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Bioactive Compounds From Spirastrella Inconstans Against Wound Infection

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Abstract

Marine invertebrates, in particular sponge (*Spirastrella inconstans*), represent a source of a wide range of secondary metabolites, many of which have been attributed to various defensive capabilities against CA-MRSA (Community associated- Methicillin Resistant *Staphylococcus aureus*) obtained from wound infection among fisherman community. In this work sponge-derived low-molecular peptide-like compounds and associated analogs are investigated for bioactivity and pharmacological targets. 3,000 Da molecular weight of protein was isolated by SDS-PAGE. Amount of protein from *Spirastrella inconstans* was estimated by UV-Vis spectrometer were analysed by IR and UV spectrum. Absorption value obtained by UV spectrum using TECHCOMP 8500 spectrophotometer was 1.7141. Further confirmation of isolated compound was carried out by HPLC analysis as 254 nm wave length and ^{13}C and ^{1}H NMR. Scanning Electron Microscope (SEM) with constant voltage of 20 kV at X 850 illumination shows 20 µm between the compounds, at X 270 illumination 50 µm, at 2,300 X illumination 10 µm, at 6,000 X illumination 2 µm, at 2,700 illumination 5 µm confirms the compound by structural elucidation as glutamic acid. The novel compound in lyophilized form can favor fibroblast proliferation with collagen production, in the therapy for the healing of tendon lesions and of wounds by acting against MRSA.

Introduction

The value of natural products can be assessed by, the rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification, the number of diseases treated or prevented by these substances, and their frequency of use in the treatment of disease. An analysis of the origin of the drugs developed between 1981 and 2002 showed that natural products or derived drugs comprised 28% of all new chemical entities (NCEs) launched onto the market (Newman *et al.,* 2003).

The increasing occurrence of infectious diseases is becoming a worldwide problem. Additionally, the resistance problem demands that renewed efforts be made to seek antimicrobial agent that are effective against pathogenic microorganisms resistant to current treatment. However, the number of reports on novel compounds from terrestrial sources has been decreasing rapidly. Marine organisms are an outstanding source of unusual, biologically active natural products, which may be considered as potential new drug leads useful for the treatment of human diseases (Ki-Bong *et al*., 2006).

Marine sponges are the source of many bioactive compounds with therapeutic potential. A critical issue in the drug development strategy for marine natural products is ensuring an adequate supply of compounds for clinical use while protecting the source organism and its habitat from over exploitation. One approach is the development of techniques for in vitro production of bioactive compounds (Pomponi *et al.*, 1996).

The search for new biomedicals from marine organisms resulted in the isolation of about 10,000 metabolites, which had pharmaco dynamic properties. A broad spectrum of biological activities has been detected, such as anti neoplastic, cytotoxic, neurotoxic, antibiotic, anti viral, anti fungal, anti mitotic and anti protozoal. Few drugs from marine sources such as the derivatives of the sponge nucleosides Ara-A (antiviral) and Ara-C (anti leukaemic) have been hitherto introduced into clinics and onto the pharmaceutical market. However, examples are known where advanced clinical or preclinical trials, carried out by using natural marine products, have led to promising results in the study of new medicines for the treatment of a variety of diseases (Ines Mancini *et al.*, 2007).

Over 12,000 new compounds had been isolated from marine sources. Of all marine organisms member of the phylum Porifera are the most productive source. In 2002 out of 677 new compounds 247 originated

from sponges. Despite the considerable amount of new bioactive metabolites that originated from marine sponges, very few compounds have reached advanced stages of clinical trials (Hadas *et al.,* 2005). Antibiotics from sponges includes plakortin and manoalide. In corals, algae, and some sponges, it is reasonable to assume that higher concentrations of bioactive compounds accumulate on their surfaces, thus providing greater defense against bacterial colonization. However, since sponges pass large volumes of water through their tissues, which contain potentially harmful bacteria, as well as encountering them on their external surfaces, it may be advantageous for sponges to distribute their antimicrobials throughout their tissues. Several studies provided evidence for the localization of natural products within the tissues of sponges. Investigations on the localization of antibiotics in tissues of sponges are therefore warranted (Dovi Kelman *et al*., 2009).

