 of the spectrum due to the C-O stretching and N-O asymmetric stretching indicative ester group [Stewart (1996)]. The absorption band at 1249 cm-1, 1259 cm-1 and 1264 cm-1 are due to S=O (sulfate esters) [Silva *et al*. (2005); Boeriu *et al*. (2004)]. The Seaweeds contain a strong absorption band at 1035 cm-1 due to S=O stretching vibration also indicates the starch and polysaccharides content in the sample. The absorption peak around 800 - 860 cm-1 may correspond to the S=O, which indicates the presence of the sulfonate group, observed generally in seaweeds [Figueira *et al*. (1999)]. The weak absorption band observed near 600 – 670 cm-1 due to C-S and C=S stretching vibrations (sulfides). The studied species contain all organic compounds, amino acids, chlorophyll, amides, lignin, carbohydrates and starch pertaining to a healthy food.

Antioxidant activities

The methanolic extracts of selected seaweeds were tested for different antioxidant activities.

Hydrogen peroxide scavenging assay

The ability of seaweeds extracts to scavenge H_2O_2 increased with elevated concentrations (Fig. 27). Though H_2O_2 itself is not very reactive, it generates highly reactive species such as OH • by interacting with metals (Fe^{2+} or Cu^{2+}) and superoxide anions in the Haber-Weiss reaction. Therefore, removal of H_2O_2 is very important for cell function and may be a valuable property of these seaweed species. The highest H_2O_2 scavenging activity was recorded in *S. tenerrimum* (32.35%) followed by *U. lactuca* (22.61 %) and *C.clavulatum* (18.51%). During the experiment it was observed that Pheophyta members have highest scavenging activity followed by Chlorophyta and Rhodophyta members. Dhinakaran *et al*. (2015)studied antioxidant activities in the marine algae *Valoniopsis pachynema* and *Sargassum swartzii* using methanol extracts. Parthiban *et al*. (2013) were studied H_2O_2 scavenging activities where maximum inhibition was observed in *Dictyota dichotoma* (45.08%) and lowest were recorded in *Enteromorpha intestinalis* (25.29%) is higher compare to studied species.

DPPH radical scavenging activity

DPPH (1,1-Diphenyl-2-picryl-hydrazyl) have been used extensively as a free radical to evaluate reducing substances [Cotelle *et al*. (1996)]. Methanol extracts of seaweeds significantly reducedDPPH radicals in dose-dependent manners. The DPPH scavenging activity was found maximum in Phaeophyta species such as *S.tenerrimum* (34.90%) followed by *D. dichotoma (32.25%) and C.indica (30.28%),* while minimum was recorded in Rhodophyta species; H. spinella (9.38%), H. venusta (12.36%) and H. pannosa (12.76%). The scavengingaction in *U. lactuca* and *S.tenerrimum* (Fig. 27) was greater thanthat of *Porphyra vietnamensis* (Pise *et al*. (2010). In agreement with our study, Wang *et al*. (2009) also found that brown algae contained higher amounts of phenols and DPPH radical scavenging activity than red and green algae. Some of the *Caulerpa*species have been reported to possess the potential to reduce free radicals [Kumar *et al*. (2011b)]. Ganesan *et al*. (2008)also observed high DPPH radical scavenging activity in methanol extract of Phaeophyta species *Turbinaria conoides*. The reducingaction of these species may be facilitated through thedonation of hydrogen to a free radical, which is reduced toa nonreactive species [Wang *et al*. (2008)]. A elevated DPPH scavenging capacity in brown and green seaweeds compared to red seaweeds has been reported in several studies [Devi *et al*. (2008); Wang*et al*. 2009; Zubia *et al*. (2009)]. However, similar tendency was observed by Jiménez-Escrig *et al*. (2001) who reported that brown seaweeds generally exhibited better DPPH scavenging capacity than red seaweeds.

Reducing power

Reductones present in seaweeds are reason for reducing capacity and are involved in the prevention of chain initiation, binding of metal ions, decomposition of peroxides and radical scavenging [Yıldırım *et al*. (2001)].The reducing power of seaweeds in methanolic extract was more in comparison with the known antioxidant ascorbic acid (ASA) (Fig. 27). Similar findings have been reported by Duh (1998) in the *Arctium lappa* L. A significant correlation ($p < 0.05$) between DPPH scavenging potential and reducing power indicates the antioxidant potential of these species (Fig. 28).

Total phenolic compound

Phenolic compounds serve as important antioxidants because of their ability to donate an electron to form stable intermediate radical as well as these compounds in plants is responsible for multiple biological effects. The total phenolic content in representative species of each group (*U. lactuca, S. tenerrimum* and *C. clavulatum)* was 18.1, 32.5 and 15 mg/g respectively (Fig.27). The phenolic content of *S. tenerrimum* is close to the *G. oxysperma* (33.91mg/g) species reported byPise *et al*. (2012)from central west coast of India. The non-enzymatic antioxidants in the form of phenolic compounds play vital role in neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides [Osawa (1994)]. The presence of more phenolic content in *S. tenerrimum* and

other species was sufficient to enhance their value as an important dietary supplement control the diseases related to mutagenesis, teratogenesis and carcinogenesis [De Flora and Ramel (1988)]. In members of Chlorophyta phenolic content was very less than in the members of Phaeophyta [Heo *et al*. (2005)]. These phenolic compounds have many hydroxyl groups in their structure and have ability to catch free radicals [Hatano *et al*. (1989)].

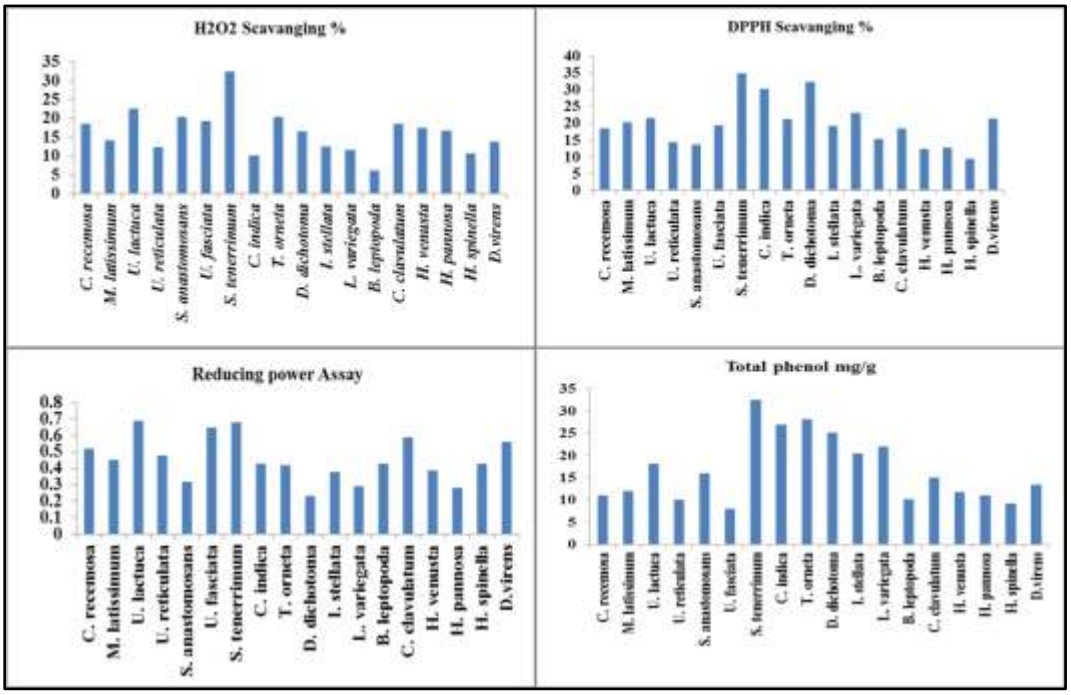


Fig27.H2O2 scavanging activity, DPPH free radical scavenging assay, Reducing power assay and Total phenol content of eighteen seaweed species.

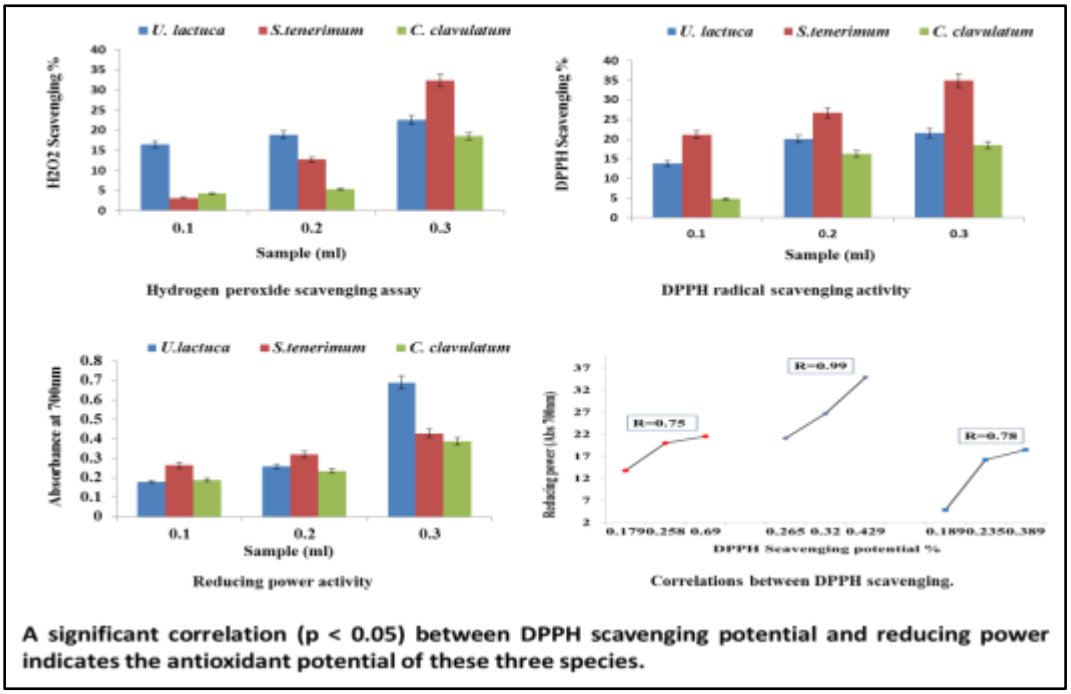


Fig 28. Dose dependent antioxidant activity of most active species of each group.

Antifungal activity

The methanolic extracts of selected species were tested against two pathogenic fungus *Aspergillus niger* and *Panicillium janthinellum.* The results highlighted the strongantifungal activity of the tested seaweeds extract (Table 6, Fig. 29). The brown seaweed *S. tenerrimum* showed maximum antifungal activity against both tested pathogenic organisms *A. niger* (17mm) and *P. janthinellum* (20mm). The highest inhibition was observed in *S. tenerrimum* (17mm) followed by *U. lactuca* (15mm) and *U. fasciata* (12 mm) against *A. niger* whereas minimum inhibition was recorded from *H. spinella* (6.3 mm) and *H. pannosa* (8.5 mm). The maximum resistance against *P. janthinellum* was registered in *S. tenerrimum* (20mm) and *C. indica* (15.8 mm) while minimum inhibition found in *H. venusta* (5.3 mm) and *D. virens* (6.4 mm). Phenolic compounds are very potent to show the antifungal activity against such pathogens [Ansari *et al*. (2013)]. It is revealed that the antifungal activity observed in the species is due to presence of phenolic and different bioactive compounds [Kumar *et al*. (2006)]. The structures of the phenolic compounds is such that they can diffuse through the microbial membrane and can penetrate into the cell, where they can interfere in the metabolic pathways by interfering with the synthesis of ergosterol, glucan, chitin, proteins and glucosamine in fungi [Brul and Klis (1999)]. The antifungal effects of extracts were comparable with the standard antifungal agent, fluconazole, ketoconazole and amphotericin B as standard antifungal agent and were establish to be active against both the fungal strain tested. The antifungal activity of *Enteromorpha* (12mm) and *Chaetomorpha* (12mm) against *A. niger* reported by Prasanna Latha and Hemalatha (2011) is comparable with present study of selected species. In the present study, the species of Phaeophyta showed the strongest activities against the test bacteria and fungi, which was in agreement with the findings of Padmakumar and Ayyakkannu (1997). Similar studies [Devi *et al*. (2008); Meenakshi *et al*. (2009)] have reported that the methanol extract of seaweeds contains phenolics, alkaloids and amino acids which may responsible for the antimicrobial activity.

Table 6.Antifungal study of methanolic extracts (100 µl) of selected seaweed species.

Seaweeds Species	***A. niger*** **(Inhibition zone mm)**	***P. janthinellum*** **(Inhibition zone mm)**	**control**
Caulerpa recemosa	**10.9**	**11.3**	**NA**
Monostroma latissimum	**9.2**	**11.9**	
Ulva lactuca	**15**	**14**	
Ulva reticulata	**10**	**12.5**	
Struviea anastomosans	**11**	**13.2**	
Ulva fasciata	**12**	**11.2**	
Sargassum tenerimum	**17**	**20**	
Cystoseira Indica	**11.3**	**15.8**	
Turbinaria orneta	**11**	**13.2**	
Dictiyota dichotoma	**11.8**	**11.9**	
Iyengaria stellata	**10.2**	**12.3**	
Lobofora variegata	**9**	**10.1**	
Botrycladia leptopoda	**11**	**11**	
Centroceras clavulatum	**10**	**12.2**	
Hypnea venusta	**NA**	**5.3**	
Hypnea. pannosa	**8.5**	**11.2**	
Hypnea spinella	**6.3**	**10.2**	
Dermonema virens	**NA**	**6.4**	
Fluconazole 10 mcg	**10**	**12**	
Ketoconazole 10 mcg	**17**	**20**	
Amphotericin B 20mcg	**18**	**19**	

NA*: No Activity.

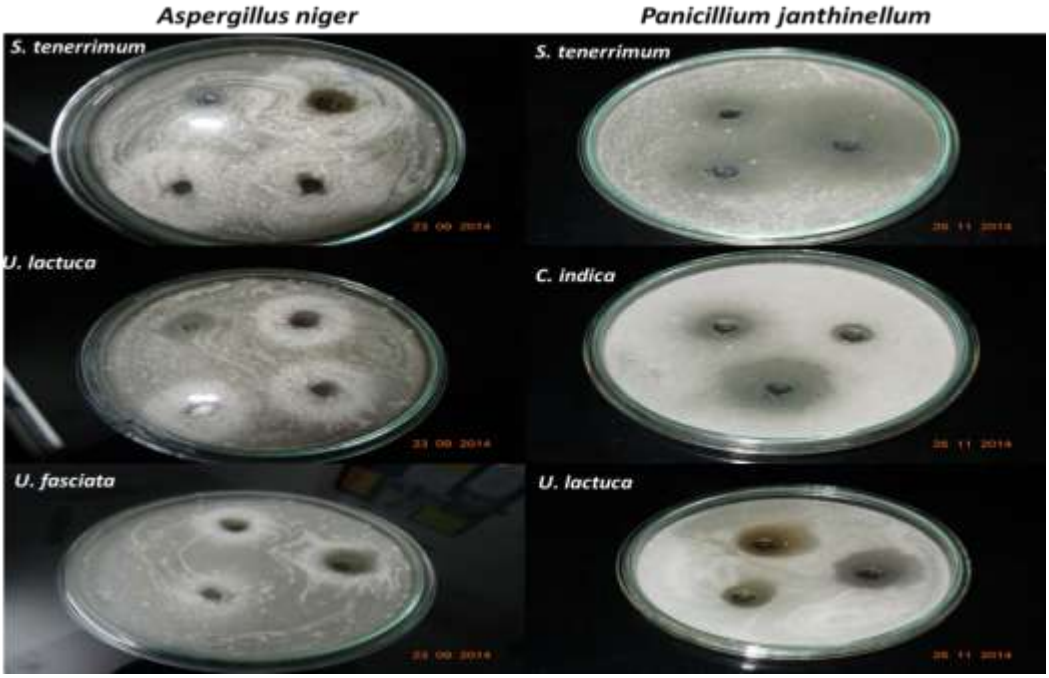


Fig 29.Antifungal activity of selected seaweeds having maximum inhibition against bacteria.

Antibacterial activity

The tested microbial organism *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus*shows varying degrees of antimicrobial activities in three studied seaweed extracts**.** Perez *et al*. (1990)observed that the extract of *U. lactuca* had no antibacterial activity. In contrast, results of our study showed that *U. lactuca* inhibited all the test organisms except *E. coli* (Table 7). This difference in results may be due to time and place of sample collection. Different species of marine algae were collected and analyzed for their antibacterial activity from different parts of the world. Reichelt and Borowitzka (1984) and Salvador *et al*. (2007), screened species of algae for their antibacterial activity. They reported that the members of the red algae exhibited high antibacterial activity. In contrast, green and brown seaweeds were most active among studied species for their antibacterial activity. The methanol extract of *U. lactuca* shows highest activity against *S. aureus* (17mm) followed by *B. subtilis* (12mm) while the extract was inactive against *E. coli*. The *C. clavulatum* methanolic extract was also inactive against *E. coli* and demonstrations activity against *B. subtilis* (15mm), *S. aureus* (14mm). The *S. tenerrimum* was most active against all tested pathogens. The greater antibacterial activity against *E. coli* was registered in *T. orneta* (14 mm) followed by *S.tenerrimum* (13) and *B. leptopoda* (13) whereas lower inhibition was reported in *U.reticulata* (9.8 mm) followed by *M. latissimum* (10.5 mm) and *I. stellate* (11 mm). Optimum activity against *B. subtilis* was registered in *C. clavulatum* (15mm) followed by *U.reticulata* (14 mm) and *U. fasciata* (13 mm) while minimum was registered in *M. latissimum* (9 mm) and *T. orneta* (11 mm). *U. lactuca* (17 mm), *S.tenerrimum* (15 mm) and *Dictyota dichotoma* (14 mm) displays significant activity against *S. aureus*, whereas least activity was found in*S. anastomosans* (8.2mm) and *H. spinella* (9 mm). (Fig 30, Table 7).

The extracts of almost all studied species shown activity against *B. subtilis* and *S. aureus* which is comparatively greater than the standard antibiotic Streptomycin 10mcg (12mm and 11mm respectively). Salvador *et al.* (2007) also reported that *E. coli* bacteria showed resistant against the extracts of *Malopteris filicine, Ulva rigida, Dictyota dichotoma, F. verticillatus* and *Cladostephus spongiosus.* The results of the present investigation revealed that Gram positive organisms were more prone to the crude extracts of studied seaweeds. Tuney *et al*. (2006) also reported that Gram-positive bacteria were more effectively controlled by the extracts of algae used in their study compared to Gram-negative bacteria. Compared to water based methods, organic solvent has a higher efficiency in extracting compounds for antibacterial activities [Tuney *et al*. (2006)]. The methanol extracts of *S. vulgare* do not have antibacterial activity against *E. coli* and *S. aureus* growth as indicated by Ibtissam *et al*. (2009). Silva *et al*. (2013) found that *E. coli* and *P. aeruginosa* were affected only by the ethanolic extract of the brown seaweed while *Padina gymnospora*. Hodgson (1984) reported antimicrobial activity of seaweeds belonging to Chlorophyta, Phaeophyta and Rhodophyta.Caccamese and Azzolina (1979) studied the antimicrobial activity of *Dictyota dichotoma, Cystoseira elegans* and *Laurencia obtuse.* Generally, the earlier studies found Gram positive was affected by algal extracts activity more than Gram negative bacteria; this may be due to the complex cell wall structure of the Gram negative bacteria [Stirk *et al*. (2007)].

Table 7.Antibacterial study of methanolic extracts (100 µl) of selected seaweed species

Seaweeds Species	***E. coli*** **(Gram -)**	***B. subtilis*** **(Gram +)**	***S. aureus*** **(Gram +)**	**control**
Caulerpa recemosa	11.2	10	13	NA
Monostroma latissimum	10.5	9	11	
Ulva lactuca	NA	12	17	
Ulva reticulate	9.8	14	12	
Struviea anastomosans	10	12	8.2	
Ulva fasciata	NA	13	9.7	
Sargassum tenerimum	13	12	15	
Cystoseira Indica	12.2	11.5	12.5	
Turbinaria orneta	14	10	13.3	
Dictiyota dichotoma	11.7	11	14	

Iyengaria stellate	11	10.3	13	
Lobofora variegate	12.5	12.4	11	
Botrycladia leptopoda	13	NA	10	
Centroceras clavulatum	NA	15	14	
Hypnea venusta	12	12.2	11.2	
Hypnea. Pannosa	12	11.2	9.6	
Hypnea spinella	11	10.2	9	
Dermonema virens	NA	10	NA	
Streptomycin 10mcg	11	12	11	
Ampicillin	14.1	12.5	14.6	

NA*: No Activity.

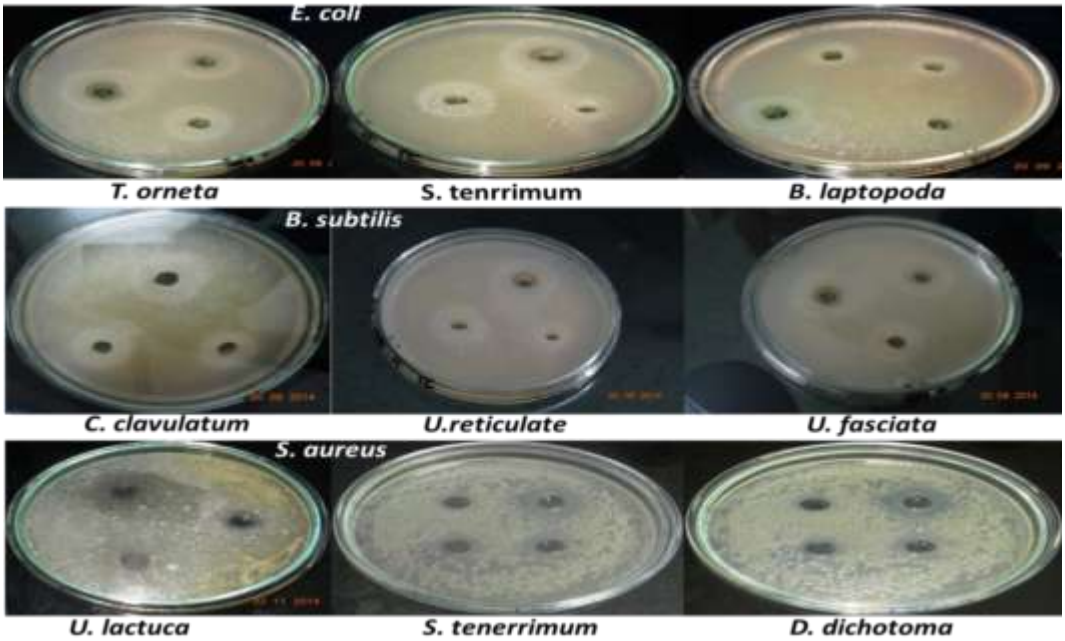


Fig.30. Antibacterial activity of selected seaweeds having maximum inhibition against bacteria.

