Journal of Marine Science and Technology

Volume 12 | Issue 1 Article 1

# Biopotentials of Ulva Fasciata and Hypnea Musciformis Collected from the Peninsular Coast of India

Joseph Selvin
*Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakavilai-629153, Kanyakumari District, India.*, selvinj@rediffmail.com

Aaron Premnath Lipton
*Marine Biotechnology Laboratory, Central Marine Fisheries Research Institute, Vizhinjam-695 521, Thiruvananthapuram, India.*

Follow this and additional works at: https://jmstt.ntou.edu.tw/journal

 Part of the Marine Biology Commons

## Recommended Citation

Selvin, Joseph and Lipton, Aaron Premnath (2004) "Biopotentials of Ulva Fasciata and Hypnea Musciformis Collected from the Peninsular Coast of India," *Journal of Marine Science and Technology*: Vol. 12: Iss. 1, Article 1.
DOI: 10.51400/2709-6998.2214
Available at: https://jmstt.ntou.edu.tw/journal/vol12/iss1/1

This Research Article is brought to you for free and open access by Journal of Marine Science and Technology. It has been accepted for inclusion in Journal of Marine Science and Technology by an authorized editor of Journal of Marine Science and Technology.

# Biopotentials of Ulva Fasciata and Hypnea Musciformis Collected from the Peninsular Coast of India

## Acknowledgements

Authors are thankful to Dr. Mohan Joseph Modayil, Director and Dr. R. Paul Raj, Head, PNP Division, CMFRI, Kochi for the facilities and encouragement.

This research article is available in Journal of Marine Science and Technology: https://jmstt.ntou.edu.tw/journal/vol12/iss1/1

# BIOPOTENTIALS OF *ULVA FASCIATA* AND *HYPNEA MUSCIFORMIS* COLLECTED FROM THE PENINSULAR COAST OF INDIA

Joseph Selvin* and Aaron Premnath Lipton**

Key words: seaweeds, secondary metabolite, *Ulva fasciata*, *Hypnea musciformis*, antibacterial, brine shrimp cytotoxicity, larvicidal, antifouling, ichthyotoxic.

## ABSTRACT

The secondary metabolites of seaweeds *Ulva fasciata* and *Hypnea musciformis*, collected form southeast and southwest coast of India, were tested for biotoxicity potential. Both species showed potent activity in antibacterial, brine shrimp cytotoxicity, larvicidal, antifouling and ichthyotoxicity assays. The green alga *U. fasciata* exhibited broad-spectrum antibacterial activity whereas the red alga *H. musciformis* showed narrow spectrum antibacterial activity. The brine shrimp cytotoxicity profile indicated that the seaweeds were moderately toxic. The overall activity profile indicated that *U. fasciata* contained more biological potency than *H. musciformis*.

## INTRODUCTION

Marine algae are not only the primary and major producers of organic matter in the sea, but they also exert profound effects on the density and distribution of other inhabitants of the marine environment. An understanding of the wide range of behavioural relationships that exist among organisms would provide us with clues to substances of biomedical interest. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds, and other marine organisms. The host organism biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment. Some of these secondary metabolites offer avenues for developing cost-effective, safe and potent drugs. Nearly 50 lakhs (50,000,00) species available in the sea are virtually untapped sources of secondary metabolites. Those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation (Elena *et al*., 2001; Kim *et al*., 1997; Okai *et al*., 1997; Premila *et al*., 1996) apart from their potential ecological/industrial significances such as controlling reproduction, settlement/biofouling and feeding deterrents (Selvin, 2002). In this background, the present study intended to evaluate the toxic influence, activity range and potency of seaweeds collected from the peninsular coast of India.

