International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 207-212
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Screening of Phytochemicals in Methanolic Extract of *Dictyota Dichotoma* (Huds.) Lamouroux From Koothankuzhi in the South East Coast of Tamil Nadu, India

John Peter Paul J*

***Assistant Professor of Botany and Director, Centre for Advanced Research in Plant Sciences (CARPS), St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.**

Received: 6 Oct 2018 / Accepted: 8 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: johnarock2008@yahoo.com

Abstract

The present study was carried out to screen the phytochemicals present in the methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux collected from Koothankuzhi in the south east coast of Tamil Nadu, India. The phytochemical screening of methanolic extract was screened using the standard procedure for UV-Visible spectroscopic, HPLC and FTIR. The UV-Visible spectrum showed the compounds separated at the nm of 450, 500, 534, 600, 655, 700, 750, 800, 850 and 900 with the absorption 4.000, 3.101, 2.048, 2.075, 3.311, 0.750, 0.101, 0.024, 0.006 and 0.003 respectively. The qualitative HPLC fingerprint profile displayed eight compounds at different retention time of 2.020min, 2.187min, 2.303min, 2.637min, 2.790min, 3.027min, 5.430min and 6.550min. The result of FTIR analysis was found the presence of functional groups such as nitrates, phenyl (C-O and C-H bonds), aliphatic ketones, sulphonic acid ester, oximes (C=N stretching), aromatic aldehyde, tertiary amine salts, alkanes, methoxy and hydroxyl group.

Keywords

Dictyota dichotoma, UV-Visible, HPLC, FTIR

*****

INTRODUCTION

It was predicted that about 90% of the species of marine plants are belonging to seaweeds or marine macro algae which contribute about 50% of the global photosynthesis [1]. Seaweeds are one of the important renewable resources of the primary producers and supply considerably to the carbon budget of the coastal ecosystem. Seaweeds are widely incorporated in Japanese and Chinese diet and traditional medicine [2,3] and have urbanized biomolecules and approaches which help the seaweeds' survival in the cruel and severe environment. Seaweeds are measured as source of bioactive compounds and fabricate a great variety of secondary metabolites characterized by a broad spectrum of biological activities [4,5]. More than 600 secondary metabolites have been isolated from some selected seaweeds.

There are a number of reports on the compounds derived from seaweeds with a broad range of biological activities such as antibiotics, antiviral diseases [6], anti-tumors and anti-inflammatory [7]

as well as neurotoxins [8]. Compounds with analgesic [9,10,11,12,13,14] diuretic [15,16], CNS depressant [17], hepatoprotective [18,19,20], muscle relaxant [21], anti-diabetic [22], anti-inflammatory [23,24, 25,26], anti-pyretic [27], anti-ulcer [28,29], anti-cancer [30] and anti-oxidant [31,32,33] have been detected in green, brown and red seaweeds.

Phytochemical types include sterols [34], isopyrenoids, amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid can be counted [35]. Consumption of seaweeds as sea vegetables in human diets has also been the common practice in several Asian countries [36]. Hence pharmacologists, physiologists and chemists have been paying increasing attention to the marine products predominantly on seaweeds for screening bioactive substances. Several works have been completed on crude and purified compounds obtained from seaweeds for evaluating the bioactive potential [37]. With this background, the present investigation was conducted to screen the phytochemicals present in the methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux collected from Koothankuzhi in the south east coast of Tamil Nadu, India.

MATERIALS AND METHODS:

Collection of Plant sample

The plant materials used in the present study was *Dictyota dichotoma* (Huds.) Lamouroux, belonging to Phaeophyceae (brown algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed separately in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. They were stored in 5% formalin solution [38].

Preparation of extracts

For the preparation of methanolic extract, the plant specimens were washed thoroughly and placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use. 30g powdered samples were packed in Soxhlet apparatus and extracted with methanol for 8h separately [39].

UV-Vis spectral analysis

The methanolic crude extract containing the bioactive compound was analyzed UV-Visible spectroscopically for further confirmation. The methanolic crude extract of *Dictyota dichotoma* (Huds.) Lamouroux was scanned in a wavelength ranging from 200-900nm using a Shimazdu spectrophotometer and characteristic peaks were detected [40].