Alkaloids form the studied marine seaweeds

The Alkaloids are important chemical compounds responsible for many of the pharmacological properties of medicinal plant, therefore in the present study chromatographic analysis of alkaloids was performed in order to know their pattern in methanolic extract of different seaweeds. The results of the preliminary phytochemical studies confirmed the presence of alkaloids in the methanolic extract of all

seaweed species accept *B. leptopoda* and *U. reticulata* species of Rhodophyta and Chlorophyta respectively. A number of solvent systems were tried, for each extract, better resolution and maximum number of spots, however the distinct resolution was obtained in the solvent of chloroform: acetone: :8:2 (Table 8-10; Fig. 31-35).

The chromatogram indicates that all samples constitute were clearly separated without any tailed and diffuseness. Bright orange coloured zone at visible light mode in reference track was observed from the chromatogram after derivatization and brown coloured zone after spraying the dragendorff reagent, which confirmed the presence of Alkaloids in the methanolic extract [Karthika and Paulsamy (2015)] (Fig.31). The methanolic extract of Chlorophyta, Phaeophyta and Rhodophyta species showed the presence of total 83 different types of alkaloids with different Rf values (Table 8-10). Total 39 different alkaloids were recorded from Chlorophyta species with Rf values ranging from 0.13 to 0.88 (Table. 8) while 30 alkaloids were encountered from methanolic extract of Phaeophyta with Rf values from 0.08 to 0.89 (Table.9) and 14 alkaloids from Rhodophyta with Rf values range from 0.13 to 0.81 (Table. 10). In general more degree of alkaloids diversity has been observed in Chlorophyta species when compared with Phaeophyta and Rhodophyta species. Among 39 different alkaloids of Chlorophyta species, nine alkaloids with Rf values 0.62, 0.40, 0.52, 0.82, 0.31, 0.46, 0.73 and 0.63 were unique to this group (Table 8). From 30 different types of alkaloids 0.77, 0.11, 0.49, 0.68, 0.77, 0.80, 0.15, 0.14, 0.28, 0.70 and 0.47 were unique occurrence to the Phaeophyta species (Table 9). The alkaloids with different Rf values 0.58, 0.71, 0.74, 0.23 and 0.54 showed their unique presence in Rhodophyta species (Table 10).

Table 8.Alkaloids profiling of the methanolic extracts of Chlorophyta species.

Track	Peak	Rf	Height	Area	Assigned substance
SA	1	0.08	19.8	252.1	Unknown
	2	0.26	38.3	1840.9	Alkaloid 1
	3	0.41	16.6	426.0	Unknown
	4	0.56	220.0	13004.1	Alkaloid 2
	5	0.62	228.7	8723.4	Alkaloid 3
	6	0.69	278.6	15281.7	Alkaloid 4
	7	0.78	31.2	427.1	Alkaloid 5
	8	0.85	78.8	1378.7	Unknown
	9	0.89	21.7	298.0	Unknown
UL	1	0.13	22.2	233.4	Alkaloid 1
	2	0.18	23.6	420.5	Unknown
	3	0.26	119.5	4161.1	Unknown
	4	0.40	37.7	934.8	Alkaloid 2

	5	0.52	82.4	4013.2	Alkaloid 3
	6	0.57	141.9	5119.8	Unknown
	7	0.67	123.3	5393.7	Unknown
	8	0.78	47.6	575.2	Alkaloid 4
	9	0.83	74.7	1383.5	Unknown
	10	0.88	46.8	699.7	Alkaloid 5
CR	1	0.13	25.1	509.5	Alkaloid 1
	2	0.21	14.1	413.6	Alkaloid 2
	3	0.27	12.3	203.9	Alkaloid 3
	4	0.56	147.4	10769.1	Alkaloid 4
	5	0.65	35.3	1565.7	Alkaloid 5
	6	0.79	45.5	647.6	Alkaloid 6
	7	0.82	29.9	643.8	Alkaloid 7
UF	1	0.22	12.5	82.1	Alkaloid 1
	2	0.31	79.9	1627.1	Alkaloid 2
	3	0.46	57.5	2837.8	Alkaloid 3
	4	0.53	90.1	3758.5	Alkaloid 4
	5	0.64	35.5	1456.1	Alkaloid 5
	6	0.73	20.9	550.5	Alkaloid 6
	7	0.80	42.4	1147.6	Alkaloid 7
ML	1	0.27	10.4	245.8	Alkaloid 1
	2	0.42	24.0	911.0	Alkaloid 2
	3	0.55	119.4	6784.0	Alkaloid 3
	4	0.63	38.0	1538.0	Alkaloid 4
	5	0.76	50.1	1976.6	Alkaloid 5
UR	1	0.43	12.9	363.0	Unknown
	2	0.78	10.9	215.6	Alkaloid 1

* SA- *Struvea anastomosans,* **UF**- *Ulva fasciata,* **UR**-*Ulva reticulata,* **UL**-*Ulva lactuca,* **ML**-*Monostroma latissimum,* **CR**- *Caulerpa recemosa.*

Table 9.Alkaloids profiling of the methanolic extracts of Phaeophyta species.

Track	Peak	Rf	Height	Area	Assigned substance
LV	1	0.08	22.3	163.4	Unknown
	2	0.18	16.7	440.1	Unknown
	3	0.30	49.3	2028.2	Unknown
	4	0.41	24.4	580.9	Unknown
	5	0.47	37.4	1631.2	Unknown
	6	0.57	39.6	1404.3	Unknown
	7	0.65	46.8	1292.4	Alkaloid 1
	8	0.77	34.4	758.6	Alkaloid 2
CI	1	0.08	56.9	603.2	Alkaloid 1
	2	0.11	15.1	141.0	Alkaloid 2
	3	0.13	19.8	138.8	Unknown
	4	0.15	19.0	145.3	Unknown
	5	0.17	13.4	74.8	Unknown
	6	0.23	27.7	420.6	Unknown
	7	0.28	41.7	1276.8	Unknown
	8	0.41	57.6	2022.9	Unknown
	9	0.49	128.7	5647.7	Alkaloid 3
	10	0.57	381.8	14333.3	Alkaloid 4
	11	0.69	68.5	1967.5	Alkaloid 5
	12	0.75	23.7	537.8	Unknown
	13	0.79	32.6	526.8	Alkaloid 6
	14	0.85	31.8	498.2	Alkaloid 7
	15	0.89	51.3	759.2	Alkaloid 8
ST	1	0.07	34.2	250.7	Unknown
	2	0.22	16.7	332.3	Alkaloid 1
	3	0.28	35.5	1288.2	Unknown
	4	0.38	12.1	288.9	Unknown
	5	0.49	107.1	3268.0	Alkaloid 2
	6	0.57	394.4	16122.3	Alkaloid 3
	7	0.68	136.8	4382.9	Alkaloid 4

	8	0.75	27.8	439.2	Unknown
	9	0.77	37.5	577.2	Alkaloid 5
	10	0.80	12.6	122.9	Alkaloid 6
	11	0.88	72.8	2204.3	Alkaloid 7
DD	1	0.15	10.8	210.1	Alkaloid 1
	2	0.26	18.8	582.7	Alkaloid 2
	3	0.32	16.9	542.6	Unknown
	4	0.50	115.5	4229.2	Unknown
	5	0.57	313.4	18741.7	Alkaloid 3
	6	0.68	106.1	4252.7	Alkaloid 4
	7	0.80	22.4	419.2	Alkaloid 5
TO	1	0.14	12.9	159.8	Alkaloid 1
	2	0.18	11.0	69.6	Unknown
	3	0.28	29.9	1611.8	Alkaloid 2
	4	0.48	79.6	2697.3	Unknown
	5	0.57	284.5	11901.1	Alkaloid 3
	6	0.70	29.8	1132.7	Alkaloid 4
	7	0.78	22.9	658.4	Unknown
IS	1	0.32	64.6	1549.3	Unknown
	2	0.47	34.3	1327.3	Alkaloid 1
	3	0.53	53.0	2037.6	Alkaloid 2
	4	0.59	34.7	747.4	Unknown
	5	0.66	20.6	555.3	Alkaloid 3
	6	0.81	47.2	944.1	Alkaloid 4

***ST-** *Sargassum tenerrimum,* **CI**- *Cystoseira indica,* **TO**- *Turbinaria ornata,* **DD**- *Dictiyota dichotoma,* **IS**- *Iyengaria stellata,* **LV**- *Lobophora variegata.*

Table 10.Alkaloids profiling of the methanolic extracts of Rhodophyta species.

Track	Peak	Rf	Height	Area	Assigned substance
CC	1	0.25	11.0	299.2	Unknown
	2	0.41	10.9	302.6	Unknown
	3	0.58	142.0	6969.7	Alkaloid 1
	4	0.66	129.0	6216.2	Alkaloid 2
	5	0.71	99.8	2774.3	Alkaloid 3
	6	0.78	21.8	416.5	Alkaloid 4
	7	0.89	27.2	376.2	Unknown
HS	1	0.21	11.2	154.1	Unknown
	2	0.24	13.0	351.8	Unknown
	3	0.38	14.6	275.1	Unknown
	4	0.43	22.0	660.0	Unknown
	5	0.55	148.9	5031.3	Alkaloid 1
	6	0.64	25.3	763.7	Alkaloid 2
	7	0.74	61.0	1520.1	Alkaloid 3
HP	1	0.08	11.4	63.0	Unknown
	2	0.23	21.5	767.4	Alkaloid 1
	3	0.39	11.6	328.2	Unknown
	4	0.49	13.8	285.2	Unknown
	5	0.56	39.2	1401.1	Unknown
	6	0.64	37.2	1254.1	Alkaloid 2
	7	0.74	139.9	3585.5	Alkaloid 3
	8	0.87	17.9	288.7	Unknown
DV	1	0.14	12.5	110.1	Unknown
	2	0.18	21.3	159.2	Unknown
	3	0.54	52.0	2226.9	Alkaloid 1
HV	1	0.13	10.1	74.7	Alkaloid 1
	2	0.29	24.4	580.2	Unknown
	3	0.42	16.5	824.6	Unknown
	4	0.54	15.5	399.9	Unknown
	5	0.58	13.9	484.9	Unknown
	6	0.68	14.7	244.8	Unknown

	7	0.69	16.9	433.7	Unknown
	8	0.81	19.2	494.4	Alkaloid 2
BL	1	0.34	31.3	1034.6	Unknown
	2	0.51	11.8	319.1	Unknown
	3	0.58	21.3	747.6	Unknown
	4	0.69	23.7	245.2	Alkaloid 1

***BL**- *Botrycladia leptopoda,* **CC**- *Centroceras clavulatum,* **HV**- *Halymenia venusta,* **HP**- *Hypnea pannosa,* **HS**- *Hypnea spinella,* **DV**- *Dermonema virens.*

The areas of detected compounds from Chlorophyta species were higher compared to species of Phaeophyta and Rhodophyta. The area of any compounds showing their concentration and prominent nature. The four species from Chlorophyta (*Struvea anastomosans, Ulva lactuca, Caulerpa racemosa* and *Ulva fasciata*) showed different alkaloids having more area than 1000 AU with Rf values, 056, 0.62, 0.69, 0.26 and 0.53 while five species from Phaeophyta (*Lobophora variegata, Cystoseira indica, Sargassum tenerrimum, Turbinaria orneta* and *Iyengaria stelleta*) with Rf values, 0.65, 0.49, 0.57, 0.68, 0.88, 0.28, 0.70, 0.47 and 0.53 whereas four species from Rhodophyta (*Dermonema virens, Hypnea pannosa , Hypnea spinella* and *Centrocerus clavulatum*) with Rf values 0.54, 0.64, 0.74, 0.55, 0.58, 0.66 and 0.71.

The HPTLC studies confirmed the presence of alkaloids in the methanolic extract of the studied seaweed species. The mobile phases used for HPTLC was relatively polar; (chloroform: acetone) to separate the alkaloids. Handful of literature also suggested mobile phase of high polarity solvents for effective separation of the bioactive compounds [Jeeshna *et al*. (2010); Alphonso and Saraf (2012); Kalaiselvi *et al*. (2012)]. Martins *et al*. (2014)studied fingerprinting of methanolic extract of *Lobophora variegata* and recorded 9 different compounds based on different Rf values from this brown seaweed species. Marimuthu *et al*. (2012)reported presence of different bioactive compounds including alkaloids from *Sargassum wightii.*Vijayraja and Jeyaprakash (2015)also described presence of alkaloids in methanolic extracts of *Sargassum myriocystum* and *Turbinaria orneta*. Their study showed polar solvents like methanol, acetone and water are most suitable for extraction of alkaloids.

Bhaigyabati and Usha (2013) recommended methanol as a best solvent for extraction of most of the bioactive compounds of the species like *Sargassum wightii*. Steiner and Hartmann (1968)recorded some brown marine algae containing Phenylethylaminealkaloids from *Desmerestia aculeata, Desmerestia viridis, Ceramium rubrum, Cystoclonium purpureum, Delesseria sanguine, Dumontia incrassata, Polysiphonia urceolata and Polyides rotundus*. The presence of Phenylethylamine alkaloids was examined in 17 marine algae and found only in six red species *Gelidium crinale, Gracilaria bursa-pastoris, Halymenia floresii, Phyllophora crispa, Polysiphonia morrowii* and *Polysiphonia tripinnata* by

Percot *et al*. (2009). Tyramine alkaloid was detected in the brown alga *Laminaria saccharina,* and red algae *Chondrus crispus* and *Polysiphonia urceolata* by Kneifel *et al*. (1977). Many scientists have reported presence of Hordenine alkaloid in different seaweeds *Phyllophora nervosa, Ahnfeltia paradoxa* , *Gigartina stellata* and *Gelidium crinale [*Kawauchi and Sasaki *(1978)*; Barwell and Blunden (1981); Percot *et al*. (2007)]. Different indole and halogenated indole alkaloids were also found from seaweeds, reviewed by Güven *et al*. (2010) and Kasım *et al*, (2010). Seaweeds contain a range of exclusive phytochemicals not present in terrestrial plants. As such, edible seaweeds may be the only relevant dietary source of some of these factors. A wide range of studies have described the high antioxidant capacity, anticancer, antibacterial, antifungal and antitumor activity of a range of edible seaweeds [Cofrades *et al*. (2010), Kolanjinathan *et al*. (2014)]. From the selected seaweeds of the present study *Sargassum tenerrimum, Ulva lactuca, Ulva fasciata, Lobofora variegata, Caulerpa racemosa, Centroceras clavulatum,* and *Hypnea* species are already being utilized and proved having great nutraceutical properties [Kolanjinathan *et al*. (2014)]. Therefore the developed HPTLC methods and finger printing are useful in differentiating the species from the adulterant and act as biochemical markers for some of this medicinally important species in the Pharma industries.

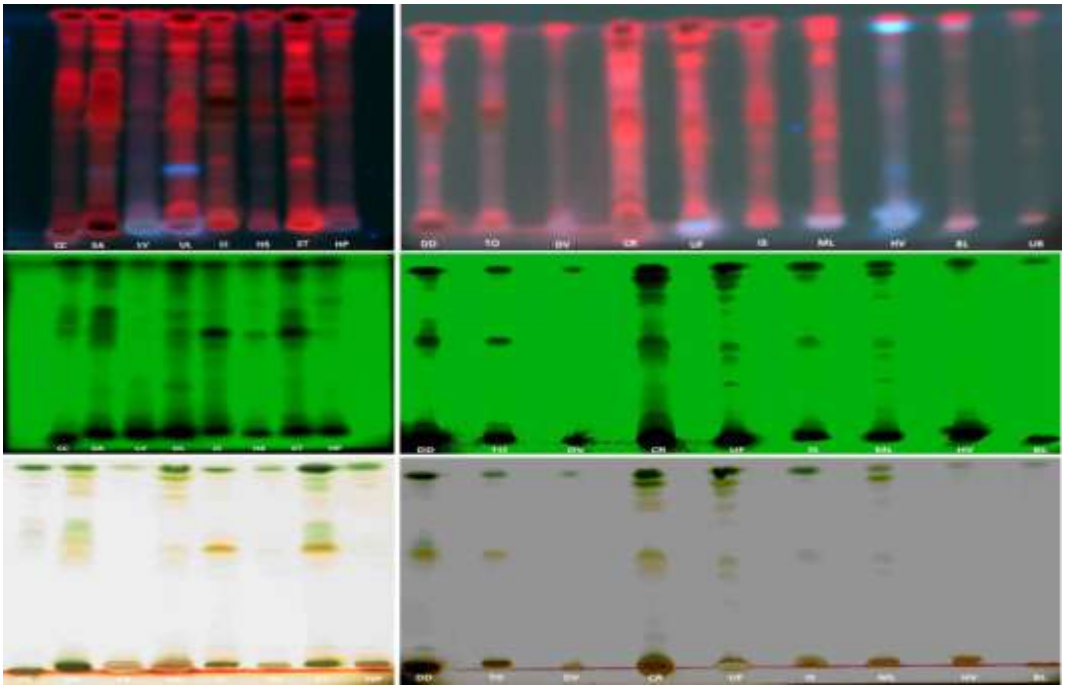


Fig 31.HPTLC profile of Alkaloids in the methanolic extract of seaweed species.under UV 366; under UV 254 and under day light after derivatization

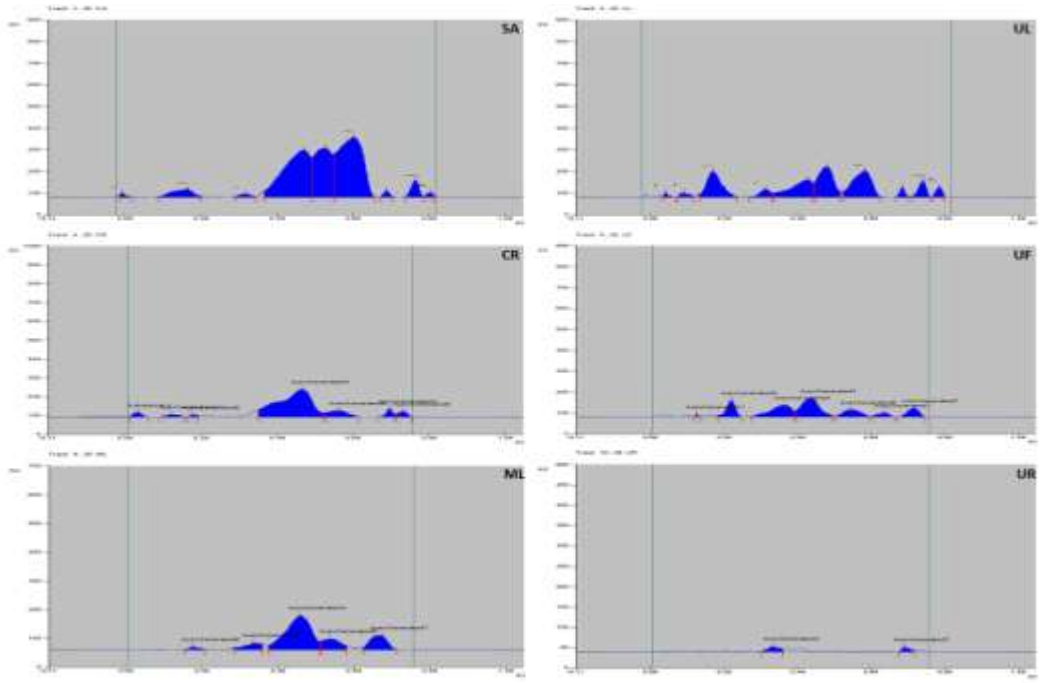


Fig 32.Alkaloids Chromatogram of Chlorophyta species.

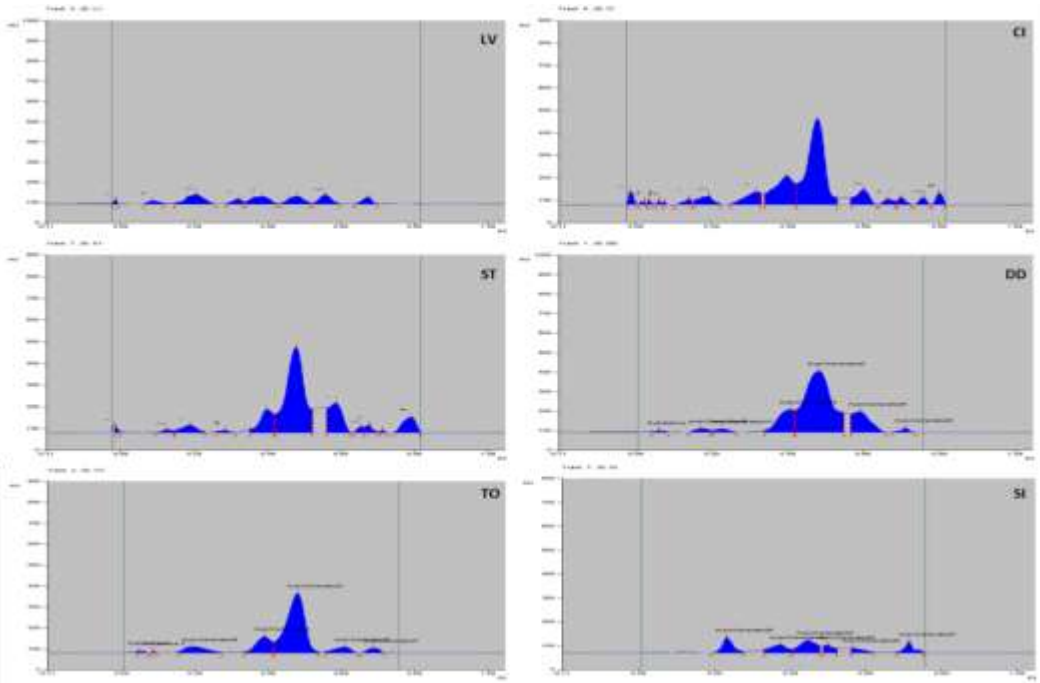


Fig 33.Alkaloids Chromatogram of Phaeophyta species.