*Paper Submitted 09/22/03, Accepted 11/24/03. Author for Correspondence: Joseph Selvin. E-mail: selvinj@rediffmail.com.*

**Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakavilai-629153, Kanyakumari District, India.*

***Marine Biotechnology Laboratory, Central Marine Fisheries Research Institute, Vizhinjam-695 521, Thiruvananthapuram, India.*

## MATERIALS AND METHODS

The algae, which inhabited exclusively on the intertidal rocky substratum, were selected for the collection in order to avoid other algal contamination (Selvin, 2002). Predominant algae such as *Ulva fasciata* and *Hypnea musciformis* were collected along the southeast and southwest of India. Immediately after collection, they were washed in fresh seawater to remove the epiphytes, sand and other extraneous matter and air-dried under shade. Completely dried material was weighed and ground finely in a mechanical grinder. The secondary metabolites of seaweeds were extracted in bulk extraction process. In this process, 500 g of finely powdered algal material was refluxed in a 5 L capacity round bottom flask using methanol-dichloromethane (1:1). The extract was filtered and concentrated to recover the excess solvents in another distillation system. Finally it was reduced to thick oily natured crude extract in a rotary vacuum evaporator (Buchi) at 40°C.

Modified cylinder plate double layer method was used for the evaluation of antibacterial potential of seaweeds. The following bacterial strains were used as test organism (i) Seven type cultures (obtained from Microbial Type Culture Collections, Chandigargh, India): *Micrococcus luteus* (ML), *Bacillus cereus* (BC), *Bacillus subtilis* (BS), *Escherichia coli* (EC), *Aeromonas hydrophila* (AH), *Pseudomonas aeruginosa* (PA), and *Vibrio fischeri* (VF), (ii) two shrimp isolates: *Vibrio alginolyticus* (QS7) and *Vibrio harveyi* (RJM5) (iii) two fish isolates: *Aeromonas* sp. (CF-1) and *Aeromonas* sp.

(CF-2). The inoculum was prepared by adding 5 ml of sterile nutrient broth onto the 18 h fresh slant culture of appropriate bacterium. The tube was gently vortexed and the suspension was collected in a sterile test tube. The first base layer was prepared with 10 mL of 1.5% agar. Six numbers of sterile porcelain beads of 7 mm diameter was placed on the base layer. The overlaid seed layer was prepared by pouring 15 mL of hot nutrient agar containing 0.2 ml of prepared inoculum (USP 1995; IP 1996; HiMedia Manual, 1998). After the seed layer solidified, the porcelain beads were removed carefully with a sterile forceps. The resultant wells were filled with 100 µL of the appropriate algal extract and vehicle control (VC). After 24 h of incubation at 37 ± 2°C the area of inhibition zone was determined as average of triplicates.

Brine shrimp cytotoxicity assay was performed using the freshly hatched free-swimming nauplii of *Artemia salina*. The assay system was prepared with 2 mL of filtered seawater containing chosen concentration of extract in cavity blocks (embryo cup) and 20 nauplii each was transferred in experimental, vehicle control and negative control wells. Invariably the concentration of the experimental systems was determined on the basis of exploratory experiments. Based on the percent mortality, the $LD_{50}$ of the test compound was determined using probit scale (Miller and Tainter, 1944). The susceptibility or resistance of the mosquito larvae (*Culex* sp.) to the selected concentration of the extracts was carried out by adopting standard bioassay protocol (WHO, 1981). Observations were made after 24 h of treatment for larvicidal activity. The anticoagulant activity was studied using whole blood clotting time method (Selvin, 2002). Fingerlings (1.5-2.0 cm) of marine acclimated *Oreochromis mossambicus* were used for evaluating the ichthyotoxic potential. Five fingerlings each were introduced in experimental and control glass bowls containing 1,000 mL seawater and chosen concentrations of extract. Immediate reflex changes and mortality were observed continuously for six hours at 1 h interval for the next 12 h. After 24 h of exposure, the number dead and live fish were counted. The acute toxicological reflexes were observed and recorded (Indap and Pathare, 1998). Antifouling activity of secondary metabolites was evaluated using newly developed 'mollusc foot adherence bioassay' (Selvin and Lipton, 2002). One of the common rock fouler *Patella vulgata* was used as test organism.

## RESULTS AND DISCUSSION

The green alga *Ulva fasciata* exhibited broad spectrum of antibacterial activity and its extract inhibited all the tested bacteria in different activity ranges. The red alga *Hypnea musciformis* showed narrow spectrum of activity as it successfully prevented the growth of gram positive bacteria more significantly than the gram negative strains (Table 1). *H. musciformis* extract inhibited the growth of gram positive strains to the extent of 66.0% at 30°C, whereas all the gram positive bacteria were susceptible at 20°C. However, the inhibitory potential was feeble towards the gram-negative strains and only 50% and 75% at 30 and 20°C, respectively, were inhibited. Based on the inhibition area, the activity range was classified as less active, moderately active, active, highly active and resistant (Selvin and Lipton, 2004).