Sponges of the genus *Erylus* have attracted the attention of natural products chemists due to the different classes of bioactive compounds present in these organisms. While glycolipids, fatty acids, and a polyketide have been described (Mostafa Fouad *et al*., 2004). Protein components found in freeze-dried specimens of the coralline sponge *Spirtastrella* (Acanthochaetetes) *wellsi* were separated and characterized. Proteins extracted from skeleton crystals (matrix proteins) contained high concentration of glycin (16%) as well as enhanced amounts of asparagin/aspartic acid (11%) and glutamine/glutamic acid (10%). At least 10 proteins could be separated by SDS-PAGE. Six of them, with molecular weights between 30 and 45kDa, may be considered as distinct matrix proteins. The bulk of total soluble proteins as well as all soluble matrix proteins are acid with *p*H values below 5.0. Results indicate that at least crystal growth is matrix mediated i.e. controlled by the sponge (Matthias Bergbauer *et al*., 1996).

Glutamic acid or glutamate can be synthesized in the body from arginine, ornithine and proline and is found in high concentration in the brain. Glutamic acid (Glu in the zwitterionic form) is a potential legend with the characteristic of having two carboxylate groups. As glutamic acid is an amino acid characterized by the presence of amino group and two carboxylic groups, it was noticed that the stretching and bending bands of the quaternary nitrogen bands wave numbers were decreased reflecting electron donation from the NH_3 (Fouad *et al.,* 2009).

The study was mainly focused on the bioactive compound isolated from *Spirastrella inconstans* remains as the drug of choice for the wound infection among fisher man community. The glutamic acid was considered as the marine drug effective in curing Methicillin Resistant *Staphylococcus aureus* (MRSA), prevalent in chronic wound.

Materials and Methods

Isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA): Community associated MRSA isolates of about different strains were isolated and characterized using standard technique from wound infection of fisherman community. The antibiogram pattern of those isolates was studied using twenty four different antibiotics using Kirby Bauer method, 1966. The presence of *mecA* gene was confirmed using PCR.

Production, Purification and characterization of *Spirastrella inconstans* protein: *Spirastrella inconstans* was collected from Gulf of Mannar, Tamilnadu, India during the period of 2008-2009. The biomolecules were precipitated using different solvents, proteins were precipitated using Ammonium sulphate precipitation method and purified using dialysis. About 1000 µl was loaded in chromatographic column and 75 fractions were collected for 20% saturation level, similarly 40%, 60% and 80% were performed. The nature of the compound was studied using thin layer chromatography. Further purification was carried out using anion exchange and gel filtration chromatography.

Purification of solvent extract showing maximum antimicrobial activity: The entire *Spirastrella inconstans* and its stony substrate were grounded and soaked in 50% Chloroform (Solvent showing maximum activity) for 24 hrs. Centrifuged for 1 hrs at 140,000 Xg and the supernatant was loaded on to a Sephadex-G 50 (fine) column equilibrated with 1% acetic acid. The column was eluted at the rate of 0.7 ml/min for 12 hrs. Then, 15 ml fractions were collected, and the elution was monitored by absorbance measurement at 280 nm. All subsequent purification steps were performed with HPLC (Jean-Luc Morel *et al*., 1997).

Ultra Violet (UV) and Infra Red (IR) spectrum: UV Spectrum were recorded on a TECHCOMP 8500 spectrophotometer, under the following conditions. Sample dilution, 0.1 ml in 3 ml of blank. Water was used as blank. λ Max, 196. Scan range used were

from 190-1100 nm. IR Spectrum were recorded on a NICOLET 360 FT-IR spectrophotometer.

High Performance Liquid Chromatography (HPLC): The sample for HPLC analysis was effective fraction obtained as a result of chromatography. A isocratic HPLC (SHIMADZu SPD-10A) with Phenomenex column (4.6X250 mm) and reverse phase Gemini 5u (Luna) C18 column was used. The mobile phase components methanol:water (50:50). The solvent reservoir was pumped at a flow rate of 0.45 ml/min. 20 µl of *Spirastrella inconstans* fraction showing efficient activity were injected using syringe.

