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Original Research Article

Phytochemical Studies on *Ulva lactuca* Linn.

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Abstract

The present study was aimed to explore the phytochemical constituents present in various extarcts of *Ulva lactuca* Linn and produce the FT-IR, TLC, UV-VIS and HPLC spectrum profile for *U. lactuca*. Phytochemical screening of the extracts was carried out according to the method described by Harborne. For the HPLC analysis, the methanol: water (45:55) was used as mobile phase. The results of phytochemical analysis revealed the presence of steroids, phenolic groups, alkaloids, saponins, tannins, glycosides and terpenoids in *U. lactuca*. The UV- VIS profile of aqueous, ethanolic, chloroform, petroleum ether and hexane confirmed various metabolites and functional groups presence in the crude extracts of *U. lactuca*. The HPLC profile of *U. lactuca* ethanolic extract showed some prominent and moderate peaks with different retention time. The results of the present study showed that *U. lactuca* may be rich sources of phytoconstituents which can be isolated and further to exploit for developing plant based drugs for various ailments.

Keywords: *Ulva lactuca*, Phytochemistry, TLC, FT-IR, UV-VIS, HPLC

Introduction

Seaweeds have been employed as a source of food, fragrance and medicine for millennia throughout the world. Seaweeds are commonly divided into three main groups based on their pigmentation. Phaeophyta, or brown seaweeds, are predominantly brown due to the presence of the carotenoid fucoxanthin and the primary polysaccharides present include alginates, laminarins, fucans and cellulose [1,2]. Chlorophyta, or green seaweeds, are dominated by chlorophyll a and b, with ulvan being the major polysaccharide component[3]. The principal pigments found in Rhodophyta or red seaweeds, are phycoerythrin and phycocyanin and the primary polysaccharides are agars and carrageenans[4]. Marine organisms are

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potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. During the last decades, numerous novel compounds have been isolated from marine organisms and many of these substances have been demonstrated to possess interesting biological activities[5-8] viz., antimicrobial [9, 10], antiviral [11], antifungal [12], anti-allergic [13], anticoagulant [14] and anticancer [15]. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties[16]. Although a number of phytochemical and bioefficacy studies were carried out at global level, only few reports are available on the bio-potential and biochemical studies on the seaweeds from Gulf of Mannar and Peninsular coast of India [17-18]. To fulfill the lacuna, the present study was aimed to explore phytochemical constituents present in *Ulva lactuca* Linn.

Materials and Methods

Collection of Materials

Ulva lactuca (Linn) was collected by handpicking at Hare Island. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The collected samples were then thoroughly washed with tap water followed by distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. The shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further use.

Preparation of extracts

30 g of air dried *U. lactuca* powder was extracted with 180 ml of solvents viz., hexane, petroleum ether, chloroform, ethanol and aqueous using soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered by using Whattman filter paper (No.1) and then concentrated in vacuum at 40ºC using Rotary evaporator. The residues obtained were stored in a freezer -20º C until further tests.

Preliminary Phytochemical Analysis

The different extracts of *U. lactuca* were tested for the presence / absence of steroids, phenolic compounds, alkaloids aminoacids, tannins, saponins, flavonoids, cardiac glycosides, anthraquinone and terpenoids. Phytochemical screening of the extracts was carried out by the method described by Harborne[19].

Proximate Analysis

The *U. lactuca* extracts were examined under visible and UV light. These powdered materials were also treated with various reagents such as 50% nitric acid, acetone, ethanol, 50% sulphuric acid, 1N HCl and 1N NaOH and changes in colour were recorded [20].

FTIR SPECTROSCOPIC ANALYSIS

FT-IR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The crude powder of *A. gangetica* was passed into the FT-IR and the peak values were recorded. Each and every analysis was repeated twice and confirmed the spectrum [21].

