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TITLE:
Marine lectins and their medicinal applications

ABSTRACT:
Marine organisms have been extensively explored for the last several decades as potential sources of novel biologically active compounds, and extensive research has been conducted on lectins. Lectins derived from marine organisms are structurally diverse and also differ from those identified from terrestrial organisms. Marine lectins appear to be particularly useful in some biological applications. They seem to induce negligible immunogenicity because they have a relatively small size, are more stable due to extensive disulfide bridge formation, and have high specificity for complex glyco-conjugates and carbohydrates instead of simple sugars. It is clear that many of them have not yet been extensively studied when compared with their terrestrial counterparts. Marine lectins can be used to design and develop new potentially useful therapeutic agents. This review encompasses recent research on the isolation and identification of marine lectins with potential value in medicinal applications.

Antibacterial activity ::: Introduction:
Innate immunity constitutes the first line of defense against pathogens depending on the pattern recognition. Host pattern recognition receptors can recognize microbial molecules that are unique to groups of related microorganisms (McGreal et al. 2004). These microbial molecules are often the cell wall components of microbes, primarily carbohydrate chains such as lipopolysaccharides, peptidoglycan, lipoteichoic acids, and β-glucans (Medzhitov 2007). Lectins, as a key member of pattern recognition receptors, could function as phagocytosis receptors, soluble opsonins, and agglutinins to mediate pattern recognition (Takano et al. 2008).
A 302-amino acid amphioxus intelectin homolog AmphiITLN-like was cloned by Yan et al. (2012). In situ hybridization studies revealed that AmphiITLN-like transcripts were expressed in the skin and digestive tract in adult amphioxus; the expression was upregulated upon challenge with Staphylococcus aureus; and agglutination with Gram-negative and Gram-positive bacteria. The agglutination was usually achieved by the interaction of lectin with lipopolysaccharide and peptidoglycan which are present on the cell walls of Gram-negative and Gram-positive bacteria. An l-rhamnose-binding lectin known as STL from Oncorhynchus mykiss (steelhead trout) eggs recognized lipoteichoic acid and lipopolysaccharides which are the major components of the outer membranes of Gram-negative and Gram-positive bacteria, respectively. It agglutinated Bacillus subtilis and Escherichia coli K-12, and the binding was inhibited by l-rhamnose (Tateno et al. 2002). Zhu et al. (2009) found an Argopecten irradians (bay scallops) C-type lectin known as AiLec using expressed sequence tag and rapid amplification of cDNA ends techniques. The amino acid sequence of the lectin had a high homology to those of the C-type lectins from other animals. C-type lectins are Ca2+-dependent carbohydrate recognition proteins with a classic domain containing four conserved disulfide bridges and two extra cysteine residues at the amino end. They take a pivotal part in the innate immunity of invertebrates. The expression of the lectin transcript was mainly detected in the hepatopancreas and was elevated 6–8 h after challenge with Vibrio anguillarum (a Gram-negative bacterium) and Micrococcus luteus (a Gram-positive bacterium). The results demonstrated that the lectin was a constitutive and inducible acute-phase protein and may be responsible for immune response toward infections caused by Gram-negative and Gram-positive bacteria. The lectin from Holothuria scabra (sea cucumber) known as HSL has been studied for its role in immune response. The results showed that a broad spectrum of bacterial challenge could induce the expression of HSL in which the glycoconjugates on the bacterial cell wall were involved. The lectin inhibited the growth of both Gram-negative (E. coli, Proteus sp., Serratia sp., and Shigella sp.) and Gram-positive bacteria (Streptococcus sp.) (Gowda et al. 2008). Saito et al. (1995) isolated a lipopolysaccharide-binding protein designated as L6 from the hemocytes of horseshoe crab (limulus). It was lectin-like in nature. The protein showed agglutinating activity on Gram-negative and Gram-positive bacteria, and inhibition on the growth of Gram-negative bacteria, including E. coli O9:K39 (K-), Salmonella minnesota R595 (Re mutant), and Klebsiella pneumonia. It presumably recognizes carbohydrate components in the bacterial cell wall. Tachycitin was purified from Tachypleus tridentatus (horseshoe crab) hemocytes which showed amino acid sequence similarity to chitin-binding lectins. It was a single-chain protein with a molecular mass of 8.5 kDa. It inhibited the growth of both Gram-negative (Escherichia, Klebsiella, and Salmonella) and Gram-positive bacteria (Staphylococcus) with IC50