HPLC Analysis

The HPLC method was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-Vis detector SPD-10AT, a Rheodyne injector fitted with a 20µl loop and an auto injector SIL-10AT. A Hypersil® BDS C-18 column (4.6 × 250mm, 5µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1ml/min at ambient temperature (25-28°C). The mobile phase consisted of 0.1% v/v methanol (solvent A) and water (solvent B). The mobile phase was prepared daily, filtered through a 0.45µm and sonicated before use. Total running time was 15min. The sample injection volume was 20µl while the wavelength of the UV-Vis detector was set at 254nm [41].

Instrumentation

An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC- 0 AT VP pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO- 10AS VP column oven (Shimadzu), a SCL-10A VP system controller (Shimadzu), a reverse phase Luna 5µl C18 (2) and Phenomenex column (250 mm X 4.6mm) were used. The mobile phase components methanol: water (45:55) were filtered through a 0.2µ membrane filter before use and were pumped from the solvent reservoir at a flow rate of 1ml/min which yielded column backup pressure of 260-270kgf/cm^2. The column temperature was maintained at 27°C. 20µl of the respective sample and was injected by using a Rheodyne syringe (Model 7202, Hamilton).

FTIR analysis

The methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux was shade dried and FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice and confirmed the spectrum [42].

RESULTS AND DISCUSSION

UV-Visible spectrum analysis

The UV-Visible spectrum of the methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux was selected at the wavelength of 200nm to 900nm due to the sharpness of the peaks and proper baseline. The profile showed the compounds separated at the nm of 450, 500, 534, 600, 655, 700, 750, 800, 850 and 900 with the absorption 4.000, 3.101, 2.048, 2.075, 3.311, 0.750, 0.101, 0.024, 0.006 and 0.003 respectively (Figure-1 and Table-1).

HPLC analysis

The qualitative HPLC fingerprint profile of the methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux was selected at a wavelength of 660nm due to the sharpness of the peaks and proper baseline. The methanolic extract prepared by hot extraction was subjected to HPLC for the separation and identification of constituents present in the *Dictyota dichotoma* (Huds.) Lamouroux. Eight compounds were separated at different retention times of 2.020min, 2.187min, 2.303min, 2.637min, 2.790min, 3.027min, 5.430min and 6.550min respectively. The profile displayed six prominent peaks at the retention times of 2.020min, 2.187min, 2.303min, 2.637min, 2.790min and 3.027min, followed by two moderate peaks were also found at the retention times of 5.430min and 6.550min (Figure-2).

FTIR ANALYSIS

The FTIR spectrum was used to predict the functional group of the active components based on the peak value in the region of infra red radiation. The crude methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux was conceded into the FTIR and the functional groups of the components were separated based on its peak ratio. As illustrated in Figure-3, FTIR spectrum of methanolic extract showed different peaks at 671.18, 1071.38, 1217.00, 1384.79, 1640.35, 1709.78, 2359.74, 2852.52, 2923.88 and 3382.91cm^{-1}. It was confirmed the presence of functional groups such as nitrates, phenyl (C-O and C-H bonds), aliphatic ketones, sulphonic acid ester, oximes (C=N stretching), aromatic aldehyde, tertiary amine salts, alkanes, methoxy and hydroxyl group respectively.

Figure-1. UV-Visible spectrum of methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux

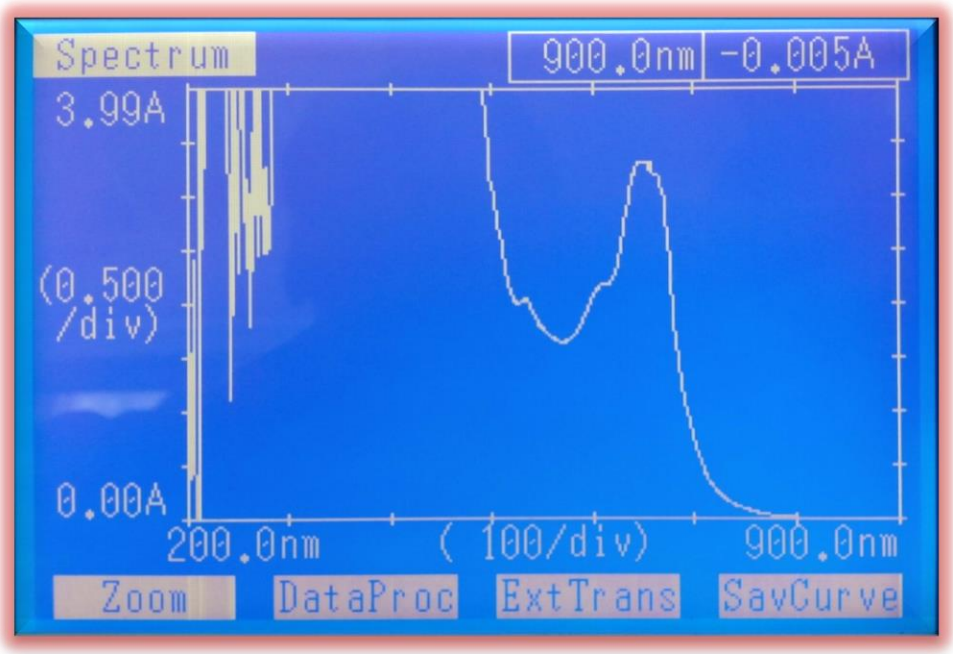


Table 1: UV-Visible spectrum of methanol extract of *Dictyota dichotoma* (Huds.) Lamouroux

Nm	450	500	534	600	655	700	750	800	850	900
Abs	4.000	3.101	2.048	2.075	3.311	0.750	0.101	0.024	0.006	0.003

Figure 2: HPLC analysis of methanolic extract of *Dictyota dichotoma* (Huds.) Lamouroux

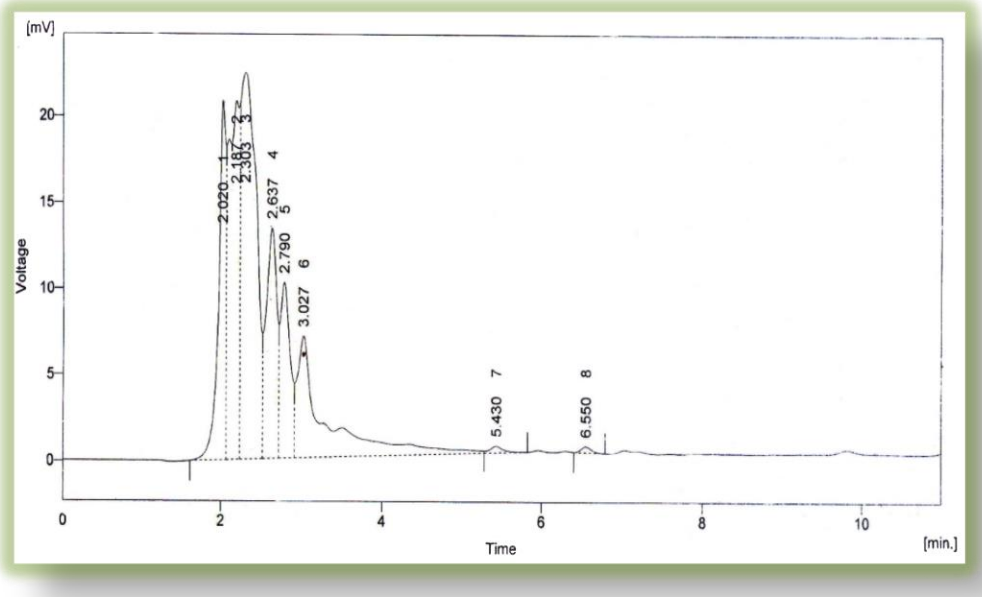
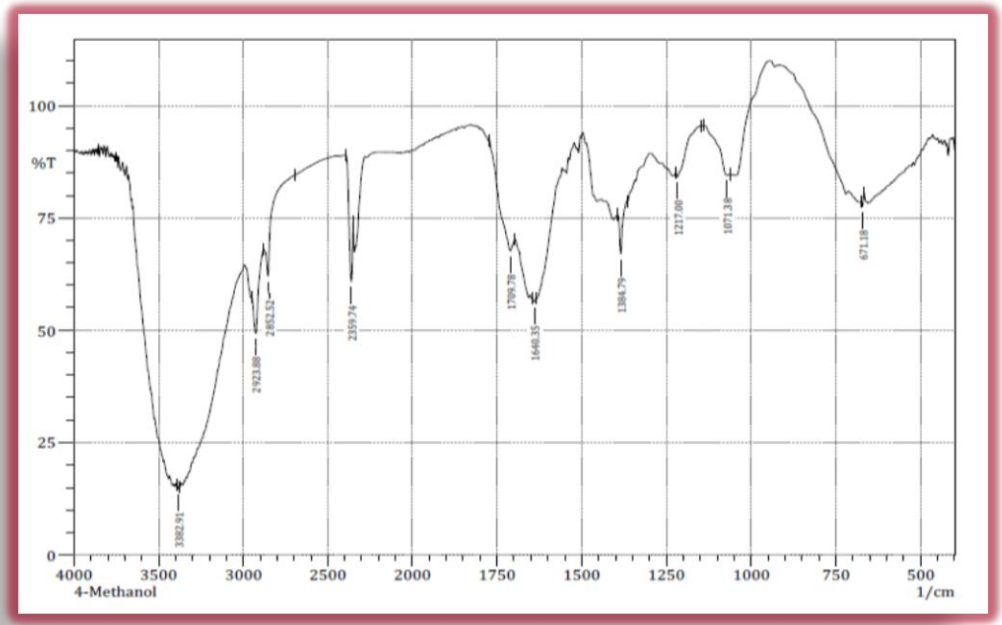