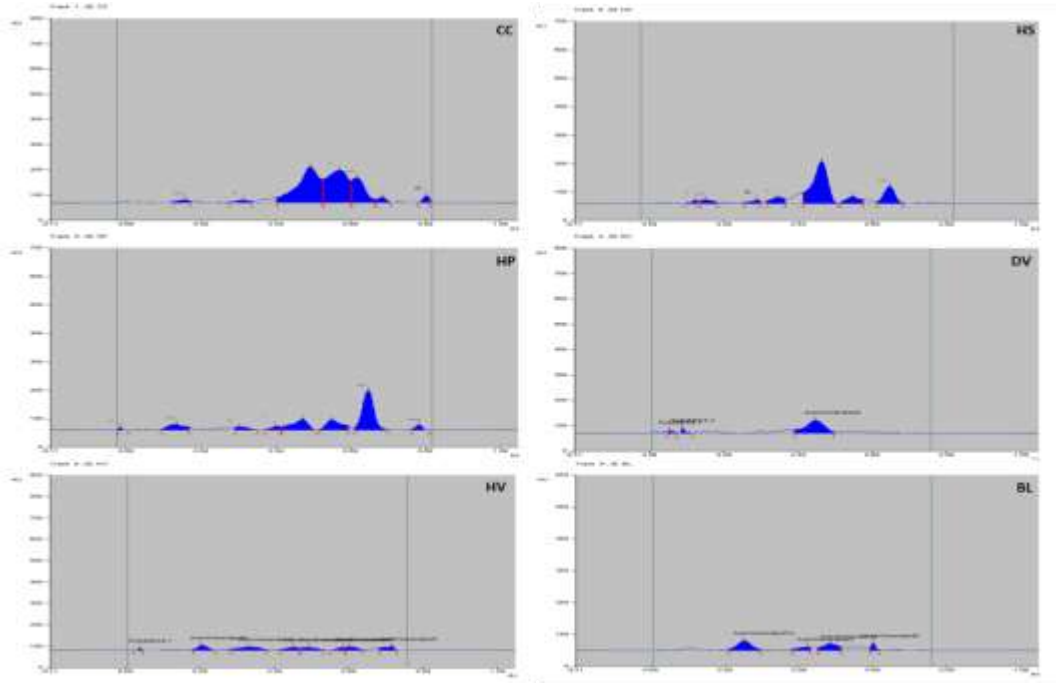
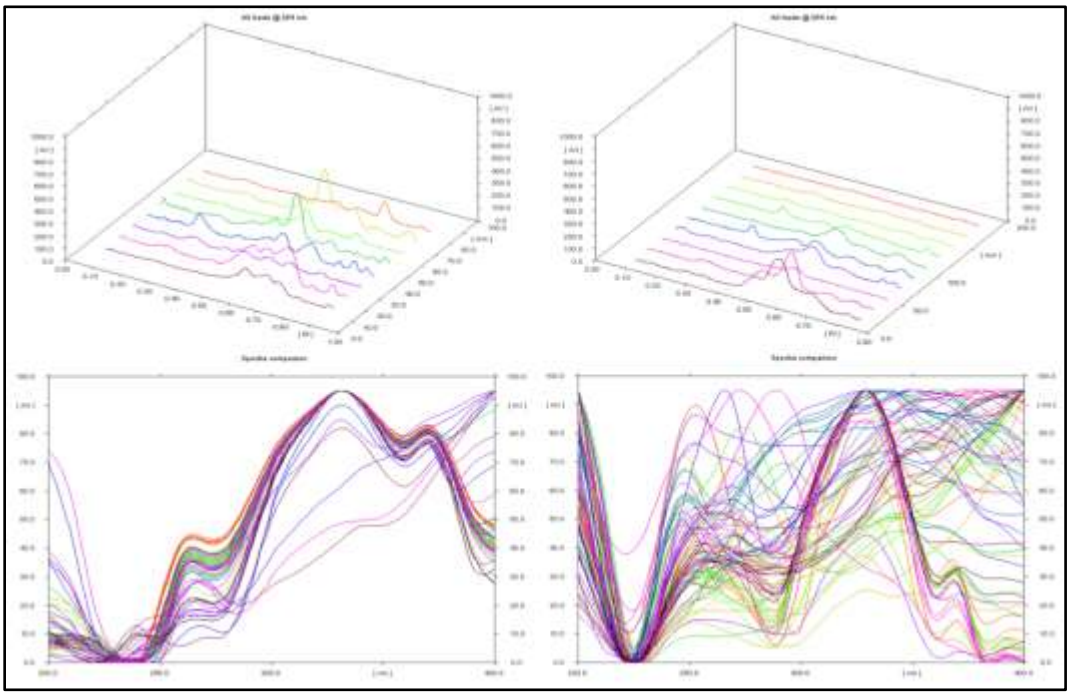


Fig 34.Alkaloids Chromatogram of Rhodophyta species.



35. 3D display of HPTLC Alkaloids chromatogram of 18 seaweeds.

Fatty Acids

In the present study fatty acids included myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1, n- 9) and linolenic (18:2, n-6) considered as dominant fatty acids in the seaweeds [Kumar *et al*. (2011a)] shown in Table 11. Among the studied fatty acids myristic acid (0.89–9.24%) found maximum in the *H.spinella* and minimum was noticed in *C.clavulatum*. Stearic (1.10- 9.23%) content was registered maximum in *I.stellate* and minimum was observed in *T.orneta*. Palmitic acid (20.17–50.02%) content

recorded higher in *C.racemosa* whereas lower values recorded in *I. stellate.* Oleic acid (2.65–17.7 %) is mono-unsaturated fatty acid encountered maximum in *U. lactuca* and minimum was recorded in *H.venusta* while, linolenic acid (0.32–12.75%) is polyunsaturated fatty acid found greater in *U.fasciata* and lower in *D. dichotoma*. The content of studied fatty acids in the *C. racemosa* recorded by Kumar *et al*. (2011a) is comparable with present study. Chakraborty and Santra (2008) reported fatty acid composition of *U.lactuca* is lower than the present study for the same species. palmitic acid and oleic acid are major acids commonly found in seaweed studied by Matanjun *et al*. (2009) and Polat and Ozogul (2008).

Table 11. Some important fatty acids (%) composition of selected seaweeds

	Fatty acids (% of Total FAs)				
	Palmitic acid (16:0)	Stearic acid (18:0)	Myristic acid (14:0)	Oleic acid (18:1, n-9)	Linolenic acid (18:2, n-6)
C.recemosa	50.02	1.14	2.00	3.78	9.10
M.latissimum	30.1	1.60	1.76	9.3	4.52
U.lactuca	30.2	4.27	2.53	17.27	8.18
U.reticulata	46.4	5.36	3.20	16.81	6.50
S.anastomosans	20.67	2.1	ND	4.76	3.86
U.fasciata	38.45	1.82	0.98	11.3	12.75
S.tenerrimum	40.8	1.23	4.61	3.58	7.59
Cystoseira indica	28.4	2.18	3.04	3.48	7.01
T.orneta	22.3	1.10	6.54	3.98	3.76
D.dichotoma	42.85	ND	1.24	3.51	0.32
I.stellata	20.17	9.23	1.12	3.60	ND
L.variegata	38.43	8.25	1.67	4.36	3.78
B.leptopoda	41.87	2.89	6.84	15.98	2.01
C.clavulatum	49.76	3.87	0.89	7.85	3.4
H.venusta	33.76	2.6	5.32	2.65	2.76
H.pannosa	46.2	4.08	7.33	6.13	2.47
H.spinella	48.9	4.78	9.24	5.35	1.80
D.virens	25.1	4.33	3.86	8.45	0.76

Free amino acids profile of selected seaweeds

Amino acids are important and essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules [Carril and García (2009)]. Different solvent systems were tried for amino acid separation, however the satisfactory resolution was obtained in the solvent of n-butanol: acetic acid: water; 8:2:2. Pink to purple coloured zone after spraying the ninhydrine reagent confirmed the presence of different amino acids in the ethanolic extract [Moore and Stein (1948)] (Fig. 36- 43). Maximum total free amino acids were detected from the *C. clavulatum* (24.37%) followed by *C. indica* (20.93%), *S. anastomosans* (13.46%) and *T. orneta* (9.35%). From the present study it was observed that essential amino acids like histidine, isoleucine and tryptophanewere present in all the selected seaweeds, whereas leucine was only found in Rhodophyta species *H. venusta* and *H.pannosa*. Many essential amino acids like isoleucine, histidine, leucine, methionine, lysine, phenylalanine tryptophane and threonine essential amino acids were detected from studied seaweeds. Nonessential amino acids like alanine, aspartic acids, cysteine, glutamic acid, proline and tyrosine were also observed from the studied seaweeds. It is promising to note that amino acid lysine, usually deficient in terresterial plants was detected in many seaweed species.

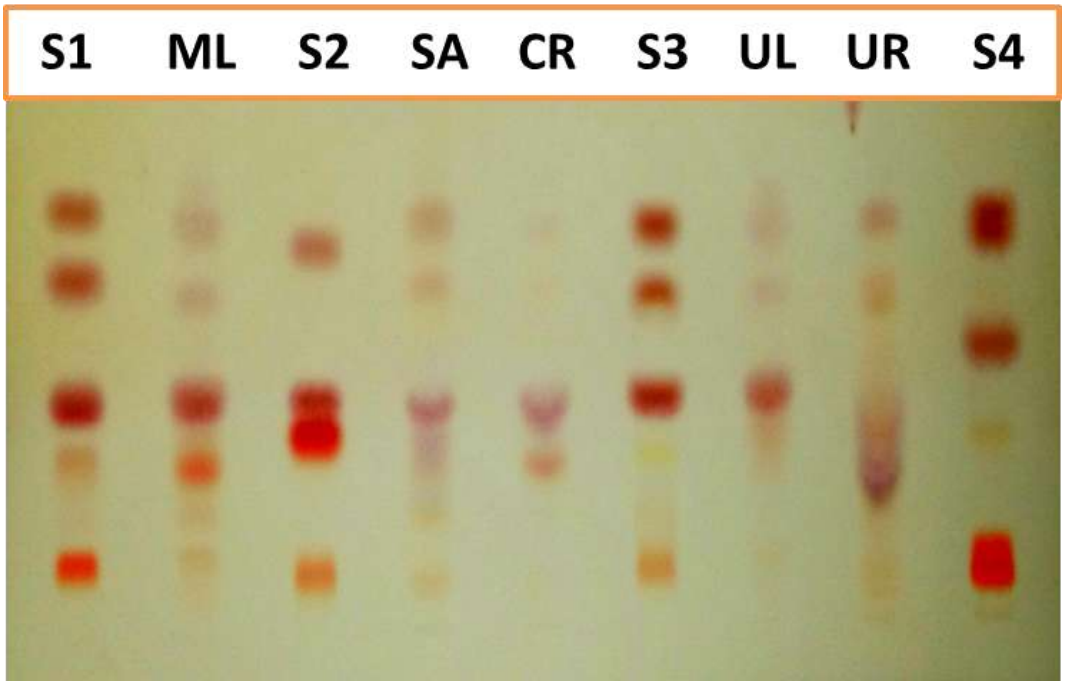


Fig. 36 SA- *Struvea anastomosans*, UR-*Ulva reticulata*, UL-*Ulva lactuca*, ML- *Monostroma latissimum*, CR- *Caulerpa recemosa*. S-1(Tryptophane, Histidine, Methionine, Threonine, Aspartic acid); S-2 (Serine, Tyrosine, Cysteine, Glutamic acid, Glycine); S-3 (Leucine, Alanine, Proline, Phenylalanine); S-4 (Isoleucine, Hydroxyproline, Arginine, Lysine, Dihydroxyphenylanine)

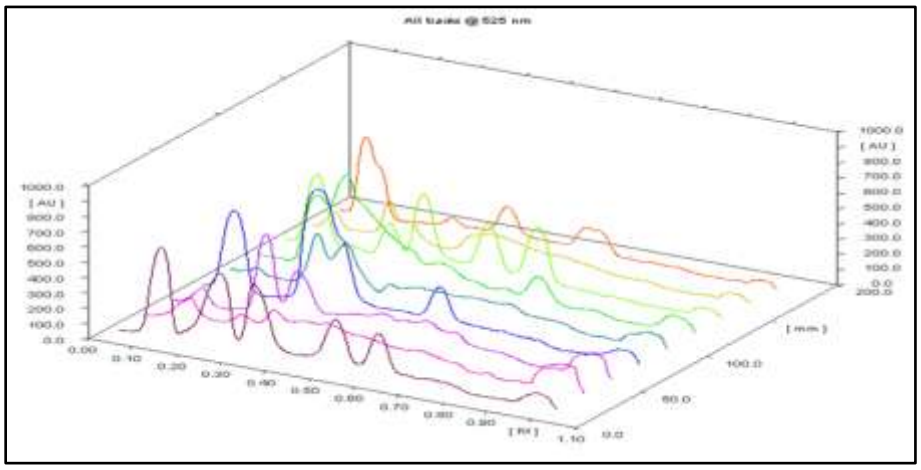


Fig 37 3D graph of amino acids spectra by HPTLC

Table 12. Amino acids (%) profile of selected species (ML,SA,UR,CR,UL).

Monostroma latissimum				
	Rf values	**Area (AU)**	**% amino acids**	**Name of amino acids**
1	0.03 Rf	3537	0.1323832	Cysteine
2	0.09 Rf	6169.7	0.2418068	Histidine
3	0.15 Rf	8184.8	0.1787519	Lysine
4	0.21 Rf	29259.2	0.4685213	Glutamic acid
5	0.30 Rf	29080.3	1.1680336	Threonine
6	0.45 Rf	3902.3	2.9411366	Tyrosine
7	0.54 Rf	3088.7	0.146185	Phenylealanine
8	0.59 Rf	5223.4	0.3847979	Tryptophane
9	0.70 Rf	723.5	2.0317327	Isoleusine
Total free amino acids			**7.693348896**	
Struvea anastomosans				
1	0.03 Rf	6109.4	0.2286632	Cysteine
2	0.13 Rf	9580.5	0.2092333	Lysine
3	0.22 Rf	13526.7	1.1898508	Proline
4	0.30 Rf	12901.2	0.5181871	Threonine
5	0.44 Rf	2716.6	2.0474827	Tyrosine
6	0.47 Rf	7523.2	0.4552342	Methionine
7	0.56 Rf	7144.5	0.5263216	Tryptophane
8	0.65 Rf	2950.4	8.2853131	Isoleusine

Total free amino acids			**13.46028594**	
Caulerpa recemosa				
1	0.03 Rf	1108	0.0414703	Cysteine
2	0.12 Rf	17006.5	0.6665295	Histidine
3	0.21 Rf	10332.1	0.1654457	Glutamic acid
4	0.27 Rf	15074.4	0.6936403	Alanine
5	0.44 Rf	3931	2.9627676	Tyrosine
6	0.49 Rf	4489.3	0.2716507	Methionine
7	0.57 Rf	2527.8	0.1862182	Tryptophane
8	0.66 Rf	896.5	2.5175512	Isoleusine
Total free amino acids			**7.505273569**	
Ulva lactuca				
1	0.03 Rf	446.2	0.0167004	Cysteine
2	0.06 Rf	3040.6	0.1191691	Histidine
3	0.18 Rf	17090.2	0.5207617	Aspartic acid
4	0.30 Rf	27173.6	1.0914495	Threonine
5	0.45 Rf	3866.6	2.9142297	Tyrosine
6	0.56 Rf	3072.7	0.2263599	Tryptophane
7	0.69 Rf	1035.6	2.9081719	Isoleusine
Total free amino acids			**7.796842197**	
Ulva reticulata				
1	0.03 Rf	1405.8	0.0526164	Cysteine
2	0.05 Rf	4303.2	0.1686537	Histidine
3	0.13 Rf	13745.9	0.3002035	Lysine
4	0.25 Rf	6962.6	0.6124521	Proline
5	0.37 Rf	2164.4	0.1205733	Dehydroxiphenylealanine
6	0.43 Rf	7261.2	0.4626353	Leusine
7	0.55 Rf	5722.2	0.2708259	Phenylealanine
8	0.65 Rf	602.8	1.6927829	Isoleusine
Total free amino			**3.680743265**	

acids			

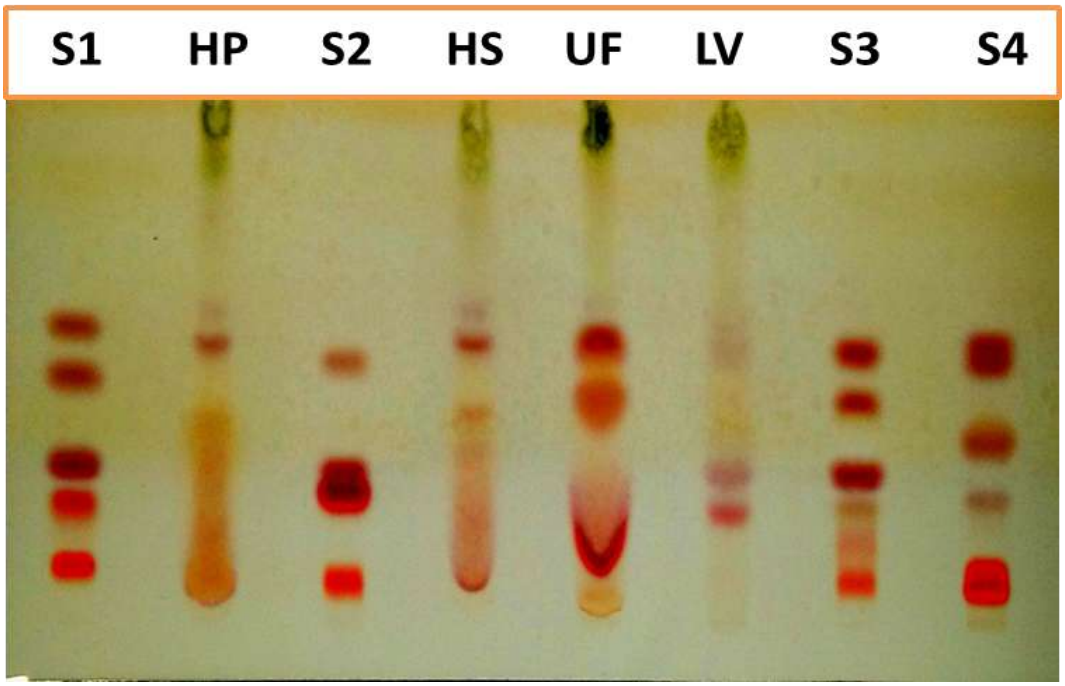


Fig 38. HP- *Hypnea pannosa,* HS- *Hypnea spinella,* UF- *Ulva fasciata,* LV- *Lobophora variegate*. S-1(Tryptophane, Histidine, Methionine, Threonine, Aspartic acid); S-2 (Serine, Tyrosine, Cysteine, Glutamic acid, Glycine); S-3 (Leucine, Alanine, Proline, Phenylalanine); S-4 (Isoleucine, Hydroxyproline, Arginine, Lysine, Dihydroxyphenylanine).

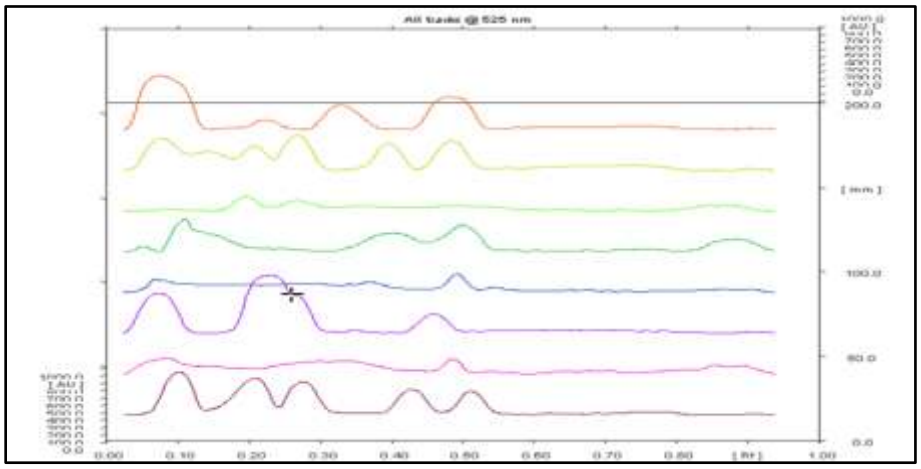


Fig 39. 3D graph of amino acids spectra by HPTLC

Table 13. Amino acids (%) profile of selected species (HP, HS, UF, LV).

Hypnea pannosa				
	Rf values	**Area (AU)**	**% amino acids**	**Name of amino acids**
1	0.03 Rf	11758.2	0.460834803	Histidine
2	0.20 Rf	11828.5	0.189407223	Glutamic acid
3	0.31 Rf	11523.9	0.462866714	Threonine
4	0.45 Rf	5602.8	0.356973107	Leusine
5	0.51 Rf	1337.2	1.007838408	Tyrosine
6	0.61 Rf	1170.5	0.086228489	Tryptophane
7	0.69 Rf	1051.5	2.952822241	Isoleusine
Total free amino acids			**5.516970984**	
Hypnea spinella				
1	0.03 Rf	6370.4	0.249672742	Histidine
2	0.27 Rf	4001.7	0.1607315	Threonine
3	0.33 Rf	1863.8	0.085761746	Alanine
4	0.35 Rf	5014.3	0.279334184	Dehydroxiphenylealanine
5	0.46 Rf	7215.9	0.436639235	Methionine
6	0.53 Rf	1701.4	0.080525541	Phenylealanine
7	0.64 Rf	433.1	0.031905646	Tryptophane
8	0.66 Rf	565.6	1.588317888	Isoleusine
Total free amino acids			**2.912888482**	
Ulva fasciata				
1	0.03 Rf	1027.3	0.040262591	Histidine
2	0.07 Rf	23054	0.862867216	Cysteine
3	0.23 Rf	1053	0.042294592	Threonine
4	0.32 Rf	16636	0.765496519	Alanine
5	0.45 Rf	17455.4	1.056238654	Methionine
6	0.56 Rf	229.5	0.010862003	Phenylealanine
7	0.59 Rf	170.1	0.012530941	Tryptophane
9	0.70 Rf	335	0.940746981	Isoleusine
Total free			**3.731299497**	

amino acids				
Lobophora variegate				
1	0.03 Rf	575.5	0.02255536	Histidine
2	0.09 Rf	366.1	0.013702424	Cysteine
3	0.15 Rf	6411.8	0.140030488	lysine
4	0.23 Rf	6520.5	0.261901128	Threonine
5	0.33 Rf	1905.3	0.087671346	Alanine
6	0.36 Rf	1351.4	0.075283133	Dehydroxiphenylealanine
7	0.45 Rf	3864.7	0.233855742	Methionine
8	0.53 Rf	1778.1	0.084155675	Phenylealanine
9	0.58 Rf	485.4	0.035758487	Tryptophane
12	0.71 Rf	440.9	1.238135355	Isoleusine
Total free amino acids			**2.193049138**	

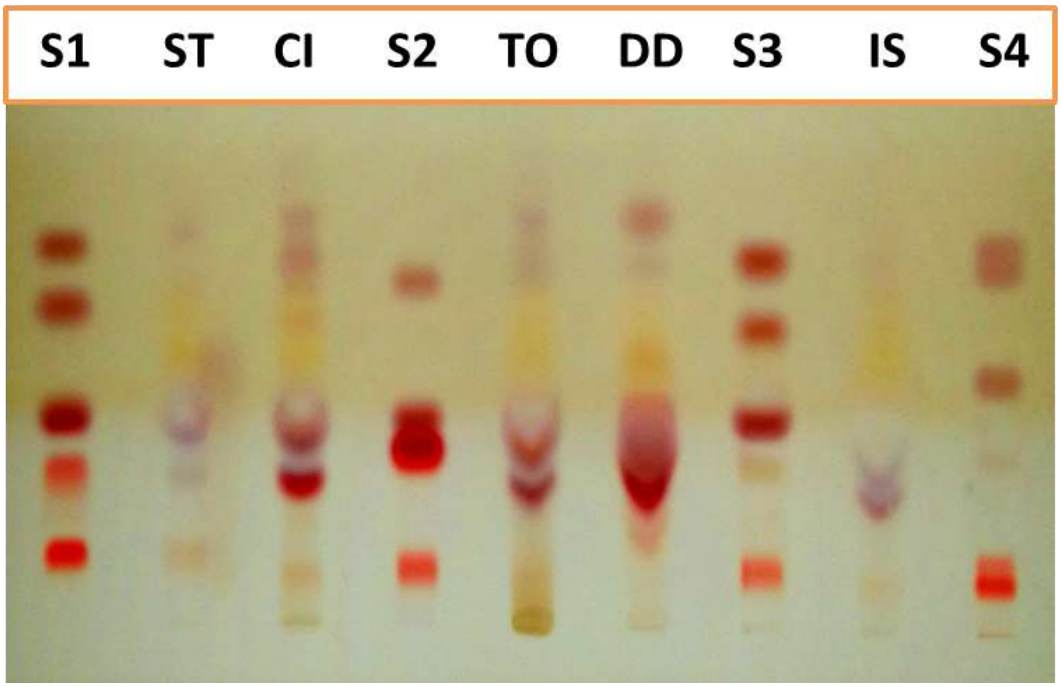


Fig 40.ST- ***Sargassum tenerrimum,*** **CI-** ***Cystoseira indica,*** **TO-** ***Turbinaria ornata,*** **DD-** ***Dictiyota dichotoma,*** **IS-** ***Iyengaria stellata,*****S-1(Tryptophane, Histidine, Methionine, Threonine, Aspartic acid); S-2 (Serine, Tyrosine, Cysteine, Glutamic acid, Glycine); S-3 (Leucine, Alanine, Proline, Phenylalanine); S-4 (Isoleucine, Hydroxyproline, Arginine, Lysine, Dihydroxyphenylanine)**

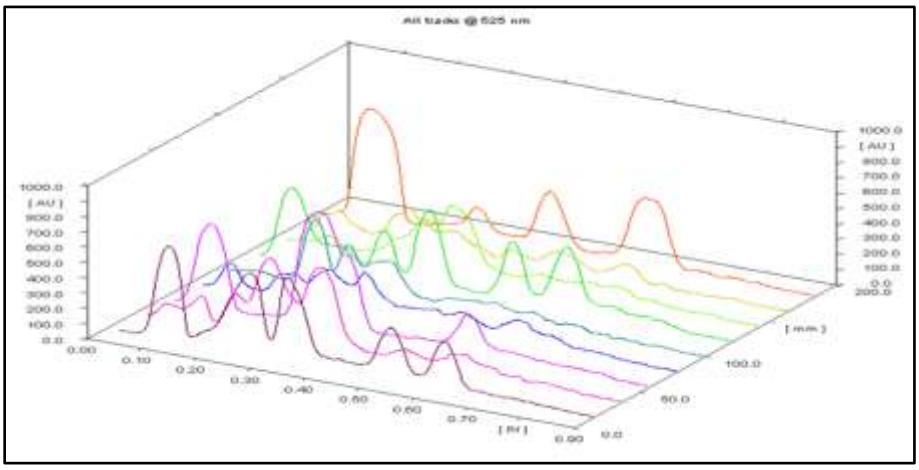


Fig 41.3D graph of amino acids spectra by HPTLC.