UV-VIS spectrophotometer and HPLC analysis

For UV-VIS spectrophotometer and HPLC analysis, the extract was centrifuged at 3000 rpm for 10 min and then filtered through Whatmann No. 1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The crude extracts containing the bioactive compound was analyzed spectroscopically for further confirmation. To detect the UV-VIS spectrum

profile of the crude extracts of *U. lactuca*, the extracts were scanned in the wavelength ranging from 200-1100 nm by using Shimadzu Spectrophotometer and the characteristic peaks were detected. The qualitative UV-VIS fingerprint profile of different extracts of was selected at a wavelength of 300-700 nm due to the sharpness of the peaks and proper baseline.

HPLC analysis:

HPLC method was performed on a Shimadzu LC-10 AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10AT. A Hypersil ® BDS C-18 column (4.6 × 250 mm, 5 µm size) with a C-18 guard column was used. An isocratic HPLC (Shimadzu HPLC Class *VP* series) with two LC-10 AT *VP* pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A *VP* (Shimadzu), CTO- 10AS *VP* column oven (Shimadzu), SCL-10A *VP* system controller (Shimadzu) and reverse phase Luna 5°C18 (2) Phenomenex column (250mm X 4.6mm) was used. The mobile phase components methanol: water (45:55) were filtered through 0.2 µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270 kgf/cm^2. The column temperature was maintained at 27°C. 20µL of respective sample was injected by using Rheodyne syringe (Model 7202, Hamilton). The elution was carried out with gradient solvent systems with a flow rate of 1 ml min-1 at ambient temperature (25-28°C). The mobile phase was prepared daily, filtered through a 0.45 µm and sonicated before use. Total running time was 15 min. The sample injection volume was 20 µL while the wavelength of the UV-Vis detector was set at 254 nm [22, 23].

TLC analysis

TLC was carried out on 10 × 20 cm silica gel plates (Merck, Germany). The phenolic and steroidal compounds present in various extracts of *U. lactuca* were tentatively detected by TLC. The mobile phase used for phenolics was chloroform and methanol at 9:1 ratio. After spraying with the solution composed of Folin-Ciocalteau reagent, the appearance of blue colour spot in the TLC chromatogram indicated the presence of phenolic compounds. The mobile phase used for steroids was benzene and methanol at 9:1 ratio. After spraying with the solution composed of 5% alcoholic sulphuric acid, the appearance of bluish green colour spot in the TLC chromatogram indicated the presence of steroidal compounds.

Results and discussion

Phytochemical activity of *Ulva lactuca*

The results of preliminary phytochemical analysis revealed the steroids, alkaloids, phenolics, saponins, cardiac glycosides and terpenoids presence in the ethanolic extract of *U. lactuca* (Table 1). However tannins were observed in hexane, petroleum ether, chloroform and aqueous extracts but not in ethanolic extract. Cardiac glycosides were observed in hexane, petroleum ether and ethanol but failed to show its presence in chloroform and aqueous. Steroids were observed in chloroform and ethanolic extracts of *U. lactuca*. Marine algae are among the richest sources of known and novel bioactive compounds [22-24]. The seaweeds are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins, tannins, steroids; related active metabolites are of great medicinal value and have been extensively used in the drug and pharmaceutical industry [25-28]. The preliminary phytochemcial results were directly coinicided and supplemented with previous observations.

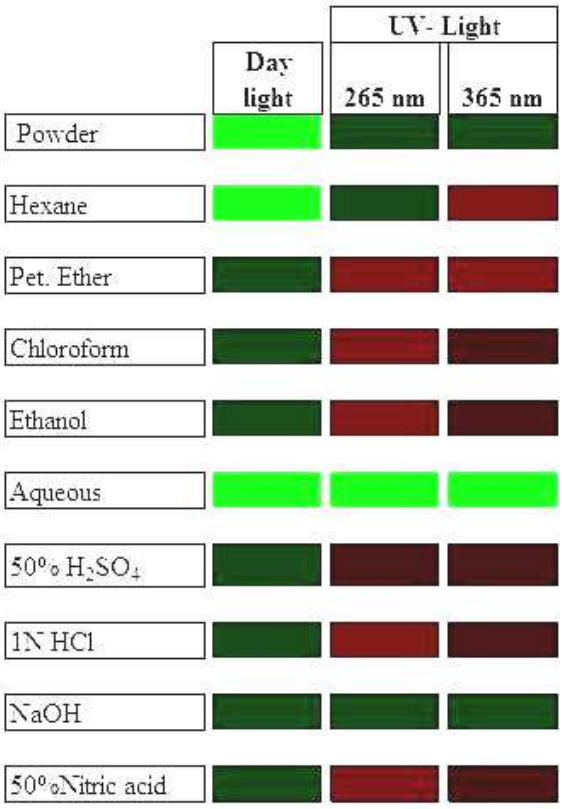
Table 1: Preliminary phytochemical screening of various extracts of *U. lactuca*

