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Research Article

Screening And Extraction Of Secondary Metabolites From Ulva Fasciata And Sargassum Tenerimum Using Soxhlation Techniques

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ABSTRACT
Limited seaweeds were used for agricultural purposes, because of the non-availability of secondary metabolite profiles. Green algae, *Ulva fasciata* (UF), a brown alga, *Sargassum tenerimum* (ST) have been used for various biological uses. In the present study, screening of secondary metabolites of these selected algae used polar hot soxhlation continuous extraction techniques to complete the process. (Petroleum Ether (PE), Chloroform (CH), Water (WA), and non-polar solvents like Acetone (AC) and Ethanol (ET) were used for extraction purpose. The WA extract of UF and ST contained flavonoids, tannins, and steroids. It is possible to extract steroids, tannins, saponins, cardiac glycosides, and phenolic chemicals using the soxhlation process. This finding envisages that secondary metabolites could be extracted with the soxhlation method and can be used for biological and agricultural lead.

Keywords: Marine algae, bioactive compounds, quantitative analysis, secondary metabolites, soxhlet method.

INTRODUCTION
Seaweeds are a significant component of the ocean's biological resources, as they are microscopic marine algae. Marine algae are abundant sources of unique and biologically active secondary metabolites that are structurally significant, making them renewable living resources. Algae are abundant and mostly encased suppliers of a wide range of physiologically significant compounds (Saz *et al*., 1963; Daoudi *et al*., 2001; Huang and Lee, 2005; Gouveia *et al*., 2008; Ghosh *et al*., 2009; Seenivasan *et al*., 2010; Chojnacka *et al*., 2012; Munirasu *et al*., 2013). Marine macroalgae are nature's most biologically active resources, as they possess a wealth of bioactive compounds like fatty acids (Quasim, 1986; Aliya *et al*., 1991; Ahmad *et al.,* 1992; Tasende, 2000; Viron *et al*., 2000), polysaccharides (O'Sullivan *et al*., 2010), alkaloids (see a review of Güven *et al.,* 2010); phenolic compounds (Athukorala *et al*., 2003; Heo *et al*., 2005), etc. The chemical compounds already isolated from algae are providing us the valuable ideas for insect pest management (Tunaz and Uygun, 2004). Seaweed dietary fibers perform a varied range of functions such as antioxidant (Elena *et al.*, 2001 and Kuda *et al.*, 2005), antimutagenic, anticoagulant, antitumor (Dhargalkar and Pereira, 2005), the alternate source for bioactive substances (Pietra, 1997; Kelecom, 2002; Watson and Cruz Rivera, 2003; Bocanegra *et al*., 2009), bactericidal (Febles *et al*., 1995; Del Val *et al*., 2001; Rouxel *et al*., 2001; Ely *et al*., 2004 and Cordeiro *et al*., 2006) fungicidal (Rajesh *et al*., 2011), source of food (Sultana *et al*., 2005; MacArtain *et al*., 2007; Sabour *et al*., 2013), feed (Ganapathy Selvam *et al*., 2013), fertilizer (Manivannan *et al*., 2008; Thirumaran *et al*., 2009; Ganapathy Selvam *et al*., 2013), medicine (Prabha *et al*., 2013; Indira *et al*., 2013; Rebecca *et al*., 2013), antimicrobial (Manilal *et al*., 2010; Premalatha *et al*., 2011; Sumathi and Krishnaveni, 2012; Prabha *et al*., 2013), pesticides (David, 2008; Sahayaraj, 2008; Sultana *et al*., 2011; Prasad *et al.,* 2012) values.

MATERIALS AND METHODS
Extraction of marine algae
A soxhlet apparatus was used for hot continuous extraction to extract secondary metabolites (Bose *et al*., 2007; Handa, 2008; Chiheb *et al*., 2009). In order to extract secondary metabolites, 750 mL of powdered algal material was subjected to a hot continuous extraction process for 24 hours at 40–50º C at room temperature using a soxhlation method that used petroleum ether, chloroform, ethanol, acetone, and water separately. Following the solvent removal, 10 mL of the residue was evaporated and dried in a desiccator with vacuum over sodium sulfate. The extracts were collected in airtight glass vials (9.4 cm) and refrigerated for future use after being concentrated under reduced pressure using a dessicator.