Table 14. Amino acids (%) profile of selected species (ST, CI, TO, DD, IS).

Sargassum tenerrimum				
	Rf values	Area (AU)	% amino acids	Name of amino acids
1	0.03 Rf	8575.8	0.336108172	Histidine
2	0.20 Rf	5274.4	0.084457831	Glutamic acid
3	0.27 Rf	8712.9	0.349960638	Threonine
4	0.35 Rf	3278.3	0.150849197	Alanine
5	0.41 Rf	9592.8	0.580467143	Methionine
6	0.50 Rf	5900.6	0.27926943	Phenylealanine
7	0.61 Rf	5053.8	0.372303748	Tryptophane
8	0.71 Rf	1340.7	3.764953665	Isoleusine
Total free amino acids			**5.918369822**	
Cystoseira indica				
1	0.04 Rf	3497.2	0.137064472	Histidine
2	0.18 Rf	20580.6	0.329552715	Glutamic acid
3	0.27 Rf	16825	0.67578966	Threonine
4	0.40 Rf	4569.4	0.27649764	Methionine
5	0.45 Rf	8781	6.618179077	Tyrosine
6	0.53 Rf	3519.1	0.166555444	Phenylealanine
7	0.56 Rf	8867.9	0.653281176	Tryptophane
8	0.64 Rf	4302.3	12.08171862	Isoleusine

Total free amino acids			**20.9386388**	
Turbinaria ornate				
1	0.04 Rf	1224.9	0.048007055	Histidine
2	0.16 Rf	12336	0.461712934	Cysteine
3	0.25 Rf	16270.4	0.355337355	Lysine
4	0.36 Rf	2914.6	0.117067254	Threonine
5	0.41 Rf	5568.1	0.256213102	Alanine
6	0.49 Rf	3692.4	0.223429747	Methionine
7	0.53 Rf	7091.8	0.451842271	Leusine
8	0.63 Rf	5824.4	0.275662961	Phenylealanine
9	0.74 Rf	2550.2	7.161471497	Isoleusine
Total free amino acids			**9.350744175**	
Dictiyota dichotoma				
1	0.10 Rf	20923.5	0.820047031	Histidine
2	0.19 Rf	59818.6	0.957862357	Glutamic acid
3	0.40 Rf	11350.1	0.686802614	Methionine
4	0.56 Rf	2668.2	0.126283207	Phenylealanine
5	0.61 Rf	9756.8	0.718764734	Tryptophane
6	0.76 Rf	1502.9	4.220443696	Isoleusine
Total free amino acids			**7.530203639**	
Iyengaria stellate				
1	0.03 Rf	3371.5	0.132137958	Histidine
2	0.14 Rf	8771.1	0.191556414	Lysine
3	0.23 Rf	4472.9	0.179657627	Threonine
4	0.33 Rf	17933.5	0.825200278	Alanine
5	0.58 Rf	4344.6	0.320058345	Tryptophane
6	0.76 Rf	824.6	2.315641674	Isoleusine
Total free amino acids			**3.964252295**	

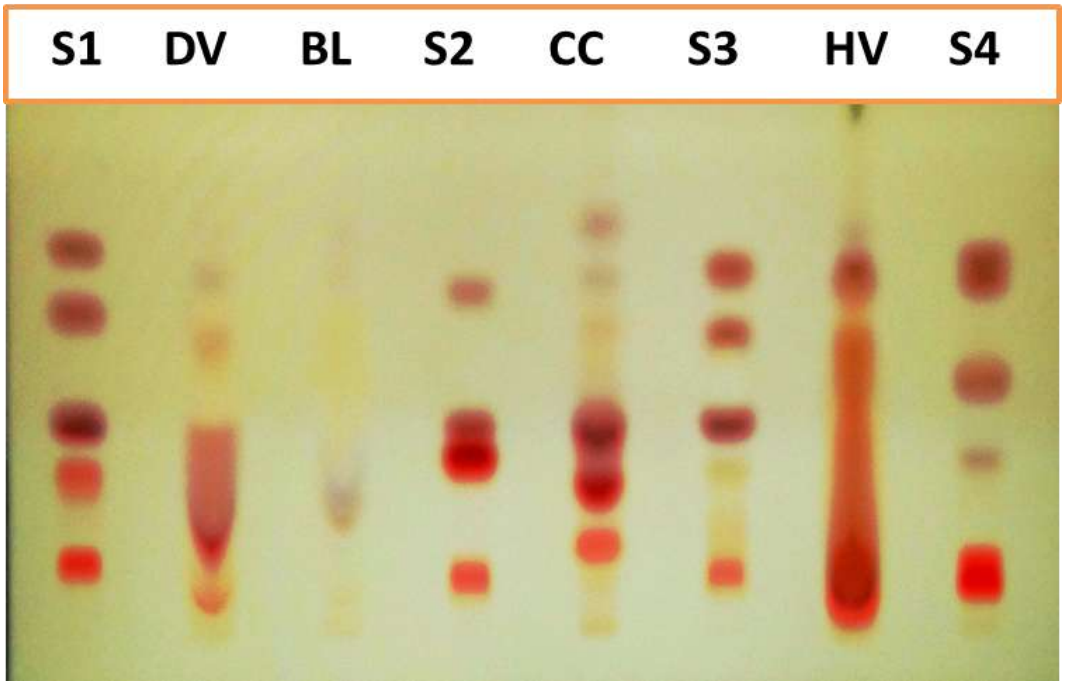


Fig 42.BL- *Botrycladia leptopoda*, CC- *Centroceras clavulatum*, HV- *Halymenia venusta*, DV- *Dermonema virens*.S-1(Tryptophane, Histidine, Methionine, Threonine, Aspartic acid); S-2 (Serine, Tyrosine, Cysteine, Glutamic acid, Glycine); S-3 (Leucine, Alanine, Proline, Phenylalanine); S-4 (Isoleucine, Hydroxyproline, Arginine, Lysine, Dihydroxyphenylanine)

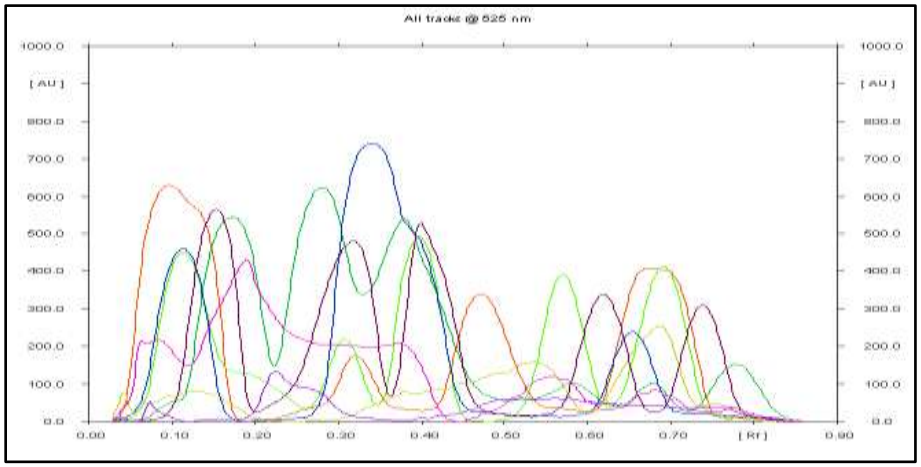


Fig 43. 3D graph of amino acids spectra by HPTLC

Table 15. Amino acids (%) profile of selected species (DV, BL,CC, HV).

Dermonema virens				
	Rf values	**Area (AU)**	**% amino acids**	**Name of amino acids**
1	0.03 Rf	3049.4	0.119514011	Histidine
2	0.07 Rf	6659.8	0.249263602	Cysteine
3	0.12 Rf	32334.8	0.706175773	Lysine
4	0.28 Rf	7307.5	0.642790542	Proline
5	0.34 Rf	9892.9	0.397356287	Threonine
6	0.48 Rf	7686.1	5.792960506	Tyrosine
7	0.64 Rf	4176.1	0.307645273	Tryptophane
8	0.76 Rf	790.3	2.219320416	Isoleusine
Total free amino acids			**10.43502641**	
Botrycladia leptopoda				
1	0.03 Rf	106.4	0.004170096	Histidine
2	0.06 Rf	781.4	0.029246311	cysteine
3	0.18 Rf	6790.1	0.206903592	Aspartic acid
4	0.43 Rf	3358	0.213949399	Leusine
5	0.57 Rf	1782.3	0.131298621	Tryptophane
7	0.72 Rf	2122.6	5.960685201	Isoleusine
Total free amino acids			**6.546253219**	
Centroceras clavulatum				
1	0.06 Rf	31053.1	1.217052714	Histidine
2	0.23 Rf	35235.1	0.564212067	Glutamic acid
3	0.33 Rf	36542.6	1.467762925	Threonine
4	0.54 Rf	5403.8	0.326987777	Methionine
5	0.64 Rf	4030.9	0.296948668	Tryptophane
6	0.72 Rf	7300.4	20.50098287	Isoleusine
Total free amino acids			**24.37394702**	
Halymenia venusta				
1	0.03 Rf	858.2	0.033635117	Histidine

2	0.06 Rf	1793.6	0.067131025	Cysteine
3	0.10 Rf	1514	0.033064999	Lysine
4	0.30 Rf	2788.3	0.111994313	Threonine
5	0.40 Rf	1812.6	0.100975439	Dehydroxiphenylealanine
6	0.43 Rf	14121.8	0.899747058	Leusine
7	0.61 Rf	13665.2	1.006689062	Tryptophane
8	0.74 Rf	1365.3	3.834035383	Isoleusine
Total free amino acids			**6.087272394**	

Present study revealed that from total free amino acids number of essential amino acids were higher compare to non-essential amino acids showing importance of the selected seaweeds for food and medicinal applications. Lourenço *et al*. (2002)reportedglutamic acid was the most abundant amino acid in species they studied except for the brown alga *Dictyota menstrualis* and the green alga *Ulva fasciata*,. Similarly in the present study glutamic acid was absent in *Ulva fasciata*. Vinoj Kumar and Kaladharan (2007) recorded amino acid content of *Sargassum wightii* constituted 12% of its dry weight and the major amino acids contributed were aspartic acid, glutamic acid, arginine, threonine, isoleucine and phenylalanine. *U. lactuca* was found to be deficient in isoleucine, leucine, lysine, methionine, cysteine amino acids reported by Vinoj Kumar and Kaladharan (2007) similarly in the present study , the same species shown presence of cysteine and isoleucine. Imbs *et al*. (2009) reported glutamic acid was the abundant free amino acid found in the brown seaweeds, *Fucus evanescens* and *Laminaria cichorioides* ranged from 26.0% to 31.8% of total free amino acid. Alanine was another main free amino acid in the brown algae, *C. costata*. Large amounts of glycine and proline were also detected in *Undaria species*. The high levels of aspartic and glutamic acids were responsible for the special flavour and taste of seaweeds [Vinoj Kumar and Kaladharan (2007); Yaich *et al*. (2011)]. Hernández-Carmona *et al*. (2009) suggested the most abundant three amino acid were glutamic acid, aspartic acid and leucine.

***4. In vitro* seed priming effect of three seaweeds extract on seed germination, viability and biochemical composition of Onion, Soyabean and Sesame seeds.**

Priming is one of the pre-sowing seed treatments among different treatments used to obtain fast and uniform germination or emergence. Seed priming is process of partially hydrate the seed at the point where germination processes begun but not completed. Very few had reported the effect of seaweed extract as a seed priming material therefore the study was conducted to explore the priming effect of seaweed extract on seed germination and overall growth.

Seaweed liquid extracts use for seed priming treatment

On the basis of their high nutraceutical properties, three seaweed species *Ulva lactuca*, *Sargassum tenerrimum* and *Centroceras clavulatum* were selected to study priming effect, seedling growth, vigour index, and metabolic response carbohydrates, amino acids, proteins and phenols of onion(*Allium cepa*), sesame (*Sesamum indicum*) and soyabean (*Glycine max*) seeds. From the current study it was observed that priming effect on the seeds is dose specific. The same extract was effective at different concentration for three seeds. The priming treatments conducted with various concentrations (5%, 10%, 20%, 30%, 40 %) of selected seaweed extract and water as a control to study the effect on seed germination for 21, 8 and 6 day for onion, soyabean and sesame, respectively. From the study, the significant difference in seed germination was observed between different extract concentration and control. Highest onion seed germination (98%) was observed by 30% *U.lactuca*, 20% *S.tenerrimum* and 10% *C.clavulatum* extracts while control shows only 92% seed germination during the experiment (Fig. 44).

Soyabean seeds germination was optimum at 40 % *C. clavulatum* extract concentration with 87 % germination followed by 20% *U.lactuca* extractwith 86% and 10% *S. tenerrimum* extract with 80% germination whereas, control shows only 59% germination (Fig. 45). Sesame seed shows significant germination at 30% *U.lactuca* and 20% *C. clavulatum* extract with 97% germination followed by 20% *S. tenerrimum* extract with 93% germination, while in control the germination was 80 % (Fig.46).

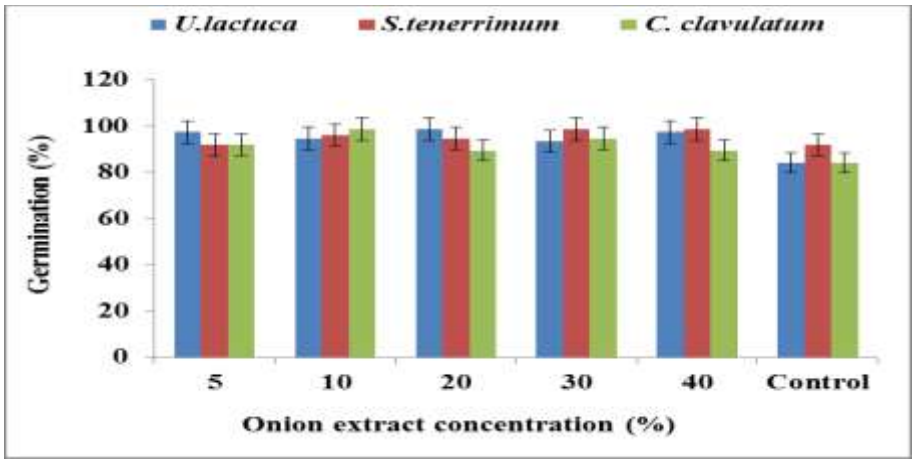


Fig 44. Priming efficiency of seaweed extract for onion seeds

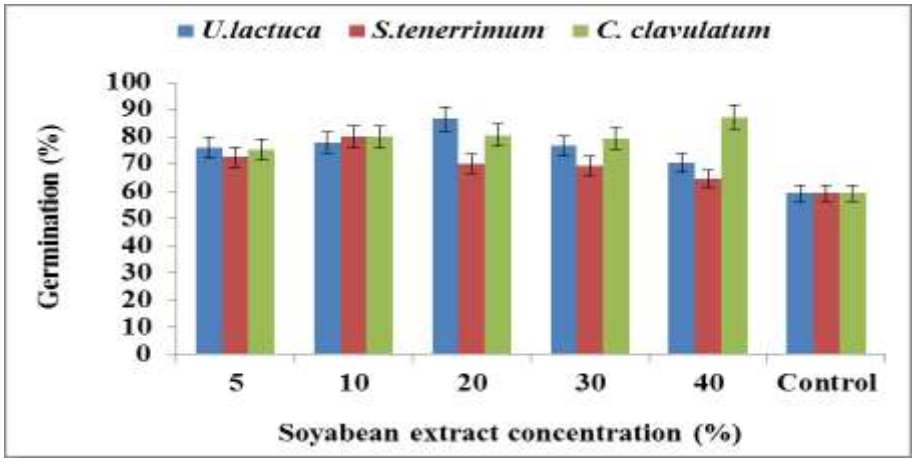


Fig 45. Priming efficiency of seaweed extract for soya bean seeds

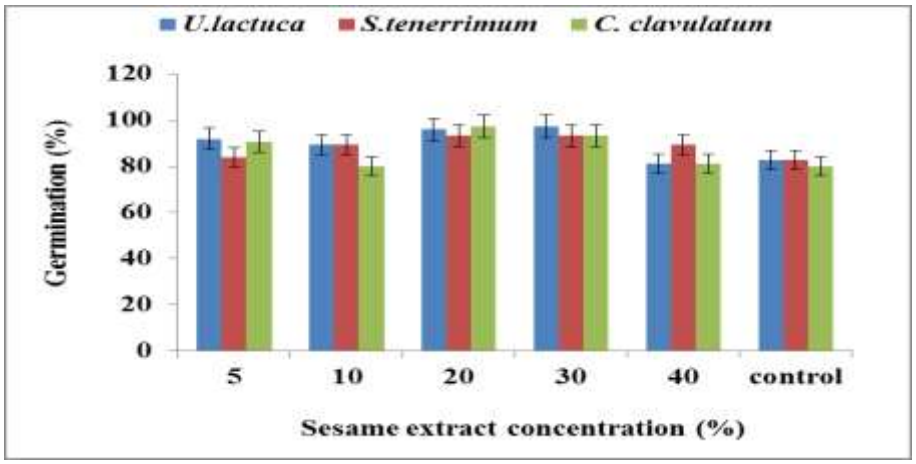


Fig. 46. Priming efficiency of seaweed extract for sesame seeds

Increased seedling growth was observed by application of seaweed extract, is agreed with earlier experiments made in *Phaseolus vulgaris* L. by Kocira *et al*. (2013). All studied seaweed extract exhibited different activity at various concentrations revealed that concentration is prime component to give significant effect due to minerals, carbohydrates and antibiotics which are active ingredients of the seaweed extract [Senn (1987)]. In the present study the selected species contain higher antioxidant and antimicrobial activity with significant amount of minerals and nutrients (R & D, Part 2) could be responsible for good priming efficiency and growth of the seedling. Seaweed extracts has been reportedly used for number of applications including enhanced crop yield and quality, resistance to cold, increased capacity of up taking inorganic nutrients from the soil, stress resistance and enhanced seed germination and growth [Craigie (2011); Sivritepe*et al*. (2015b); Lutts *et al*. (2016)]. Seeds priming generally show high germination rate, greater uniformity and total percent germination Basra *et al*. (2004). Improved germination was reported in table beet [Wilczek and Ng (1982)] and lettuce [Möller and Smith (1998)] by applying the seaweed extracts.

Effect of seaweed liquid extracts on seed germination and seedling growth of the selected seeds.

Our study on seed germination of onion, soya bean and sesame recorded with better result than control. The germination percentage was significantly influenced by different treatments and shows positive influence on study parameter. The germination of seeds was recorded at different days interval as a first and second count according to seed germination testing chart, germination of onion count at 6^{th} and 21^{st} days; soyabean at 5^{th} and 8^{th} days and sesame at 3^{rd} and 6^{th} days. The seedling and vigour was calculated by randomly selected seedlings, 5 seedlings were selected and average was taken in to consideration for each parameter to minimize the error (Fig. 47 - 48&49). Onion seed germination was recorded maximum as 96% and 98% on 6^{th} and 21^{st} day, respectively by 30% *U.lactuca*, 20% *S.tenerrimum* and 10% *C.clavulatum* extract. Control treatment in onion seeds was effective at initial stage on 1^{st} count after 6^{th} day with 82% and after 21 days, it was observed 92% germination. Similarly soyabean was noticed to have highest germination with 92% by 40 % *C. clavulatum* extract after 5 days and 87 % on 8^{th} day while control shows 59% germination during both counting. Sesame seed shows significant germination with 92 % on 3^{rd} day and 97% on 6^{th} day at 30% *U. lactuca* and 20% *C. clavulatum* extract.