Metabolites	Hexane	Pet. ether	Chloroform	Ethanol	Aqueous
Steroids	-	-	+	+	-
Alkaloids	-	-	-	+	-
Phenolics	-	-	-	+	-
Flavonoids	-	-	-	-	-
Saponins	-	-	-	+	-
Tannins	+	+	+	-	+
Amino acids	-	-	-	-	-
Cardiac glycosides	+	+	-	+	-
Anthraquinone	-	-	-	-	-
Terpenoids	-	-	-	+	-

Fluorescence analysis of *U. lactuca*

The behavior of the drug powder with different reagents will also be helpful in characterization of the crude drug. The result of the present study revealed the various behavior character of *U. lactuca* crude drug. The result of the present study is depicted in Fig. 1.

Fig. 1: Fluorescence analysis of *U. lactuca*



FT-IR analysis of *U. lactuca*

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FT-IR analysis showed different peaks values viz., 3315.41, 3197.76, 2975.96, 2885.31, 2268.13, 2106.12, 1670.24, 1454.23, 1400.22, 1334.65, 1195.78, 1112.85, 806.19, 752.19, 651.89 and 605.61. It confirmed the presence of functional groups such as carboxylic acids, alcohols and phenolics, alkanes, aldehydes, alkynes and allenes, compounds containing nitrogen- oxygen band, alcohols and phenolics, esters, secondary alcohols, halogen compounds, alkynes and allenes/sulfonic acids in the crude powder of *U. lactuca* (Table 2; Fig. 2).