The secondary metabolites of *U. fasciata* successfully inhibited the growth of *B. cereus* and *E. coli* to the extent of 132.66 $mm^2$ and 112.56 $mm^2$ respectively at 20°C. It was active (94.98 $mm^2$) against *M. luteus*, *A. hydrophila* and *P. aeruginosa* at 20°C (Fig. 1). The range was extended against *B. cereus* (78.5 $mm^2$), *E. coli* (98.06 $mm^2$) at 30°C and a clown fish isolate, CF1 (63.58 and 94.98 $mm^2$) at 30 and 20°C respectively. A gram-positive bacterium, *B. subtilis* and a secondary pathogenic shrimp isolate, *V. alginolyticus* (QS7) were found to be moderately sensitive to *U. fasciata* (50.24 and 38.46 $mm^2$ and 38.46 and 50.24 $mm^2$ inhibition area respectively at 30 and 20°C). The same range of inhibition (50.24 $mm^2$) was observed in the case of *A. hydrophila* and *V. fischeri* at 30°C whereas opportunistic shrimp pathogen, *V. harveyi* (RJM5) exhibited 38.46 $mm^2$ inhibition at 20°C. The activity range was reduced to less than 30 $mm^2$ inhibition area against *M. luteus* (28.26 $mm^2$) and RJM5 (28.26 $mm^2$) at 30°C, *V. fischeri* (28.3 $mm^2$) at 20°C and a clown fish isolate, CF2 (12.86 and 19.26 $mm^2$) at both the incubation temperatures.

The secondary metabolites of *H. musciformis*

**Table 1. Antibacterial activity of algal extracts**

| Source organism of algal extracts | % antibacterial activity | | | | | |
|---|---|---|---|---|---|---|
| | Total (%) | | Gram positive (%) | | Gram negative (%) | |
| | 30°C | 20°C | 30°C | 20°C | 30°C | 20°C |
| *Ulva fasciata* | 100 | 100 | 100 | 100 | 100 | 100 |
| *Hypnea musciformis* | 54.0 | 50 | 66.0 | 100 | 50 | 75 |