values of 2.0–50 μg/ml. Furthermore, tachycitin was found to agglutinate both Gram-negative and Gram-positive bacteria and the activity was higher toward Escherichia strains (Kawabata et al. 1996). Schroder et al. (2003) purified a tachylectin-like protein from a demosponge, Suberites domuncula. The 27-kDa protein manifested antibacterial activity against E. coli (Gram-negative bacteria) and S. aureus (Gram-positive bacteria). The antibacterial activity resulted was about 16 and 81 % inhibition of E. coli, at lectin concentrations of 10 and 300 μg/ml, respectively. The inhibition percentage for S. aureus was relatively low which only exhibited 15 % inhibition at 300 μg/ml. The lectins present in the hemolymph of many bivalve mollusks are known to have an important involvement in host defense mechanisms. Takahashi et al. (2008) isolated a lectin known as MCL-4 from the plasma of Ruditapes philippinarum (Manila clam) and demonstrated its bacteriostatic and opsonizing properties against invading bacteria in R. philippinarum. The phagocytic activity of its hemocytes for the lectin-opsonized Vibrio tubiashii was markedly higher than that toward untreated bacteria. Besides, the lectin significantly inhibited the growth of Alteromonas haloplanktis. A tetrameric lectin was isolated from the ovaries of a teleost, the cobia Rachycentron canadum. The lectin exerted antibacterial activity against E. coli with 50 % inhibition at 250 μg/ml (Ngai and Ng 2007). A lectin (CvL) from the marine sponge Cliona varians displayed a cytotoxic effect on Gram-positive bacteria, such as B. subtilis and S. aureus. The results showed that the lectin at a concentration of 25 μg/ml exhibited strong antibacterial activity (75 % inhibition) on B. subtilis, which increased with higher lectin concentrations, reaching 90 % inhibition at 100 μg/ml. Potent antibacterial activity was also observed when S. aureus was incubated with the lectin, reducing bacteria growth by 90 % at 50 μg/ml. However, it showed a relatively low inhibitory effect on the growth of Gram-negative bacteria, Pseudomonas aeruginosa and E. coli (Moura et al. 2006). Holanda et al. (2005) purified a lectin from Solieria filiformis (red algae). It inhibited the growth of Gram-negative bacteria like Serratia marcescens, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus sp., and P. aeruginosa though no changes in growth phases (log, exponential, and decline) were observed. It was postulated that the binding of the lectin to the cell surface receptors on Gram-negative bacteria enhanced the changes in nutrient flow. Thus, it would explain the bacteriostatic effect and the involvement of such interactions of the lectin in this effect were suggested. Tunkijjanukij and Olafsen (1998) purified a heterogeneous sialic acid-binding lectin from Modiolus modiolus (horse mussel) hemolymph. It recognized bacterial lipopolysaccharides, and its antibacterial activity was tested against different marine bacteria. The sensitive strains included Vibrio anguillarum, Vibrio ordalii, Vibrio salmonicida, Vibrio viscosus, Vibrio wodanis, Aeromonas salmonicida, and Shewanella putrefaciens. The purified lectin expressed stronger activity than the whole hemolymph. This lectin reacted with several sialoglycoconjugates and purified lipopolysaccharides from marine Vibrios and with dead and resting cells of a number of bacteria. This suggested that the lectin may play a role in recognition and elimination of bacteria.

Antifungal activity ::: Introduction:
An enormous number of lectins and hemagglutinins have been isolated from marine organisms, but only few of them manifested antifungal activity. Agglutination of fungi is normally tested as part of lectin characterization, as agglutination may aid its clearance by facilitating phagocytosis. The lectins usually bind and agglutinate fungi or their expression level is upregulated when challenged with fungi, and they act in a similar manner to eliminate invading pathogenic bacteria. The antifungal lectins discussed under this topic are all C-type lectins. They are important in innate immunity, pathogen recognition, and cell-cell interactions. A C-type lectin was dramatically upregulated when the amphioxus (Brachiostoma belcheri) was challenged with Saccharomyces cerevisiae. The lectin (AmphiCTL1) directly killed S. cerevisiae in a Ca2+-independent fashion by cell wall permeabilization. The antimicrobial activity of AmphiCTL1 was dose-dependent with AmphiCTL1 at 200 μg/ml strongly suppressed microbial growth and its binding to microbial cell wall polysaccharides such as peptidoglycans and glucans preceded microbicidal activity. These results disclosed that the lectin interacted with the peptidoglycans and glucans, bound to the yeast, and killed it directly (Yu et al. 2007). In a study of another C-type lectin known as AiCTL-7 purified from Argopecten irradians (bay scallop), the expression level of AiCTL-7 was upregulated considerably in the hepatopancreas and hemocytes after challenge with Pichia pastoris GS115. The agglutination of P. pastoris with the lectin was Ca2+-dependent and inhibited by d-mannose. The collective results revealed that AiCTL-7 played a role in the primitive acute-phase response to the yeast. It acted as a key pattern recognition receptor in the innate immune system of the bay scallop (Kong et al. 2011). Another C-type lectin with antifungal effect was demonstrated in a lectin known as Ec-CTL, which was purified from Epinephelus coioides (orange-spotted grouper)

by Wei et al. (2010). It bound to and aggregated S. cerevisiae in a Ca2+-dependent manner. The expression was upregulated when challenged with S. cerevisiae. A serum lectin purified from Lampetra japonica (lamprey) by Xue et al. (2013) was classified as an intelectin. The interlectin was a new type of extracellular animal lectins, being glycan-binding receptors which bind glycan epitopes on foreign pathogens in the host systems. Its expression was induced by bacterial stimulation in vivo, and the lectin showed agglutinating activities against Candida albicans. These findings suggested that it plays an important role in innate immunity against yeast in the internal circulatory system of the lamprey.
A repertoire of natural bioactive compounds has been isolated from marine organisms which showed promising medicinal potential (Cheung et al. 2014). The marine lectins exhibited strong antimicrobial activity as aforementioned, implying that they can be potential candidates for drug design and development in the future. Although the mechanisms of the marine organism defense systems have been revealed for some lectins, the possibility to apply those to other antimicrobial mechanisms remains to be examined. Further studies on molecular mechanisms of action (Li et al. 2015; Ng et al. 2014), structure–function relationships (Huang et al. 2015; Zhou and Sun 2015), and clinical trials can help researchers in investigating the therapeutic effects and toxicity of lectins. It is also worthwhile to check if lectins have synergism with existing antimicrobial agents. Results of the investigation do not only contribute to knowledge corresponding to antimicrobial mechanisms but also have applications from a clinical viewpoint and the perspective of drug design. It also starts a new perspective on the potential of lectins from the marine species.