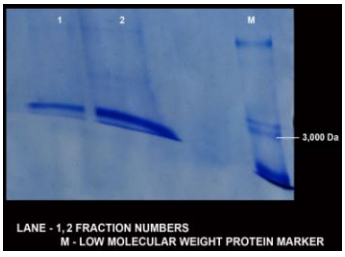
Nuclear Magnetic Resonance (NMR): ^{1}H and ^{13}C NMR experiments were performed on a Bruker advance DPX300 spectrometer. Chemical shift values (δ) were reported in parts per million (ppm) relative to appropriate internal solvent standard and coupling constants are given in hertz. Bruker (300 Mhz) NMR spectroscopy was used for analysis of the 12 culture supernatants. The sample were dissolved using deuterated chloroform ($CDCI_3$) as solvent **(**Asha Devi *et al.,* 2008).

Scanning Electron Microscope (SEM): Lyophylised form of purified fraction was observed through Scanning Electron Microscope with constant voltage of 20 kV at X 850 illumination shows 20 µm between the compounds.

Results

The fractions bearing anti MRSA activity were collected by column chromatography and the nature of compound was detected by TLC, whereas the molecular weight was detected by SDS-PAGE as 3,000 Da. Further purification was carried out by IR, UV and HPLC.

Figure 1 Separation of protein content in *Spirastrella inconstans* by SDS-PAGE



IR Spectrum were recorded on a NICOLET 360 FT-IR spectrophotometer (Figure: 2). Absorption value obtained by UV spectrum using TECHCOMP 8500 spectrophotometer was 1.7141 (Figure: 3). Wave length obtained by HPLC analysis was 254 nm (Figure: 4).

Figure 2 Infra spectrum of compound from *Spirastrella inconstans*

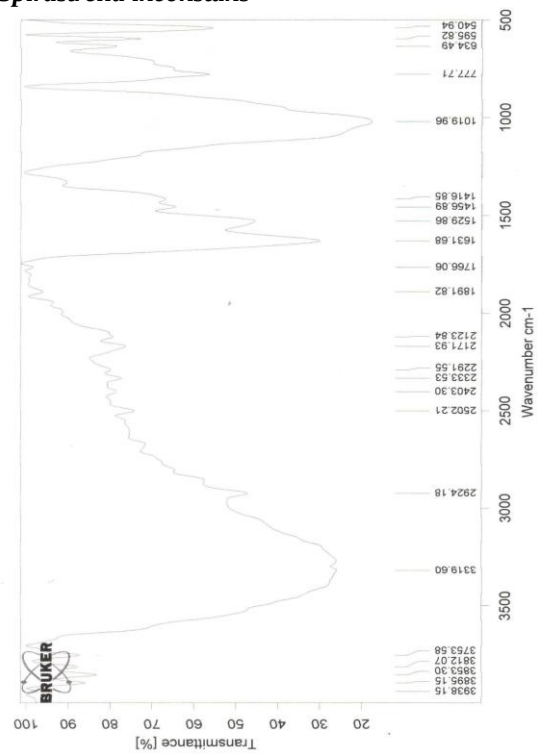


Figure 3 Ultra violet spectrum of compound from *Spirastrella inconstans*

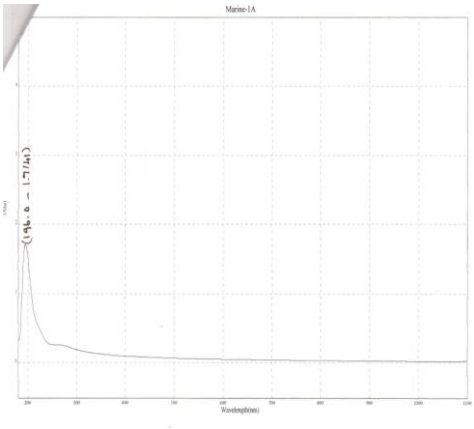
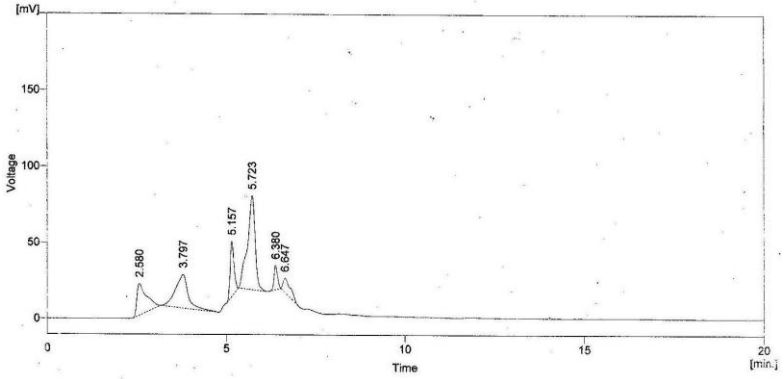


