American Journal of Pharmacology and Toxicology 5 (2): 95-102, 2010
ISSN 1557-4962

Antibacterial Activity of Marine Source Extracts Against Multidrug Resistance Organisms

[1,2]Nagi A. AL-Haj, [2]Nurmas I. Mashan, [2,3]Mariana N. Shamsudin,
[4]Habsah Mohamad, [5]Charles S. Vairappan and [2]Zamberi Sekawi
[1]Department of Medical Microbiology, Faculty of Medicine and Health Sciences,
Sana'a University, P.O. Box 13078, Sana'a, Yemen
[2]Department of Microbiology and Parasitology, Faculty of Medicine and Health Sciences,
[3]Department Marine Science and Aquaculture, Institute of Bioscience,
University Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4]Department of Chemical Sciences, Faculty of Science and Technology,
University Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[5]Laboratory of Natural Products, Department of Chemistry,
Institute for Tropical Biology and Conservation, University Malaysia Sabah,
88999 Kota Kinabalu, Sabah, Malaysia

Abstracts: Problem statement: Antimicrobial resistance is the major problem of global dimensions with a significant impact on morbidity, mortality and healthcare-associated costs. The problem has recently been worsened by the steady increase in multiresistant strains and by the restriction of antibiotic discovery and development programs. Methicillin-resistant *Staphylococcus aureus*, *pseudomonads* and *Escherichia coli* are a major nosocomial and community-acquired pathogens for which few existing antibiotics are efficacious. The current study was conducted to investigate antibacterial activity of natural seaweed sources. **Approach:** *Gracilaria changii Euchema denticulatum* and sea cucumbers extracts against Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. **Results:** The Minimal Inhibitory Concentration (MIC) values and Minimal Bactericidal Concentration (MBC) values of methanol extract were used against all assayed bacteria. Results indicated that *G. changii*, *E. denticulatum* and sea cucumbers extracts must possess major antibacterial components against infectious microorganisms. **Conclusion:** The results obtained indicate that *Gracilaria changii* and *Euchema denticulatum* could be a source of natural products with antibiotic modifying activity to be used against multidrug resistant bacteria.

Key words: Methicillin Resistant *Staphylococcus Aureus* (MRSA), Extended Spectrum Beta Lactamase (ESBL), Vancomycin Resistant Enterococci (VRE), Multiple Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC), Community Acquired Pathogens (CAP)

INTRODUCTION

Even control continuous use of antibiotics has resulted in multi-resistant bacterial strains all over the world and as expected, hospitals have become breeding grounds for human-associated micro organisms (Mainous and Pomeroy, 2001). There is an urgent need to search for alternatives to synthetic antibiotics. The revaluation of the discovery of new groups of antimicrobial peptides make natural antibiotics the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections (De Lucca, 2000; Hancock, 2000; Selitrennikoff, 2001; Welling *et al*., 2000). Even some of the wide spectrum of antimicrobial activities it has a potential benefit in the treatment of cancer (Tanaka, 2001) and viral (Andersen *et al*., 2001; Chernysh *et al*., 2002; Chinchar *et al*., 2001). These features make the antibiotic of natural product resources as a powerful arsenal of molecules that could be the antimicrobial

Corresponding Author: Nagi A. AL-Haj, Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a University, P.O. Box 13078, Sana'a, Yemen Tel: 00967-773579777