Fig. 2: FTIR peak values on crude powder of *U. lactuca*

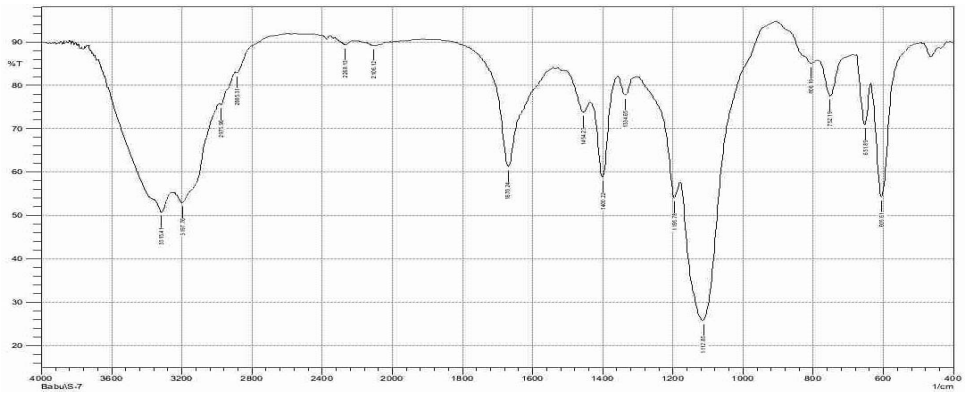


Table 2: FTIR peak values on crude powder of *U. lactuca*

Frequency (cm^{-1})	Bond/stretching	Functional groups
3315.41	O- H stretch	Carbonyl compounds (carboxylic acids)
3197.76	O- H stretch	Alcohols and Phenolics
2975.96	C-H stretch	Alkanes
2885.31	C-H stretch	Alkanes
2268.13	-N=C=O stretch	Unsaturated nitrogen compounds (Isocyanates)
2106.12	C-H def.	Alkynes and allenes (monosubstituted)
1670.24	N=O stretch	Compounds containing nitrogen- oxygen band
1454.23	C-H	Alkanes
1400.22	O-H def.	Alcohols and Phenolics
1334.65	S=O stretch	Sulfur compounds
1195.78	C-O stretch	Esters (Fonnates)
1112.85	C-OH stretch	Secondary alcohols
806.19	Skeletal	Alkanes
752.19	C-Cl stretch	Halogen compounds
651.89	S= O stretch	Alkynes and allenes/Sulfonic acids
605.61	C-H def.	Alkynes

In the present study, the crude powder subjected to FT-IR analysis is used for the identification of functional constituents present in *U. lactuca*. From the spectra we can see clearly that although they show substantial overlap of each absorption spectrum of various components, each band represents an overall overlap of some characteristic absorption peaks of functional groups in the samples. Similar to the present study many researchers used FT-IR as a tool for classifying the plants and other organisms [29, 30].

UV-VIS analysis of *U. lactuca*

The qualitative UV-VIS fingerprint profile of different extracts of *U. lactuca* was selected at the wavelength of 190 to 1100 nm due to sharpness of the peaks and proper baseline. The qualitative UV-VIS spectroscopic profile of aqueous extract of *U. lactuca* showed the metabolites and functional compounds presence at the nm of 268.84, 360.20, 382.76 and 811.25 with the absorption of 0.609, 0.253, 0.240 and 0.169 (Fig. 3 A). The qualitative UV-VIS spectroscopic profile of ethanolic extract showed the metabolites and functional compounds presence at the nm of 222.01, 371.64, 665.06 and 852.95 with the absorption of 0.302, 0.285, 0.225 and 0.232 respectively (Fig. 3 B). The qualitative UV-VIS spectroscopic profile of chloroform extract demonstrated the metabolites and functional compounds at the nm of 413.26 and 666.32 with the absorption of 0.810 and 0.386 respectively (Fig. 3 C). The qualitative UV-VIS spectroscopic profile of petroleum ether extract displayed the metabolites and functional compounds at the nm of 273.13 and 408.04 with the absorption of 0.085 and 0.071 respectively (Fig. 3 D). The qualitative UV-VIS spectroscopic profile of hexane extract of *U. lactuca* illustrated the metabolites and functional compounds at the nm of 216.04, 221.36, 254.27, 408.96 and 668.67 with the absorption of 0.210, 0.202, 0.131, 0.100 and 0.085 respectively (Fig. 3 E).

Phenolic and steroid TLC profile of *U. lactuca*

TLC separation of phenolics and steroids present in ethanolic extract of *U. lactuca* were tabulated (Table 3). The results showed 5 phenolic bands with different R_f values viz., 0.15, 0.32, 0.35, 0.53 and 0.65. Steroidal profiles present in ethanolic extracts of *U. lactuca* illustrated 5 bands with different R_f values viz., 0.20, 0.30, 0.37, 0.51 and 0.63. TLC is one of the chemotaxonomical method, many workers followed this method for distinguish one species from other species [31-33]. In the present study also we develop the phenolics and steroids TLC profile of ethanolic extract.