Table 1: Result showing HPLC analysis of compound from *Spirastrella inconstans*

Result Table (Uncal - E:\Asthagiri-HPLC-10A\Job Order\marine-JON 1)

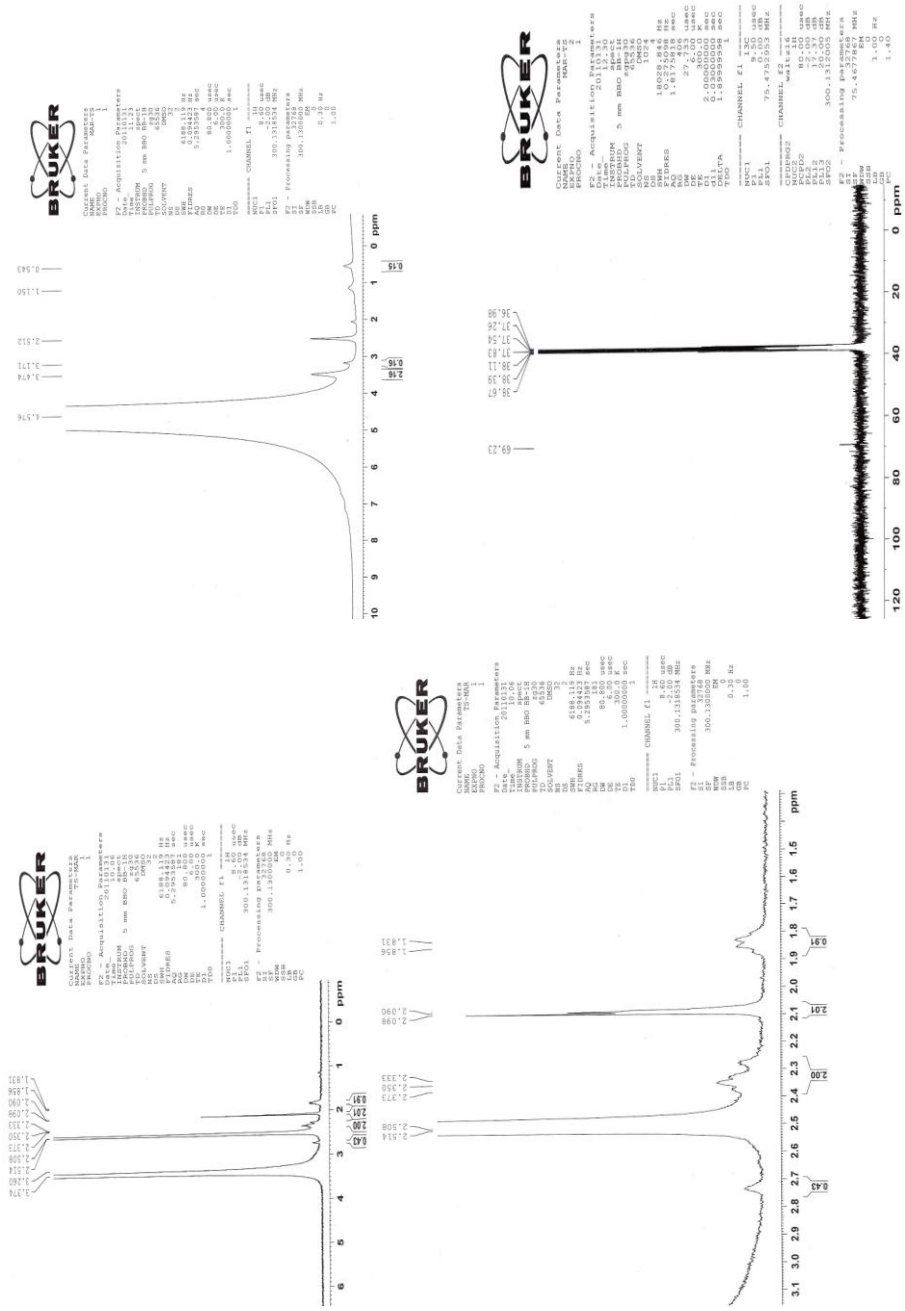
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.580	387.102	20.775	16.2	12.5	0.40
2	3.797	548.668	22.120	22.9	13.3	0.36
3	5.157	266.405	36.108	11.1	21.8	0.12
4	5.723	938.873	61.638	39.2	37.1	0.21
5	6.380	108.037	15.884	4.5	9.6	0.11
6	6.647	145.306	9.474	6.1	5.7	0.17
	Total	2394.391	165.999	100.0	100.0	