Screening of secondary metabolites
Standard protocols were used to determine the quantities of tannins (Aparna, 2000), total phenols, ortho-di-hydric phenols, bound phenols (Harbone, 1973), total flavonoids (Suresh Chand and Muralirangan, 2010), and total saponins (Obadoni and Ochuko, 2001).

Total alkaloids: A 250 mL conical flask containing 5 g of the plant sample was filled with 200 mL of 10% acetic acid in ethanol, capped, and left to stand for 4 hours. After filtering, the extract was concentrated to a fourth of its original volume in a water bath at 90º C. The extract was gradually mixed with concentrated ammonium hydroxide until the precipitation was fully formed. After letting the entire mixture settle, the precipitation was collected and cleaned using

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filtered Whatman No. 1 filter paper and diluted ammonium hydroxide (1%). Weighing and drying the leftover alkaloid (Edeoga *et al*., 2005).

Total flavanoids: For extraction of flavanoids, 1 gm of powered algal material was macerated with 30 mL of 2N HCl. The mixture was incubated in an oven (Kemi, India) for 4 hours at 100^0C. Flavanoids were subsequently extracted with equal volumes of ethyl acetate. This was repeated thrice. The ethyl acetate fractions were pooled, dried over anhydrous sodium sulphate, and evaporated. The residue was weighed and represented as flavanoids (mg/g fresh weight) (Suresh Chand and Muralirangan, 2010).

Total phenols: 10 mL of 80% ethanol were used to extract 0.5 g of the powder at 40 °C for four hours. Following a cooling period to ambient temperature, the samples were centrifuged for 15 minutes at 4500 rpm. A 10 mL test tube was filled with aliquots of the extracts and then topped up with 3 mL of distilled water. Each test tube received sequential additions of 2 mL sodium carbonate (20%) and 0.5 mL Folinciacalteau reagent (1:1 with water). They created a blue hue. Following a minute of warming, the test solutions were cooled, and the absorbance at 650 nm was measured in comparison to the blank reagents. Standard curves were created using known catechol concentrations. The total phenol content was expressed as mg catechol equivalent of phenols/gram of sample (Malick and Singh, 1980).

Total saponins: After grinding the dried algae, 20 g of each was put to a conical flask along with 100 mL of 20% aqueous ethanol. Using constant stirring, the samples were heated to approximately 55°Cover a 4-hour period in a hot water bath. After the mixture was filtered, 200 mL more of 20% ethanol was used to extract the residue once again. At roughly 90°C, the mixed extracts were boiled down to 40 mL in a water bath. After transferring the concentrate into a 250 mL separating funnel, 20 mL of diethyl ether was added, and the mixture was violently agitated. The ether layer was disposed of, and the aqueous layer was recovered. There was another purifying procedure. N-butanol (60 ml) was added. Two washes using 10 ml of 5% aqueous sodium chloride were performed on the combined n-butanol extracts. In a water bath, the residual solution was heated. Following evaporation, the samples were dried in an oven to a set weight, at which point the percentage of saponin was determined (Obadoni and Ochuko, 2001).

Total tannins: In a 100 mL volumetric flask, 1 mL of sample extract and 75 mL of distilled water were combined. 5 mL of the Folin-Danis Reagent and 10 mL of the sodium carbonate solution were added. After adding distilled water to reach the desired volume, giving it a good shake, and letting it stand for half an hour. The optical density measured at 700 nm in relation to the reagent blank (1 mL of distilled water in place of sample) after the necessary amount of time. Using the same process, a standard graph of known samples was created. Tannic acid content in the test sample was determined using the standard graph (Aparna, 2000).