drugs of the new century as an innovative response to the increasing problem of MDR. Anti Microbial Peptides (AMPs) known as new generation native peptide molecules isolated from a full range of organisms and species from bacteria to man, seem to fit this description. As a consequence, they have been termed "natural antibiotics", because they are active against a large spectrum of microorganisms, including bacteria and filamentous fungi-in addition to protozoan and metazoan parasites (Liu *et al*., 2000). A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. The evolution of antibiotic-resistant pathogenic bacteria has stimulated the search for alternative antimicrobial agents from alternative sources including sources from the ocean. The powers of marine organisms have been realized for thousands of years and its potential as producers of pharmaceutical products have been reviewed (Baker, 2004). Red algae that are used in this study are *Gracilaria changii*, which grow mild in Pantai Morib, Selangor and *Euchema denticulatum* which grow mild and cultivated in Pantai Sabah. Sea cucumber or Holothurian belongs to phylum of echinodermata. Cured products termed beche-de-mer (meaning seaworm), trepang (meaning sea slug) and in Chinese it is called hoi-sum. Sea cucumbers are believed to have some aphrodisiac qualities as well as curing effects on variety of elements. According to Ridzwan *et al*. (1995) the current research focus on sea cucumber in Malaysia are based on studies which include identification of species, their distributions, nutrition evolution, kinetic properties of crude lactate dehydrogenase and medicinal value. In microbiology term, antibacterial agent is defined as any compound that is clinically useful in the treatment of bacterial infections which may derive from a natural source, synthetic or produced semi synthetically. The increasing prevalence of multi-drug resistant organisms with few or no treatment options such as Methicillin Resistant *Staphylococcus Aureus* (MRSA), Vancomycin Resistant Enterococci (VRE) and the Extended Spectrum Beta-Lactamase (ESBL) producing gram-negative bacilli both in hospitalized patients and to a lesser extent, in the community are a serious cause for concern and have become a global problem. A member of the *Staphylococci* group, the *S. aureus* is perhaps the pathogen of the greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life threatening infections and its capacity to adapt to different environmental conditions (Lowy, 1998). Although it is a part of our natural microflora, however, some strains of *S. aureus* are capable of producing a highly heat-stable toxin that is the main cause of illness in humans (Washington *et al*., 2006). It can multiply in food held at room temperature and produced the enterotoxins which is resistant to heat, refrigeration and freezing (Schlievert, 1993) causing gastroenteritis or inflammation of the lining of the intestinal tract. It is also released pyrogenic exotoxins into the blood stream and causing toxic shock syndrome. *S. aureus* grows to higher numbers in pimples, sores and when a person is down with a cold and can causes variety of suppurative (pus forming) infections such as boils and furuncles and deep-seated infections such as osteomyelitis and endocarditis pneumonia. Other infections are mastitis, phlebitis, meningitis and urinary tract infections. This study is in search for the antibacterial properties of natural product such as red algae and Sea cucumber in order to find a new antibacterial agent and peptide that can inhibit or reduce the growth of bacteria in human body. In the present study is designed based on the bioassay and molecular assay for determining antibacterial activity of *G. changii* and *E. denticulatum* extracts and peptide gene of Sea cucumber respectively. The investigation could scientifically proof the natural products to be potentially potent antibacterial agents.

MATERIALS AND METHODS

Bacteria sources: Bacteria used in this study, *S. aureus*, *Streptococcus pyogenes*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* were obtained from several hospitals in Malaysia. All of the isolates were provided in the form of pure bacterial stock culture. Bacterial isolates were maintained at -20°C in Luria Bertani broth (Invitrogen Inc.) containing 15% (vol/vol) glycerol.

Sea cucumber sources: Live specimens of sea cucumber (5 species) and tunicate (1 species) were collected from Beting Darat, Gelang Patah, Johore. For genomic extraction, samples were cut in to small pieces and preserved in TNEAS buffer.

Genomic extraction and organic extraction: Genomic extraction was carried out using Master Pure^{TM} Complete DNA and RNA Purification Kit, following Tissue Samples and Precipitation of Total DNA protocols. The samples were blended in the solvent, stirred for 48 h and kept at 4°C until further used.

Molecular screening: Polymerase Chain Reaction (PCR) amplification and primers were design using Primer Premium 5 software based on region of interest. The reaction mixture volume of 25 µL

contained 1X BST buffer (Biosyntech Inc.), 1.8 mM $MgCl_2$, 200 μm dNTPs, 20 pmole of reverse primer and forward primer, 1U taq polymerase. The PCR programs were, initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 5 sec, annealing temperature depended on the particular primer for 1 min, extension at 72°C for 2 min and final extension at 72°C for 3 min.

Disc diffusion test: Whatman paper No. 1 filter paper was used to make sterile discs in order to screen for the antibacterial activities of *G. changii* and *E. denticulatum*. Filter paper was punctured to the shape of commercial antibiotic disc and discs were autoclaved at 121°C for 15 min. The methanol extract of either *G. changii* or *E. denticulatum* was solubilized in the 60% methanol. The suspension of bacteria culture was prepared according to the MacFarland standard 0.5 and was lawned onto the Mueiller Hinton agar plate to produce the bacteria field. A sterile of punctured filter papers was placed on the bacteria field by a sterile forceps and the solubilized extract then pipettes out onto the surface of filter paper on the bacteria field. 60% methanol was used as a negative control while the commercial antibiotic discs were used as a positive and negative control. Finally, the plate was incubated at 37°C and the zone of inhibition is observed after 24-48 h.