In the present investigation, 20% *U. lactuca* extract showed maximum onion seed germination 98%, 15.86 cm seedling growth and 1565.2 Vigour index. Similarly 40% *S. tenerrimum* extract treatment recorded maximum germination 98%, 16.05 cm seedling growth and 1583.48 vigour index in onion seed. While, 10% *C. clavulatum* extract was efficient to induce maximum germination (98%) but the seedling (15.48 cm) and vigour (1424.46) index but growth was higher at 5% extract for the same species. Control was noticed with 92% germination, 10.43 cm seedling growth and 959.86 vigour index in onion seeds. The same extract concentration of each species shows similar pattern for fresh weight and dry weight. During the experiment it was observed that lower seaweed extract concentration (5% and 10%) of *U. lactuca* was less effective to give maximum growth while 20% proved to be the best for maximum growth, further the growth was decreased at higher concentration. *S. tenerrimum* extract induced maximum growth at high concentration, with increase in extract concentration the growth was increased while, in lower concentration of *C. clavulatum* extract was effective to give maximum growth and the growth was decreased with increasing concentration (Fig 47&48).

Soyabean seeds grown well by seaweed extract treatment compared to control where, maximum germination (86%), seedling (20.51 cm) and vigour (1778.61) was registered at 20 % *U. lactuca extract* whereas *10% S. tenerrimum* treatment recorded with 80% germination, 19.15 cm seedling growth and 1530 vigour index while *C. clavulatum* was most efficient at 40% extract to give maximum germination (87%), seedling 19.48 cm) and vigour (1701.90). However, control treatment recorded with 59 % germination, 17.89 cm seedling and 1061.64 vigour for soyabean seeds. The fresh weight and dry weight followed the same pattern for maximum values accept *U. lactuca* shows greater fresh and dry

weight at 40% concentration while the seedling was encountered maximum at 20% concentration. This was may be due to high biomass and nutrient content compare to seedling growth. The *U. lactuca* was effective at lower concentration on the soyabean seeds where maximum growth was recorded at 20 % and growth was decreased with escalated extract concentration. Similarly, *S. tenerrimum* extract showed decreasing seedling growth with increasing extract concentration and highest growth was obsereved at 10 % concentration. On the other hand, *C. clavulatum* induced higher seedling growth at 40 % extract, showing increasing growth with increasing concentration (Fig 49&50).

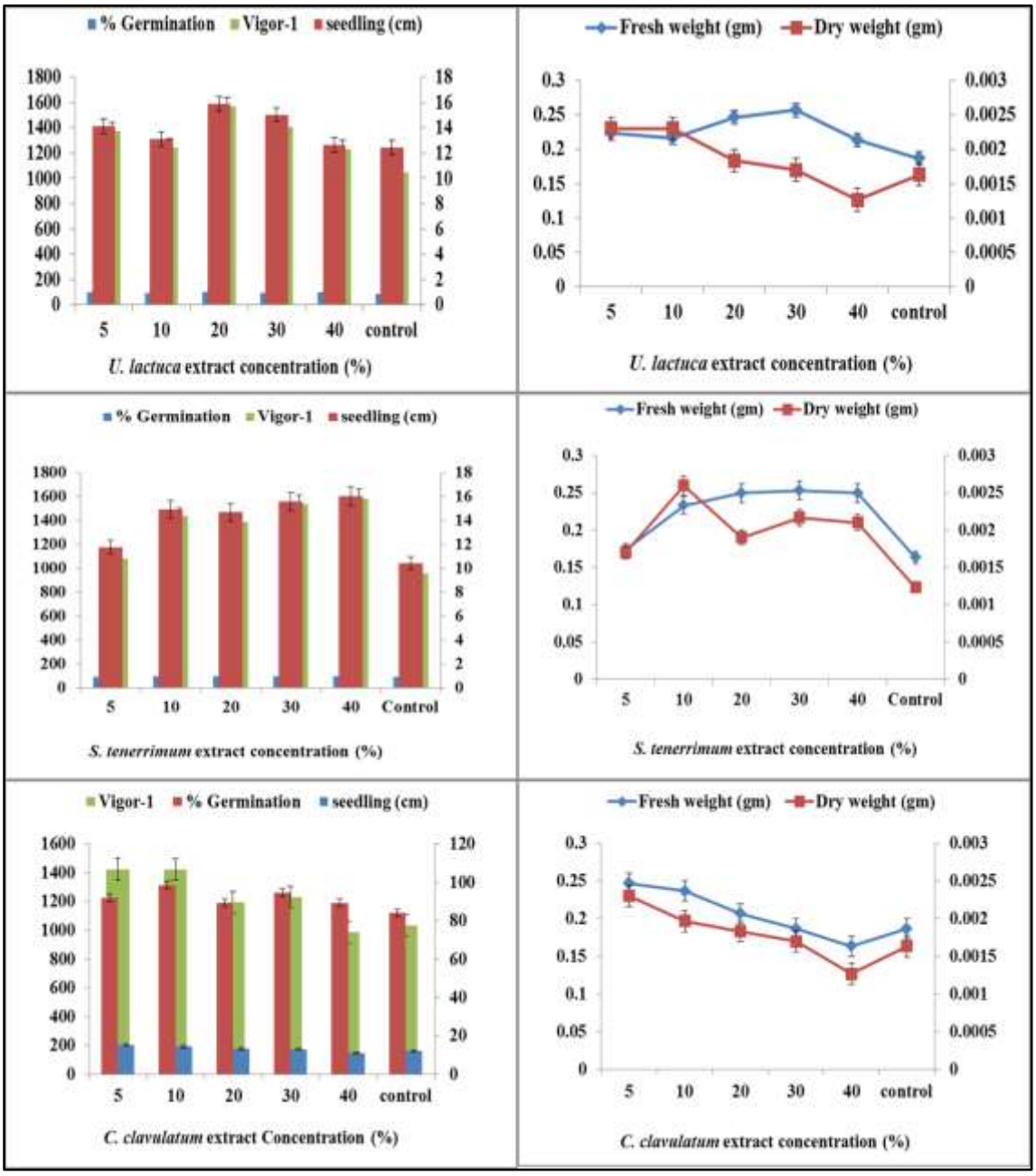


Fig 47. Different seaweed extracts efficiency for onion seed germination and growth.

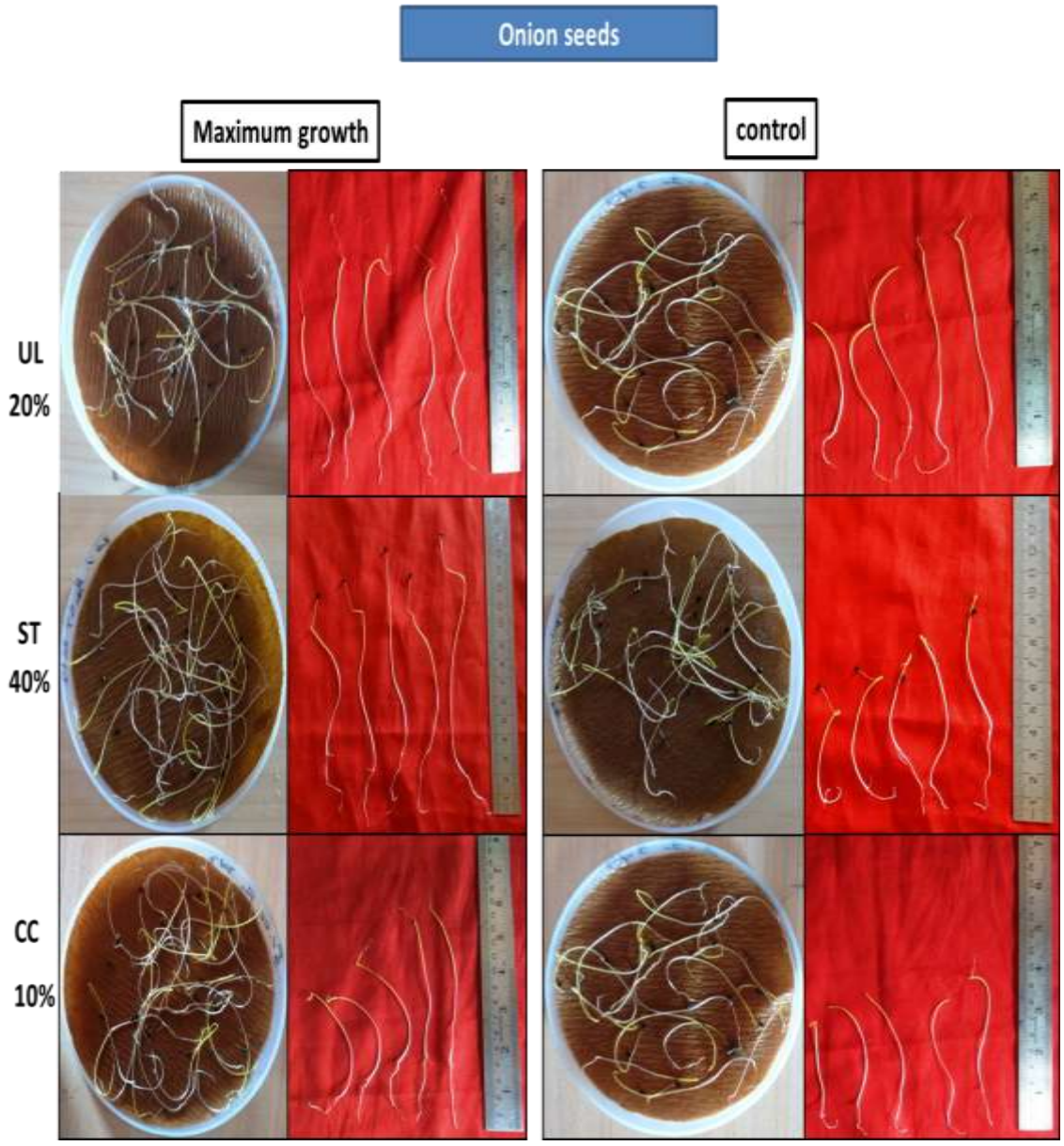


Fig 48. Different seaweed extracts showing maximum seedling growth of onion seeds.

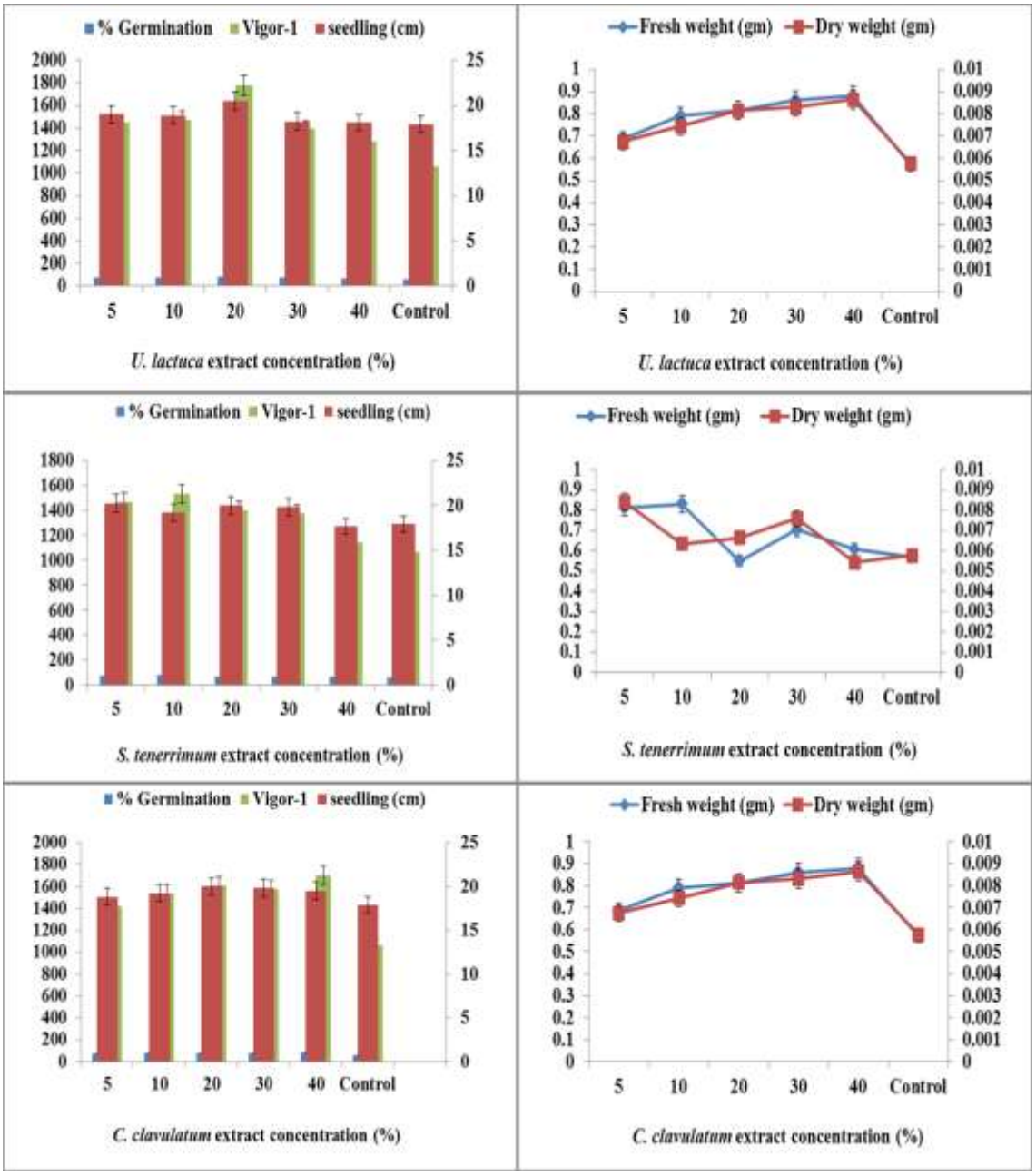


Fig 49. Different seaweed extracts efficiency for soyabean seed germination and growth.

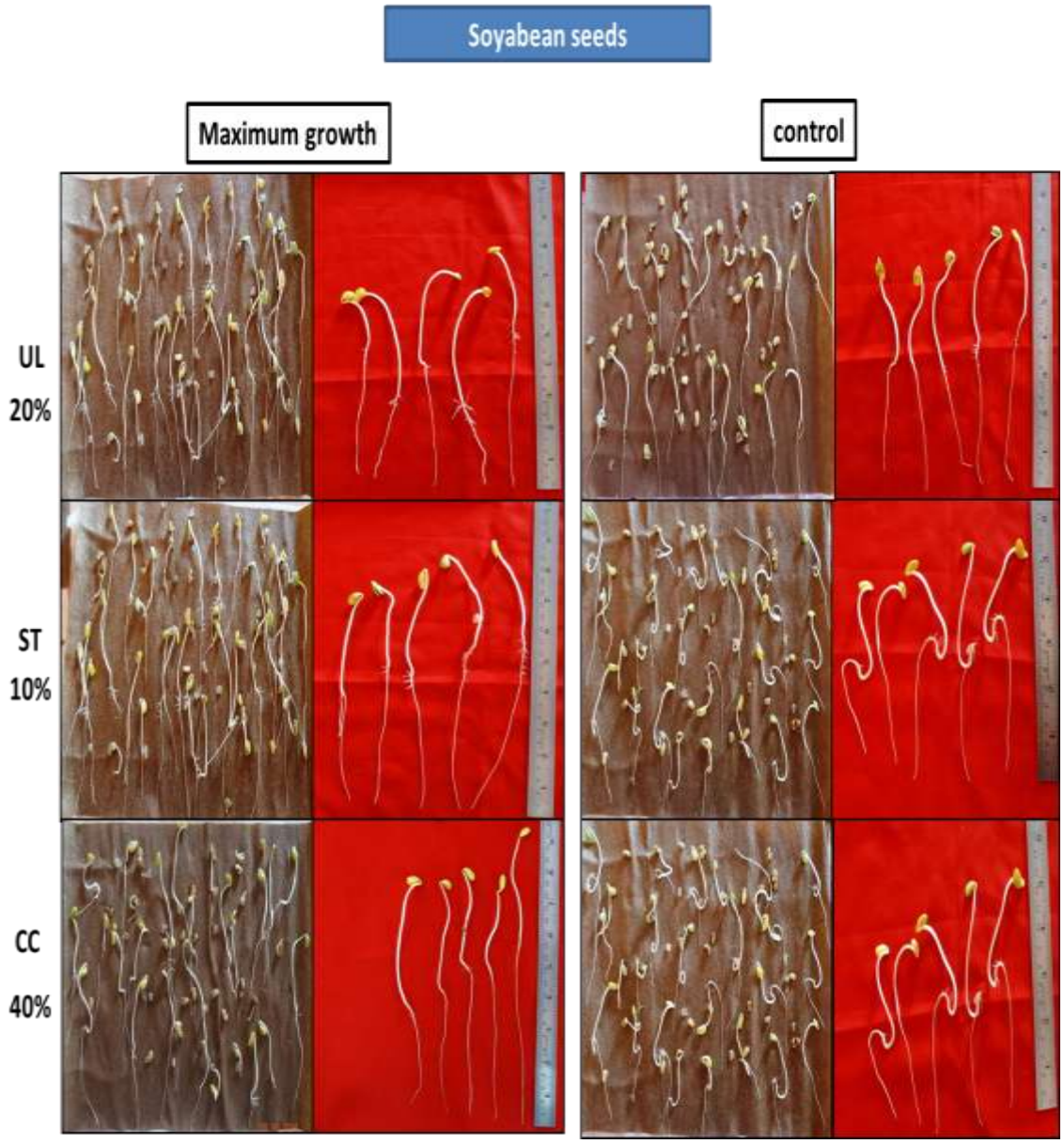


Fig 50. Different seaweed extracts showing maximum seedling growth of soyabean

The maximum sesame seed germination (97%, 93%, 90%), seedling (2.38 cm, 2.66 cm, 2.22cm) and vigour (231.49, 373.33, 201.29) were encountered in 30% *U. lactuca*, *20% S. tenerrimum* and 5% *C. clavulatum*, respectively. In *C. clavulatum* species at 20% extract maximum germination was observed but seedling growth and vigour was high at 5% compare to other extract concentration in sesame seeds. *U lactuca* was effective at 30% extract concentration and was less effective at higher concentration. Moreover, *S. tenerrimum* showed increased growth at lower extract concentration and growth was decreased as the extract concentration escalated, whereas *C. clavulatum* was most effective at lower extract concentration (5%) and growth was decreased with higher concentration (Fig 51&52).

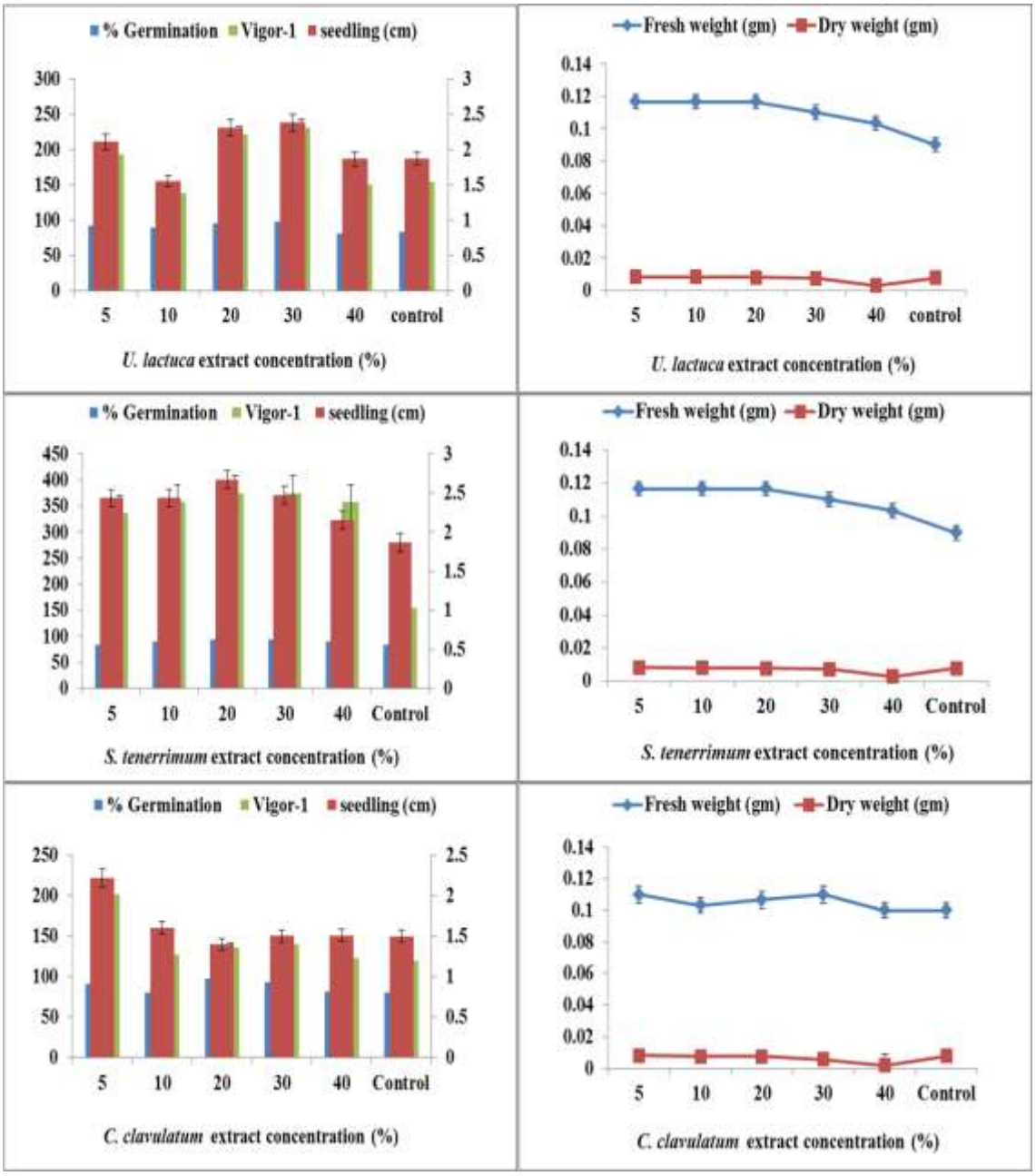


Fig 51. Different seaweed extracts efficiency for sesame seed germination and growth.