Total Ortho-dihydric (O.D.) phenols: The pipette 1 mL of 0.5 N HCl and 1 mL of the alcohol extract were added to a test tube. Mix one millilitre of Arnow's reagent (ten grammes of sodium molybdate) with one millilitre of distilled water. Reagent should be kept in a brown bottle. Hcl, 0.5 N NaOH, and 1 mL of Hcl are stable for a year (Nickson Prabhu, 1998), after which 10 mL of distilled water and 2 mL of 1 N NaOH are added. Not long after the alkali was added. The produced pink colour keeps the reagent blank in place in the absence of the extract. If the colour intensity is strong, dilute it to 25 millilitres and measure the solution's absorbance at 530 nm. Determine the O.D. of the phenols in the samples using a catechol-prepared standard curve.

Total Bound Phenol: Chop the leaves into 0.5–1 cm pieces. Using a pestle and five millilitres of sodium lauryl sulphate, grind a 100 mg sample in a mortar. After transferring the suspension to centrifuge tubes, centrifuge for five minutes at 2000 rpm. Throw away the supernatant and rinse the residue twice, using 5 mL of sodium lauryl sulphate and 5 mL of diethyl ether each time. Centrifuge and discard the supernatant following each washing. After letting the residue dry, suspend it in centrifuge tubes with 3 mL of 0.5 N NaOH. After leaving it overnight at room temperature, centrifuge it and gather the supernatant. The liberated phenols, which have absorbance maxima at 530 nm, are present in the alkali extract. Utilising 0.5 N NaoH to dilute 1 mL to 3 mL, determine the absorbance at 530 nm. Absorbance values can be used to express bound phenol concentrations. Alternatively, the Folin- Phenol Reagent can be used to measure the concentration calorimetrically (Nickson Prabhu, 1998).

Statistical analysis

Using the statistical software SPSS (20.0 version), the data were reported as mean ± standard error (SE), and the statistical significance was evaluated by comparing the means using One Way Analysis of Variance (ANOVA) and a "p" value that was reached at 5%.

RESULTS

Quantitative estimation of secondary metabolites

In Figures 1 and 2, the chemical composition of the chosen species of marine algae that were screened is quantitatively estimated. *S. tenerimum* has the highest tannin content, followed by *U. fasciata*, *G. corticata*, and *U. fasciata* had high total phenol contents. *S. tenerimum* had the highest bound phenol concentration, followed by *G. corticata* and *U. lactuca* (Figure 1). High levels of ortho-dihydric phenols were found in *S. tenerimum*, *G. corticata*, and *U. fasciata*. S. *tenerimum* has the highest concentration of flavanoids, followed by *G. corticata* and *U. fasciata. G. corticata, S. tenerimum,* and *U. fasciata* have high saponin contents. The contents of *G. corticata* and *S. tenerimum* were substantially higher than those of flavanoids, phenolic compounds, total tannins, and saponins (F= 2364.87; df= 8,1; P= 0.016). (Figure 2).