Minimal Inhibitory Concentration Test (MIC) and Minimal Bactericidal Concentration Test (MBC): Sensitivity of bacteria to either *G. changii* or *E. denticulatum* methanol extracts can be measured by using a tube dilution technique, which determines the MIC ad MBC of seaweed used in this study in vitro. These tests were done to determine the lowest concentration of either *G. changii* or *E. denticulatum* extract, where it can show the bactericidal and bacteriostatic effect. Test was performed in 96-well microtitre plates, so that, several replicates of each sample can be run. All isolates were grown until the concentration is equal to 0.5 MacFarland standard at 37°C and diluted in Mueller Hinton Broth (MHB; Difco Laboratories, Detroit, USA) supplemented with 2% NaCl (Thornsberry and McDougal, 1983) to a concentration of 50 mg mL^{-1} for *G. changii* and 40 mg mL^{-1} for *E. denticulatum* and serial two-fold dilutions were made.

Table 1: Minimum Inhibitory Concentrations (MIC) of the extracts against gram-positive bacterial growth

Microorganism	MIC 70% MeOH SC3 (mg mL^{-1})	MIC 70% MeOH SC5 (mg mL^{-1})
S. aureus (STR9)	125	125
S. aureus (N8)	250	125
S. pyogens	125	125

Then, the suspension of *S. aureus* culture was added into the 96-well microtitre plates containing diluted sample of either *G. changii* or *E. denticulatum* extract. Finally, the 96-well microtitre plates containing diluted sample of either *G. changii* or *E. denticulatum* and bacteria was then incubated overnight at 37°C with constant shaking on the shaker. On the next day, the diluted sample of *S. aureus*-*G. changii* or *S. aureus*-*E. denticulatum* in the 96-well microtitre plates were plated out onto the Mueiller-Hinton agar (Merck, Germany) plate according to the concentration of either *G. changii* or *E. denticulatum* extract. The plate was incubated at 37°C for 24 h in the incubator. Finally, the number of bacteria colonies developed on each agar plates was counted. MIC for Sea cucumber extracts against bacterial growth were determined using the serial dilution method. 900 μL of freshly prepared Mueller Hinton broth was placed in 2 mL of eppendorf tube (Table 1). An aliquot of 100 μL of bacterial suspension at a concentration of 0.5 Mc Farland were pipette into the first tube, followed by 1.0 mL of the respective extracts and mixtures were re suspended accordingly. Then 1 mL of mixture were taken out and re suspend in another tube contain the same mixture and repeat 10 times.

RESULTS

Clear inhibition zones around the antibiotic discs showed the sensitivity to the antibiotic compounds. Seven antibiotic discs were used in each plate including erythromycin (15 μg), methicillin (5 μg), mupirocin (5 μg), gentamycin (10 μg), vancomycin (30 μg), penicillin (10 μg) and oxacillin.

Disc diffusion test: In this test, filter paper disc impregnated with *G. changii* extract showed clear inhibition zone to *S. aureus* and *S. pyogenes* (Fig. 1). The clear inhibition zones are seen in both MRSA and non-MRSA isolates. For isolates of *E. coli*, *P. aeruginosa* and *K. pneumoniae* (Fig. 2 and 3) there are no inhibition zones around discs impregnated with methanol extract of *G. changii*. The antibiotic disc which was used as positive control vancomycin showed clear inhibition zones around both MRSA and non-MRSA isolates.

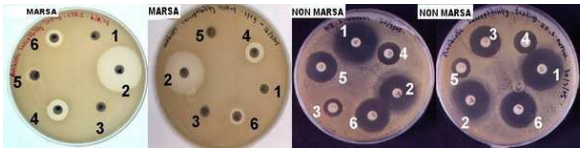


Fig. 1: The disc diffusion assay for determination of MRSA and non-MRSA isolates: (1) *methicillin*, (2) *mupirocin*, (3) *gentamycin*, (4) *vancomycin*, (5) *penicillin*, (6) *erythromycin*