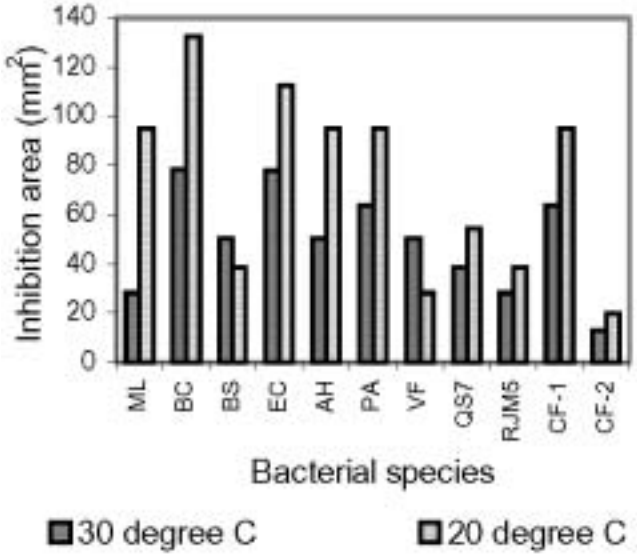


**Fig. 1. Antibacterial activity of *Ulva fasciata*.**

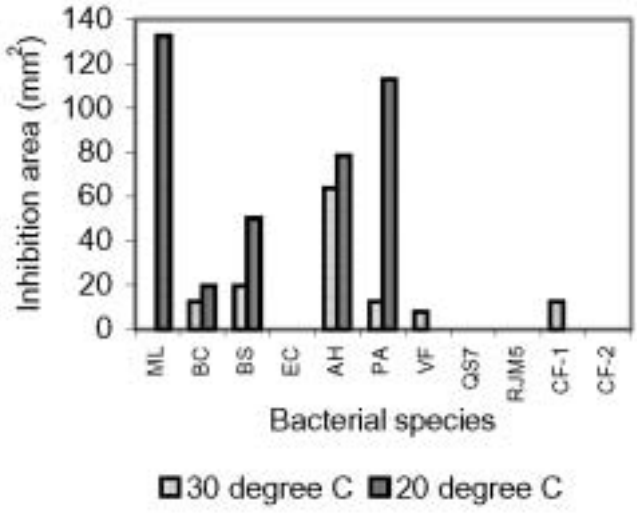


**Fig. 2. Antibacterial activity of *Hypenea musciformis*.**

showed antibacterial activity to the extent of 132.60 and 113.04 mm$^2$ respectively at 20°C against *M. luteus* and *P. aeruginosa* (Fig. 2). However, *M. luteus* was totally resistant at 30°C. It successfully prevented the growth of *A. hydrophila* at a rate of 63.6 and 78.50 mm$^2$ in both the temperatures. In terms of activity range, it was active against *B. subtilis* (50.24 mm$^2$) at 20°C. However, at 30°C, it exhibited less activity (19.62 mm$^2$). Such less activity was also extended against *B. cereus* at both temperatures and *V. fischeri* and CF1 at 30°C. The strains such as *E. coli*, QS7, RJM5 and CF2 showed total resistance to *H. musciformis* extract at both temperatures followed by *V. fischeri* and CF1 at 20°C. The

**Table 2. *Artemia* cytotoxicity profile of algal extracts at 30 and 20°C**

| Algal extracts | Concentration | Mortality (%) | |
|---|---|---|---|
| | (mg/mL) | 30 ± 2°C | 20 ± 2°C |
| *U. fasciata* | 1 | 10.2 ± 2.6 | 0 |
| | 2 | 50.0 ± 7.0 | 20.0 ± 3.4 |
| | 4 | 91.66 ± 1.26 | 40.0 ± 5.6 |
| | 6 | 97.5 ± 4.8 | 60.0 ± 7.37 |
| *H. musciformis* | 1 | 20.2 ± 3.6 | 0 |
| | 2 | 60.0 ± 7.0 | 0 |
| | 4 | 90.6 ± 3.2 | 20.0 ± 1.26 |
| | 6 | 100 ± 0.0 | 40.0 ± 2.19 |

Mean ± SD n=10

results clearly indicated the incubation temperature considerably influenced the antibiosis of algal extracts. In certain cases the activity was significantly increased at 20°C. The slow growth rate of bacteria at 20°C may be the reason for the increased activity of algal extracts at low temperature. Antibiosis of algal extracts revealed that gram positive bacterial strains were more susceptible. It was reported that the gram positive strains were more susceptible to seaweed extracts than gram negative bacterial strains (Rao and Parekh, 1981; Pesando and Caram, 1984; Rosett and Srivastava, 1987).

Invariably, seaweeds have been proven to be potent source of antimicrobial compounds. This potential have also been proved in 'in captivity' control of shrimp bacterial pathogens (Selvin, 2002). Ethanolic extract of 8 species of seaweeds belonging to the groups of Chlorophyta, Pheophyta and Rhodophyta exhibited broad-spectrum antibacterial and antifungal activities (de-Campos *et al*., 1998). The algal extracts such as *Enteropmorpha compressa, Cladophorosis zoolingeri, Padina gymnospora, Sargassum wightii* and *Gracilaria corticata* were active against gram positive and gram negative bacteria (Rao *et al*., 1991). Based on the present findings, it could be inferred that the bioassay guided fractionation and purification may come up with potent antibacterial compounds.

Based on the brineshrimp lethality potential, the medium lethal dose (1.8 mg/mL) extrapolated from the graph indicated that *U. fasciata* extract was moderately toxic. The $LC_{50}$ value of *H. musciformis* extract was 4.2 mg/mL. Temperature had significant influence on the toxicity of algal extracts. The toxicity profile of algal extracts considerably decreased at 20 ± 2°C (Table 2). At this temperature, the toxicity of *U. fasciata* extract was reduced to 60% mortality at 6 mg/mL while the same concentration produced 97.5% mortality at 30 ± 2°C. Schmitz *et al*. (1993) reported the compounds