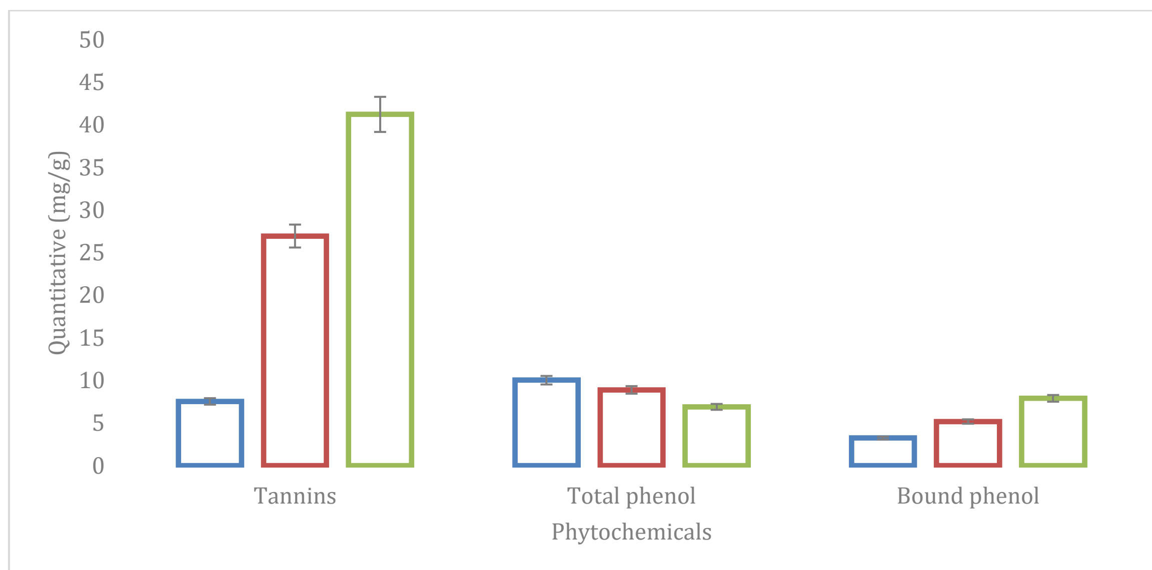

Figure 1. Quantitative determination of tannins, total phenol, and bound phenol content of selected species of marine algae (UL- *Ulva fasciata*, GC-*Gracilaria corticata*, SW- *Sargassum tenerimum*).

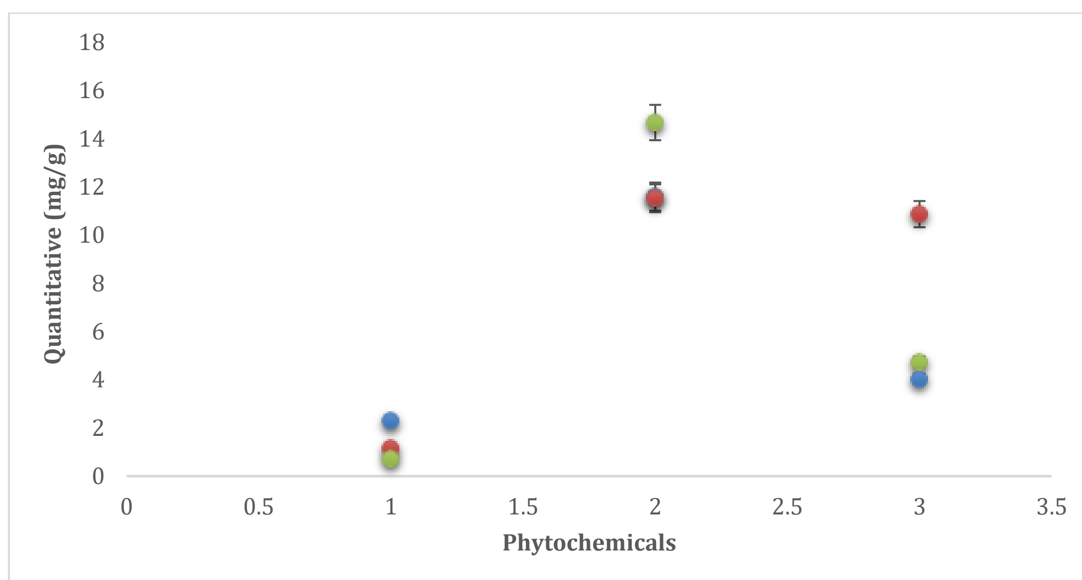

Figure 2. Quantitative determination of ortho-dihydric phenol, flavanoids and saponins (mg/g) of selected species of marine algae (UL- *Ulva fasciata*, GC-*Gracilaria corticata*, SW- *Sargassum tenerimum*).