Antiviral activity ::: Introduction:
In recent years, marine lectins gave important clues for the development of antiviral drugs against different viral infections, particularly human immunodeficiency virus type 1 (HIV-1) infection. They bind to the high mannose sugars which is the key compoenent of the gp120 on the HIV envelope and inhibit the conformational change that keep it in an inactive, nonfusable state (Koharudin and Gronenborn 2014). Due to this breakthrough, a number of antiviral therapeutic agents based on marine lectins have potential medicinal application as new effective drugs for antiviral treatment. Liu et al. (2013) found that expression of the galectin-1 gene from Paralichthys olivaceus (flounder) was reduced after poly I:C challenge in the first 8 h and then increased significantly beyond 24 h. Galectin is a class of Ca2+-independent lectins that bind to β-galactosides. Recombinant galectin-1 could neutralize the lymphocystis disease virus (LCDV) and inhibit cytopathy of the infected cells. It could also prevent inflammation against LCDV infection, and the expression of TNF-α and mx (an antiviral protein) in the head kidney and the gill and IL-1β in the head kidney would be significantly downregulated when LCDV and galectin-1 were injected. These findings showed that galectin-1 was related to the antiviral activity, controlled mx gene overexpression, and alleviated LCDV pathogenicity. Luo et al. (2003) studied an antiviral gene (PmAV) found in Penaeus monodon (shrimp), which encodes a 170 amino acid protein with a C-type lectin-like domain. The PmAV gene was cloned, and the protein was expressed in E. coli. The purified recombinant protein was tested for antiviral activity in vitro. The grouper iridovirus was inoculated into grouper embryo cells for 3 days. It was found that the cells were all killed by the virus. When the inoculated cells were incubated with the PmAV protein, around 50 % of the grouper embryo cells remained viable with an EC50 value of about 6.25 μg/ml. The protein also evinced potent antiviral activity on virus-induced cytopathy inhibition in the fish cell line. Neither the recombinant nor the native form demonstrated agglutination activity toward the virus. This suggested that the antiviral mechanism of the PmAV protein does not entail the inhibition of virus and target host cell interaction. Lectins can recognize the specific carbohydrate structures such as proteoglycans, glycoproteins, and glycolipids resulting in the regulation of various cells via glycoconjugates and their physiological and pathological phenomena such as apoptosis induction, cancer metastasis and differentiation, carbohydrate recognition and binding, cell to cell communication, cell targeting, and host–pathogen interactions. Recently, lectins were also known to block the binding of virus to its target cells which would prevent viral infection and dissemination (Sato and Hori 2009). Geyer et al. (1988) observed that the gp120 on HIV-1 envelope was heavily glycosylated with about 24 possible N-linked glycosylated sites. Approximately 50 % of the molecular weight of the gp120 was attributed to glycans located at these glycosylation sites. The gp120 glycans were high-mannose and complex glycoproteins and could act as binding sites for various lectins. The significance of involvement of gp120 glycans in HIV-1 infection makes them a possible clue for development of anti-HIV-1 agents (Balzarini et al. 2007).

Microcystis viridis is a unicellular freshwater bloom-forming cyanobacterium. It showed transient hemagglutinating activity in laboratory culture during the stationary phase under nonaeration conditions. This lectin is a single 13-kDa polypeptide with 113 amino acid residues and has two tandemly repeated homologous domains of 54 amino acid residues. M. viridis lectin binds oligomannosides with submicromolar affinities and that two novel carbohydrate recognition domains are composed of four noncontiguous regions. The residues make numerous intermolecular contacts with their carbohydrate ligands (Yamaguchi et al. 1999). The lectin inhibited HIV type 1 envelope-mediated cell fusion with an IC50 value of 30 nM (Bewley et al. 2004). Lectin purified from the red alga, Kappaphycus alvarezii, known as KAA-2, exhibited antiviral activity against a number of influenza strains including swine influenza virus H1N1. This algal lectin suppressed virus infection by its specific interaction with target high mannose type N-glycans on viral surfaces. The anti-influenza virus activity was tested against different strains including swine influenza virus H1N1-2009 with EC50 values of 1.71–68.56 nM. By using an anti-influenza antibody, immunofluorescence microscopy results showed that the lectin interfered with the virus entry to the host cells. ELISA assay results demonstrated that the lectin bound directly to the viral envelope protein (Sato et al. 2011b). Lectin purified from the green alga Boodlea coacta known as BCA was also found to be active against influenza viruses. Like KAA-2, it also bound to high mannose-type N-glycans. Besides, BCA has a high specificity for α1-2-linked mannose at the nonreducing end of the glycans. As the number of nonreducing end substitution of α1-2-linked mannose increased, the binding specificity was also increased. The mechanism for suppression of virus infection is same as that of KAA-2. BCA could also interfere with the entry of HIV-1 virus to the host cells with an EC50 value of 8.2 nM. The surface plasmon resonance assay showed a high association constant with a value of 3.71 × 108 M−1 in BCA with gp120 on the HIV envelope. Additionally, BCA demonstrated anti-influenza virus activity against H3N2 subtypes and H1N1 subtypes with EC50 values of 18.8–74.2 and 79.3–1590.2 nM, respectively (Sato et al. 2011a). Griffithsin (GRFT) was a lectin-like protein purified from the the red alga Griffithsia sp. aqueous extract with 121 amino acids in length. GRFT was regarded as an anti-HIV protein for its prevention of HIV entry to the host cells. The native and recombinant protein showed strong antiviral activity against different primary isolates and laboratory strains of T cell and macrophage-tropic HIV-1 with EC50 values at a range of 0.043 to 0.63 nM. It also stopped HIV-1 infection transmission and cell-to-cell fusion among infected and uninfected cells at this concentration range. Different tests on the interactions of GRFT and gp120 were done. It was found that GRFT bound to glycoproteins (gp120, gp41, and gp160) on the virus envelope in a glycosylation-dependent manner, blocked CD4-dependent gp 120 binding to receptor-expressing cells, and mildly inhibited gp120 and soluble CD4 interaction. The soluble gp120 binding occurred in a monosaccharide-dependent manner in which mannose was the most potent inhibitor and inhibited gp120/monoclonal antibody 2G12 and gp120/monoclonal antibody 48d binding. All these findings demonstrated that GRFT bound to different viral glycoproteins and could be a potential lead for anti-HIV agent (Mori et al. 2005). GRFT also demonstrated synergistic activity with tenofovir, maraviroc and enfuvirtide against HIV-1 clade C. The combined use of the two drugs enhanced their antiviral potency and supports further clinical investigations in pre-exposure prophylaxis (Ferir et al. 2011). Besides HIV, GRFT was also active against hepatitis C virus (Meuleman et al. 2011), Ebola virus (Barton et al. 2014), atypical pneumonia, and other pathogenic coronaviruses (O'Keefe et al. 2010), herpes simplex virus 2 (Nixon et al. 2013), and Japanese encephalitis virus (Ishag et al. 2013).

