

HAEMAGGLUTINATION ACTIVITY OF THE EXTRACTS FROM SOME VIETNAM MARINE INVERTEBRATES

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Received: 16.11.2016

Accepted: 29.7.2017

SUMMARY

Aqueous extracts from 21 species of Vietnam marine invertebrates, including 11 bivalve and 10 gastropod species, were examined for haemagglutination activity using native and enzyme-treated different animal and human erythrocytes. The 8 bivalve and 10 gastropod species were found to have haemagglutination activities toward at least one type of erythrocyte tested. A total of 86% of marine invertebrate species surveyed were active. Strong activity was detected in extracts from two bivalve species (*Tridacna squamosa* and *Geloina coaxans*) and three gastropod species (*Tutufa rubeta*, *Pleuroploca trapezium* and *Tectus conus*) with enzyme-treated rabbit, horse and human A, B, O erythrocytes. In a haemagglutination–inhibition test with various monosaccharides and glycoproteins, haemagglutination activities of two extracts from *T. rubeta* and *P. trapezium* had no affinity for any of the monosaccharides and glycoproteins tested, while activities of the extracts from *T. squamosa* and *T. conus* were strongly inhibited by porcine stomach mucin tested, suggesting the presence of lectins specific for O-glycans of these species. The activities of four marine invertebrate extracts were stable over a wide range of pH and temperature. The haemagglutination activities of *T. rubeta* and *P. trapezium* extracts were independent of the presence of divalent cations, whereas the haemagglutination activity of extracts from *T. squamosa* and *T. conus* were slightly dependent on the presence of divalent cations. The results suggest that Vietnam marine invertebrates may be good sources of useful lectins for biochemical and biomedical applications.

Keywords: Carbohydrate-binding specificity; Haemagglutination activity; Lectins; Marine invertebrates; Stability; Survey

INTRODUCTION

Marine bioresources such as marine cyanobacteria, algae, invertebrate animals, and fishes produce a great variety of specific and potent bioactive molecules, such as fatty acids, polysaccharides, polyether, peptides, proteins, and enzymes. To date, many researchers focused on the marine natural products and their various pharmacological functions to develop new potent drugs including antimicrobials, anti-human immunodeficiency virus (HIV), anticancer, and Alzheimer's therapeutics (Vo, Kim, 2010). In the drug discovery from natural resources, lectins are one of the promising candidates for useful therapeutic agents because they can recognize carbohydrate structures such as proteoglycans,

glycoproteins and glycolipids, and have been implicated in certain cell types and their physiological and pathological functions including host-pathogen interactions and cell-cell communications (Lakhtin *et al.*, 2011). Although in most cases physiological functions of invertebrate lectins are not completely clear, there are increasing data suggesting the involvement of these lectins in processes of differentiation and development of organisms as well as in elimination of foreign substances through binding to their carbohydrate structures (Kawsabata, Tsuda, 2002; Arreguin-Espinosa *et al.*, 2001). Marine invertebrates survive in environments rich in bacterial populations, many of which may be pathogenic (Austin, 1988). The immune defense of these animals is non-specific with responses against microbial organisms based on

both cellular and humoral activities (Chu, 1988; Canesi *et al.*, 2002). In marine animals, humoral lectins have been shown to participate actively the same as opsonins (Bayne, 1990). Lectins are ubiquitous proteins in the hemolymph and cells of many invertebrates (Renwrautz, 1986; Cooper *et al.*, 1992) with the activity based in the carbohydrate-binding protein that produces the agglutination of many cells, such as erythrocytes, bacteria, and others through interaction with specific complementary ligands (Nesser *et al.*, 1986). Interestingly, it has been theorized that some marine invertebrate lectins mediate the interaction between symbiont and host (Vasta, 1992). They represent a heterogeneous group of oligomeric proteins that vary widely in size, structure, molecular organization, and constitution of their combining sites. In recent years, there have been several surveys on distribution of haemagglutinins in marine invertebrates from Brazil (Moura *et al.*, 2015) and the Philippines (Mojica *et al.*, 2005) and the variety of lectins have been purified and characterized from marine invertebrates (Moura *et al.*, 2006; Xiong *et al.*, 2006; Molchanova *et al.*, 2007; Kawsar *et al.*, 2009). Characterization studies reveal that animal lectins are classified into several categories: C-type lectins (CTLs), galectins, I-type lectins, pentraxins, P-type lectins, tachylectins, and so forth (Gabiuss, 1997). Among lectins isolated from marine invertebrates, lectins from the marine worms *Chaetopterus variopedatus* and *Serpula vermicularis* inhibited cytopathic effect induced by HIV-1 and could block the cell-to-cell fusion process of HIV infected and uninfected cells (Wang *et al.*, 2006); lectins from mussel *Crenomytilus grayanus* and acidium *Didemnum ternatanum* inhibited the HIV-1 IIIB-induced syncytium formation in C8166 cells and had activity against cellular fusion between the H9/HIV-1 chronically infected cells and the C8166 uninfected cells (Luk'yanov *et al.*, 2007). Thus, marine invertebrate lectins may promise as a potential candidate for the development of novel antiviral agents.