Figure 4 HPLC analysis of compound from *Spirastrella inconstans*



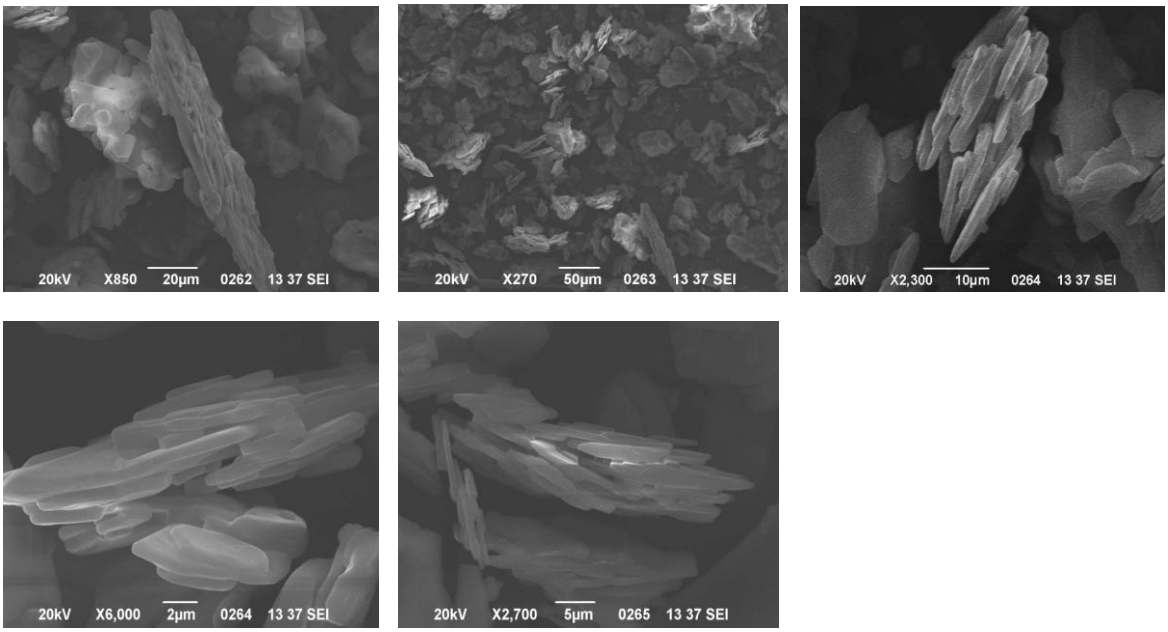
^{1}H and ^{13}C NMR experiments were performed on a Bruker advance DPX300 spectrometer. Thus the bioactive compound was identified as Glutamic acid by structural elucidation using ^{1}H and ^{13}C NMR, from the marine source against dread full wound caused by MRSA among fisherman community (Figure: 5).

Figure 5 NMR spectrum of compound from *Spirastrella inconstans*



Scanning Electron Microscope (SEM): At X 270 illumination 50 µm, at 2,300 X illumination 10 µm, at 6,000 X illumination 2 µm, at 2,700 illumination 5 µm and the compound was conformed as glutamic acid (Figure: 6).