Other lectins from cyanobacteria such as cyanovirin-N (Chen et al. 2014; Xiong et al. 2010), microvirin (Huskens et al. 2010; Shahzad-ul-Hussan et al. 2011), scytovirin (Alexandre et al. 2013; Garrison et al. 2014), and Oscillatoria agardhii agglutinin (Ferir et al. 2014; Koharudin et al. 2011) are considered as potential candidates to combat HIV or other virus-infected diseases with the same mechanism, i.e., interacting with the glycans on HIV gp120. They show specificity for high mannose carbohydrates on the surface of the heavily glycosylated envelope of HIV and are endowed with potent anti-HIV activity. However, these lectins do have unique properties, including the number of carbohydrate recognition sites and their specificity for the oligosaccharides. These differences may account for the differences in antiviral activity (Huskens and Schols 2012). They are usually divided into two or more internal tandems or repeats, and they display pronounced homology to each other. The domains formed are stabilized by disulfide bridges. Their amino acid sequences and internal alignments between the repeats are presented in Table 2. Overall, these lectins have a broad antiviral activity; however, GRFT and cyanovirin-N exhibited superior anti-HIV activity which are more potent (Huskens and Schols 2012; Woodrum et al. 2013), were studied extensively, and also showed antiviral activity against other enveloped

viruses. This benefits their usage as anti-HIV agents because HIV-1 infection is commonly associated with other sexually transmitted viruses, such as hepatitis C virus and herpes simplex virus (Helle et al. 2006; Meuleman et al. 2011; Nixon et al. 2013; Yu et al. 2010). Irrespective of the potent antiviral activity of an anti-HIV agent candidate, safety issues are extremely importanct and can also contribute to a lack of efficacy. The use of cyanovirin-N as a safe microbicide raises questions because it has clearly stimulatory/mitogenic activity and induces elevated amounts of a large number of cytokines (Buffa et al. 2009). However, when it was tested preclinlically against HIV in vaginal and rectal transmission models, it was proved to be effective and safe (Tsai et al. 2003, 2004). In contrast, GRFT with its broad and potent antiviral activity is devoid of stimulatory properties (Kouokam et al. 2011). However, it is less chemically and physically stable than cyanovirin-N. To sum up, these two lectins have disadvantages. Nevertheless, they still stand out as potential candidates for development of anti-HIV agents.