Vietnam is located in the tropical and subtropical zone with a long coast line of about 3,260 km where there is a rich marine biodiversity of algae, invertebrates, bacteria, viruses, etc. Such species may be potential sources for studies and application of lectins. However, only little information is known about lectins from Vietnam marine organisms, including several reports on lectins from marine invertebrates (Cao Dang Nguyen, 1998), (Cao Dang Nguyen, Nguyen Quoc Khang,

1998), (Cao Dang Nguyen *et al.*, 2000, 2008), (Nguyen Quoc Khang *et al.*, 2000) and from marine algae (Le Dinh Hung *et al.*, 2009a, 2009b, 2011, 2012, 2015a, 2015b). Thus, the objective of this research was to survey haemagglutinins from Vietnam marine invertebrates, which will provide more valuable information for future applications.

MATERIALS AND METHODS

Materials

Twenty one specimens of marine invertebrates including 11 bivalve and 10 gastropod species were collected at Khanh Hoa province of Vietnam from July, 2015 to May, 2016. After collection, they were immediately transferred to the laboratory on ice and kept at -20°C until used. The marine invertebrate samples were collected and identified by MSc. Bui Quang Nghi – Institute of Oceanography, Vietnam. Blood from rabbit, sheep and horse was obtained from the Institute of Vaccine – Nha Trang, Vietnam, and human A, B, and O blood were obtained from Khanh Hoa General Hospital, Vietnam. Transferrin, fetuin, porcine thyroglobulin, porcine stomach mucin (Type III), D-glucose, D-mannose, D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, N-acetyl-D-galactosamine and Tris (hydroxymethyl)-aminomethane (Tris) were purchased from Sigma Chemical Co. Yeast mannan and N-acetylneuraminic acid were obtained from Nakarai Chemical Co.

Preparations of invertebrate extracts and ammonium sulfate-precipitates

A 20 g sample of each invertebrate was cut into small pieces, homogenized for 1 min in a blender with 2 volumes of Tris-HCl buffer 20 mM, pH 7.5 containing NaCl 150 mM (TBS), and kept at 4°C for 12 h with occasionally stirring. After filtration through a cheese cloth, the filtrate was centrifuged at 10,000 rpm for 30 min. The supernatants were recovered and stored at -20°C until used. Haemagglutination tests were carried out with erythrocytes of various human and animals in a native state or enzyme-treated with trypsin and papain. To the supernatant (extract), solid ammonium sulfate was slowly added to attain 75% saturation. The mixture was gently stirred and then kept at 4°C for 18 h. Precipitates were recovered by centrifugation at 10,000 rpm for 20 min, dissolved in a small volume of TBS, and thoroughly dialyzed against the same buffer. The non-dialyzable fraction

was recovered as an ammonium sulfate-precipitate (Kawsar *et al.*, 2009). The ammonium sulfate-precipitates obtained were used for both haemagglutination-inhibition and stability tests.

Preparation of a 2% suspension of native or enzyme-treated erythrocytes

Each blood sample was washed three to five times with 50 volumes of saline. After washing, a 2% erythrocyte suspension (v/v) was prepared in saline and used as native erythrocytes. Trypsin- or papain-treated erythrocytes were prepared as follows. One-tenth volume of 0.5% (w/v) trypsin or papain solution was added to a 2% native erythrocyte suspension, and the mixture was incubated at 37°C for 60 min. After incubation, the erythrocytes were washed three to five times with saline and a 2% suspension (v/v) of trypsin- or papain-treated erythrocytes was prepared in saline.