Figure 3: FT-IR spectrum of methanol extract of *Dictyota dichotoma* (Huds.) Lamouroux



CONCLUSION

From the present study, it was concluded that UV-Visible spectrum showed the compounds separated at the nm of 200, 250, 331, 398, 433, 450, 500, 550, 606, 659, 700, 750, 862, 909, 975, 996, 1053 and 1100 with the absorption 4.000, 0.384, 1.342, 1.207, 4.000, 4,000, 4,000, 2.960, 3.612, 0.424, 0.954, 0.331, 0.130, 0.127, 0.111, 0.104, 0.043 and 0.003 respectively. The qualitative HPLC fingerprint profile displayed eight compounds at different retention time of 2.020min, 2.187min, 2.303min, 2.637min, 2.790min, 3.027min, 5.430min and 6.550min respectively. The profile displayed six prominent peaks at the retention time of 2.020min, 2.187min, 2.303min, 2.637min, 2.790min and 3.027min, followed by two moderate peaks were also found at the retention time of 5.430min and 6.550min. The result of FTIR analysis was found the presence of functional groups such as nitrates, phenyl (C-O and C-H bonds), aliphatic ketones, sulphonic acid ester,

oximes (C=N stretching), aromatic aldehyde, tertiary amine salts, alkanes, methoxy and hydroxyl group.

ACKNOWLEDGEMENT:

The author is thankful to Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Government of India, New Delhi for providing the financial assistance through Major Research Project (Ref. No. EMR/2017/004009) sanctioned July 2018.

CONFLICT OF INTEREST:

The author declares that he has no conflict of interest.