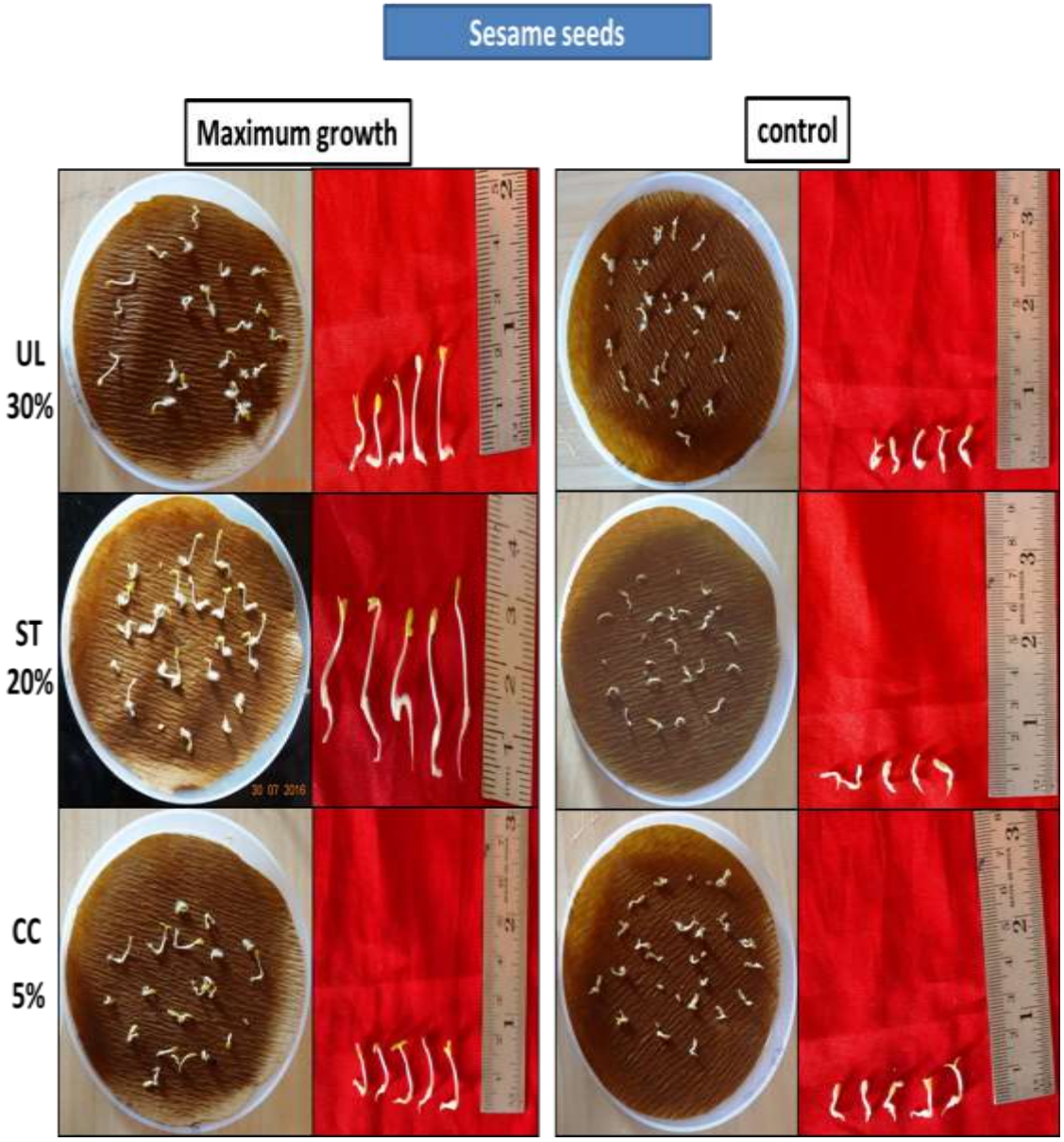


Fig 52. Different seaweed extracts showing maximum seedling growth of sesame seeds

It was reported that the presence of plant growth regulators, trace elements, vitamins, micronutrients and amino acids in the low concentration of seaweed liquid fertilizer enhance the growth of root and shoot [Thomas *et al*. (2013)]. Ruan *et al*. (2002) reported that primed seeds showed better germination pattern and higher vigour levels than non- primed. The results of present study agreed with those of earlier studies on wheat [El-Din (2015)] showing 93% germination when soaked in lower concentrations of seaweed extracts while at increased concentrations of the extract inhibits germination in onion seeds. Similarly seaweed liquid extract treated seeds of green gram [Ganapathy Selvam *et al*. (2013)] shows good result with 100% germination in lower concentration of extract prepared from *Ulva reticulata* and in cluster bean.

Thirumaran *et al*. (2009) also reported similar findings while using brown seaweed extract from *Rosenvingea intricate*. Seaweed liquid extract treatment enhanced the rate of seed germination in green

chilies and turnip [Dhargalkar and Untawale (1983)] and found that lower concentrations increase the germination percentage than the higher concentration. Thirumaran *et al*. (2009) reported that seaweed extract prepared from brown algae *Rosenvingea intricata* have good result on shoot length (33.96cm) and root length (17.23cm) on cluster bean at the 20% extract solution. This result coincides with our current study on onion where maximum seedling length and vigour was recorded in 20% *U. lactuca* and 5 % *C. clavulatum* extracts which is significantly higher than control. Similar finding were reported by using seaweed liquid fertilizer from different algae [Dhargalkar and Untawale (1983)] on the growth of crops such as *Capsicum frutescens*, *Brassica rapa* and *Anans comosus*. Similar results were recorded in *Cajanus cajan* [Mohan *et al*. (1994)] , red gram [Venkataraman Kumar *et al*. (1993)], *Rosenvigea intricata* [Thirumaran *et al*. (2009)], *watermelon* [Abdel-Mawgoud *et al*. (2010)] and maize [Stephenson (1974)]. The growth promoting factors like IAA and IBA, Gibberlins (A&B), micronutrients, vitamins and amino acids might influence on the germination rate whereas retarded growth effects at higher concentration can be seaweed liquid extract recognized by excessive hormones or high concentration of minerals [Challen and Hemingway (1966)]. In addition, Stephenson (1974) suggested seaweeds contain precursors of elicitor compounds that promote germination.

Effect of seaweed liquid extracts on metabolites of the selected seeds.

Primed seeds usually have better and more synchronized germination [Farooq *et al*. (2008)], owing simply to less imbibition time [Brocklehurst and Dearman (1983)] and build-up of germination enhancing metabolites [(Basra *et al*. (2004)]. Seed priming induce some of the metabolic changes necessary for germination. Increased germination rate and uniformity have been attained due to metabolic repair during imbibition [(Bray *et al*. (1989)], a buildup of germination enhancing metabolites [(Basra *et al*. (2004)]. In the present study variation in carbohydrate, protein, amino acids and phenol concentration was recorded at different extract treatment when compared with control. In onion seedling maximum carbohydrate concentration was recorded at 40% *S.tenerrimum* (17.1 mg/g) followed by 20% *U. lactuca* (16.67mg/g) and 10% *C. clavulatum* extracts (16.1mg/g). Protein content was greater at the 40% *S.tenerrimum*, 20% *U. lactuca* and 10% *C. clavulatum* treatments recorded 10.95 mg/g, 9.96 mg/g and 9.78 mg/g, respectively. Amino acids was recorded 6.1 , 5.76 and 5.12 mg/g while phenol registered with 4.16, 3.87 and 3.24 mg/ g at 40% *S.tenerrimum*, 20% *U. lactuca* and 10% *C. clavulatum*, respectively. The carbohydrate, protein, amino acids and phenol content in control treatment seedlings was recorded 14.98, 8.5, 4.3 and 1.52 mg/g, respectively (Fig.53).

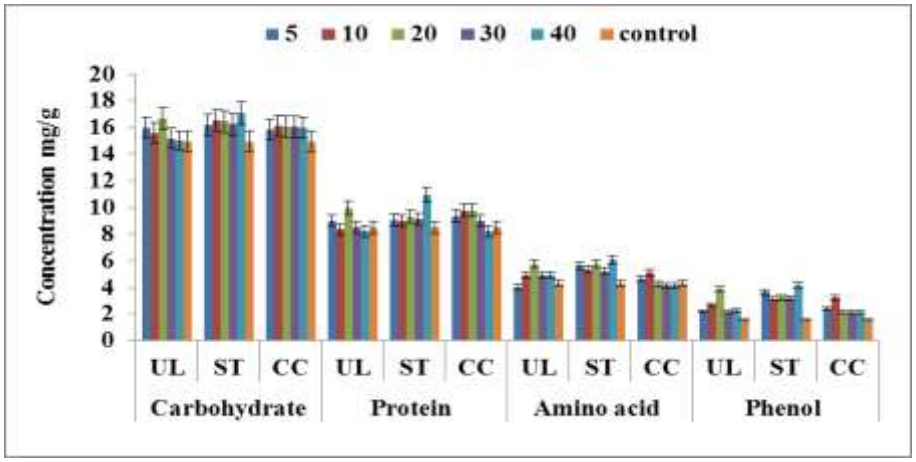


Fig. 53 Biochemical variation in onion seedling at different treatments (%) of seaweeds-*U. lactuca, C. clavulatum* and *S.tenerrimum*.

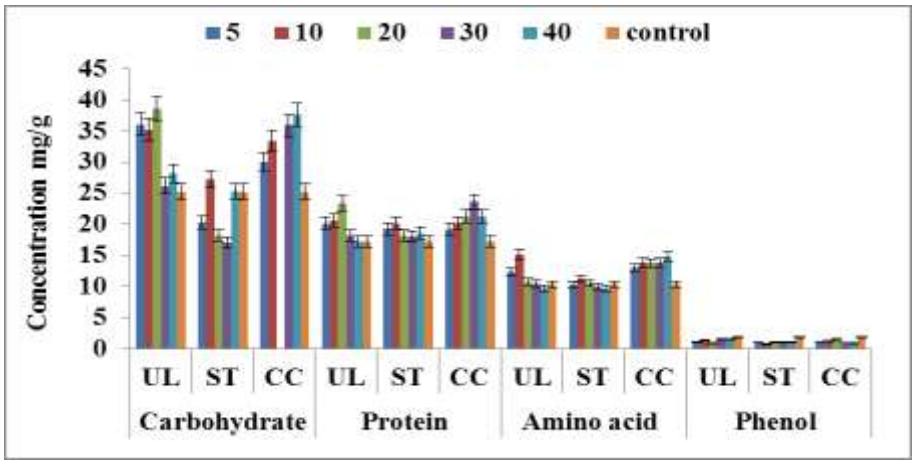


Fig. 54 Biochemical variation in soyabean seedling at different treatments (%) of seaweeds- *U. lactuca, C. clavulatum* and *S.tenerrimum*.

Soyabean showed the maximum biochemical constituents at 20% *U. lactuca*, 40% *C. clavulatum* and 10 % *S.tenerrimum* seaweed extract. TheCarbohydrate noticed 38.6, 37.65 and 27.33 mg/g; maximum protein was 23.4, 21.32 and 20.21mg/g; amino acids content was 15.2, 11.2 and 14.87 mg/g and phenols content was 0.97, 0.7 and 0.87mg/g whereas control was registered with 25.23mg/g carbohydrate, 17.2mg/g protein, 10.4 mg/g amino acids and 1.78 mg/g phenol (Fig. 54).

The Sesame seed were influence by 20 % *S. tenerrimum*, 30% *U. lactuca* and 5% *C. clavulatum* where maximum growth in terms of germination, seedling, vigour and biochemical component was observed.Carbohydrate concentration at these concentration was recorded as 26.4, 24.6 and 22.5 mg/g; protein by 11.3, 10.5 and 10.37 mg/g; amino acid 8.76, 6.87, 6.65 mg/g and phenol content was 0.23, 0.13 and 0.42 mg/ g with respective to 20 % *S.tenerrimum*, 30% *U. lactuca* and 5% *C. clavulatum seaweed extracts* . In control treatment seedling was registered with 18.4mg/g carbohydrate, 9.5 protein, 5.3 mg/g amino acid and 0.52 mg/g phenol content which was comparatively lower than seedling treated with seaweed extracts (Fig. 55).

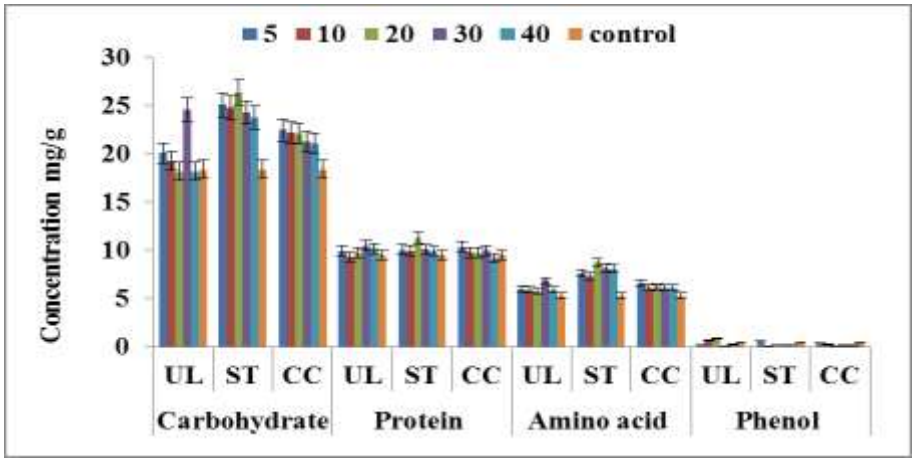


Fig. 55 Biochemical variation in sesame seedling at different treatments (%) of seaweeds- *U. lactuca, C. clavulatum* and *S.tenerrimum*

Seaweed formulations as biostimulants in crop production are well established which are serving as fertilizer and metabolic enhancers [(Zhang and Schmidt (1997)]. Different concentrations (10, 15, 20, 25 and 30%) of seaweed liquid extract were used and better results were obtained in lower doses (10, 15, 20%) which was also reported by Selvaraj *et al*. (2004). The improved germination, growth and biochemical composition of some vegetables and fruits was observed using seaweed extract [Khan *et al*. (2009)]. Moreover, lower concentration of 1% *Padina boergesenii* and *Ulva lactuca* extract significantly increased the shoot length, leaf breadth, leaf length, root length and number of roots in *Rhizophora mucronata* [(Pise and Sabale (2010)] and *Arachis hypogea* plants [(Sridhar and Rengasamy (2010b)] respectively. On the contrary, it has been reported that concentration at 20% of *S. wightii* [Jothinayagi and Anbazhagan (2009)]; [Thirumaran *et al*. (2009)] and *Rosenvingea intricate* [Thirumaran *et al*. (2009)] promoted shoot, root length, fresh and dry weight of *Abelmoschus esculentus* and *Cyamopsis tetragonolaba* respectively.

In present study also higher concentration 5, 10, 20 and 40 % of different extract was effective for (onion, soya bean and sesame) growth and metabolites. Growth enhancement by seaweed extracts may be due to biostimulant components such as macro and microelement, amino acids, vitamins, cytokinins, auxins and abscisic acid (ABA)-like growth substances which affects cellular metabolism in treated seedlings and plants leading to enhanced growth and crop yield [Ördög *et al*. (2004); Stirk and Van Staden (1997); Durand *et al*. (2003)]. This is in accordance with the earlier reports that lower concentrations of seaweed extracts enhanced the biochemical constituents in *C. cajan* [Erulan *et al*. (2009)], *B. nigra* [Kalidass *et al*. (2010)], *Citrullus lanatus* [Abdel-Mawgoud *et al*. (2010)], *Trigonella foenum-graecum* [Pise and Sabale (2010)], *Solanum melongena* [Bozorgi (2012)], *Abelmoschus esculentus* [Sasikumar *et al*. (2011)]. Moreover, it has been reported that seaweed liquid fertilizer at 10% extracted from brown alga *S. wightii* increased the content of photosynthetic pigments, protein and total sugars in *Vigna radiata* [Sivasankari *et al*. (2006a)] and seaweed extract of *Rosenvingea intricate* at

20% extract enhanced the photosynthetic pigments and carotenoids in *C. tetragonoloba* [Thirumaran *et al*. (2009)].

Hence, in the current study the stimulation effect of the onion, soya bean and sesame seeds clearly revealed that the soaking/ priming of seeds in different concentration of seaweed extract had provided better growth compare to control or nonprimed seeds. Seeds primed for 10 h with 40% *S. tenerrimum* extract for onion, 6 h with 20% *U.lactuca* extract for soyabean and 10 h with 20% *S. tenerrimum* were highly suitable for getting quality seedlings and higher vigour.

Examination of seaweeds as bio-fertilizer in *Ex-situ* experiment on Fenugreek and Spinach seeds.

Three seaweed species *Ulva lactuca*, *Sargassum tenerrimum* and *Centroceras clavulatum* were selected to study the effect of seaweed as liquid fertilizer on seed germination, growth, biochemical and pigment content of Fenugreek (*Trigonella foenum-graecum*) and Spinach(*Spinacia oleracea*) seeds. The concentration of 20%, 40%, 60% and 80% extract was prepared from selected seaweed species *Ulva lactuca*, *Sargassum tenerrimum* and *Centroceras clavulatum*, whereas untreated plants were considered as control. By soil drench method each extract concentration was provided to the seeds at three intervals at first day of sowing, after 5 days and after 15 days. 1ml of each extract concentration was added at the base of every seedling.

Effect of seaweed liquid fertilizer on seed germination and growth.

The 86% fenugreek seed germination registered by 80% *S. tenerrimum* and *C. clavulatum* extract, whereas 100% germination was recorded in spinach by all three extracts at different concentration. *U. lactuca* at 20 and 40%, *C. clavulatum* at 60 and 80% while each concentration (20, 40, 60, 80%) of *S. tenerrimum* induced 100% germination. Control was recorded with 46 % and 66 % germination in fenugreek and spinach seed, respectively. In both seeds higher concentration of seaweeds extract was found effective to induce maximum germination except in fenugreek by *U. lcatuca* extract was effective at 20 and 40% concentration. The effect of seaweed extract on seedling growth was observed initially at 10^{th} day and then after 30 day. Initially at 10^{th} day Shoot length of fenugreek seedlings was recorded maximum at 60% *U. lactuca* (5.5 cm) extract treatment followed by 60% *C. clavulatum* (5 cm) and 80% *S. tenerrimum* (4.9cm) whereas control showed maximum 2.8 cm shoot length. Besides, Root length was noticed maximum at 80% *S.tenerrimum* (6.5cm), 60% *C. clavulatum* (5cm) and 40% *U.lactuca* (4.2cm) extract treatment, while control was registered with 2 cm fenugreek seedlings. Shoot in spinach seedling showed maximum length at 20% *S.tenerrimum* (5.9cm),20% *U.lactuca* (5.7 cm) and 80% *C. clavulatum* (5.7cm) extract treatment .Moreover, Root length was higher at 20% *U.lactuca* (4.5 cm), 20% *S.tenerrimum* (4.3cm) and 80% *C. clavulatum* (3.8cm) extract treatment. However, in control, shoot and root length was registered as 3.2 and 1.9 cm, respectively (Fig. 56, 57&58).

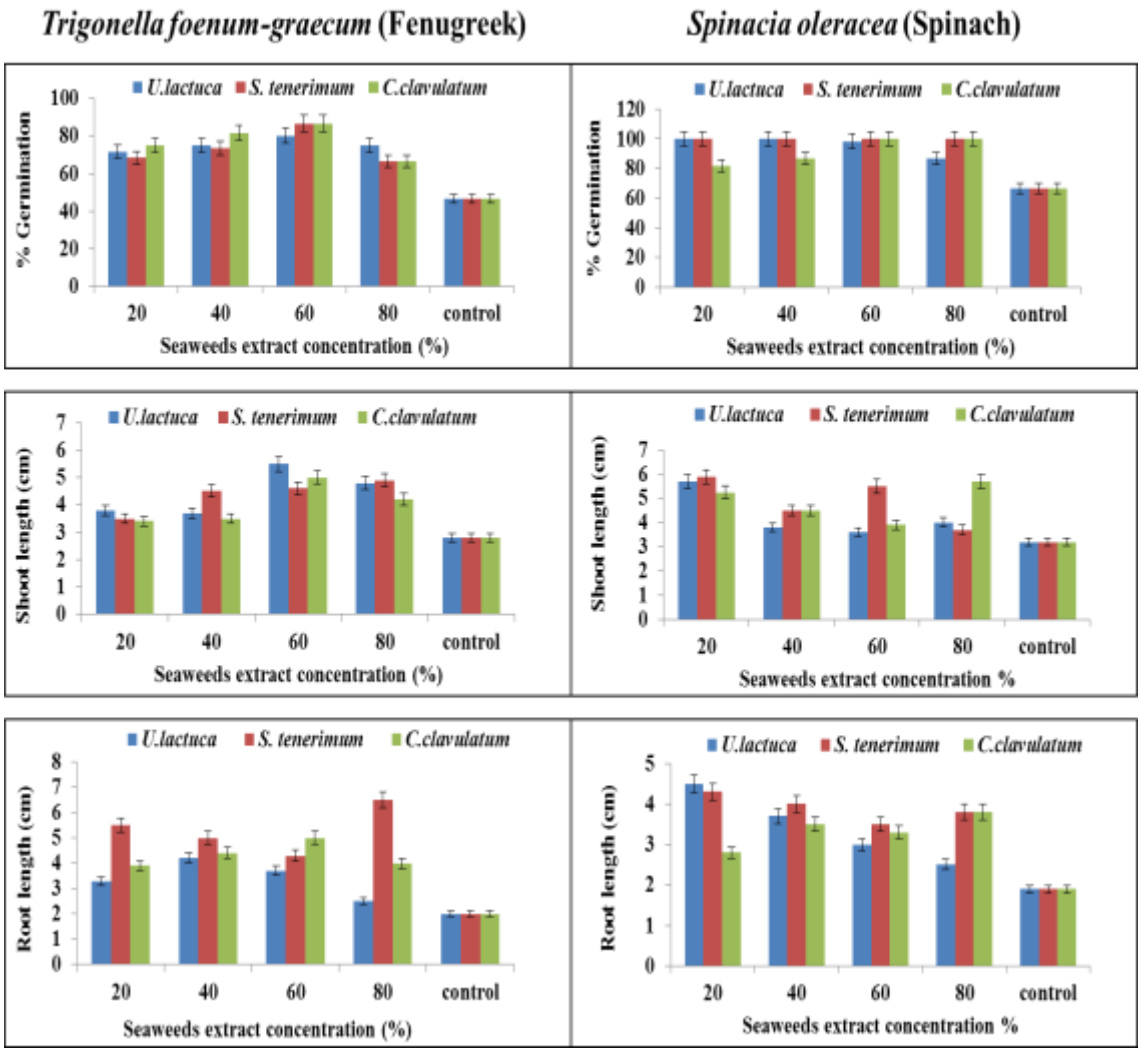


Fig 56. Effect of seaweed extract on germination, shoot length and root length of fenugreek and spinach plant after 10 days.

After 30 days the highest shoot length of fenugreek was recorded at 60% *U. lactuca* with 14.5 cm followed by 60% *C. clavulatum* extract treatment with 13.7cm and 80% *S. tenerrimum* 13.2cm but control showed maximum 9.0cm shoot length. The root length was noticed maximum at 80% *S.tenerrimum* (8cm), 60% *C. clavulatum* (7.5cm) and 40% *U.lactuca* extract treatment (7.1cm), however, control was observed to be 5 cm in fenugreek seedlings. The shoot length in spinach plant showed maximum length at 20% *S.tenerrimum* (17.1cm),20% *U.lactuca* (15.3 cm) and 80% *C. clavulatum* extract treatment (15.1cm). Whereas root length was noticed higher at 20% *U.lactuca* (5 cm), 20% *S.tenerrimum* (4.3cm) and 80% *C. clavulatum* extract treatment (4.2cm). But in control shoot and root length was registered as 9.5 and 2.8cm, respectively (Fig. 56 to 59).