As the glycans are commonly found on the virus surface, the aforementioned glycan-binding lectins are promising antiviral agents that could be used for preventing and controlling virus infections. Although many other high mannose-binding lectins (such as legume family lectins) have been demonstrated to exhibit anti-HIV activity (Balzarini et al. 2007), algal and cyanobacterial lectins are the most potent compounds reported thus far compared with other plant lectins, because they inhibit HIV entry into the host cells with EC50 values in the picomolar to rnanomolar ange by directly binding to envelope gp120 compared with plant lectins which generally show a higher (micromolar) range of EC50 values. These lectins also demonstrated powerful activity against HIV and influenza viruses regardless of different strains and subtypes. Effective antiviral agents are always in short supply because vaccine production is sometimes not on time, but there will be a high demand when there is an outbreak. The antiviral mechanism of the lectins is strain-independent. They may be more practical for medicinal use when compared with antibody-based antiviral agents that are prone to antigenic shift or antigenic drift. The substantial amount of carbohydrate residues on the virus envelope may be good targets for antibody treatment. However, some virus strains are continually changing their antigenicity by making an increased number of glycosylation sites in order to evade the antibody actions. As a result, it may be advantageous to use lectins as preventive drugs since they inactivate different virus strains and subtypes collectively. The results from research on these lectins have uncovered their potential as novel antiviral agents for the prevention of infection.
Besides the ability to block HIV entry into host cells, lectins from other sources can inhibit the cytopathic effect induced by HIV-1 and production of HIV-1 p24 antigens, thus replication of virus in host cells. Wang et al. (2006) purified a β-galactose-specific lectin with antiviral activity known as CVL from a polychaete marine worm Chaetopterus variopedatus. Its size is 30-kDa, and the anti-HIV-1 activity was tested. It was demonstrated that at the early virus replication stage, the lectin inhibited production of the viral p24 antigen and HIV-1-induced cytopathy with EC50 values of 0.057 and 0.0043 μM, respectively. It also stopped the cell-to-cell fusion with an EC50 of 0.073 μM among HIV-infected and normal cells. The anti-HIV-1 action started at the early virus replication phase and may be due to blocking of HIV-1 from gaining entry into the target cells. By using fluorescence-based real-time quantify PCR, CVL blocked 21 and 86 % of virus attachment at a concentration of 0.07 and 0.33 μM, respectively. Molchanova et al. (2007) purified an N-acetylglucosamine-specific lectin (SVL) from another polychaete Serpula vermicularis which also showed anti-HIV activity. It inhibited HIV-1-induced cytopathy and viral p24 antigen production in host cells with EC50 values of 0.15 and 0.23 μg/ml, respectively. Luk'ianov et al. (2007) purified three lectins from Crenomytilus grayanus (designated as CGL) and Didemnum ternatanum (designated as DTL and DTL-A). CGL manifested high specificity to glycoproteins of the mucin type. DTL was N-acetylglucosamine-specific with a shorter carbohydrate-binding site which bound to N-acetylglucosamine residues at the terminal. DTL-A was an N-acetylglucosamine/N-acetylgalactosamine and heparin-specific lectin. CGL, DTL, and DTL-A inhibited syncytium formation in C8166 cells induced by the HIV-1 IIIB with EC50 values of 27.88, 0.002, and 0.36 μg/ml, respectively.

Antitumor activity ::: Introduction:
Tumors and normal cells have different surface structures as shown by the selective agglutination of malignant tumor cells by some lectins. Nearly all types of malignant cells demonstrate alterations in their glycosylation patterns when compared to their normal counterparts (Powlesland et al. 2009). Meticulous studies of cell surface carbohydrates from human and experimental tumors showed that a prominent alteration in glycoproteins is the presence of larger

and extensively branched N-linked β-1,6-GlcNAc oligosaccharides (Couldrey and Green 2000). The β-1,6-GlcNAc branched N-glycans are tri- or tetra-antenna oligosaccharides that increase the total cell surface terminal sialylation in malignant cells and are typically found in the initial stages of carcinogenesis induced by oncogenic viruses or by oncogenes (Dennis et al. 1989). The lectin that recognizes carbohydrate moieties on the N-glycans on tumor cells would function as a therapeutic agent via apoptosis induction.
Yao et al. (2012) studied the antitumor activity of bighead carp (Aristichthys nobilis) lectin known as GANL purified from its gills. Six human tumor cell lines were tested for antiproliferative activity, and it was found that the lectin exhibited strong antitumor activity against the HeLa cell line with an IC50 value of 11.86 μg/ml. Rabelo et al. (2012) investigated the mechanism of apoptosis induction in human tumor cells from a marine sponge lectin. They isolated a marine sponge Cinachyrella apion lectin (CaL) and studied its antiproliferative activity against three human tumor cell lines. The results showed that the lectin exhibited the highest antiproliferative activity toward HeLa cells at a dose-dependent manner. It was also found that the lectin probably induced apoptosis in HeLa cells by increasing expression of the proapoptotic protein Bax, enhancing mitochondrial membrane permeabilization, activating caspase cascades, and inducing cell cycle arrest at the S phase. Matsumoto et al. (2012) isolated a lectin known as HOL-18 from Halichondria okadai (demosponge). It is divalent and cation-independent and exhibited cytotoxic activity against K562 erythroleukemia cells and Jurkat leukemia T cells in a carbohydrate- and dose-dependent manner. N-acetylgalactosamine and N-acetyl-d-glucosamine residues are known to be expressed on the surface of these two cell lines, and the cytotoxic activity was inhibited by the presence of these two amino sugars. The results suggested that lectin-glycan interaction plays a pivotal role in its cytotoxicity to the tumor cell lines. Bah et al. (2011) isolated a rhamnose-binding lectin from Oncorhynchus tshawytscha (chinook salmon) roe. It was demonstrated that the lectin exhibited cytotoxic activity toward human hepatoma Hep G2 and breast cancer MCF-7 cells. Queiroz et al. (2009) investigated the antitumor activity of a lectin known as CvL isolated from Cliona varians (sponge). It was tested on a few cancer cell lines and found that Jurkat cells and K562 cells were sensitive with IC50 values of 100 and 70 μg/ml, respectively. These human leukemia cells were killed due to induction of apoptosis after addition of lectin. Experimental results from confocal fluorescence microscopy revealed that the lectin induced cathepsin B release; flow cytometry disclosed an increase in apoptotic index; Western blot demonstrated that the expression of tumor necrosis factor receptor 1 was upregulated and that p65 subunit of nuclear factor kappa B was downregulated in lectin-treated cells. These results suggested that cathepsin B played a role in an undefined association with the apoptotic pathway. A 17-kDa lectin known as MytiLec was purified by (Fujii et al. 2012) from the Mediterranean mussel, Mytilus galloprovincialis. It is an α-d-galactose-binding lectin and could also bind to globotriose (Gb3; Galα1-4Galβ1-4Glc) specifically. This lectin exhibited antiproliferative activity on human Burkitt lymphoma Raji cells in which Gb3 was expressed on their surfaces. The activity was inhibited in the presence of α-galactoside. Experimental results demonstrated that the lectin was related to late-stage apoptosis and triggered the loss of membrane integrity and cell membrane inversion. Another example of Gb3-specific lectin is Silurus asotus (catfish) egg lectin designated as SAL. It also bound to Gb3 expressed on the surfaces of the Burkitt's lymphoma cells. It was found that the size of the lectin-treated cells was 10 % smaller than that of untreated cells. Gb3 present in the glycosphingolipid-enriched microdomain was involved in the lectin-Gb3 interaction which brought about a reduction in cell size through activation of potassium channel Kv1.3. These results provided some new approaches into the understanding of glucosphingholipid-targeting cancer therapy (Kawano et al. 2009).