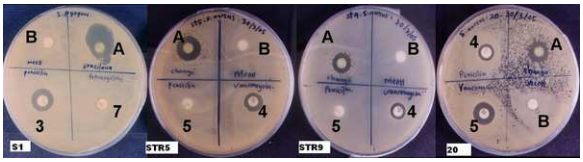


Fig. 2: Disc susceptibility testing of *Gracilaria changii* on *Streptococcus pyogenes* (S1), *S. aureus* (STR5), *S. aureus* (STR9) and *S. aureus* (20). Filter paper disc impregnated with extract (A) and (B) 60% methanol. Antibiotics *gentamycin* (3), *vancomycin* (4) *penicillin* (5) and *tetracycline* (7) were used as positive control

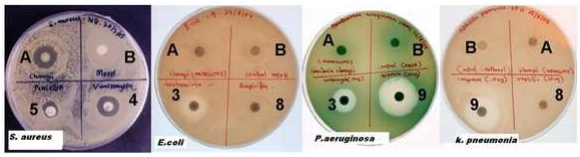


Fig. 3: Disc susceptibility testing of *G. changii* on *S.aureus* (N8), *E. coli*, *P. aeruginosa* and *K. pneumonia*. Filter paper disc impregnated with extract (A) and (B) 60% methanol. Antibiotics *gentamycin* (3), *vancomycin* (4) *penicillin* (5), *ampicillin* (8) and *Imipenem* (9) were used as positive control

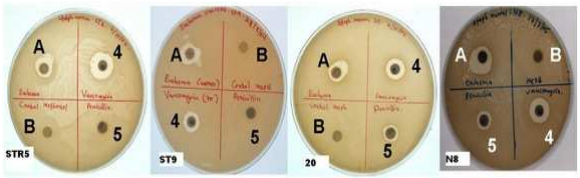


Fig. 4: Disc susceptibility testing of *E. denticulatum on S. aureus* (STR5), *S. aureus* (STR9), *S. aureus* (20) and *S. aureus* (N8). Filter paper disc impregnated with extract (A) and (B) 60% methanol. Antibiotics *vancomycin* (4) and penicillin were used as positive control.

Discs impregnated with methanol extract of *E. denticulatum* similar clear inhibition zone around discs are seen in the MRSA and lawn of non-MRSA isolates (Fig. 4). Clear inhibition zones are also seen in isolates lawned with *S. pyogenes*. Plates lawned with gram negative bacteria namely *E. coli*, *V. cholerae*, *P. aeruginosa* and *K. pneumoniae* (Fig. 5) showed no inhibition zone around discs. The results of inhibition and no inhibition zones represented for tests that were performed twice. The zones of diameter values are as average of two readings.

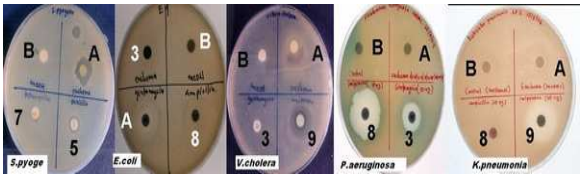
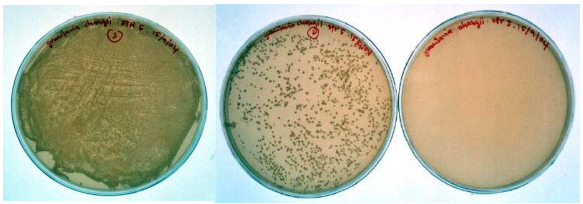


Fig. 5: Disc Susceptibility Testing of *E. denticulatum* on *S. pyogens*, *E. coli*, *V. cholera*, *P. aeruginosa* and *K. pneumonia*. Filter paper disc impregnated with extract (A) and (B) 60% methanol. Antibiotics *gentamycin* (3), *penicillin* (5), *tetracycline* (7) *ampicillin* (8) and *Imipenem* (9) were used as positive control



12.5 mg mL^{-1} 25 mg mL^{-1} 50 mg mL^{-1}

Fig. 6: MIC and MBC test for MRSA isolate with *G. changii* extract. The reducing of colonial growth of *STR5* isolates in different concentrations of *G. changii* methanol extract. MBC-50 mg mL^{-1} MIC-25 mg mL^{-1}

Table 2: Minimal bactericidal concentration and minimal inhibitory concentration test for *G. changii* extract on MRSA and non-MRSA