DISCUSSION

Previously, preliminary secondary metabolites of Pakistan seaweeds, namely *Spatoglossum asperum* (J. Agardh) and *Caulerpa racemosa* (Forsskal) Webber V. Bosse (Quasim, 1986; Aliya *et al.,* 1991; Ahmad *et al*., 1992) and Indian seaweeds namely, Kappaphycus sp. (Rao and Pullaiah, 1982; Lahaye and Kaffer, 1997; Muthuraman and Ranganathan, 2004; Rajasulochana *et al*., 2009; Varghese *et al*., 2010; Manilal *et al*., 2010); *Ulva reticulate* Forsskal (Varghese *et al*., 2010); *Gracilaria fergusonii* J. Agardh (Renuka Bai, 2010); *Caulerpa racemosa* (Forsskal) webber V. Bosse and *Grateloupia lithophila* Boergesen (Rao and Pullaiah, 1982; Lahaye and Kaffer, 1997; Muthuraman and Ranganathan, 2004; Manilal *et a*l., 2010; Srivastava *et al*., 2010) were available in the literature.Terpenoids have been identified in *P. pavonica* and *C. scalpelliformis* water extracts. Marine algal seaweeds contain a large number of steroids (Rajasulochana *et al*., 2009). Sterols are an important class of lipids, just like they are in many other eukaryotic cells. Algal cells typically contain a combination of sterols, including cholesterol, 24-methylenecholesterol, stigmasterol, and β-sitosterol (Nabil and Cosson, 1996). In this investigation, β-sitosterol a sterolic compound was found in *U. fasciata*. In a similar vein, steroids in *U. lactuca* were found by Kapetianovic *et al*. (2005). The absence of alkaloids was demonstrated by both qualitative and quantitative phytochemical testing on *U. fasciata* and *U. lactuca*. On the other hand, *Ulva reticulata* ethanol and aqueous extracts (Varghese *et al*., 2010) revealed alkaloids. The amount of total phenolic content in the algae under study ranged from 2 to 16 mg/g. Nevertheless, phenolic compounds were discovered in Brazillian red algae, according to a prior investigation (Rajasulochana et al., 2009). Brown algae had significant concentrations of tannins, total phenols, and bound phenols (Craigie, 2011). On the other hand, green algae had large levels of flavonoids and O. D. phenol.

CONCLUSION

Finally, it can be said that a higher number of secondary metabolites from marine algae may be extracted using the soxhlation approach. This discovery aids in the ongoing study of other marine algae and their biological activities using various solvent extracts, as well as the potential use of these algae as a food and medicinal source.