**Table 3. Larvicidal profile of algal extracts on second and fourth instar larvae of *Culex* sp.**

| Algal extracts | Concentration (mg/mL) | Mortality (%) | |
|---|---|---|---|
| | | Second instar larvae | Fourth instar larvae |
| *U. fasciata* | 10 | 100 ± 0.0 | 20.2 ± 1.72 |
| | 6 | 60.2 ± 2.6 | 0 |
| *H. musciformis* | 10 | 100 ± 0.0 | 10.2 ± 2.71 |
| | 6 | 61.2 ± 3.0 | 0 |

Mean ± SD n = 10

**Table 5. General behavioural changes observed in *Oreochromis mossambicus* exposed to ichthyotoxic algal extracts**

| Stages | Behavioural changes |
|---|---|
| State I: | a) Increased in ventilatory frequency |
| Initial signs | b) Erratic/rapid movements |
| Stage II: | a) Inclined towards one side |
| Secondary signs | b) Loss of swimming activity |
| State III: | a) Rapid surface respiration |
| Advanced signs | b) Inclined to bottom |
| | c) Start of sporadic uncontrollable swimming with non directional bursts |

**Table 4. Ichthyotoxicity profile of algal extracts to *Oreochromis mossambicus* fingerlings**

| Species | Concentration (mg/mL) | Mortality (%) | Time of death (h) |
|---|---|---|---|
| *U. fasciata* | 8 | 100 | 2 |
| | 4 | 60.4 ± 3.0 | 6 |
| | 2 | 0 | - |
| *H. musciformis* | 4 | 100 | 6 |
| | 2 | 20.0 ± 2.89 | 6 |
| | 1 | 0 | - |

Mean ± SD n = 10

that showed activity in the brineshrimp could be correlated some extent to their potent cytotoxicity. Therefore the secondary metabolites of *U. fasciata* could be used for the isolation of cytotoxic fractions. Literature also evidenced the anticancer potentials of secondary metabolites isolated from seaweeds (Harada *et al*., 1997; Prabhadevi *et al*., 1998; Elena *et al*., 2001).

The results of larvicidal activity indicated that the second instar larvae were more susceptible than the fourth instar larvae (Table 3). Similar observations were reported in the earlier works by Purohit *et al*., (1983) and Krishnamoorthy (1996). The seaweeds (*U. fasciata* and *H. musciformis*) produced 100% mortality at 10 mg/mL. The activity profile considerably deviated among the different larval stages. Notably the fourth instar larvae were resistant at the concentration that produced 100% mortality for the second instar larvae exposed to *U. fasciata* and *H. musciformis*. The extracts might possess larvicidal and growth regulatory activities when the treatment was effected to different larval stages (Rao *et al*., 1991). The acetone extract of seaweeds *C. scalpelliformis* and *Dictyota dichotoma* exhibited mosquito larvicidal activity against *Aedes aegypti* (Thangam and Kathiresan, 1991). Albeit the larvicidal potential of seaweeds was well established, no evidence was available on the field trials of seaweed-based mosquito repellents.

The secondary metabolites of *U. fasciata* effectively prevented the blood clotting to the extent of 120 seconds while the control blood clotted within 40 seconds. The secondary metabolites of *H. musciformis* and Methanol (VC) did not significantly influence the clotting time. It was reported that seaweeds are excellent source of anticoagulants (Lara-Isassi and Alvarez, 1995). Sulfated xylogalactam isolated from a semi-perennial red alga *Delesseria sanguinea* and fucoidaw from the brown algae *Fucus vesiculosus* and *Pelvetia canaliculata* showed significant anticoagulant activity (Nabil and Cosson, 1996; Nishino *et al*., 1995; Collinec *et al*., 1994). The secondary metabolites of *U. fasciata* were proven to be a potent immunomodulatory agent (Selvin *et al*., 2004).

Antifeedant activity (ichthyotoxicity profile) of algal extracts is presented in Table 4. *U. fasciata* was toxic at 4 mg/mL while 2 mg/mL was less toxic and did not influence mortality within 6 h. *H. musciformis* extract was less toxic as 20.0% mortality was recorded only at 2 mg/mL. Both the algal extracts exhibited more or less same sort of behavioural changes (Table 5). Initially the fishes exhibited erratic movements and then inclined towards one side. Later, they rapidly went for surface respiration followed by settling at bottom or rapid swimming activity with non-directional bursts, which culminated in dwelling at bottom and mortality (Indap and Pathare, 1998). The present findings suggested that the seaweeds might possess secondary me-

tabolites to ward off predators. However in the 'mollusc foot adherence assay', it was found that the seaweeds were not molluscicidal. Both species prevented the foot adherence of limpets to extent of 70% at 10 mg/mL. Literature indicated that the antifouling property of seaweeds were associated with epiphytic microorganisms (Boyd *et al*., 1998). Based on the present findings it could be inferred that bioassay guided fractionation and purification of *U. fasciata* and *H. musciformis* may give forth potent bioactive compounds.