Antinociceptive and anti-inflammatory activities ::: Introduction:
Different harmful stimuli provoke a strong inhibition of pain sensation evoked at a distant region of the body. After tissue injury and inflammation, nociceptors are sensitized in such a way that an ineffective or a weak stimulation becomes painful. The sensitization of primary afferent nociceptors is normal for all inflammatory pain types that lead to hyperalgesia and/or allodynia states in humans and is known as nociception in animal models (Kurihara et al. 2003). At present, nonsteroidal anti-inflammatory drugs and analgesia-inducing drugs such as opioids are not suitable for all cases because of their low potency and side effects (Ahmadiani et al. 1998). As a consequence, development of alternative drugs has become necessary and beneficial.

Holothuria grisea agglutinin (HGA) from the sea cucumber Holothuria grisea was used to test for antinociceptive and anti-inflammatory activities in rats. It was a dimeric, mucin-specific lectin with

a molecular mass of 228 kDa. HGA was injected intravenously, and the results showed that neutrophil migration induced by carrageenan into the peritoneal cavity was inhibited. As selectins were known to induce neutrophil migration into the inflammatory tissues, it was proposed that HGA could block the selectin which shares a common carbohydrate ligand; thus, the selectin action was stopped. HGA also exhibited a strong antinociceptive effect in acetic acid-induced writhing test, and the number of abdominal constrictions was reduced significantly in the HGA-treated group. Intraperitoneal injection of acetic acid caused local irritation and induced the release of different endogenous mediators such as prostaglandins, bradykinin, substance P, tumor necrosis factor-α, interleukin-1β, and interleukin-8. They activated the chemosensitive nociceptors that caused inflammatory pain (Collier et al. 1968). The results suggested that the antinociceptive effect may be caused by decreasing the number of leukocytes in the peritoneal cavity or the inhibition of the endogenous mediators release induced by HGA administration. HGA showed antinociceptive effect only in phase 2 of the inflammatory phase in formalin tests, indicating that the effect was only associated with inflammatory pain. However, in the hot-plate tests, the lectin did not exhibit any nociceptive action, demonstrating that HGA did not show a central antinociceptive action, and that a peripheral mechanism may be involved instead, and verifying what was observed in the formalin test (antinociceptive effect in inflammatory phase). The treatment with HGA caused an increase in the circulating nitric oxide levels and inhibition of myeloperoxidase activity. Myeloperoxidase is a peroxidase usually expressed in neutrophils and regarded as a direct marker of neutrophils. The decrease in the myeloperoxidase activity agreed with the results of neutrophil migration test. The inhibition of the migration may be associated with increased circulating nitric oxide levels caused by HGA. It has been demonstrated that the expression of adhesion molecules was downregulated by nitric oxide in the vascular endothelium, hence decreasing the number of neutrophil that migrated to the inflammatory region (Lavich et al. 2006). The results, show that HGA has the ability to modulate the inflammatory response in in vivo models (Moura et al. 2013).

Bitencourt Fda et al. (2008) tested the mucin-specific Hypnea cervicornis agglutinin (HCA) previously purified from the red marine algae Hypnea cervicornis for antinociceptive and anti-inflammatory activities in mouse and rat models. HCA smoothened the paw edema in rats induced by carrageenan but not that induced by dextran. The edema induced by carrageenan is a multimediated and temporal incident, distinguished by massive neutrophil migration (Srinivasan et al. 2001). On the contrary, dextran is a pro-inflammatory mediator distinguished by an increase in vascular permeability without involving neutrophil migration (Lo et al. 1982). These results demonstrated that its action involved an inflammatory process related to neutrophil migration. The results of an investigation have shown that there was a strong link between the inflammation and pain development, and the inhibition of neutrophil migration alleviated the hypernociception induced by different inflammatory stimulations (Cunha et al. 2005). The effects were inhibited after pre-incubation with mucin, suggesting that the lectin was mucin-specific, and the participation of its carbohydrate-binding site in its actions. When it was intravenously injected to mice, no signs of toxicity were observed. It did not cause renal or hepatic malfunctions, affect body mass and macroscopy of the liver, kidney or heart. Further investigations demonstrated that the anti-hypernociceptive effect of HCA was not related to increase in nitric oxide production. Nitric oxide alleviated hyperalgesia by activating the l-arginine/NO/cGMP pathway. It caused direct blocking of persistent and acute hypernociception by opening K+
(ATP) channels through the stimulation of protein kinase G (Nunes et al. 2009; Sachs et al. 2004). Experimental results disclosed that the antinociceptive effect of HCA on carrageenan-induced hypernociception was reduced by treatment with nitric oxide synthase inhibitors which demonstrated that nitric oxide may be associated with the action of HCA (Figueiredo et al. 2010). Another lectin purified from the marine alga Amansia multifida is mannose-specific, and its actions were tested in mice. An interesting finding was observed in the formalin and hot-plate tests. The antinociceptive action of the lectin was blocked by naloxone, indicating possible involvement of the opioid system (Neves et al. 2007). Similar results were obtained in tests of antinociceptive and anti-inflammatory activity for the red marine alga Pterocladiella capillacea lectin known as PcL (Silva et al. 2010) and the green marine alga Caulerpa cupressoides lectin known as CcL (Vanderlei et al. 2010).