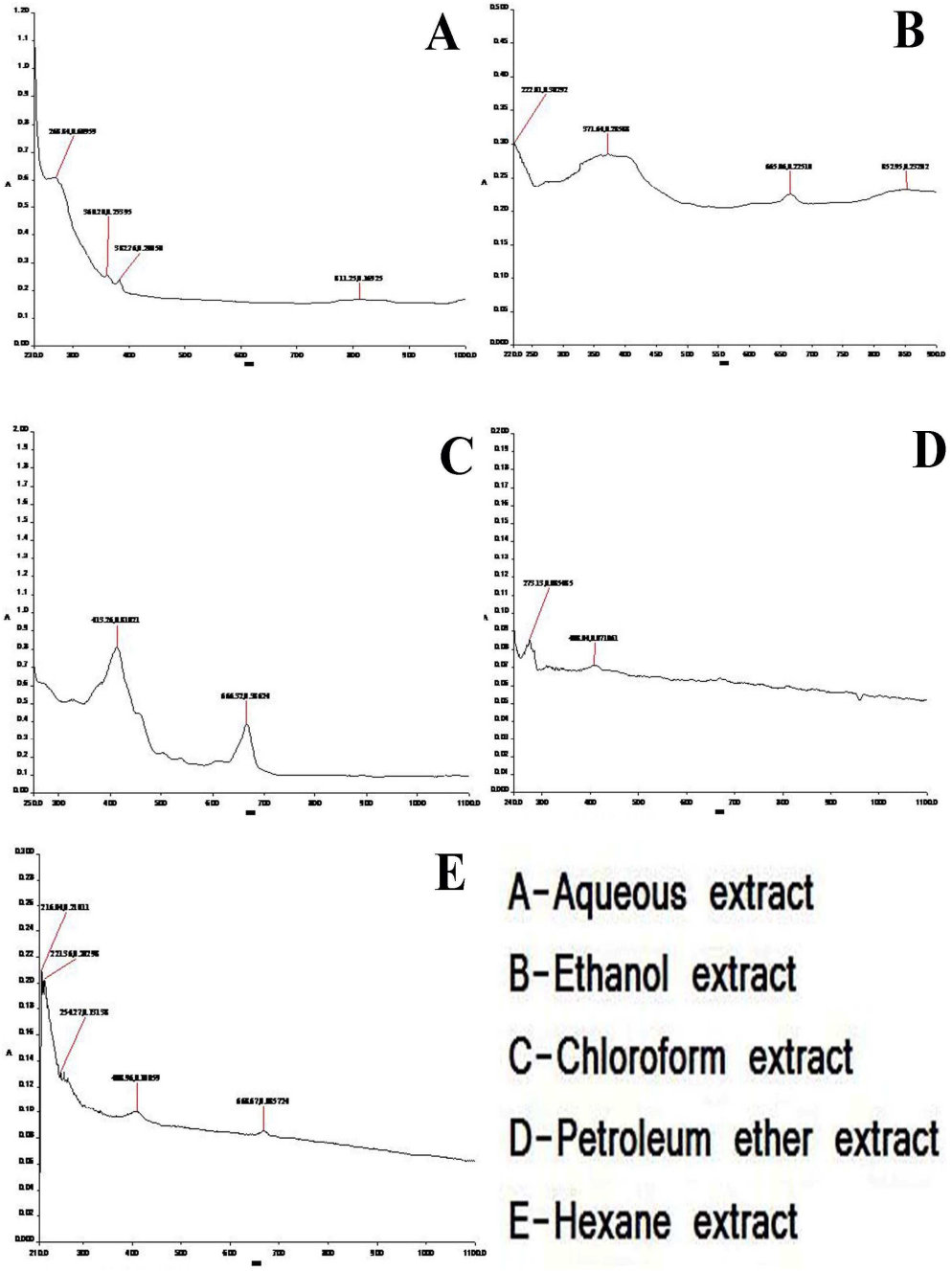


Fig. 3: UV- VIS spectrum of different extracts of *U. lactuca*

Table 3: Steroids and phenolics profiles of ethanolic extract of *U. lactuca* using TLC

R_f value	Steroids	Phenolics
0.15	-	+
0.20	+	-
0.30	+	-
0.32	-	+
0.35	-	+
0.37	+	-
0.51	+	-
0.53	-	+
0.63	+	-
0.65	-	+

HPLC analysis of *U. lactuca*

The ethanolic extracts of *U. lactuca* showed four metabolites and functional compounds with different retention time's viz., 0.077, 1.970, 2.130 and 2.787 min. The profile displayed two prominent peaks with the retention time of 2.130 and 2.787 min some fair peaks were also observed with the retention time 0.077 min and 1.970 min (Fig. 4).