		Gracilaria changii (mg mL^{-1})			
Concentration	Isolate	50	50	12.5	6.25
No. of colony	STR5	No colony	400-500	*	*
	STR9	No colony	300-400	*	*
	20	No colony	5	20	*
	N8	No colony	6	13	*

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) determined based on the lowest *G. changii* and *E. denticulatum* extract concentration reducing colonial growth or killing all bacteria cells. The MIC and MBC tested for *G. changii* and *E. denticulatum* are for two isolates of MRSA and 2 isolates of non-MRSA. Table 2and 3 showed the MIC and MBC of *G. changii* or *E. denticulatum* extract against *S. aureus* while Fig. 6-9 showed the comparison between the numbers of colonies growth in different concentration of either *G. changii* (Fig. 6 and 7) or *E. denticulatum* (Fig. 8 and 9) extracts.

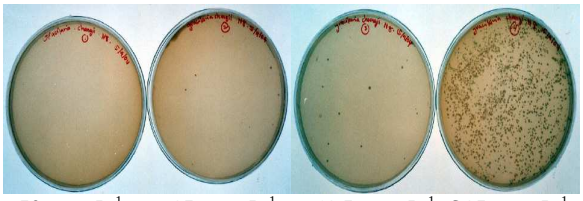


Fig. 7: MIC and MBC test for Non-*MRSA* isolate with *G. changii* extract. The reducing of colonial growth of N8 (non-*MRSA*) isolates in different concentrations of *Gracilaria changii* methanol extract. MBC-50 mg mL^{-1} MIC-12.5 mg mL^{-1}

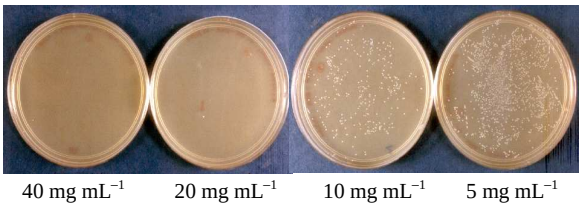


Fig. 8: MIC and MBC test for *MRSA* isolate with *E. denticulatum* extract. The reducing of colonial growth of STR5 (*MRSA*) isolates in different concentrations of *E. denticulatum* methanol extract. MBC-40 mg mL^{-1} MIC-20 mg mL^{-1}

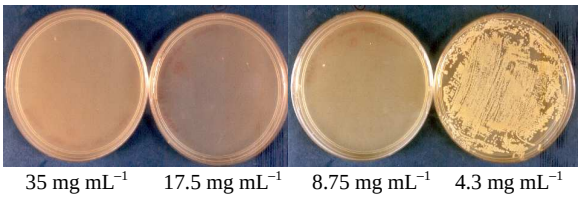


Fig. 9: MIC and MBC test for Non-MRSA isolate with *E. denticulatum* extract. The reducing of colonial growth of N8 (non-*MRSA*) isolates in different concentrations of *E. denticulatum* methanol extract. MBC-17.5 mg mL^{-1} MIC-8.75 mg mL^{-1}

According to the Table 2 and 3, methanol extract of *G. changii* and *E. denticulatum* showed strong activity irrespective of MRSA and non-MRSA isolates, whereas the MIC and MBC level for *G. changii* extract are 25 and 50 mg mL^{-1} for MRSA isolates and 12.5 and 50 mg mL^{-1} for non-MRSA isolates, respectively, while the MIC and MBC level for *E. denticulatum* extract are 20 and 40 mg mL^{-1} for MRSA isolates and 8.75 and 17.5 mg mL^{-1} for non-MRSA isolates, respectively.

Table 3: Minimal Bactericidal Concentration and Minimal Inhibitory Concentration Test for *E. denticulatum* Extract on MRSA and Non-MRSA

		Gracilaria changii (mg mL^{-1})			
Concentration	Isolate	40	20	10	5
No. of colony	STR5	No colony	1	300-400	*
	STR9	No colony	1	300-400	*
		35	17.5	8.75	4.38
	20	No colony	No colony	1	*
	N8	No colony	No colony	2	*

Note: For the test of *E. denticulatum* extract on non-MRSA isolate, the MIC and MBC level are different due to different starting concentrations of the extract *: Uncountable colonies