Figure 6 Scanning Electron Microscope (SEM) view of purified compound from *Spirastrella inconstans*



Carbon skeletal structure of Glutamic acid, $C_5H_9NO_4$ (2-aminopentadioic acid or 2-aminoglutaric acid)

Thus the bioactive compound was identified as Glutamic acid by structural elucidation using ^{1}H and ^{13}C NMR, from the marine source against dread full wound caused by MRSA among fisherman community.

Discussion

Marine organisms are yielding more variety and quantity of bioactive compounds over the terrestrial organisms. Among different marine organisms, which yield bioactive compounds, sponges have a high potentiality for the production of marine bioactive compounds such as antibiotics, enzymes, vitamins, minerals, etc (Rajagopal *et al*., 2008), whereas in our report glutamic acid remains as bioactive compound.

Secondary Metabolites produced by sponges have ecologically significant antimicrobial effects. Organic extracts from 33 species of Caribbean sponges were assayed for antibiotic activity against 8 strains representing 6 genera of Marine bacteria, opportunistic pathogen, fouling bacteria. The concentrations of extracts used were volumetrically equivalent to whole tissue concentration of sponge. Bio assay results revealed that 16 species exhibited antibiotic activity against atleast one bacterial isolate and two bacteria isolated from necrotic sponge tissues. Extracts from A*mphimedon* species, *Aplysina lacunose* and *Ptilocaulis spiculifera* exhibited the most antibiotic activities exceeding those of a control antibiotic (Gentamycin). Caribbean Sponge communities do not produce similar antibacterial or broad-spectrum metabolites (Newbold *et al.,* 1999). But *Spirastrella inconstans* which was collected from India bears antimicrobial compound against MRSA (Methicillin Resistant *Staphylococcus aureus*).

In the extracted soluble matrix enclosed within skeleton crystals, protein concentration was 35µg/100mg freeze dried skeleton material as determined by the nano Orange method. Analysis of amino acid composition revealed high concentration of glycin (16.1%) and enhanced amounts of asparagin/aspartic acid (Asx, 11.7%) and

glutamine/glutamic acid (Glx, 10.2%). Due to method inherent limitations, it could not be distinguished between aspartic acid and glutamic acid on the one hand and asparagin and glutamine on the other hand (Matthias Bergbauer *et al.,* 1996) , since the glutamic acid have antibacterial activity, it may be used for wound infection.

According to Davies- Coleman and Beukes, 2004 the ubiquitous wall sponge *Spirastrella spinispirulifer* bears a sources of spongiostatin 4(1) which is the anti-cancer compound yet to be discovered from South African marine organisms. The C21 norsesterterpenoid originally reported with conjugated double bonds **423** has been revised to **424** on the basis of more complete spectroscopic data obtained from a sample isolated from an Australian specimen of *Spirastrella papilosa* (Capon *et al*., 2001).

Over 10,000 biologically active molecules have been isolated from sponges, making them the highest biosynthesizing organisms. Sessile and unarmoured body propels the synthesis of secondary metabolites to defend the predators and competitors. Since sponges are voracious filter feeders, they take in along with regular food, the toxic chemicals excreted by other plants and animals, such as corals. They then modify and reuse these chemicals for their defense purposes. Thus, these sponge-produced and sponge-modified metabolites form the chemical defense mechanism of sponges (Limna Mol *et al*, 2010).

Marieke Koopmans *et al.,* (2009) showed that sponges produce secondary metabolites because they have to compete for space with other organisms, they have to prevent fouling by other organisms and they have to keep predators away. The highest frequency of toxic or deterrent metabolites is found in high competing environments.

A standardized protocol enabling rapid NMR data collection for high-quality protein structure determination is presented that allows one to capitalize on high spectrometer sensitivity: a set of five G-matrix Fourier transform NMR experiments for resonance assignment based on highly resolved 4D and 5D spectral information is acquired in conjunction with a single simultaneous 3D ^{15}N, $^{13}C^{aliphatic}$, $^{13}C^{aromatic}$-resolved [^{1}H, ^{1}H]-NOESY spectrum providing ^{1}H-^{1}H upper distance limit constraints. The protocol was integrated with methodology for semi automated data analysis and used to solve eight NMR protein structures of the Northeast Structural Genomics Consortium pipeline. The molecular masses of the hypothetical target proteins ranged from 9 to 20 kDa with an average of ≈14 kDa. Between 1 and 9 days of instrument time were invested per structure, which is less than ≈10-25% of the measurement time routinely required to date with conventional approaches. The protocol presented here effectively removes data collection as a bottleneck for high-throughput solution structure determination of proteins up to at least ≈20 kDa, while concurrently providing spectra that are highly amenable to fast and robust analysis (Gaohua Liu et al., 2005).