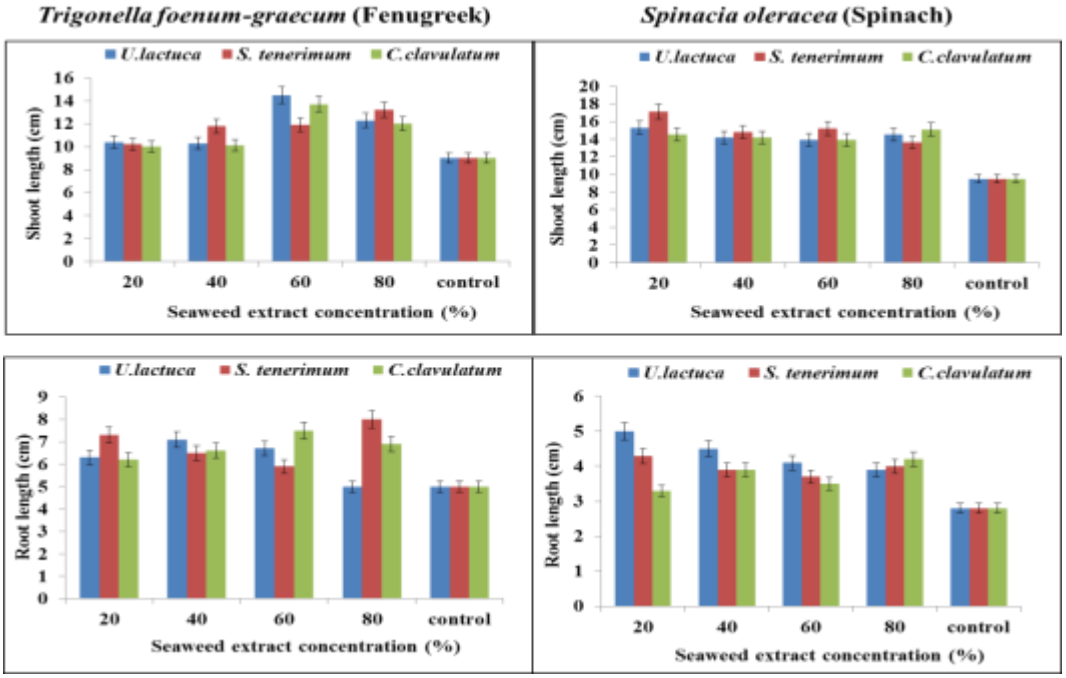


Fig 57.Effect of seaweed extract on shoot length and root length of fenugreek and spinach plant after 30 days.

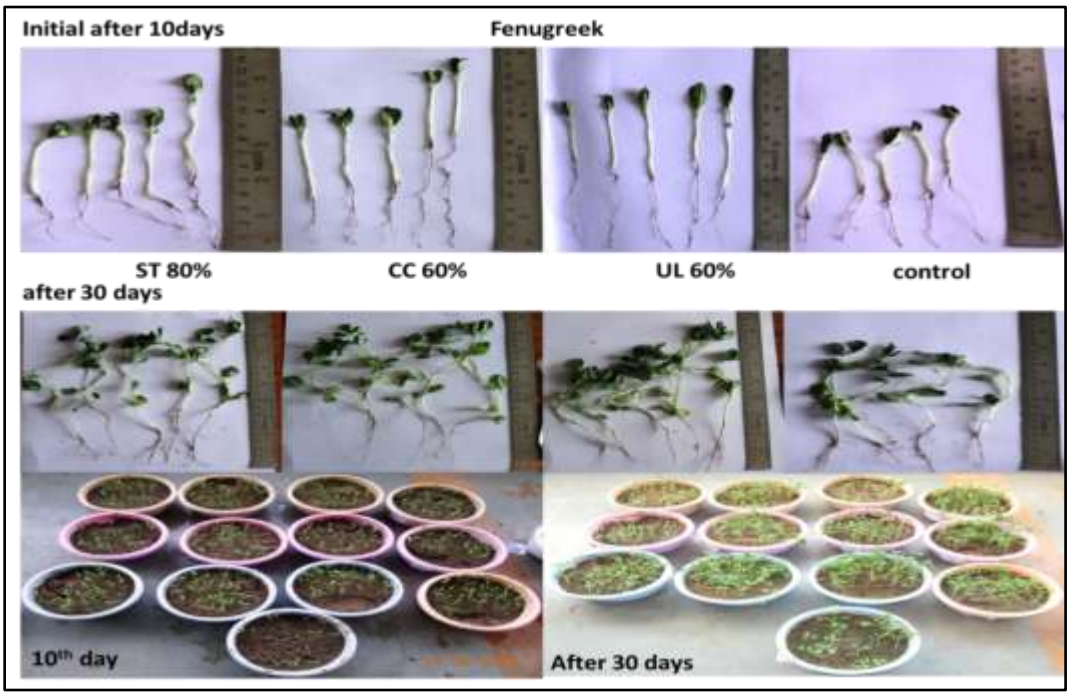


Fig 58. Physiological changes in fenugreek plant at different day's interval shown with different concentration of seaweed extract.

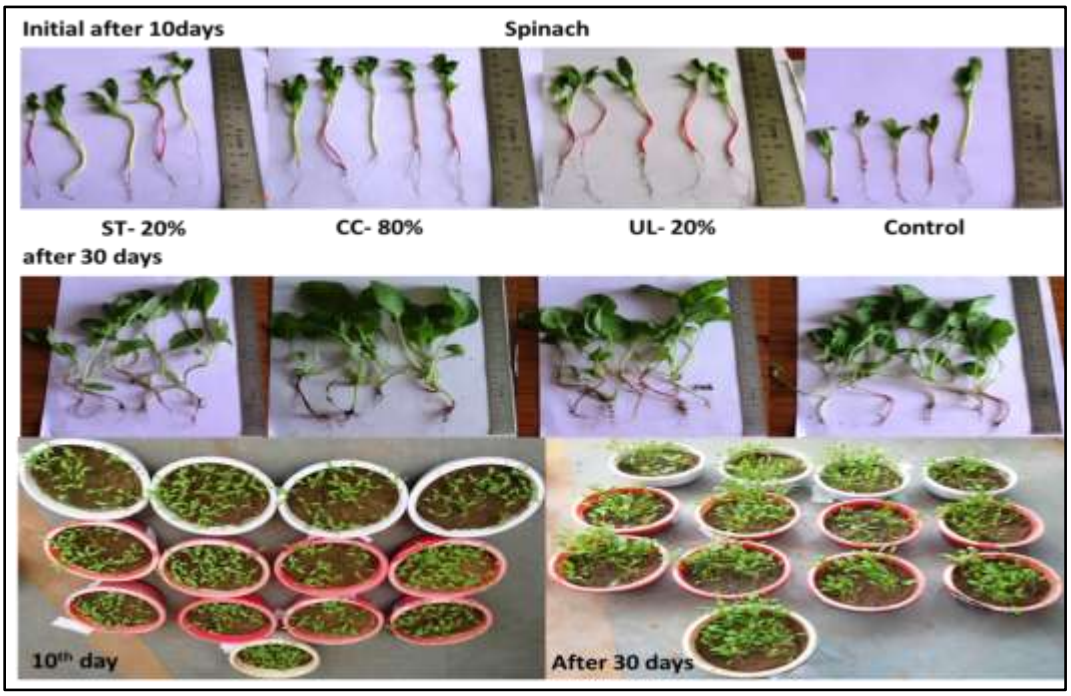


Fig 59. Physiological changes in spinach plant at different day's interval shown with different concentration of seaweed extract.

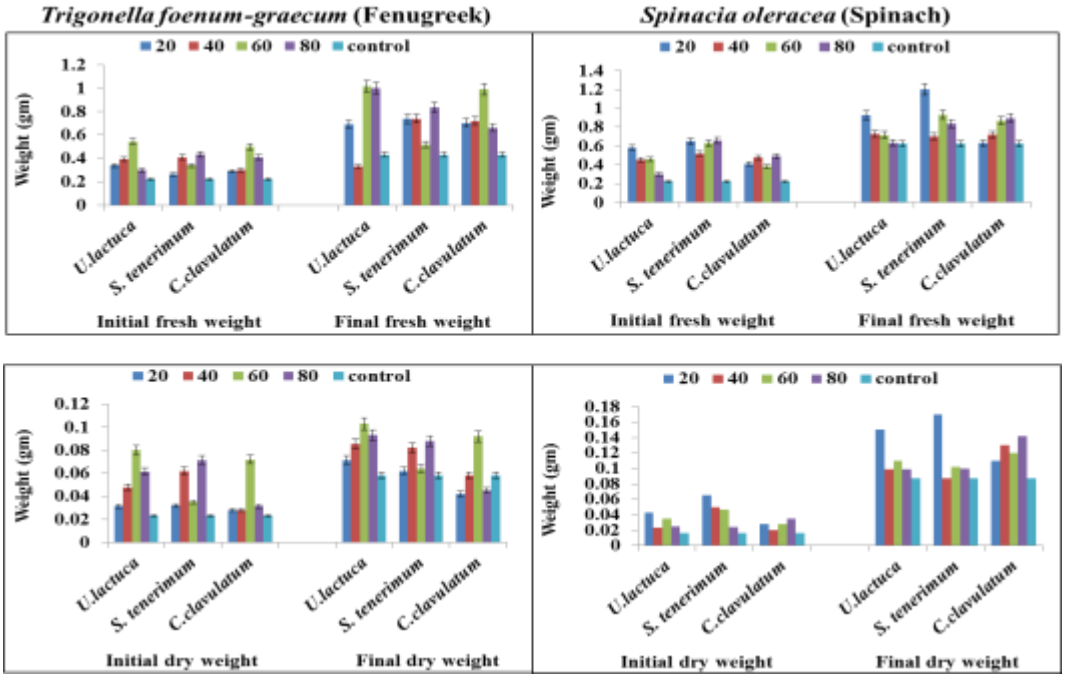


Fig 60. Effect of seaweed extract on fresh and dry weight of fenugreek and spinach plant.

Fresh weight of fenugreek plant recorded in the range from 0.26 to 0.544 gm and 0.33 to 1.015 gm at initial and final stage. But fresh weight at initial and final stage respectively was registered with 0.22 and 0.43 gm in control. The dry weight varies from 0.028 to 0.08 gm and 0.042 to 0.10 gm range in treated plants whereas in control dry weight was encountered as 0.023 gm and 0.058 gm at initial and final stage, respectively.

The maximum value of fresh weight as well as dry weight was recorded in the 60% *U.lactuca*, 60% *C. clavulatum* and 80% *S. tenerrimum* extract treated plant as compare to control. In the spinach fresh weight at seedling stage (after 10 days) from the treatment was observed in the range of 0.3 to 0.66 gm whereas at final stage (after 30 days) it was in the range from 0.63 to 1.201 gm. But the fresh weight of control seedlings at initial and final stage was 0.22 and 0.625 gm, respectively. Dry weight varies from 0.02 to 0.065 gm and 0.087 to 0.17 gm at initial and final stage. However, control plants showed dry weight as 0.016 and 0.087 gm. The maximum values were obtained from 20 % *S. tenerrimum*, 20 % *U. lactuca* and 80% *C. clavulatum* of extract concentration(Fig. 60).

Khan *et al*. (2009) reported that seaweed extracts are commercially available as liquid bio fertilizers due to presence of growth promoting compounds such as betaines, cytokinins and auxins. Seaweed fertilizer efficiency was explored in different vegetables, pulses and cereals crops [Kalidass *et al*. (2010); Sasikumar *et al*. (2011); Zodape *et al*. (2011) ; Bai *et al*. (2013); Parthiban *et al*. (2013); Kalaivanan *et al*. (2012)]. The responses from seaweed treated plants in terms of growth, physical and chemical properties and yield have been documented; seaweed fertilizer effect in *Brassica nigra* [Kalidass *et al*. 2010)], *Abelmoschus esculentus* [(Sasikumar *et al*. 2011)], *Lycopersicon esculentum* [Zodape *et al*. 2011)], *Vigna radiata* [Bai *et al*. (2013); Parthiban *et al*. (2013)], *Vigna mungo* [Kalaivanan *et al*. (2012)], *Solanum lycopersicum*Hernández-Herrera *et al*. (2014), *Mangifera indica* [Mohamed and El-Sehrawy (2013)] and *Fagopyrum esculentum* [Anisimov *et al*. (2013)]. It has been reported that 20 % *Sargassum wightii* [Jothinayagi and Anbazhagan (2009)] and *Rosenvingea intricata* extract [Thirumaran *et al*. (2009)] stimulated shoot length, root length, fresh and dry weight of *Abelmoschus esculenthus* and *Cyamopsis tetragonolaba*, respectively. Growth improving effect of seaweed extracts might be due to presence of active compounds such as macro and micro nutrients, amino acids, vitamins and growth hormones (cytokinins, auxins and abssisic acid) which affect the treated plants leading to improved growth and crop yield [Ördög *et al*. (2004; Thirumaran *et al*. (2009); Durand *et al*. (2003)]. Similarly, in present study, the increased growth of fenugreek and spinach plants might be the results of growth stimulating substances present in the selected seaweed extract as recorded in other macro algal extract [Mooney and Van Staden (1986)].

Selvaraj *et al*. (2004) studied effect of seaweed extract using different concentration (10, 15, 20, 25 and 30%) and better results was noticed in lower concentration (10,15,20 %) . The low concentration (10%) of seaweed liquid extract induced higher seedling growth, fresh weight and dry weight in *Vigna catajung* [Anantharaj and Venkatesalu (2002)]. In the present study 60 %*U. lactuca*, 60% *C. clavulatum* and 80% *S.tenerrimum* extract was effective to promote maximum growth in fenugreek plant whereas20% *U.lactuca*, 20 % *S.tenerrimum* and 80% *C. clavulatum* extract was most effective

concentration recorded for growth of spinach plant. However, Ashok *et al*. (2004) reported the higher concentrations (2, 2.5 and 5%) proved to be the inhibitory doses on *Sorghum*.

Effect of seaweed liquid fertilizer on biochemical and pigment concentration of fenugreek and spinach plant.

The changes in biochemical and pigment concentration at different extract treatments were recorded in both selected fenugreek and spinach plants after 30 days when compared with control. The carbohydrate concentration in fenugreek was observed in the range from 1.32 to 5.3 mg/g, where maximum 5.3 mg/g carbohydrate content showed at 60% *U. lactuca* treatment followed by 4.2 and 3.1 mg/g carbohydrate content in 60% *C. clavulatum* and 80% *S. tenerrimum* extract treatments but the control plants showed 1.32 mg/g carbohydrate content. Protein content varies from 1.13 to 1.76 mg/g, while phenol ranged from 0.12 to 0.43 mg/g and amino acids found in the range from 0.23 to 0.85 mg/g where all these biochemical compounds were recorded maximum in 60% *U.lactuca,* 60% *C. clavulatum* and 80% *S. tenerrimum,* extract treatments,respectively. The control plants recorded with 1.13 mg/g protein, 0.12 mg/g phenol and 0.23 mg/g amino acids. It was observed that all the biochemical compounds were recorded in higher concentration in plants treated with seaweed liquid fertilizer as compared to control. The total chlorophyll and carotenoids registered in the range from 2.0 to 2.31mg/g and 0.88 to 1.98 mg/g, respectively, however not much variations in the total chlorophyll was noticed compare to control whereas significant difference was observed in the carotenoid content. Highest value was recorded in 60% *U. lactuca*, 60% *C. clavulatum* and 80% *S. tenerrimum* extracttreatment (Fig 61).

In spinach plant, the maximum biochemical compounds and pigments were recorded from 20 % *S. tenerrimum,* 20% *U. lactuca* and 80% *C. clavulatum* compare to control. The carbohydrate valued from 8.5 to 10.65 mg/g, where maximum was recorded from 20 % *S. tenerrimum* and minimum was registered in control. Protein content varies from 1.08 to 2.45mg/g; 20 % *S. tenerrimum* extract treatment showedmaximum value and minimum was recorded in control. Phenol content was noticed in the range of 0.76 to 1.01 mg/g, here the difference in control and treated plant is not much, while 20 % *S. tenerrimum* extract treated plants showed 1.01 mg/g phenolic content. Amino acids were recorded higher in the same treatment with 1.083 mg/g while, amino acid was registered0.57 mg/g in the control. In pigment concentration, chlorophyll content was recorded maximum 12.32 mg/g whereas carotenoid content was valued as 1.5 mg/g from 20 % *S. tenerrimum* extract treatment. But control was encountered with 10.65 mg/g and 1.2 mg/g chlorophyll and carotenoid, respectively (Fig 61).

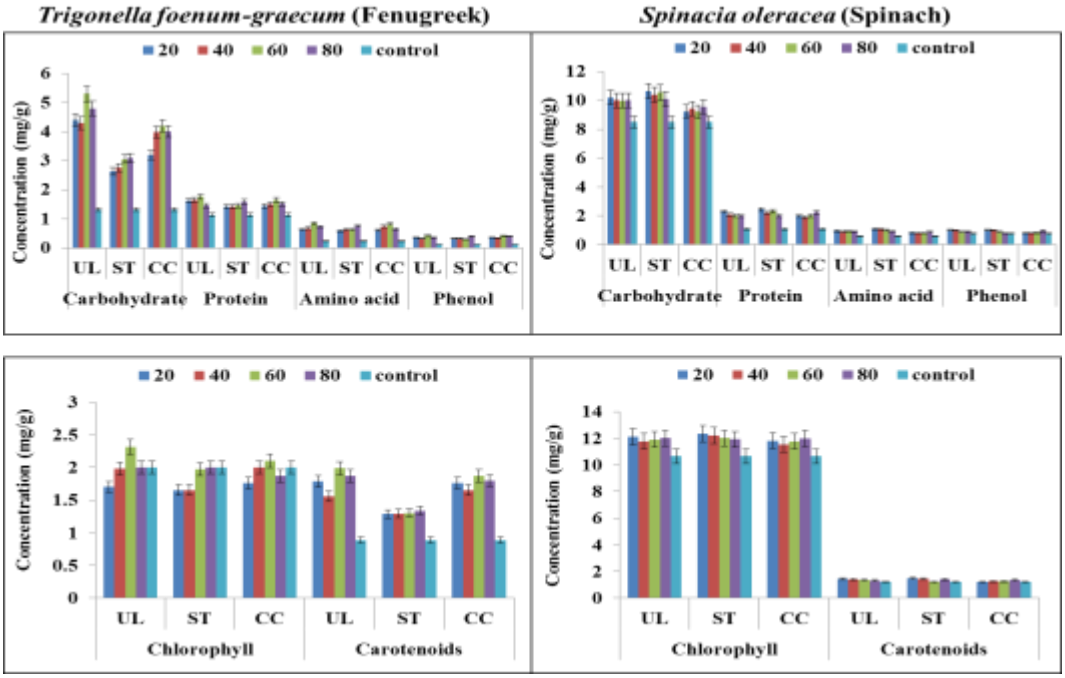


Fig 61. Effect of seaweed extract on biochemical and pigment composition of fenugreek and spinach.

The low seaweed extracts concentrations improved the biochemical and pigment constituents in *Cajanus cajan* [Erulan *et al*. (2009)], *Brassica nigra* [Kalidass *et al*. (2010)], *Citrullus lanatus* [Abdel-Mawgoud *et al*. (2010)], *Trigonella foenum graecum* [Pise and Sabale (2010)], *Solanum melongena* [Bozorgi (2012)] and *Abelmoschus esculentus* [Sasikumar *et al*. (2011)]. The rise in photosynthetic pigments may be due to the presence of betaines [Blunden *et al*. (1996)], improved size and number of the chloroplast and healthier grana development [Atzmon and Van Staden (1994)] in the seaweed liquid extract treated plants. Furthermore, the elevated levels the protein concentration and nitrate reductase enzyme activity at lower dose of seaweed liquid extract proved the efficiency of foliar spray as it improved the absorption and uptake of most of the essential elements by the seedlings.

The higher chlorophyll content could also be a result of reduction in degradation of chlorophyll, influenced by presence of betaines in the seaweed liquid fertilizer [Whapham *et al*. (1993)]. Sridhar and Rengasamy (2010a) suggested that 1 % *Ulva lactuca* extract along with 50 % recommended rate of chemical fertilizers increased the protein, carbohydrate and lipid content in *Tagetus erecta*. Conversely, it has been also reported that at 10 % *Sargassum wightii* (brown alga) extract elevated the content of photosynthetic pigments, protein and total sugars in *Vigna radiata* [Sivasankari *et al*. (2006b)] and seaweed liquid extract of *Rosenvingea intricata* at 20 % extract of the same species enhanced the photosynthetic pigments and carotenoids in *Cyamopsis tetragonoloba* [Thirumaran *et al*. (2009)]. Sivasankari *et al*. (2006a) studied the effect of seaweed extract where the low (10%) concentration increased the biochemical, pigment content and enzymes activity. In our study too, the high biochemical and pigment content was improved at 20, 60 and 80 % of selected seaweed extracts. The 50 %

concentration of seaweed liquid fertilizer improved the protein content in fenugreek plant as per Pise and Sabale (2010) , similarly in our study 60% *U. lactuca* extract was most effective than other treatment to escalate protein content. Tarraf *et al*. (2015)concluded that seaweed extract applied at 30 days after sowing fenugreek seeds are helpful in obtaining higher yield and quality.

Effect of seaweed liquid fertilizer on soil physicochemical properties.

In the present study sandy loam soil was selected to grow fenugreek and spinach plants. The soil was suitable to better growth of fenugreek with low moisture retention capacity and slightly acidic to neutral pH. The soil texture and chemical properties was measured before the soil drenching treatment with seaweed extract and after the harvesting of plant. The seaweed extract of different species was applied to the seedlings by soil drenching three times on first day of sawing, after 5 days and 15 days. The increase in soil quality and texture was proved effect of seaweed extract to enhance the plant growth. After soil drenching the significant increase in physiochemical parameters was observed (Table-16).

Table 16.Physico-chemical properties of experimental soil.Values are mean ± SE (n=3)

Soil properties	**Farm soil before treatment**	**Soil treated with *U. lactuca***	**Soil treated with *S. tenerrimum***	**Soil treated with *C. clavulatum***
Texture class	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Moisture content (%)	13.49±0.1	18.09±0.5	18.5±1	18.8±0.1
Bulk density (g mL–1)	0.68±0.02	0.9±0.01	0.95±0.04	0.9±0.02
Porosity (%)	69.95±0.3	66.3±0.4	65.2±0.2	65±0.1
Sand (%)	55±0.1	55±0.6	52±0.1	54±0.2
Silt (%)	30±0.1	30.5.±0.2	32±0.2	32±0.1
Clay (%)	15±0.01	14.5±0.1	16±0.1	14±0.08
pH	7.2±0.1	6.8±0.1	6.9±0.1	6.8±0.3
Total organic matter (%)	2.5±0.02	4.1±0.02	4.5±0.3	4.2±0.1
Sulphate (mg kg–1)	429±1.2	743±2.4	746±1.4	720±1.3
Phosphate (mg kg–1)	376±3.2	412±1.4	410±1.2	408±1.4
Nitrate (mg kg–1)	324±1.2	485±2.3	498±1.8	482±2

The seaweeds are rich source of essential nutrients and bioactive compounds make them suitable to use as organic fertilizer. The increase in moisture retention capacity (increased by 5%) compare to control or untreated soil was observed whereas average increased in 1.7%. The phycocoloids mainly alginic acid combine with ions in soil to form high molecular weight complex that absorb moisture and improve

crump formation causing in enhanced aeration and capillary action of pores, which result in induce the growth of plant root system and microbial activity [Gandhiyappan and Perumal (2001)]. Total organic matter was recorded. The pH of the soil was found to become slightly acidic after the treatment. The presence of biochemical like carbohydrates, protein, amino acids and other essential element may be contributing in increasing organic matter and inorganic substances. Sulphate, phosphate and nitrate content were observed in high concentration compared to untreated soil this is coincide with the studies of seaweed extract composition [Khan *et al*. (2009)]. The seaweed extract trigger the microbial growth in the soil, moreover beneficial organism like Rhizobium when applied along with seaweed extract, enhanced the prompt growth and yield quality legume plants like *Arachis hypogea* and *Vigna mungo* and the plant response was 12-25% higher than that of control [Sethi and Adhikary (2009)]. Seaweed compost and extract have been widely used in agricultural applications [Wosnitza and Barrantes (2006); Khan *et al*., 2010)] because of its properties to improve soil biological, chemical and physical properties which enhanced the plant growth [Khan *et al*. (2009)], soil fertility and quality and nutrient-rich biomass. Seaweed fertilizers have been explored to use for different agricultural purposes, including the prompt plant growth and defence response, soil nutrient enrichment, and elevation of microbial activity and mycorrhizal fungi [Khan *et al*. (2009)]. Seaweeds have been used for centuries as organic matter, soil conditioners and fertilizer nutrients [Blunden and Gordon (1986); Metting B (1988); Temple and Bomke (1988)]. The seaweed extract and seaweeds enhance the growth of beneficial soil microorganisms and secretion of soil conditioning substances by these microbes.