DISCUSSION

Research approaches used in this study was bioassays or preliminary screening which included the screening of antibacterial activity through disc diffusion test and minimal inhibitory concentration tests. A fundamental concept of *in vitro* susceptibility testing is the measurement of Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of either *G. changii* or *E. denticulatum* extract that exhibit their antibacterial activity against Methicillin Resistant *S. Aureus* (MRSA) and non-Methicillin Resistant *S. Aureus* (non-MRSA). Both methods were chosen in order to point out if there might be some significant difference between the lowest concentration of both extracts required to inhibit growth of *S. aureus* and the lowest concentration at which both extracts can kill 99.9% of the initial *S. aureus* inoculums. These were indicated by an absence of visible turbidity of the Mueiller-Hinton broth and an absence of bacterial colonies on the Muieller-Hinton agar. Since the "bactericidal" and "bacteriostatic" terminology originates from either the antibacterial's mechanism is based on inhibiting cell wall formation ("bactericidal") or inhibiting bacterial metabolism or ribosomal protein synthesis ("bacteriostatic") (Zarakolu *et al*., 1999), the idea is that if cell wall formation is blocked, the organisms will lyse and perish, but if metabolism or protein synthesis is blocked, the organisms merely slow down. While this is true to some degree, bactericidal or bacteriostatic outcomes are dependent on the concentration of the antibacterial agent as well. A low dose of a "bactericidal" antibacterial agent may only inhibit bacterial growth, while a high dose of a "bacteriostatic" antibacterial agent will be bactericidal. Additionally, according to Zarakolu *et al.* (1999), organisms which are not proliferating may not be significantly affected by anti-cell wall antibiotics, in

which case anti-ribosomal antibiotics would be more effective. Therefore, in this study, MIC would be taken as the reference point for the study designations "susceptible or resistant", while MBC determinations would be more certain for the prediction of susceptibility in the cases that required bactericidal therapy rather than bacteriostatic theraphy. MBC and MIC tests were done carefully since it is easy affected by the nature of the bacteria used, the inoculums size and the composition of the culture medium, the incubation time and the conditions of incubation, such as temperature, pH and aeration. In the present study, the reading of MIC and MBC which were based on the number of colonies growing on the Mueiller-Hinton agar is constant for both seaweeds after repeated twice. The methanol extract of *G. changii* reduced the number of colonial growth for MRSA isolates at the extract concentration of 25 mg mL^{-1}, while in non-MRSA isolates, the reduction in colonial growth can be observed at the extract concentration of 12.5 mg mL^{-1}. The concentration levels were considered as the MIC level. The cidal effect of *G. changii* extract was indicated by no colonial growth of colonies which was observed when the extract concentration was at 50 mg mL^{-1} for all *S. aureus* isolates. These concentration levels are considered as the MBC level. The present study also revealed that methanol extract of *E. denticulatum* inhibited or reduced the growth of *S. aureus* isolates better than the inhibitory activity showed by the methanol extract of *G. changii.* Since the inhibitory effect of *E. denticulatum* extract to *S. aureus* isolates is greater if compared to the inhibitory effect of *G. changii* extract to *S. aureus* through the disc diffusion test as discussed above, the experimental concentration level for *E. denticulatum* extract was tested at a lower concentration of *G. changii* extract. In this study, the MIC level of *E. denticulatum* extract, which is indicated by the reduced in number of MRSA colonial growth was at 20 mg mL^{-1} while for non-MRSA was at 8.75 mg mL^{-1}. The MBC effect of the extract was at 40 mg mL^{-1} for MRSA isolates and 17.5 mg mL^{-1} for non-MRSA isolates. Preliminary screening of this study revealed the significant finding of antibacterial activity of *G. changii* and *E. denticulatum* extracts against *S. aureus* isolates. Several significant findings of the present study were found whereby the methanol extract of *G. changii* and *E. denticulatum* exhibited the inhibitory activity against *S. aureus* and *S. pyogenes*. Inhibitory activities of both extracts were indicated both in MRSA and non-MRSA isolates. In comparison to the *G. changii* extract, the methanol extract of *E. denticulatum* exhibited more intense activity against MRSA and non-MRSA isolates. The current study revealed that the extract inhibited a MRSA isolate, STR9 and a non-MRSA isolate, N8, with zones of about 3 and 2 mm more than the inhibition zones around the vancomycin disc, respectively. The isolate, STR5, was also inhibited by the extract but with a smaller inhibition zone which is 1mm less than the zone around the vancomycin disc. The extract can be considered valuable as an alternative in the treatment of MRSA and non-MRSA infection substituting penicillin, since this extract inhibited both MRSA and non-MRSA with inhibition zones of 6 mm and 1mm more than the inhibition zone developed around penicillin disc. In addition, both seaweeds extracts also indicated significant inhibitory activity against *S. pyogenes* isolates. However, further research on *S. pyogenes* isolates, was not performed, since this current study is focused on the inhibitory activity of both extracts against *S. aureus*. Ballantine *et al*. (1987) and Reichelt and Borowitzka (1984), the higher frequency of activity against gram positive bacteria has been observed in most of the surveys of antimicrobial activities from seaweeds if compared with activity against gram negative bacteria. Thus present study also showed the same inhibitory pattern whereby the antibacterial activity of *G. changii* and *E. denticulatum* extracts were only observed in gram positive bacteria and no inhibitory activity against tested gram negative bacteria such as *E. coli*, *V. cholerae*, *P. aeruginosa* and *K. pneumoniae* were indicated. According to Michael *et al*. (2000), there is several reasons why certain bacteria may develop resistant to certain antimicrobial agents that may explain the findings of the current study on gram negative bacteria. Although, the methanol extract of *G. changii* and *E. denticulatum* can be classified into the narrow spectrum antimicrobial agents, which acts on only a single group of organism, both extracts are still quite valuable for the control of microorganisms especially MRSA and non-MRSA that fail to respond to available antibiotics. The incorporation of molecular methods for typing of nosocomial pathogens has assisted in efforts to obtain a more fundamental assessment of strain interrelationship (Cockerill and Smith, 2004; Emori and Gaynes, 1993). Two methods of extraction were utilized, methanol extraction and Phosphate Buffer Saline (PBS). The aim was to find the most suitable and effective extraction method of sea cucumber extract to yield active antibacterial substances. Differences in methods used were in obtaining water soluble or hydrophobic fraction. Antibacterial activity was only found using methanol solvent, whereby inhibition in the growth of gram positive pathogens, *S. aureus* and *S. pyogenes*, were observed. The identity of the active compound in the extract cannot be confirmed in current report.