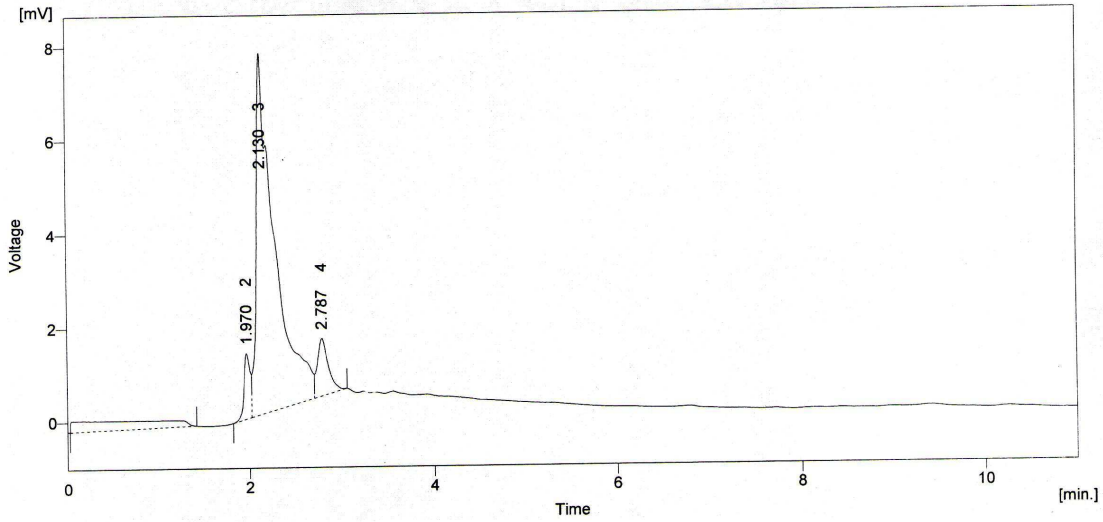


Fig. 4: HPLC chromotogram of the ethanolic extract of *U. lactuca*

In pharmacognosy, the phytochemical evaluation is one of the vital tools for quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques such as fluorescence, UV-VIS, FT-IR, HPLC, HPTLC and GC-MS[34]. In the present study also we made an attempt to produce the chemoprofiling of *U. lactuca* using UV-VIS, FT-IR, HPLC and TLC. The results of the present study exhibited novel markers in standardization as useful analytical tools to check not only the quality of the powder but also the presence of adulterants in ayurvedic drugs for *U. lactuca*. Fluorescence, UV-VIS and HPLC profiles can be used as effective markers in identifying authentic *U. lactuca* from its adulterants. Therefore using newer analytical techniques as markers can be generated for the researchers as a chain of markers for use of the common man to evaluate the quality of herbal drug and also incorporated in pharmacopoeias [35-37].