REFERENCES

1. Dhargalkar, V.K., Neelam, P. Seaweed: Promising plant of the millennium. *Science and culture*, 2005; 71:60-66.
2. Fujiwara, A, T., Mino, N., Kuroda, M. The protein value in human nutrition of edible marine algae in Japan. *Hydrobiologia,* 1984; 116/117:513-516.
3. Chengkui, Z., Tseng, C.K., Junfu, Z., Chang, C.F. Chinese seaweeds in herbal medicine. *Hydrobiologia,* 1984; 116/117:152-154.
4. Lindequist, U., Schweder, T. Marine Biotechnology. In: Rehm, H.J., Reed, G. (Eds). *Biotechnology,* 2001; 10:441-484.
5. Newman, D.J., Gordon M.C., Kenneth, M.S. Natural products as sources of new drugs over the period 1981-2002, *Journal of Natural products,* 2003; 66(7):1022-1037.
6. Trono, J.G.C. Diversity of the seaweed flora of the Philippines and its utilization. *Hydrobiologia,* 1999; 398/399:1-6.
7. Scheuer, P.J. Some marine ecological phenomena: chemical basis and biomedical potential. *Science,* 1900; 248:173-177.
8. Kobashi, K. Pharmacologically active metabolites from symbiotic microalgae in Okinawan marine invertebrates. *J. Nat. Prod.*, 1989; 52:225-238.
9. Ana, I.F.V., Carlos, M.D.S., Neivys, G.D., Ana, M.S.A., Yulica, S.O., Hiran, C.S. Anti-inflammatory and analgesic activities of red seaweed *Dichotomaria obtusata. Brazilian Journal of Pharmaceutical Sciences,* 2011; 47(1):111-118.
10. Dang, D.H., Hoang, M.H., Hoang, T.L.A. Studies on the analgesic and anti-inflammatory activities of *Sargassum swartzii* (Turner) C. Agardh (Phaeophyta) and *Ulva reticulata* Forsskal (Chlorophyta) in experiment animal models. *African Journal of Biotechnology,* 2011; 10(12):2308-2314.
11. Jiyoung, K., Quochai, L., Mohammed, N., Jaesuk, C., Yongki, H. *In vivo* anti-inflammatory, antipyretic and analgesic activities of the aquaculturable green seaweed *Codium fragile* extracts in mice. *Journal of Life Science*, 2012; 22(6):852-856.
12. Saurabh, B., Kiran, S., Ajay, S., Kalpana, N., Tanmoy, B. Anti-inflammatory, analgesic and antiulcer properties of *Porphyra vietnamensis. Avicenna Journal of Phytomedicine,* 2015; 5(1):69-77.
13. Iniya Udhaya, C., John Peter Paul, J. Analgesic activity of methanolic extract of *Gracilaria corticata* J.Ag. (Red seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India. *American Journal of Biological and Pharmaceutical Research.* 2015; 2(4):157-160.
14. John Peter Paul, J., Muthu Sheeba, M. Screening of analgesic activity of methanolic extract of *Chaetomorpha litorea* Harvey (green seaweed) in Koothankuzhi coast, Tamil Nadu, India. *International Journal of Biopharmaceutics,* 2016; 7(2):59-62.
15. John Peter Paul, J. Screening of Diuretic Activity of Methanol Extract of *Gracilaria corticata* J.Ag. (Red Seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India. *American Journal of Biological and Pharmaceutical Research.* 2014; 1(2):83-87.
16. John Peter Paul, J., Shri Devi, S.D.K. Screening of Diuretic Activity of Methanol Extract of *Gracilaria dura* (Ag.) J.Ag. (Red Seaweed) on Mice. *International Journal of Pharmacological Screening Method,* 2014; 4(3):113-118.
17. Rahila, N., Shahida, P., Iqbal, A. Pharmacological activities of *Hypnea musciformis. African J. Biomed. Res.*, 2010; 13(1):69-74.
18. Vasquez, R., Apostol, J., Ramos, J.D., Morales, M., Padiernos, K., Pangilinan, C., Payuran, C., Princesa, J. Hepatoprotective effects of aqueous sulfated polysaccharide extract from *Sargassum siliquosum* Agardh on Paracetamol-induced oxidative liver toxicity and antioxidant properties. *International Journal of Pharmaceutical Frontier Research,* 2012; 2(1):15-27.
19. Mahsa, M., Mahdi, E., Yong, M.G., Hemn, H.O., Mohd, H.B., Suhaila, M. Effects of brown seaweed (*Sargassum polycystum*) extracts on kidney, liver and pancreas of type-2 diabetic rat model. *Evidence-Based Complementary and Alternative Medicine.* 2014; Doi:10.1155/2014/379407.
20. Kulandhaisamy, A.S., Annappan, M. Antiulcer, wound healing and hepatoprotective activities of the seaweeds *Gracilaria crassa, Turbinaria ornata* and *Laurencia papillosa* from the southeast coast of India. *Brazilian Journal of Pharmaceutical Sciences,* 2013; 49(4):669-678.
21. John Peter Paul, J. Skeletal muscle relaxant activity of methanolic extract of *Gracilaria corticata* J.Ag. (Red Seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India. *International Journal of Phytopharmacy Research,* 2017; 8(1):1-3.
22. Palanisamy, S., Sellapa, S. Evaluation alpha-amylase and alpha-glucosidase inhibitory properties of selected seaweeds from Gulf of manner. *International Research Journal of Pharmacy*, 2012; 3(8):128-130.
23. Radhika, D., Veerabahu, C., Priya, R. Anti-inflammatory activities of some seaweed collected from the Gulf of Mannar coast, Tuticorin, South