REFERENCES

1. Abirami, R. G. and Kowsalya, S. 2012. Phytochemical screening, microbial load and antimicrobial activity of under exploited seaweeds. *International Research Journal of Microbiology*, 3(10): 328-332.
2. Ahmad, V. U., Rehman, A., Perveen, S. and Shameel, M. 1992. A sterol glycoside from marine green alga, *Codium iyengarii. Phytochemistry*, 31*:* 1429 - 1431.
3. Aliya, R., Shameel, M., Usmanghani, K. and Ahmad, V. U. 1991.Analysis of fatty acids from *Codium iyengarii* (Bryopsidophyeae). *Pakistan Journal of Pharmacological Science,* 4: 103 - 111.
4. Asha, A., Rathi, J. M., Patric Raja, D. and Sahayaraj, K. 2012. Biocidal activity of two marine green algal extracts against third instar nymph of *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae). *Journal of Biopesticide*, 5 (Suppl.): 129-134.
5. Athukorala, Y., Lee, K.W., Shahidi, F., Heu, M. S., Kim, H. T., Lee, J. S. and Jeon, Y. J. 2003.Antioxidant efficacy of extracts of an edible red alga (*Grateloupia wlicina*) in linoleic acid and wash oil. *Journal of Food Lipidology*, 10: 313 - 327.
6. Bocanegra, A., Sara, B., Juana, B., Sofia, R., Francisco and Sanchez-Muniz, J. 2009.Characteristics and nutritional and cardiovascular health properties of seaweeds. *Journal of Medicinal Food,* 12 (2): 236-258.
7. Chojnacka, K., Saeid, A., Witkowska, Z. and Tuhy, L. 2012. Biologically active compounds in seaweed extracts - the prospects for the application. *The Open Conference Proceedings Journal*, 3 (1-M4): 20-28.
8. Cordeiro, R. A., Gomes, V. M., Carvalho, A. F.U. and Melo, V. M. M. 2006. Effect of proteins from the red seaweed *Hypnea musciformis* (Wulfer) Lamouraux as the growth of human pathogen yeasts. *Brazilian Archives of Biology and Technology*, 49 (6): 915-921.
9. David, B. 2008. Biotechnological approaches in IPM and their impact on environment. *Journal of Biopesticides*, 1: 1-5.
10. Del Val, A. G., Platas, G. and Basilio, A. 2001.Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *International Microbiology*, 4: 35-40.
11. Dhargalkar, V. K. and Pereira, N. 2005. Seaweed: Promising plant of the millennium. *Science and Culture*, 71 (3-4): 60-66.
12. Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. 2005.Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4 (7): 685-688.
13. Elena, M., Francisco, Y. and Erickson, K. L. 2001.Mailiohydrin, a cytotoxic chamigrenedibromohydrin from a Philippine *Laurencia* species. *Journal of Natural Products*, 64 (6): 790-791.
14. 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 Biology and Ecology,* 309: 121-127.
15. Febles, C. I., Arias, A. and Gil-Rodriguez, M. C. 1995. *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife. *Anuario del Estudios Canarios*, 34: 181-192. (In Spanish).
16. Ganapathy Selvam, G., Balamurugan, M., Thinakaran, T. and Sivakumar, K. 2013. Developmental changes in the germination growth and chlorophyllase activity of *Vigna mungo* L. using seaweed extract of *Ulva reticulate* Forsskal. *International Journal of Pharmacy*, 4 (1): 252-254.
17. Ghosh, T., Chattopadhyay, K., Marschall, M., Karmakar, P., Mandal, P. and Ray, B. 2009. Focus on antivirally active sulfated polysaccharides: From structure–activity analysis to clinical evaluation. *Glycobiology*, 19 (1): 2–15.
18. Gouveia, L., Batista, A. P., Sousa, I., Raymundo, A. and Bandarra, N. M. 2008.Microalgae in novel food products. In: *Food chemistry research developments* (Konstantinos and Papadopoulos, N., eds.) Nova Science Publishers, Inc. 1-37 PP.
19. Guven, K. C., Percot, A. and Sezik, E. 2010.Alkaloids in marine algae. *Marine Drugs*, 8: 269-284.
20. Hebsibah Elsie, B., Dhanarajan, M. S. and Sudha, P. N. 2011.*In vitro* screening of secondary metabolites and antimicrobial activities of ethanol and acetone extracts from red seaweed *Gelidium acerosa. International Journal of Chemical Research*, 2 (2): 2729.
21. Heo, S. J., Park, E. J., Lee, K. W. and Jeon, Y. J. 2005.Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresources Technology*, 96: 1613 - 1623.
22. Huang, R. and Lee, H. T. 2005.Immunological properties of the marine brown alga *Endarachne binghamiae* (Phaeophyceae). *International Journal of Applied Science and Engineering*, 3 (3): 167-173.
23. Indira, K., Balakrishnan, S., Srinivasan, M., Bragadeeswaran, S. and Balasubramanian, T. 2013. Evaluation of *in vitro* antimicrobial property of seaweed (*Halimeda tuna*) from Tuticorin coast, Tamil Nadu, Southeast coast of India.*African Journal of Biotechnology*, 12 (3): 284-289.
24. Kapetanovic, R., Dusan, S., Simeon, P., Mario, Z., Zoran, K. and Gasic, J. G. 2005. Sterol composition of the adriatic sea algae *Ulva lactuca, Codium dichotomum, Cystoseira adriatica* and *Fucus virsoides*. *Journal of the Serbian Chemical Society*, 70 (12): 1395.