Reference

1. Haugan, J.A. and Liaaenjensen, S. 1994. *Algal Carotenoids 54. Carotenoids of Brown-Algae (Phaeophyceae)*. Biochem. Syst. Ecol., *22*: 31–41.
2. Goni, I., Valdivieso, L. and Gudiel-Urbano, M. 2002. *Capacity of edible seaweeds to modify in vitro starch digestibility of wheat bread.* Nahrung, 46: 18–20.
3. Robic, A., Gaillard, C., Sassi, J.F., Lerat, Y. and Lahaye, M. 2009 *Ultrastructure of Ulvan: A Polysaccharide from Green Seaweeds*. Biopolymers, 91: 652–664.
4. McHugh. D.J. 2003 *A guide to seaweed industry*. FAO Fish. Tech. Pap., T441: 118.
5. Duarte, M. E. R., Noseda. D. G., Noseda. M. D., Tulio. S., Pujol . C. A. and Damonte. E. B. 2002. *Inhibitory effect of sulfated galactans from the marine alga Bostrychia montagnei on herpes simplex virus replication in vitro*. Phytomedicine, 8: 2002, 53–58.
6. Faulkner, D. J. 2002. *Highlights of marine natural products chemistry.* Nat. Prod. Rep. 17: 1-6.
7. Ely, R., Supriya. T. and Naik, C.G. 2004. *Antimicrobial activity of marine organisms collected off the coast of South East India*. Journal of Experimental Marine Biology and Ecology, 309: 2004, 121–127.
8. Dubber. D. and Harder. T. 2008. *Extracts of Ceramium rubrum, Mastocarpus stellatus and Laminaria digitata inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations*. Aquaculture, 274: 196–200.
9. Chiheb, I., Riadi, H., Martinez-lopez, J., Dominguez-seglar, J. F., Gomez-vidal, J. A., Bouziane, H. and Kadiri, M. 2009. *Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco*. African Journal of Biotechnology, 8: 1258–1562.
10. Bouhlal, R., Haslin. C., Chermann, J.C., Colliec-jouault, S., Sinquin, C., Simon, G. Cerantola, S., Riadi, H. and Bourgougnon, N. 2011. *Antiviral activities of sulfated polysaccharides isolated from Sphaerococcus coronopifolius (Rhodophytha, Gigartinales) and Boergeseniella thuyoides (Rhodophyta, Ceramiales).* Marine Drugs, 9: 1187–1209.
11. Bouhlal, R., Riadi, H. and Bourgougnon. N. 2010. *Antiviral activity of the extracts of Rhodophyceae from Morocco*. African Journal of Biotechnology, 9: 7968–7975.
12. De Felício, R., De Albuquerque, S., Young, M.C.M., Yokoya, N.S. and Debonsi, H.M. 2010. *Trypanocidal, leishmanicidal and antifungal potential from marine red alga Bostrychia tenella J. Agardh (Rhodomelaceae,*

Ceramiales). Journal of Pharmaceutical and Biomedical Analysis, 52: 763–769.
13. Na, H. J., Moon, P. D., Lee, H. J., Kim, H. R., Chae, H. J., Shin, T., Seo,Y., Hong, S. H. and Kim, H.M. 2005. *Regulatory effect of atopic allergic reaction by Carpopeltis affinis*. Journal of Ethnopharmacology, 101: 43–48.
14. Dayong, S., Jing, L., Shuju, G. and Lijun, H. 2008. *Antithrombotic effect of bromophenol, the alga-derived thrombin inhibitor*. Journal of Biotechnology, 136: 577–588.
15. Kim, S.K., Thomas, N.V. and Li, X. 2011. *Anticancer compounds from marine macroalgae and their application as medicinal foods*. Advanced Food and Nutrition Research, 64: 213–224.
16. De-Fatima, A., Modolo, L.V., Conegero. L. S., Pilli, R. A., Ferreira, C. V. and Kohn, L.K. 2006. *Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design*. Curr Med Chem, 13: 3371-3384.
17. Eluvakkal, T., Sivakuamr, S. R. and Arunkumar, K. 2010. *Fucoidan in some Indian Brown Seaweeds found along the coast of Gulf of Mannar*. International Journal of Botany. 6(2): 176-181.
18. Manivannan, K., Thirumaran, G., Karthikai Devi, G. and Anantharaman, P. 2009. *Seaweeds from Vedalai Coastal Waters (Gulf of Mannar): Southeast Coast of India*. Middle-East Journal of Scientific Research 4 (2): 72-77.
19. Harbone, J. P. 1998. *Phytochemical Methods*: *A guide to modern techniques of plant analysis* 3rd edn. Chapman and Hall, New York, 1-150.
20. The Pharmacopoeia of India. 1996. Controller of Publication, Government of India.
21. Sharanabasappa, G. K., Santosh, M. K., Shaila, D., Seetharam, Y. N. and Sanjeevarao, I. 2007. *Phytochemical Studies on Bauhinia racemosa* Lam. *Bauhinia purpurea* Linn. and *Hardwickia binata* Roxb. E-Journal of Chemistry, 4(1):21-31.
22. Mallikharjuna, P.B., Rajanna, L.N., Seetharam, Y. N. and Sharanabasappa, G.K. 2007. *Phytochemical Studies of Strychnos potatorum L. f. - A Medicinal Plant*. E-Journal of Chemistry, 4(4): 510-518.
23. Blunt, J. W., Copp, B. R., Hu, W. P., Munro, M.H.G., Northcote, P.T., Prinsep, M. R. 2007. *Marine natural products*. Nat. Prod. Rep. 24: 31-86.
24. Prabha, V., Prakash, D.J. and Sudha P.N. 2013. *Analysis of bioactive compounds and antimicrobial activity of marine algae Kappaphycus alvarezii using three solvent extracts*. IJPSR, 4(1): 306-310
25. Poppy Mary Vimalabai, C. and Mary Phoebe M. 2003. *Distribution of trace metals in red algae, seawater and sediment of Tuticorin coast. Seaweed Res*. Utiln. 25(1&2): 63-63.
26. Balandrin, M.J. and Klocke, J.A. 1988. *Medicinal, aromatic and industrial materials from plants*. Springer-Verlag, Berlin, Heidelberg: pp. 1-36.
27. Djeridane, A., Yousfi, M., Nadjemi, B., Maamrim, S., Djireb, F. and Stocker, P. 2006. *Phenolic extracts from various Algerian plants as strong inhibitors of porcine liver carboxylesterase*. J. Enzym. Inhib. Med. Chem. 21: 719-726.
28. Lamien-Meda, A., Lamien, C.E., Compaoré, M.M.., Meda, R.N.T., Kiendrebeogo M., Zeba, B., Millogo J.F. and Nacoulma O.G. 2008. *Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso*. Molecules, 13: 581-594.
29. Meda, N.T.R., Bangou, M.J., Kiendrebeogo, M., Compaoré, M., Coulibaly, A.Y., Almaraz-Abarca, N., Zeba, B., Millogo-Rasolodimby, J. and Nacoulma, O.G. 2011. *Enzyme Inhibition Effect and Polyphenolic Content of Medicinal Plant Extracts*