India. *International Journal of Pharma and Bio Sciences,* 2013; 4(1):39-44.
24. Krishnankartha, P., Kajal, C. Antioxidant and anti-inflammatory potential of the aqueous extract and polysaccharide fraction from brown marine macroalgae *Padina* sp. from Gulf of Mannar of Peninsular India. *Journal of Coastal Life Medicine,* 2013; 1(1):38-48.
25. Khadija, O., Nabila, B., Samira, E., Omar, A. Anti-inflammatory and antimicrobial activities of twenty-three marine red algae from the coast of Sidi Bouzid. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2013; 5(3):145-149.
26. Neelakandan, Y., Venkatesan, A. *In vitro* anti-tumor, anti-inflammatory, antioxidant and antibacterial activities of marine brown alga *Sargassum wightii* collected from Gulf of Mannar. *Global Journal of Pharmacology,* 2014; 8(4):566-577.
27. John Peter Paul, J. Screening of antipyretic activity of methanol extract of *Gracilaria corticata* J.Ag. (red seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India. *Asian Journal of Pharmaceutical Clinical Research,* 2014; 7(3):142-144.
28. Doungporn, A., Yuwadee, P., Tawat, T., Thidarat, N., Duangta, K. Gastroprotective activity of *Padina minor* Yamada. *Chiang Mai. J. Sci.*, 2009; 36(1):92-103.
29. Ellya, S., Rosmawaty, D. Evaluation of fucoidan bioactivity as anti gastric ulcers in mice. *Procedia Environmental Sciences,* 2015; 23:407-411.
30. Neelakandan, Y., Venkatesan, A. *In vitro* anti-tumor, anti-inflammatory, antioxidant and antibacterial activities of marine brown alga *Sargassum wightii* collected from Gulf of Mannar. *Global Journal of Pharmacology,* 2014; 8(4):566-577.
31. Indu, H., Seenivasan, R. *In vitro* antioxidant activity of selected seaweeds from south east coast of India. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2013; 2(2):474-484.
32. Parthiban, C., Saranya, C., Girija, K., Hemalatha, A., Suresh, M., Anantharaman, P. Biochemical composition of some selected seaweeds from Tuticorin coast. *Advances in Applied Science Research,* 2013; 4(3):362-366.
33. Devaraj, I.D., Paramasivam, G., Rajalakshmi, S., Solomon, J. Antioxidant activities of marine algae *Valoniopsis pachynema* and *Sargassum swartzii* from the south east coast of India. *International Journal of Fisheries and Aquatic Studies,* 2015; 3(2):426-430.
34. Ahmad, V.U., Aliya, R., Perveen, S., Shameel, M. Sterols from marine green alga *Codium decortacatum*. *Phytochemistry,* 1993; 33:1189-1192.
35. Mtolera, M.S.P., Semesi, A.K. Antimicrobial activity of extracts from six green algae from Tanzania. *Curr. Trends Marin Bot. East Afr. Region.* 1996; P.211-217.
36. Nisizawa, K. Seaweeds Kaiso. Japan Seaweed Association Publication, Kochi, Japan. 2002; P.23.
37. Faulkner, D.J. Biomedical uses for natural marine chemicals. *Oceanus,* 1992; 35:29-35.
38. Iniya Udhaya C, John Peter Paul J. Screening of preliminary phytochemicals of *Gracilaria cylindrica* Boergesen in Koothankuzhi, Tirunelveli district, Tamil Nadu, India. *Indo American Journal of Pharmaceutical Sciences,* 2017; 4(12):4590-4594.
39. Iniya Udhaya C, John Peter Paul J. Screening of anti-oxidant activity of methanolic extract of *Gracilaria fergusonii* J.Ag. (Red seaweed) in Hare Island, Thoothukudi, Tamil Nadu, India. *Indo American Journal of Pharmaceutical Sciences,* 2017; 4(9):2724-2727.
40. Amster Regin Lawrence, R., Iniya Udhaya, C., John Peter Paul, J. Phytochemical analysis of chloroform extract of *Sargassum linearifolium* (Turner) C.Ag. (Brown seaweed) using UV-VIS, FTIR and HPLC. *Indo American Journal of Pharmaceutical Research,* 2017; 7(9):390-395.
41. John Peter Paul, J., Shri Devi, S.D.K. Phytochemical studies on *Padina pavonica* (L.) Thivy ex Taylor. *Indo American Journal of Pharmaceutical Research,* 2013; 3(6):4452-4459.
42. John Peter Paul, J. Phytochemical analysis of methanolic extract of *Caulerpa scalpelliformis* (R.Br.) Web. V. Bosse collected from Hare Island, Thoothukudi, Tamil Nadu, India. *Indo American Journal of Pharmaceutical Sciences,* 2018; 5(3):1905-1908.