In conclusion, all aforementioned marine lectins exhibited strong antinociceptive and anti-inflammatory activities. However, the detailed mechanism has yet to be clarified. Further investigations could help in acquiring more information for their development into alternative therapies for pain and inflammatory processes.

Other medicinal applications ::: Introduction:
Cutaneous wounds originate from the disruption of skin integrity and could be a major trouble in public health. Postponed healing or lack of proper treatment will lead to complex therapeutic problems, particularly in diabetic patients. The healing process is a complex arrangement, but well-ordered phases overlap in which highly specialized cells interact with the extracellular matrix to result in growth and tissue repair (Diegelmann and Evans 2004). A lectin was purified from Bryothamnion seaforthii (marine red algae) known as BSL, and its healing potential was studied. Cutaneous wounds were made in mice in the healing test, and the mice were topically treated with BSL for 12 days. In the first few days of treatment, edema and hyperemia were observed in the treated group. There were indicative signs of a pro-inflammatory effect of BSL, and a decrease in the wound area was observed. The results of histopathological examination revealed that the BSL-treated animals experienced epithelial covering restructure with keratin production, cutaneous immature annex formation, and active collagen formation in reticular dermis region with fibroblastic activity. All these observations were signs of good healing. The portion of wound closures was assessed in terms of the wound exposed areas with the progress of time. The results showed that the BSL-treated mice experienced faster healing and an increased closing in the wound areas. The results demonstrated that the lectin had a wound healing enhancing effect and can be used as a potential acute wound treatment. It can enhance the healing process and expedite wound closure. The mechanism probably involves triggering of the immune system in the inflammatory phase, followed by modulation of growth factors and cytokines, activation of collagen synthesis by fibroblasts and their differentiation into myofibroblasts used for traction and wound contraction (do Nascimento-Neto et al. 2012).
In an effort to halt transmission by inhibiting the development of malarial parasite, lectin (CEL-III) isolated from the sea cucumber Cucumaria echinata was expressed in genetically modified mosquitoes. This lectin elicited both cytotoxic and hemolytic activities. The transgenic mosquitoes expressed CEL-III after gene modification and managed to impair the formation of ookinetes. The ookinetes failed to migrate into the mosquito midgut, reproduce to form daughter cells, which will then invade the salivary glands and consequently infect a human. Positive results were reported by observing ookinete inhibition by CEL-III and erythrocyte lysis in the midgut of mosquitoes after ingestion of infectious blood, suggesting a novel approach incorporating genetic engineering to control the disease (Yoshida et al. 2007).
By using drug delivery systems to transport pharmaceutical products to targeted tissues are much better than traditional nontargeted therapeutics. Smart drug delivery efficiently delivers the medication to the diseased tissues, localizes drug exposure to diseased tissues, and reduces the side effects of medications on healthy tissues, thus increases treatment efficiency. Limiting or preventing side effects in treatments is important because side effects typically lead to reduction in dosage, delay in treatment and therapy termination (Minko 2004; Rek et al. 2009). The lectin–sugar interaction can be used to trigger vesicular transport into or across epithelial cells. Many proteins and lipids on cell membranes are glycosylated, and these glycans are binding sites for lectins. Different cell types express different glycan arrays and in particular, diseased cells, often express different glycans compared with their normal counterparts. Therefore, lectins could be used as carrier molecules to target drugs specifically to different cells and tissues. This kind of receptor-mediated bioadhesion may also be used to convey signals to cells in order to trigger vesicular transport processes into or across polarized epithelial cells. When coupled to macromolecular drugs or particular drug carriers, the selection of a suitable lectin may perhaps allow the cellular uptake and subsequent intracellular routing of such delivery systems to be controlled (Haltner et al. 1998).
Lectin may also be used to target therapeutic agents for different cells due to their property of increasing microparticle adherence to the target cells and of enhancing penetration of drugs. One application is used in the novel lipidic microcapsule drug delivery system for transporting and delivering anticancer drugs for the treatment of cancer. Eucheuma serra agglutinin (ESA) derived from a marine red alga, Eucheuma serra, is a lectin that specifically binds to mannose-rich carbohydrate chains. It specifically bound to human colon adenocarcinoma and cervical squamous cell carcinoma but did not bind to normal human lymphocytes and fibroblasts. It can also induce apoptotic cell death in carcinoma and sarcoma cells. The specific binding of the lectin to the cancer cell lines is based on unique interactions between ESA and the high mannose type sugar chains on tumor cell surface (Sugahara et al. 2001). When ESA was incorporated into Span 80 vesicles and use in drug delivery system, the ESA bound on the vesicles surface can act as a targeting ligand. ESA bound to the osteosarcoma cell at 2 μg/ml and induced apoptosis after 48 h. The results showed that the ESA-incorporated Span 80 vesicles have the potential to be used

in osteosarcoma treatment via drug delivery system. In addition, encapsulation of anticancer drugs into the ESA-incorporated Span 80 vesicles is expected to provide extra antitumor action on sarcoma when compared with blank vesicles (Hayashi et al. 2012). Some problems associated with drug delivery system still have to be tackled, notably those of toxicity and immunogenicity. It is hoped that some of these problems might be overcome in the future by the application of biotechnology techniques to produce quantities of smaller fragments of lectins that will retain the high target specificity that these fascinating molecules possess, but will be easier to manipulate (Bies et al. 2004).