from Burkina Faso. Journal of Biological Sciences, 11 (1): 31-38.
30. Yamunadevi, M., Wesely, E.G. and Johnson, M. 2012. *FTIR spectroscopic studies on Aerva lanata (L.) Juss. ex schult*. Asian Journal of Pharmaceutical and Clinical Research 5 (2): 82-86.
31. Janakiraman, N., Sahaya Sathish, S. and Johnson M. 2011. *UV-VIS and FTIR Spectroscopic Studies on Peristrophe bicalyculata (Retz.) Nees*. Asian Journal of Pharmaceutical and Clinical Research, 4(4): 125-129.
32. Harish kumar, A., Hullatti, B., Sharanappa, P. and Paras Sharma, C. 2010. *Comparative antimicrobial activity and tlc bioautographic analysis of root and aerial parts of Andrographis serpyllifolia.* International Journal of Pharmacy and Pharmaceutical Sciences 2(1): 52- 54.
33. Roy, S., Sen, A. K., Sayantan Dey. and Haraprasad Pal. 2012. *Isolation of steroids from Tinospora malabarica stem* International Journal of Research in Pharmaceutical and Biomedical Sciences, 3 (2): 453-455.
34. Mathekaga, A.D. and Meyer, J.J.M. 1998. *Antibacterial activity of South African Helichrysum species.* South African Journal of Botany, 64: 293-295.
35. Javed Kamal, 2011. *Quantification of alkaloids, phenols and flavonoids in sunflower (Helianthus annuus L.)* African Journal of Biotechnology 10(16): 3149-3151.
36. Krishnaveni, E. and Johnson, M. 2012. *Preliminary Phytochemical, UV-VIS, HPLC and Anti-bacterial Studies on Gracilaria corticata J. Ag* Asian Paicfic Journal of Tropical Biomedicine, S568-S574.
37. Johnson, M. and Krishnaveni, E. 2012. *UV- VIS Spectroscopic and HPLC Studies on Dictyota bartayresiana Lamour* Asian Pacific Journal of Tropical Biomedicine S514-S518.