25. Kelecom, A. 2002.Secondary metabolites from marine microorganism. *Annals of Brazilian Academy of Sciences,* 74 (1): 151-170.
26. Krishnaiah, D., Sarbatly, R. and Bono, A. 2007.Phytochemical antioxidants for health and medicine - a move towards nature. *Biotechnology and Molecular Biology- Review,* 1 (4): 97-104.
27. Krishnaiah, D., Devi, T., Bono, A. and Sarbatly, R. 2009.Studies on phytochemical constituents of six Malaysian medicinal plants. *Journal of Medicinal Plants Research,* 3 (2): 67-72.
28. Kuda, T., Tsunekawa, M. and Goto, H. 2005.Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan.*Journal of Food Composition and Analysis,* 18: 625-633.
29. Lahaye, M. and Kaffer, B. 1997. Seaweed dietary fibre's structure physiochemical and biological properties relevant to intestinal physiology. *Science Ailments,* 17: 563 - 564.
30. MacArtain, P., Christopher, I. R., Gill Brooks, M., Campbell, R. and Rowland, I. R. 2007. Nutritional value of edible seaweeds**.***Nutrition Reviews*, 65 (12): 535-543.
31. Manilal, A., Sujith, S., Sabarathnam, B., Kiran, G. S., Selvin, J., Shakir, C. and Lipton, A. P. 2010. Bioactivity of the red algae, *Asparagopsis taxiformis* collected from the South-Western Coast of India. *Brazilian Journal of Oceanography*, 58 (2): 93 - 100.
32. Manivannan, K., Thirumaran, G., Karthikai Devi, G., Hemalatha, A. and Anantharaman, P. 2008.Biochemical composition of seaweeds from Mandapam coastal regions along Southeast Coast of India.*American-Eurasian Journal of Botany*, 1 (2): 32-37.
33. Munirasu, S., Ramasubramanian, V., Uthayakumar, V. and Muthukumar, S. 2013. Bioenrichment of live feed *Daphnia magna* for the survival and growth of freshwater fish *Catla catla*. *International Journal of Current Research Review*, 5 (8): 20-31.
34. Muthuraman, B. and Ranganathan, R. 2004. Biochemical studies on some green algae of Kanyakumari coast. Seaweed Research and Utilization, 26 (1, 2): 69 - 71.
35. Nabil, S. and Cosson, J. 1996. Seasonal variations in sterol composition of *Delesseria sanguinea* (Ceremiales: Rohodophyta). *Hydrobiologia*, 326/327: 511- 514.
36. O'Sullivan, L., Murphy, B., McLoughlin, P., Patrick, D., Peadar, G. Lawlor, Helen, H. and Gardiner, G. E. 2010.Prebiotics from marine macroalgae for human and animal health applications.*Marine Drugs*, 8: 2038 - 2064.
37. Pietra, F. 1997. Secondary metabolites from marine microorganisms; bacteria, protozoa, algae and fungi: Achievements and perspective. *Natural Product Reports,* 14 (5) 453-464.
38. 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.*International Journal of Pharmaceutical Sciences and Research*, 4 (1): 306-310.
39. Prasad, A. K., Kumar, S., Iyer, S. V., Sudani, J. R. and Vaidya, S. K. 2012. Pharmacognostical, phytochemical and pharmacological review on *Bryophyllum pinnata.International Journal of Pharmaceutical and Biological Archives,* 3 (3): 423-433.
40. Premalatha, M., Dhasarathan, P. and Theriappan, P. 2011. Phytochemical characterization and antimicrobial efficiency of seaweed samples, *Ulva fasciata* and *Chaetomorpha antennina.International Journal of Pharmacology and Bio Sciences*, 2 (1): 288-293.
41. Quasim, R. 1986. Studies on fatty acid composition of eighteen species of seaweeds from the Karachi coast. *Journal of Chemical Society of Pakistan,* 8: 223 - 230.
42. Rajasulochana, P., Dhamotharan, R. and Krishnamoorthy, P. 2009. Primary phytochemical analysis of *Kappaphycus* sp. *Journal of American Sciences*, 5 (2): 91 - 96.
43. Rajesh, S., Asha, A., Kombiah, P. and Sahayaraj, K. 2011.Biocidal activity of algal seaweeds on insect pest and fungal plant pathogen. In: Proceedings of the National seminar on Harmful/beneficial insects of agricultural importance with special reference to the nuisance pest *Luprops tristis* in rubber plantations, 86-91 PP.
44. Rao, C. B. and Pullaiah, K. C. H. 1982. Chemical examination of marine algae of Visakhapatnam coast: Part II constituents of *Padina tetrastraomatica* Hauck. *Indian Journal of Chemical Sciences*, 21: 605 - 606.
45. Rebecca, J., Dhanalakshmi, L., Sharmila, V. and Merina Paul Das, S. 2013.*In vitro* antimicrobial activity of *Gracilaria* sp and *Enteromorpha* sp. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 4 (1): 693-697.
46. RenukaBai, N., 2010. Evaluation of *Gracilaria fergusonii* for phytochemical analysis and antibacterial activity. *Plant Archives*, 10 (2): 711 - 713.
47. Rouxel, C., Bonnabeze, E., Daniel, A., Jerome, M., Etienne, M. and Fleurence, J. 2001. Identification by SDS PAGE of green seaweeds (*Ulva* and *Enteromorpha*) used in the food industry. *Journal of Applied Phycology*, 13: 215-218.
48. Sabour, B., Reani, A., Magouri, M. E. L. and Haroun, R. 2013. *Sargassum muticum* (Yendo) Fensholt (Fucales, Phaeophyta) in Morocco, an invasive marine species new to the Atlantic Coast of Africa.*Aquatic Invasions*, 8: 1-6.
49. Sahayaraj, K. 2008. Common plant oils in agriculture and storage pests' management. *Green Farming*, 1 (2): 48–49.