Seaweed extract root-growth-promoting activity was detected when the seaweed extracts were applied either to the roots or as a foliar spray [Biddington and Dearman (1982); Finnie and Van Staden (1985)]. The effectiveness of seaweed extract is mainly depends on dose, the high concentration of kelp extract inhibited root growth of tomato plants however, stimulatory effects were found at a lower concentration [Finnie and Van Staden (1985)]. Seaweed bioactive and biostimulating substances are capable to improve root system [Vernieri *et al*. (2005) ; Mancuso *et al*. (2006)], improved root system could be influenced by growth promoting hormons like auxins as well as other elements in the extracts [Crouch *et al*. (1992)]. The seaweed extract improve nutrient uptake by roots [Crouch *et al*. (1990)], as a result improved water and nutrient efficiency, thereby causing greater plant growth and vigour.

Seaweed extracts of *Sargassum tenerrimum*, *Ulva lactuca* and *Centroceras clavulatum* was clearly showing biofertilizer effect on germination, growth and biochemical composition of selected crops *Trigonella foenum-graecum* and *Spinacia oleracea* at different concentration compared to control. Soil analysis was indicated that seaweeds extract improved soil quality by adding minerals and important nutrients required for better growth and yield. *Exsitu* study suggested that seaweed extracts can be used as substitute for chemical fertilizers to improve the sustainable crop growth and yield of

tested crops. Further, the study also emphasizes that the application of seaweed extracts can be effectively used as eco-friendly approach to organic farming.

5. Study of phylogenetic relationships among seaweed species and genetical identification by DNA barcoding by using *tufA* gene for green and COI gene for brown and red algae.

The genetical identification of species is carried out by DNA barcoding by comparisons of short, standardized DNA sequences [*tufA* (plastid elongation factor) and COI (Cytochrome c oxidase subunit 1) primers].

Barcoding analysis

The sequences obtained from the individual species were matched with accessions in the NCBI (National Center for Biotechnology Information) using BLAST analysis (Table.17). A total of 6 *tufA* sequences for Chlorophyta species and 24 COI sequences for Phaeophyta and Rhodophyta species were aligned to construct the Neighbor- Joining tree and to calculate pairwise distance. The analysis involved total 30 nucleotide sequences and codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position.The Neighbor- Joining tree (Fig. 62) showed the presence of only single clade of 6 different species of Chlorophyta phylum. The average corrected divergence over all sequence pairs was 0.11. From the tufA sequence alignment (distance analysis) intraspecific and interspecific genetic divergence was range from 0.040 to 0.044 and 0.089 to 0.24, respectively. The result clearly indicates the overlapping of maximum The Neighbor- Joining tree obtained from COI sequence alignment (Fig.63) showed the presence of two clades of 24 different species of Rhodophyta and Phaeophyta phylum. The average corrected divergence over all sequence pairs was 0.12. The COI gene distance analysis record data showed the intraspecific difference ranged from 0.011 to 0.280 while interspecific difference ranged from 0.011 to 0.080. The presence of introns in rbcL-3P leads to moderate success in amplification reduced their use as universal marker wheras *tufA* marker had 95 % amplification success proved to use this marker for green algal amplification [Saunders and Kucera (2010)]. Different markers have been proposed where to use for animals, red and brown algae, the COI-5P (5’ end of the cytochrome c oxidase 1 gene) provides high resolution at the species level between most tested groups, and it was accepted as the barcode marker and is now being useful to biodiversity and taxonomic studies across the globe [Hebert *et al*. (2004); Saunders (2005); McDevit and Saunders (2009); Ferri *et al*. (2009)].

Table 17. Species identification and similarity results from BLAST analysis.

Sr. No	Genetically Identified species	Similarity (%)	Accession Number
1	*Caulerpa scalpelliformis*	99	KF840150.1
2	*Caulerpa sertuloides*	99	KJ957123.1

3	*Caulerpa racemosa*	100	KJ957088.1
4	*Caulerpa sertuloides*	83	AJ417944.1
5	*Caulerpa verticillata*	100	KU761495.1
6	*Ulva ohnoi*	99	KF195549.1
11	*Chordariaceae sp.*	89	LM995226.1
12	*Padina boergesenii*	90	HF559122.1
13	*Colpomenia sinuosa*	81	KF281125.1
14	*Amphiroa anceps*	90	LC071725.1
15	*Scinaia furcata*	92	HQ422967.1
16	*Lithophyll umhibernicum*	100	KR733541.1
17	*Sargassum phyllocystum*	93	HQ416039.1
18	*Sargassum zhangii*	93	KJ653272.1
19	*Acinetosporaceae sp*	83	LM995275.1
20	*Padina boergesenii*	87	HF559121.1
21	*Lobophora variegata*	96	HF559116.1
22	*Scinaia confusa*	91	HQ603251.1
23	*Jania sp.*	99	LC071780.1
24	*Polysiphonia breviarticulata*	83	HM573497.1
25	*Scinaia interrupta*	91	HQ603259.1
26	*Heterosiphonia crispella*	88	KC795909.1
27	*Lithothamnion sp*	86	KJ710337.1
28	*Champia expansa*	83	KF356140.1
29	*Gracilaria flabelliformis*	92	KP210178.1
30	*Laurencia crustiformis*	93	GU223895.1
31	*Chondrophycus dotyi*	94	HQ422621.1
32	*Solieria filiformis*	93	KJ202080.1
33	*Hypnea sps*	97	HQ422821.1
34	*Hypnea cornuta*	90	KF714858.1

Phylogenetic analysis

The evolutionary history was inferred using the Neighbor- Joining method [Saitou and Nei (1987)]. The confidence probability (multiplied by 100) that the interior branch length is greater than 0, as estimated using the bootstrap test (500 replicates is shown next to the branches [Dopazo (1994), Rzhetsky and Nei (1992)]. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. There were a total of 523 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [Kumar *et al*. (2016)].

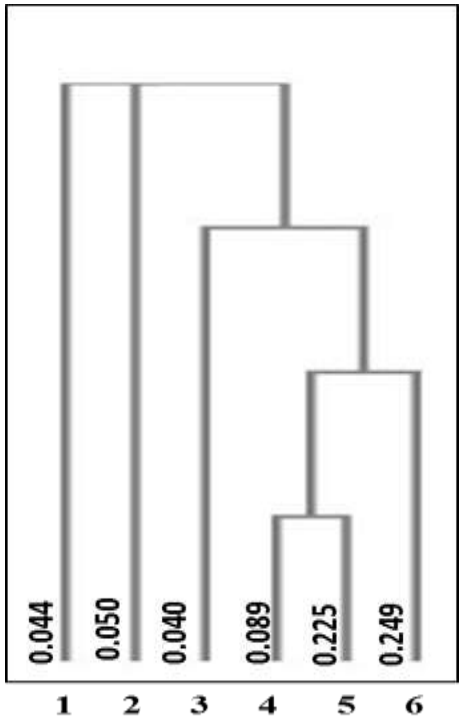


Fig. 62 Phylogenetic tree based on *tufA* marker sequence data.

From the Neighbor- Joining tree of tufA gene revealed that 5 morphologically similar species (*C. racemosa, C. scalpelliformis*, *C. sertuloides*, *C. verticillata* and *C.sertuloides*) are actuly sister species belonging to same family (Caulerpaceae) and one species similar to *U.lactuca* was genetically identified as *U. ohnoi* fromUlvaceae family (Table 18)**.** It was observed that all identified species on the bases of *tufA gene* was fall under one clade where they all belonging to same phylum Chlorophyta; 5 species are from the same family and class but the *U.ohnoi* species is belonging to different class was genetically more similar to *C. verticillata.* The genetical divergen between these two species is 0.089 to 0.22.

The phylogeny concrete from genetical sequencing from COI for Rhodophyta and Phaeophyta phylum was revealed that studied species are fall under three clades, where species *H. crispella, S. interrupta, S. furcate, Jania sp., G. flabelliformis* from the Florideophyceae and Rhodophyceae was fall nunder the one clade, the distance range from 0.011 to 0.124. From the analysis clear species level classification was observed between sister species from the same family and class. The second clade distributaed *C. dotyi, L.crustiformis, H. sps, S. filiformis and H. cornuta* species from the Florideophyceae and Rhodophyceae where the distance varied from 0.071 to 0.157. The clades showed closely related species with same class but different familes from these 5 species. Third clade includes *A. anceps, P.breviarticulata, L. hibernicum, Lithothamnion sp, C. expansa, C. sinuosa, S. zhangii, S. phyllocystum, L. variegate, Acinetosporaceae sp, Chordariaceae sp., P. boergesenii, P. boergesenii* and *S. confuse* from Florideophyceae and Rhodophyceae with different families and orders. The

divergen ranged from 0.080 to 0.280, where *S. zhangii* and *S. phyllocystum* siter species showd close relation with genetical divergen from 0.128 to 150 while *P. boergesenii and P. boergesenii* two species identified as same species with genetical distance 0.080 to 0.104. The phylogeny using COI is clearly revealed close relation between species belonging to different class and the their distance with sister species as well as intraspecific relationship was measured. (Table 19).

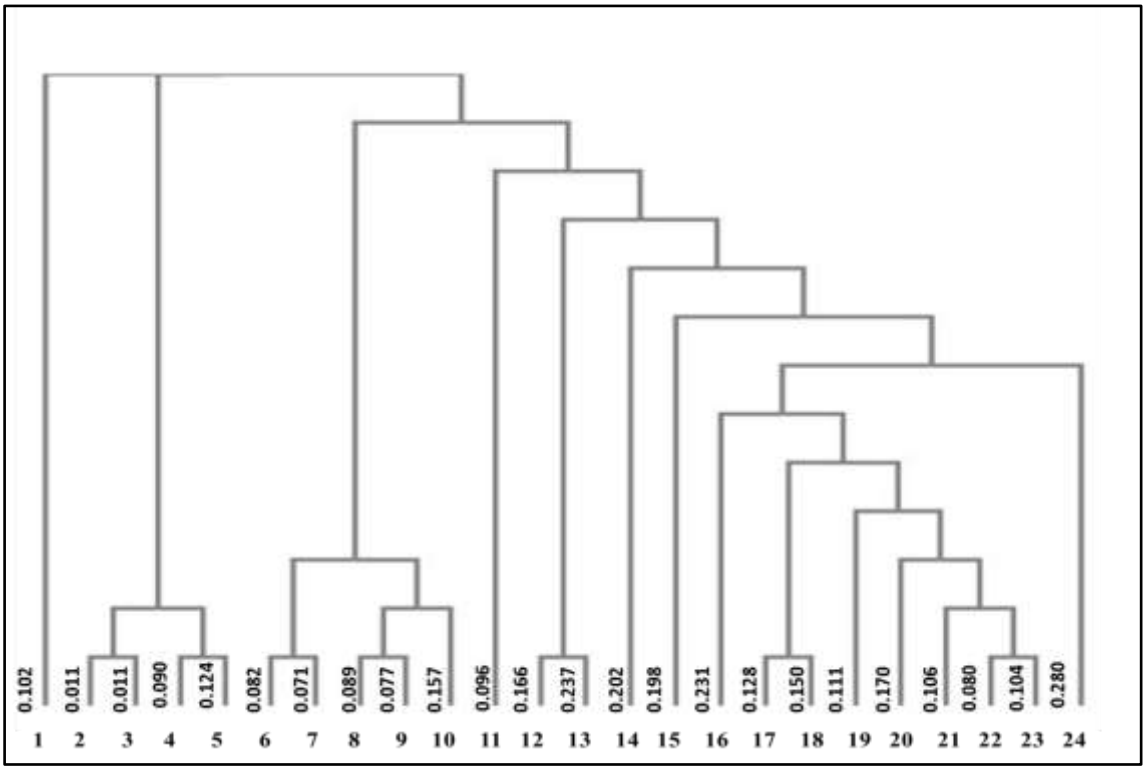


Fig. 63. Phylogenetic tree based on COI marker sequence data.

Table 18 Detailed classification of the species identified by *tufA*.

Sr.No	Species	Family	Order	Class
1	*Caulerpa racemosa*	Caulerpaceae	Bryopsidales	Bryopsidales
2	*Caulerpa scalpelliformis*	Caulerpaceae	Bryopsidales	Bryopsidales
3	*Caulerpa sertuloides*	Caulerpaceae	Bryopsidales	Bryopsidales
4	*Caulerpa verticillata*	Caulerpaceae	Bryopsidales	Bryopsidales
5	*Ulva ohnoi*	Ulvaceae	Ulveles	Ulvophyceae
6	*Caulerpa sertuloides*	Caulerpaceae	Bryopsidales	Bryopsidophyceae

Table 19.Detailed classification of the species identified by COI.

Sr.No	Species	Family	Order	Class
1	*Heterosiphonia crispella*	Dasyaceae	Ceramiales	Florideophyceae
2	*Scinaia interrupta*	Scinaiaceae	Nemaliales	Florideophyceae
3	*Scinaia furcata*	Scinaiaceae	Nemaliales	Florideophyceae
4	*Jania sp.*	Corallinaceae	Corallinales	Rhodophyceae
5	*Gracilaria flabelliformis*	Gracilariaceae	Gracilariales	Florideophyceae
6	*Chondrophycus dotyi*	Corallinaceae	Ceramiales	Rhodophyceae
7	*Laurencia crustiformis*	Rhodomelaceae	Ceramiales	Rhodophyceae
8	*Hypnea sps*	Cystocloniaceae	Gigartinales	Florideophyceae
9	*Solieria filiformis*	Solieriaceae	Gigartinales	Florideophyceae
10	*Hypnea cornuta*	Cystocloniaceae	Gigartinales	Florideophyceae
11	*Amphiroa anceps*	Corallinaceae	Corallinales	Florideophyceae
12	*Polysiphonia breviarticulata*	Rhodomelaceae	Ceramiales	Florideophyceae
13	*Lithophyllum hibernicum*	Corallinaceae	Corallinales	Rhodophyceae
14	*Lithothamnion sp*	Dasycladaceae	Dasycladales	Florideophyceae
15	*Champia expansa*	Champiaceae	Rhodymeniales	Florideophyceae
16	*Colpomenia sinuosa*	Scytosiphonaceae	Scytosiphonales	Phaeophyceae
17	*Sargassum zhangii*	Sargassaceae	Fucales	Phaeophyceae
18	*Sargassum phyllocystum*	Sargassaceae	Fucales	Phaeophyceae
19	*Lobophora variegata*	Dictyotaceae	Dictyotales	Phaeophyceae
20	*Acinetosporaceae sp*	Acinetosporaceae	Ectocarpales	Phaeophyceae
21	*Chordariaceae sp.*	Chordariaceae	Chordariales	Phaeophyceae
22	*Padina boergesenii*	Dictyotaceae	Dictyotales	Phaeophyceae
23	*Padina boergesenii*	Dictyotaceae	Dictyotales	Phaeophyceae
24	*Scinaia confusa*	Scinaiaceae	Nemaliales	Florideophyceae

The study revealed the efficiency of two selected universal markers *tufA* and COI for successful amplification and phylogeny of selected species. The morphologically similar species can be identified on the bases of their genetical make up and intraspecific relationship was possible by using these universal markers. Multiple markers approach to construct the phylogenetic relationship is needed to study the phylogeny or genetically relatedness of among the species.

13. ACHIEVEMENTS FROM THE PROJECT

- Presented research paper in **India International Science Festival (Young Scientist Meet) 2016**, 7th to 11th December, 2016, NPL, New Delhi, India.
- Poster presentation in one day seminar on Integrating Climate change, Energy, transformation and Youth (ICE TRAY-2016) on **1st October, 2016 at Gujarat University, Gujarat, India.**
- Presented research paper in **India International Science Festival (Young Scientist Meet) 2015**, 4th to 8th December, 2015, IIT, New Delhi, India.
- Presented paper at **Science Excellence 2015**, organized by Department of Botany, Gujarat University, Ahmedabad on **26th September,2015**
- Oral presentation in **28th Gujarat Science congress** on February 22&23, 2014.
- Achieved **First prize** for Poster presentation in National seminar on **Climate Change in Indian context** on 14th December, 2013 conducted by Department of Environmental science, MS. University Baroda.

14. SUMMARY OF THE FINDINGS
(IN 500 WORDS)

The geography of studied coastal area is most suitable for seaweed to grow, Okha coast is rich with number of various group of seaweeds. Long wide reef of this study site and long intertidal zone provide suitable habitat for most of the seaweeds. In the present study seasonal variation in seaweed diversity and influence of environmental ingredients was studied. The eighteen seaweed species of three algal groups selected from this site were analyzed for nutraceutical properties. Efficiency of seaweed extract for better germination and growth, and role of seaweed to improve soil fertility and quality was also explored. The seaweeds species identification and phylogenetic tree was developed by using DNA barcoding method.

A total of 121 species has been recorded, with highest number of Rhodophyta (55%) species than Chlorophyta (23%) and Phaeophyta (22%). Okha Coast is unique in terms of seaweed diversity and water quality due to environmental factors and several anthropogenic activities. Temperature, dissolved oxygen, light intensity and salinity mainly support the growth of seaweeds. From the habitat of the seaweeds, species distribution pattern at selected site suggest influence of different environmental factors like low temperature, light intensity, high DO triggered rich seaweed species diversity and distribution during cooler months.

The results of the present study revealed that seaweeds are a rich with the important metabolites which vary from one species to another and can provide a dietary alternative due to its nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of seaweed-based products. More research is needed to evaluate the nutritional value of marine algae, besides; seaweeds can be regarded as an under-exploited source of health benefit molecules for food processing and nutraceutical industry.

In the current study the stimulation effect of the onion, soyabean and sesame seeds clearly revealed that the soaking/ priming of seeds in different concentration of seaweed extract had provided better growth compare to control or nonprimed seeds. Seeds primed for 10 h with 40% *S. tenerrimum* extract for onion, 6 h with 20% *U. lactuca* extract for soyabean and 10 h with 20% *S. tenerrimum* were highly suitable for getting quality seedlings and higher vigour. It was concluded that seaweed extract could be used as an osmotic agent in Organic priming.

Seaweed extracts of *Sargassum tenerrimum*, *Ulva lactuca* and *Centroceras clavulatum* was clearly showing biofertilizer effect on germination, growth and biochemical composition of selected crops *Trigonella foenum-graecum* and *Spinacia oleracea* at different concentration compared to control. Soil analysis was indicated that seaweeds extract improved soil quality by adding minerals and important nutrients required for better growth and yield. *Exsitu* study suggested that seaweed extracts can be used as substitute for chemical fertilizers to improve the sustainable crop growth and yield of tested crops. Further, the study also emphasizes that the application of seaweed extracts can be effectively used as eco-friendly approach to organic farming.

The study revealed the efficiency of two selected universal markers *tufA* and COI for successful amplification and phylogeny of selected species. Species of Chlorophyta, Phaeophyta and Rhodophyta were identified genetically to know the relationships between the classes and genera. Both the markers are clearly revealed genetical differences between morphologically similar species.

15. CONTRIBUTION TO THE SOCIETY
(GIVE DETAILS)

Biochemical properties of selected seaweeds, which are dominant species in Eco-sensitive Zone, Okha coast, were tested to nutritive value. The species has sufficiently low lipid content for human nutrition, in addition to good carbohydrates and proteins. The presence of elevated concentrations of Ca, Fe, K and other essential minerals confirms its potential importance as a valuable nutrient source. Presence of important fatty acids, alkaloids and essential amino acids showing the significance of

studied seaweeds. Similarly, the antioxidant, antimicrobial and antifungal activities might usefulness in potentially controlled serious cell disorders. Thus the study suggests that some of the seaweeds could safely be utilized in pharmaceutical as well as cosmetic preparations and human diet. Its commercial value can be enriched by improving the quality and increasing the range of seaweed-based products.

Exsitu study on seaweed fertilizer effect on different crops suggested that seaweed extracts can be used as substitute for chemical fertilizers to improve the sustainable crop growth and yield of tested crops. Soil analysis was indicated that seaweeds extract improved soil quality by adding minerals and important nutrients required for better growth and yield.Further, the study also emphasizes that the application of seaweed extracts can be effectively used as eco-friendly approach to organic farming.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT yes
    Ms.Megha Barot research fellow got registered for Ph.D in 2013 under the Guidance and of Prof.Nirmal Kumar,J.I , Principal and PI ,ISTAR and got Ph.D in the month of February,2018.

17. NO. OF PUBLICATIONS OUT OF THE PROJECT : **Fiv**e
    (PLEASE ATTACH)

Nirmal Kumar, J.I., **Megha Barot** ., Rita N. Kumar. 2014. Phytochemical analysis and antifungal activity of selected seaweeds from Okha coast, Gujarat, India.**Life Sciences Leaflets. 52 (57 -70)** ISSN 0976–1098

Megha Barot, Nirmal Kumar J.I., Rita N. Kumar.*2015.*Seaweed species diversity in relation to hydro chemical Characters of Okha coast, western India. **International Journal of Recent Research and Review. 8 (3).** ISSN 2277 – 8322

Megha Barot, Nirmal Kumar J.I., Rita N. Kumar. 2015.Nutraceutical properties of green, red and brown seaweeds from the eco-sensitive zone, India as potential dietary and pharmaceutical applications.**Proceedings of the India International Science Festival- Young Scientists' Meet Department of Science and Technology, Government of India – Dec 4-8.**

Megha Barot, Nirmal Kumar J.I., Rita N. Kumar. 2016. Bioactive compounds and antifungal activity of three different seaweed species *Ulva lactuca, Sargassum tenerrimum* and *Laurencia obtusa* collected from Okha coast, Western India. **Journal of Coastal life medicine.** 4(4): 284-289.