The H-NMR spectra and colors of fractions 1,2,3 and 5 suggested the presence of carotenoids, fatty acids, sterols and sterols endoperoxides respectively, and hence these fractions were not investigated further (Piggott *et al.,* 2005). But the presence of amino acid was detected in our study. Yong, 2008, suggested that structural HPLC and NMR elucidation by fatty acid esters were identified in Australian sponge. Tiiu Roovere, 2006 identifed sponges using SEM. The sponge *Spirastrella abata* collected from Korean waters yielded four phosphatidylcholines **206**-**209** of which **208** and **209** showed an inhibitory effect on the biosynthesis of cholesterol (Alam *et al*., 2001). SEM observation of purified compound, L-glutamic acid of sponge had large orthorhombic shape particles (Fouad *et al.,* 2009), which is similar to the present study.

Conclusion

Although substantial progress has been made in identifying novel drug leads from the sponge's resources, great efforts are still needed to advance to clinical applications. This work has produced new results regarding the potent bioactivity of compound; glutamic acid derived from the sponges *Spirastrella inconstans* against MRSA (Methicillin Resistant *Staphylococcus aureus*). The predominant microorganism in wound infection among fisherman community can be treated by using this novel compound as the drug in future.

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References

Alam, N., B. H., Bae, J., Hong, C. O., Lee, B. A., Shin, K. S., Im and J. H. Jung (2001) "Additional bioactive Lyso-PAF congeners from the sponge *Spirastrella abata".* J. Nat.Prod Vol 64, pp. 533.

Asha Devi, N. K., Balakrishnan, K., Gopal, R. and Padmavathy, S. (2008) "*Bacillus clausii* MB9 from the east coast regions of India: Isolation, biochemical characterization and antimicrobial potentials". Current Science Vol. 95, No. 5, pp. 627-635.

Bauer, A. W., Kirby, W. M., Sherris, J. C., Turck, M. (1966) "Antibiotic susceptibility testing by a standardized single disk method". Am. J. Clin. Pathol Vol. 45, No. 4, pp. 493-496.

Capon, R. J., A. Jenkins., F. Rooney and E. L. Ghisalberti (2001) "Structure revision and assignment of absolute stereochemistry of a marine C21 bisfuranoterpene". J. Nat. Prod Vol. 64, pp. 638.

Davies-Coleman M. T. and Denzil R. Beukes (2004) "Ten years of marine natural products research at Rhodes University". South African Journal of Science Vol. 100, pp. 539-544.

Dovi Kelman, Yoel Kashman, Russell T. Hill, Eugene Rosenberg and Yossi Loya (2009) "Chemical warfare in the sea: The search for antibiotics from Red Sea corals and sponges". Pure Appl. Chem Vol. 81, No. 6, pp. 1113-1121.

Fouad, E. A., Mahmoud El-badry, Fars K. Alanazi, Maha M. Arafah, Ibrahim A. Alsarra (2009) "Preparation and investigation of acetyl salicylic acid glutamic acid complex: a novel oral delivery system". Digest Journal of Nanomaterials and Biostructures Vol. 4, No. 2, pp. 299-308.

Gaohua Liu, Yang Shen, Hanudatta S. Atreya, David Parish, Ying Shao, Dinesh K. Sukumaran, Rong Xiao, Adelinda Yee, Alexander Lemak, Aneerban Bhattacharya, Thomas A. Acton, Cheryl H. Arrowsmith, Gaetano T. Montelione, and Thomas Szyperski (2005) "NMR data collection and analysis protocol for high-throughput protein structure determination". PNAS Vol. 102, No. 30, pp. 10487-10492.