## ACKNOWLEDGEMENTS

Authors are thankful to Dr. Mohan Joseph Modayil, Director and Dr. R. Paul Raj, Head, PNP Division, CMFRI, Kochi for the facilities and encouragement.

## REFERENCES

1. Boyd, K.G., Mearns-Spragg, A., Brindley, G., Hatzidimitriou, K., Rennie, A., Bregu, M., Hubble, M.O., and Burgese, J.G., "Antifouling Potential of Epiphytic Marine Bacteria from the Surfaces of Marine Algae," *Proc. of the Meeting on Marine Microorganism for Industry*, Brest, Vol. 21, pp. 128-186 (1998).
2. Collinec, S., Boisson, V.C., and Jozefonvicz, J., "A Low Molecular Weight Fucoidan Fraction from the Brown Seaweed *Pelvetia canaliculata*," *Phytochemistry*, Vol. 35, No. 3, pp. 697-700 (1994).
3. de-Campos, T.G.M., Diu, M.B.S., Koening, M.L., and Periera, E.C., "Screening of Marine Algae from Brazilian Northeastern Coast for Antimicrobial Activity," *Bot. Mar.*, Vol. 31, No. 5, pp. 375-377 (1998).
4. Elena, M., Francisco, Y., and Erickson, K.L., "Mailiohydrin, a Cytotoxic Chamigrene Dibromohydrin from a Phillippine *Laurencia* Species," *J. Nat. Prod.*, Vol. 64, No. 6, pp. 790-791 (2001).
5. Harada, H., Noro, T., and Kamei, Y., "Selective Antitumour Activity *in vitro* from Marine Algae from Japan Coasts," *Boil. Pharm. Bull*., Vol. 20, No. 5, 541-546 (1997).
6. HiMedia Manual, *HiMedia Manual for Microbiology Laboratory Practice*. HiMedia Laboratories Pvt. Limited, Mumbai. pp. 524 (1998).
7. Indap, M.M. and Pathare, S.P., "Cytotoxicity and Bioactivity of Some Marine Animals," *Ind. J. Mar. Sci.*, Vol. 27, pp. 433-437 (1998).
8. IP (Indian Pharmacopoeia), *Microbiological Assays and Tests*, Appendix 9, Ministry of Health and Family welfare, Govt. of India, New Delhi (1996).
9. Kim, J.H., Hudson, J.B., Huang, A.M., Bannistes, K., Jin, H., Choi, T.J., Towers, G.H.N., Hong, Y.K., and DeWreede, R.E., "Biological Activities of Seaweed Extracts from British Columbia, Canada, and Korea. I. Antiviral Activity," *Can. J. Bot. Rev. Can. Bot.*, Vol. 75, No. 10, pp. 1656-1660 (1997).
10. Krishnamoorthy, N., "Mosquito Control Through Neem Based Insecticides," *Fourth Seminar cum Exhibition on Student Project Scheme*, Madurai, India, (1996).
11. Lara-Isassi, G. and Alvarez, H.S., "Anticoagulant Properties of Mexican Marine Algal Extracts: Heparin-like Potency of *Halimeda discoidea* (Chlorophyta) Extract," *Cryptogame: Algol*., Vol. 16, No. 3, pp. 199-205 (1995).
12. Miller, L.C. and Tainter, M.L., "Estimation of the ED50 and Its Error by Means of Logarithmic-probit Graph Paper," *Proc. Soc. Exp. Bio. Med*., Vol. 57, pp. 261-264 (1944).
13. Nabil, S. and Cosson, J., "Seasonal Variations in Sterol Composition of *Delesseria sanguinea* (Ceremiales, Rohodophyta)," *Hydrobiologia*, Vol. 326/327, pp. 511-514 (1996).
14. Nishino, T., Ura, H., and Nagumo, T., "The Relationship Between the Sulfate Content and the Antithrombin Activity of an Alpha (1 arrow right 2)-fucoidan Purified from a Commercial Fucoidan Fraction," *Bot. Mar.*, Vol. 38, No. 3, pp. 187-193 (1995).
15. Okai, Y., Higashi, O.K., Ishizaka, S., and Yamashita, U., "Enhancing Effect of Polysaccharide from an Edible Brown Alga, *Hijikia fusiforme* (Hijiki), on Release of Tumour Necrosis Factor Alpha from Macrophages of Endotoxin Nonresponder C3H/HeJ Mice," *Nutr. Cancer*, Vol. 27, No. 1, pp. 74-79 (1997).
16. Pesando, D. and Caram, B., "Screening of Marine Algae from the French South Eastern Mediterranean Coast for Antibacterial and Antifungal Activities," *Bot. Mar.*, Vol. 27, pp. 381-386 (1984).
17. Prabhadevi, Solimabi, W., Desouza, L., and Kamat, S.Y. "Larvicidal activity of some marine macrophytes against *Artemia salina*," *Adv. Biosci.*, Vol. 17, No. 2, pp. 75-84 (1998).
18. Premila, J.C., Raviraja, N.S., and Sridhar, K.R., "Antimicrobial Activity of Some Marine Algae of Southwest Coast of India," *Indian J. Mar. Sci*., Vol. 26, No. 2, pp. 201-205 (1996).
19. Purohit, P.M., Mutafa, N.C., and Osmani, Z., "Insecticidal Properties of Plant Extract of *Cuminum cyminum*," *Lim. Sci. Cul.*, Vol. 4, pp. 101-103 (1983).
20. Rao, O.S., Girijavallaban, K.G., Muthusamy, S., Chandrika, V., Gopinathan, C.P., Kalimuthu, S., and Najmuddin, M., "Bioactivity in Marine Algae. In: Bioactive Compounds from Marine Organisms with Emphasis on the Indian Ocean," *Proc. of an Indo-United Sates Symposium*, pp. 373-377 (1991).
21. Rao, P.S. and Parekh, K.S., "Antibacterial Activity of Indian Seaweed Extracts," *Bot. Mar*., Vol. 24, pp. 577-582 (1981).
22. Rosett, R.G. and Srivastava, L.M., "Fatty Acids as An-