50. Sahayaraj, K. and Kalidas, S. 2011. Evaluation of nymphicidal and ovicidal effect of seaweed, *Padina pavonica* (Linn.)(Pheophyceae) on cotton pest, *Dysdercus cingulatus* (Fab.).*Indian Journal of Geo Marine Sciences*, 40 (1): 125-129.
51. Saz, A. K., Watson, S., Brown, S. R. and Lowery, D. L. 1963.Antimicrobial activity of marine waters.Macromolecular nature of anti staphylococcal factor. *Limnolitical Oceanography,* 8: 63-67.
52. Seenivasan, R., Indu, H., Archana, I. G. and Geetha, S. 2010. The antibacterial activity of some marine algae from south east coast of India.*American-Eurasian Journal of Agricultural and Environmental Sciences*, 9 (5): 480-489.
53. Srivastava, N. K., Saurav, V., Mohanasrinivasan, Kannabiran, K. and Singh, M. 2010. Antibacterial potential of macroalgae collected from the Madappam Coast, India. *British Journal of Pharmacology and Toxicology*, 1 (2): 72 - 76.
54. Sultana, V., Ehteshamul-Haque, S., Ara, J. and Athar, M. 2005. Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra.*International Journal of Environmental Science and Technology*, 2: 129-132.
55. Sumathi, S. and Krishnaveni, M. 2012. Preliminary screening, antioxidant and antimicrobial potential of *Chaetomorpha antennina* and *Caulerpa scalpelliformis in vitro* study. *International Journal of environmental sciences*, 2 (3): 2312-2320.
56. Tasende, M. G. 2000. Fatty acids and sterol composition of gametophytes and saprophytes of *Chondrus crispus*.*Scientia Marina*, 64 (4): 421 - 426.
57. Thirumaran, G., Arumugam, M., Arumugam, R. and Anantharaman, P. 2009. Effect of seaweed liquid fertilizer on growth and pigment concentration of *Cyamopsis tetrogonolaba* (L.)Taub.*American-Eurasian Journal of Agronomy*, 2 (2): 50-56.
58. Tunaz, H. and Uygun, N. 2004. Insect growth regulators for insect pest control. *Turkey Journal of Agriculture*, 28: 377-387.
59. Varghese, K. J., Sukhumaran, T. P. M., Asokan, M. A. and Syama, H. S. 2010. Phytochemical investigation of seaweeds *Ulva reticulate* from the coast of Bakel, Kasaragod of Kerala State in India. *International Journal of Pharmacology World Research*, 1 (2): 1-11.
60. Viron, C., Saunois, A., Andre, P. Perly, B. and Lafosse, M. 2000. Isolation and identification of unsaturated fattyacid methyl esters from marine micro-algae.*Analytical Chemical Acta*, 409: 257-266.
61. Watson, S. B. and Cruz Rivera, E. 2003. Algal chemical ecology: an introduction to the special issue. *Phycologia,* 42: 319-323.