Lectins show high affinity for specific carbohydrate structures, and therefore, they can be used as specific probes for both intracytoplasmic compartments and cell surface glycoconjugates in histopathological and histological applications. As some carbohydrates do not elicit antibody production due to their less complex structure, the use of lectin, instead of traditional antibodies, in histochemical studies of carbohydrates and membrane glycoconjugates has become more common. Transformed cells present wide qualitative and quantitative variations in the glycosylation pattern of the membrane and the formation of carbohydrate clusters that occupy restricted areas of the cell surface, which allows their interaction with lectins. By contrast, this distribution is homogeneous in normal cells. Lectins may be important tools for obtaining information regarding the development, differentiation stage, malignancy level, and capacity for metastasis of tumor cells (Wu et al. 2009). The lectin (designated ACL-1) isolated from aqueous extracts of Axinella corrugata (a marine sponge) displayed strong agglutinating activity with rabbit erythrocytes. The hemagglutination was inhibited by N-acetyl derivatives like N-acetyl-d-glucosamine, N,N′,N″-triacetylchitotriose, N-acetyl-d-galactosamine, and N-acetyl-d-mannosamine. ACL-1 was able to bind to N-acetyl-d-galactosamine, and N-acetyl-d-glucosamine glycans present on the surfaces of several tumor cells. These included the bladder (T24), colon (HT-29), breast (T-47D, MCF7), ovary (OVCAR-3), and lung (H460) cancer cell lines, and thus, biotinylated ACL-1 could stain them. The lectin can be used as a marker diagnostic and also tissue mapping tool in suspicious neoplasms. As biotinylated ACL-I could stain the tumor cells, it might be used to identify transformed cells and study the glycan structures synthesized by such cells (Dresch et al. 2013). One characteristic of malignant cells is related to abnormal cell surface protein glycosylation. It was shown that some of these abnormal glycosylations are common in the development of distant metastases and tumor progression (Corfield et al. 1999). The expression of these glycoproteins was associated with malignant transformation. For example, when compared with normal colon cells, expression of MUC1 and MUC13 (mucin) in colorectal neoplasia were overexpressed (Baldus et al. 2004). Pinto et al. (2009) have purified two lectins known as BSL and BTL from two red marine algae under the same genus, Bryothamnion seaforthii and Bryothamnion triquetrum, respectively. These carbohydrate-binding lectins could be used to discriminate human colon carcinoma cell variants regarding glycoprotein types on their cell membrane. The two lectins could bind to mucin found on the cell surface of human colon adenocarcinoma cell line LS-180. The interaction occurred in a dose-dependent manner, and the fluorescence spectra could discriminate them clearly from the flow cytometry results. Besides, these two lectins bound to glycoproteins on cell surface experienced extensive internalization as observed under the confocal microscope. It showed that the lectins could be used as targeting tools.

Discussion:

Nowadays, many investigators are studying natural products isolated from marine species and their diverse pharmacological roles, hoping to develop new antimicrobials, and antiviral especially anti-HIV agents and antitumor agents (Smith et al. 2010; Vo and Kim 2010). Lectins which recognize and bind to specific carbohydrate structures such as glycoproteins, glycolipids, and proteoglycans are one of the investigated targets. Such interactions mediated via the glyco-conjugates induce cell regulation, pathological and physiological actions through cell-cell communications, and host–pathogen interactions. Investigations on these phenomena and their applications are now becoming popular. A large number of marine lectins have been isolated, and the number is growing, and we need to investigate and elucidate the details of structure/interaction/activity relationships in marine lectins in order to develop new therapeutic agents. One reason for the urgency in development of new therapeutic drugs is the problem of drug resistance. Pathogens that have developed drug resistance to a specific antimicrobial or antiviral may not develop drug resistance to its natural derivatives which have similar actions (Yasuhara-Bell and Lu 2010). Hence, the different antimicrobial or antiviral derivatives that are produced by other species may be a breakthrough in the discovery of new drugs. Furthermore, the countless

undefined unique compounds in the marine world form an attractive reserve for novel medications against drug-resistant pathogens.

With respect to the question regarding the potential value of the aforementioned marine lectins in medicinal applications, the answer is not yet certain. Lectin resources from marine species are rather new. To date, relatively few have received commercial consideration. At this stage, it is hard to know which of these lectins might persist and meet with success in the drug market. Many may not pass muster because of the problems of toxicity, antigenicity, and production costs. Others might show promise initially but have low stability in the human body. Compounds derived from marine species that are used as anti-HIV agents have been shown to play an important role against the disease recently. It is encouraging to know that the use of marine lectins as possible drug candidates to reduce or control HIV infection-related chronic symptoms (Vo and Kim 2010). Furthermore, these results imply that these anti-HIV agents have potential to be use as active ingredients for new drug discovery. Up till now, nearly all investigations on marine lectins derived anti-HIV agents were tested only in vitro or in an animal model. Hence, the final step of the novel drug development must be the study of their activities in clinical trials.