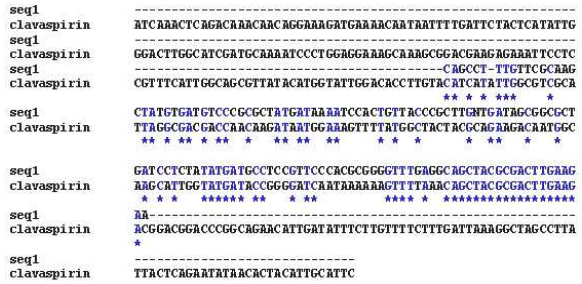


Fig. 10: Alignment between sequencing result of permeabilization gene size 222 bp (seq1) and clavaspirin gene

However, potential antimicrobial agent in tissue of sea cucumber was investigated by molecular screening. The study successfully designed a primer pair for amplifying an antimicrobial peptide gene encoding for membrane permeablization as evidenced from the amplification of the gene at the expected size of 222 bp. Commercialized sequencing further confirmed the identity of the gene after Blast analysis with the published gene in the public domain. The memebrane permeablization gene primers were design from tunicates but could amplify the gene from tissue of sea cucumber 5 as shown in (Fig. 10) alignment between the sequenced product and the permeabilize gene from tunicates showed minor changes in sequenced product due to different species. The finding of antibacterial activity by both extracts against MRSA and non-MRSA strains is hoped to have potential in producing alternative antibacterial agents from natural resources, against resistant *S. aureus* to reduce the infections and fatality.

REFERENCES

Andersen, J., S. Osbakk, L. Vorland, T. Traavik and T. Gutteberg, 2001. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. Antiviral Res., 51: 141-149. PMID: 11431038

Baker, J.T., 2004. Seaweeds in pharmaceutical studies and applications. Hydrobiology, 116/117: 29-40. DOI: 10.1007/BF00027635

Ballantine, D.L., W.H. Gerwick, S.M. Velez, E. Alexander and P. Guevara, 1987. Antibiotic activity of lipid-soluble extracts from Caribbean marine algae. Hydrobiology, 151-152: 463-469. DOI: 10.1007/BF00046168