Hadas, E., Shpigel, M. and Ilan, M. (2005) "Sea ranching of the marine sponge *Negombata mangnifica* (Demospongiae, Latrunculiidae) as a first step for latrunculin B mass production". Vol. 244, pp. 159-169.

Ines Mancini, Andrea Defant and Graziano Guella (2007) "Recent Synthesis of Marine Natural Products with Antibacterial Activities". Anti-Infective Agents in Medicinal Chemistry. Vol. 6, pp. 17-48.

Jean-Luc Morel, Herve Drobecq, Pierre Sautiere, Andre Tartar, Jean Mironneau, Janti Qar, Jean-Louis and Hugues (1997) "Purification of a new Dimeric Protein from *Cliona vastifica* Sponge, which specifically Blocks a Non-L-Type Calcium Channel in Mouse Duodenal Myocytes". Molecular Pharmacology Vol. 51, pp. 1042-1052.

Ki-Bong, O. H., Woongchon, M. A. R., Sanghee, K. I. M., Ji-Yun, K. I. M., Tae-Hoon, L. E. E., Jae-Gyu, K. I. M., Daehyun, S. H. I. N., Chung J. S. I. M. and Jongheon, S. H. I. N. (2006) "Antimicrobial Activity and Cytotoxicity of Bis (indole) Alkaloids from the Sponge *Spongosorites* sp". Biol. Pharm. Bull. Vol. 29, No. 3, pp. 570-573.

Limna Mol, V. P., T. V., Raveendran, Abhilash, K. R. and P. S., Parameswaran. (2010) "Inhibitory effect of Indian Sponge extracts on bacterial strains and larval settlement of the barnacle, *Balanus Amphitrite".* Int. Biodeterior. Biodegrad. Vol. 64, No. 6, pp. 506-510.

Marieke Koopmans, Dirk Martens and Rene H. Wijffels (2009) "Towards Commercial Production of Sponge Medicines". Mar. Drug Vol. 7, pp. 787-802.

Matthias Bergbauer, Robert Lange and Joachim Reitner (1996) "Characterization of Organic Matrix Proteins Enclosed in High Mg-Calcite Crystals of the coralline Sponge *Spirastrella* (Acanthochaetetes) *wellsi".* Reef evolution research report pp. 9-12.

Mostafa Fouad, Khaled Al-Trabeen, Mohammad Badran, Victor Wray, RuAngelie Edrada, Peter Proksch and Rainer Ebel. (2004) "New Steroidal saponins from the sponge *Erylus lendenfeldi".* Issue in honor of prof. Karsten Krohn Vol. 13. pp. 17-27.

Newbold, R. W., Paul R. Jensen, William Fenical and Joseph R. Pawlik (1999). "Antimicrobial activity of Caribbean sponge extracts". Aquat Microb Ecol. Vol. 19, pp. 279-284.

Newman, D. J., Cragg, G. M., Snader K. M. (2003) "Natural products as sources of new drugs over the period 1981-2002". J Nat Prod. Vol. 66, pp. 1022-1037.

Piggott, A. M. and Peter Karuso. (2005) "9-Hydroxyfurodysinin-*O*-ethyl Lactone: A New Sesquiterpene Isolated from the Tropical Marine Sponge *Dysidea arenaria*". Molecules Vol 10, pp. 1292-1297.

Pomponi, S. A., Robin Willoughby, Edward Kaighn, M. and Amy E. Wright (1996) "Development of Techniques for In Vitro Production of Bioactive Natural Products from Marine Sponges". pp. 231-237.

Rajagopal, B., Jegan, S. R., Seena, R.V., Suma, S., Angiesh, T. K. and Baby Joseph (2008) "Bioactive screening of Peninsular Indian Marine Sponges on Selected Microorganisms". Journal of Basic & Applied Biology Vol. 2, No. 1, pp. 56-64.

Tiiu Roovere, Annika Lopp, Tonu Reintamm, Anne Kuusksalu, Evelyn Richelle-Maurer and Merike Kelve (2006) "Freshwater sponges in Estonia: genetic and morphological identification". Proc. Estonian Acad. Sci. Biol. Ecol. Vol. 55, No. 3, pp. 216-227.