timicrobial Substances in Brown Algae," *Hydrobiologia*, Vol. 151/152, pp. 47-475 (1987).
23. Schmitz, F.J., Bowden, B.F., and Toth, S.I., *Marine Biotechnology*, *Vol. I., Pharmaceutical and Bioactive Natural Products*, Plenum Press, New York, Vol. 1, pp. 197-308 (1993).
24. Selvin, J., "Shrimp Disease Management Using Secondary Metabolites Isolated from Marine Organisms," Ph.D. Dissertation, Manonmaniam Sundaranar University, Tirunelveli, India, p. 204 (2002).
25. Selvin, J. and Lipton, A.P., "Biopotentials of Secondary Metabolites Isolated from Marine Sponges" *Hydrobiologia*, Vol. 513, 231-238 (2004).
26. Selvin, J. and Lipton, A.P., "Development of a Rapid 'Mollusc Foot Adherence Bioassay' for Detecting Potent Antifouling Bioactive Compounds," *Curr. Sci.*, Vol. 83 pp. 735-737 (2002).
27. Selvin, J., Huxley, A.J., and Lipton, A.P., "Immunomodulatory Potential of Marine Secondary Metabolites Against Bacterial Diseases of Shrimp," *Aquaculture*, Vol. 230, pp. 241-248 (2004).
28. Thangam, T.S. and Kathiresan, K., "Mosquito Larvicidal Activity of Marine Plant Extracts with Synthetic Insecticides," *Bot. Mar.*, Vol. 34, No. 6, pp. 537-539 (1991).
29. USP (United States Pharmacopoeia/National Formulary), (USP 23/NF 18), US Pharmacopoeia Convention, Inc., Rockville, MD (1995).
30. WHO, *Instructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticides*, WHO/VBC/81-807, rm100 (1981).