Chernysh, S., S.I. Kim, G. Beckeer, V.A. Pleskach and N.A. Filatova *et al*., 2002. Antiviral and antitumor peptides from insects. Proc. Natl. Acad. Sci. USA., 99: 12628-2632. DOI: 10.1073/pnas.192301899

Chinchar, V.G., J. Wang, G. Murti, C. Carey and L. Rollins-Smith, 2001. Inactivation of frog virus 3 and channel catfish virus by esculentin-2P and ranatuerin-2P, two antimicrobial peptides isolated from frog skin. Virology, 288: 351-357. PMID: 11601906

Cockerill, F.R. and T.F. Smith, 2004. Response of the clinical microbiology laboratory to emerging (new) and reemerging infectious diseases. J. Clin. Microbiol., 42: 2359-2365. PMID: 15184405

De Lucca, E.A.J., 2000. Antifungal peptides: Potential candidates for the treatment of fungal infections. Expert Opin. Investig. Drugs, 9: 273-299. PMID: 11060677

Emori, T.G. and R.P. Gaynes, 1993. An overview of nosocomial infections, including the role of the microbiology laboratory. Clin. Microbiol. Rev., 6: 428-442. PMID: 8269394

Hancock, R.E.W., 2000. Cationic antimicrobial peptides: Towards clinical applications. Expert Opin. Investig. Drugs, 9: 1723-1729. http://journals.tubitak.gov.tr/medical/issues/sag-99-29-6/sag-29-6-2-98180.pdf

Liu, Y., J. Luo, C. Xu, F. Ren and C. Peng *et al*., 2000. Purification, characterization and molecular cloning of the gene of a seed-specific antimicrobial protein from pokeweed. Plant Physiol., 122: 1015-1024. PMID: 10759497

Lowy, F.D., 1998. *Staphylococcus aureus* infections. Natl. English J. Med., 339: 520-532. PMID: 9709046

Mainous, A.G. and C. Pomeroy, 2001. Management of Antimicrobials in Infectious Diseases: Impact of Antibiotic Resistance. Humana Press, ISBN: 0-89603-821-1, pp: 349.

Michael, T.M., M.M. John and P. Jack, 2000. Brock Microbiology of Microorganism. 9th Edn., New Jersey, ISBN: 10: 0130662712, pp: 1104.

Reichelt, J.L. and M.A. Borowitzka, 1984. Antimicrobial activity from marine algae: Results of a large scale screening program. Hydrobiology, 116-117: 158-167. DOI: 10.1007/BF00027657

Ridzwan, B.H., M.A. Kaswandi, Y. Azman and M. Fuad, 1995. Screening for antibacterial agents in three species of sea cucumbers from coastal areas of Sabah. Gen. Pharmacol., 26: 1539-43. PMID: 8690242

Schlievert, P.M., 1993. Role of superantigens in human disease. J. Infect. Dis., 167: 997. PMID: 848697

Selitrennikoff, C.P., 2001. Antifungal proteins. Applied Environ. Microbiol., 67: 2883-2894. PMID: 11425698

Tanaka, K., 2001. P-I3-kinase p85 is a target molecule of proline-rich antimicrobial peptide to suppress proliferation of ras-transformed cells. Jap. J. Cancer Res., 92: 959-967. PMID: 11572764

Thornsberry, C. and L.K. McDougal, 1983. Successful use of broth microdilution in susceptibility tests for methicillin-resistant (heteroresistant) Staphylococci. J. Clin. Microbiol., 18: 1084-1091. PMCID: PMC272846

Washington, W., D.A. Stephen, M.J. William, W.K. Elmey and W.P. Gary *et al*., 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Edn., United States, America, ISBN: 13: 978-07817-3014-3.

Welling, M.M., A. Paulusma-Annema, H.S. Balter, E.K. Pauwels and P.H. Nibbering, 2000. Tenehtium-99m labeled antimicrobial peptide discriminate between bacterial infection and sterile inflammations. Eur. J. Nuclear Med., 27: 292-301. DOI: 10.1007/s002590050036

Zarakolu, P., N. Coplu, A. Arslanturk, B. Levent and E. Guvener, 1999. Comparison of inhibitory and bactericidal activities of cefoperazone + sulbactam against *Pseudomonas aeruginosa* strains, from 1992-1994. J. Med. Sci., 29: 607-609. http://mistug.tubitak.gov.tr/bdyim/abs.php?dergi=sag&rak=98180