Haemagglutination assay

Haemagglutination assays were carried out using a microtiter method in a 96-well microtiter V-plate (Le Dinh Hung *et al.*, 2009a). First, 25 µL of serially two-fold dilutions of a test solution were prepared in saline on a microtiter V-plate. To each well, 25 µL of a 2% erythrocyte suspension was added and the mixtures gently shaken and incubated at room temperature for 2 h. A positive result was indicated by formation of a uniform layer of coagulant over the surface of the well. On the other hand, a negative test result was indicated by the formation of a discrete "button" at the bottom of the well. Haemagglutination activity was expressed as a titer, the reciprocal of the highest two-fold dilution exhibiting positive haemagglutination. The assay was carried out in duplicate for each test solution.

Haemagglutination-inhibition test

Of the six erythrocytes used, human O erythrocytes were selected for haemagglutination-inhibition tests, using ammonium sulfate-precipitates according to the method previously described (Kawsar *et al.*, 2009). First, 25 µL of serially two-fold dilutions of sugar or glycoprotein were prepared in saline. To each well, an equal volume of extract solution with a haemagglutination titer of eight was added, and the plate was mixed gently and allowed to stand at room temperature for 1 h. Finally, 25 µL of a 2% suspension of trypsin-treated human O erythrocytes was added to each well, and the plate gently shaken and incubated for a further 1 h. Inhibition was observed macroscopically and

inhibition activity was expressed as the lowest concentration of sugar or glycoprotein at which complete inhibition of haemagglutination was achieved. The assay was performed in duplicate per sugar and glycoprotein. The following sugars and glycoproteins were tested: the monosaccharides: D-glucose, D-mannose, D-galactose, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, N-acetyl-D-galactosamine, and N-acetylneuraminic acid; and the glycoproteins: transferrin, asialo-transferrin, fetuin, asialo-fetuin, yeast mannan, porcine stomach mucin, asialo-porcine stomach mucin and thyroglobulin. Asialo-transferrin, asialo-fetuin and asialo-porcine stomach mucin were prepared by hydrolyses of their parent sialoglycoproteins with 0.05 M HCl for 1 h at 80°C followed by dialysis against saline overnight.

Effects on hemagglutination activity of divalent cations, pH, and temperature

To examine the effects of divalent cations on hemagglutination activity, a 1 mL aliquot of an extract solution was dialyzed at 4°C overnight against 100 mL of 50 mM EDTA in TBS. The non-dialyzable fraction was recovered and haemagglutination activity in the presence or absence of divalent cations (10 mM CaCl₂ or MgCl₂) was determined. To examine the effect of temperature, a 1 mL aliquot of an extract solution was heated at various temperatures (30 - 100°C) for 30 min, then immediately cooled on ice, and haemagglutination activity was determined as above. To examine the effect of pH, a 1 mL aliquot of an extract solution was dialyzed at 4°C overnight against 0.05 M buffers of various pH from 3 to 10 and then dialyzed against saline to eliminate the pH effect. The non-dialyzable fractions were assayed for haemagglutination activity. The following buffers were used; acetate buffer for pH from 3 to 5, Tris-HCl buffer for pH from 6 to 10 (Le Dinh Hung *et al.*, 2009a). Haemagglutination activity was determined with trypsin-treated human O erythrocytes.

RESULTS AND DISCUSSION

The results of the screening for haemagglutination activity from extracts of some marine invertebrates are summarized in table 1. The extract amount from marine invertebrate samples agglutinated the different types of erythrocytes expressed as % (Figure 1). Sheep erythrocytes gave the most detection of the haemagglutination activity of crude extracts, followed by rabbit, horse and human A, B, O blood erythrocytes.

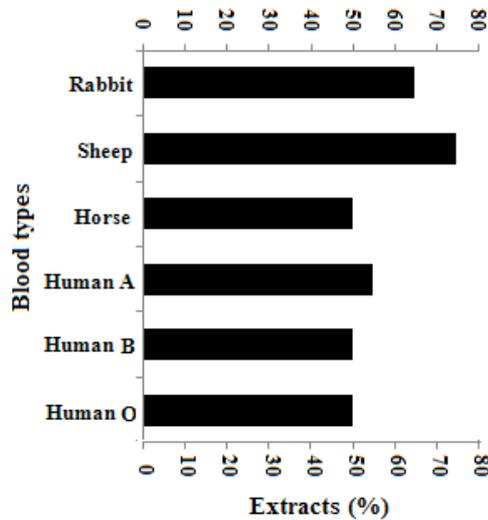


Figure 1. The extract amount agglutinated the different types of erythrocytes expressed as %.

Bivalves

Of the 11 bivalve species surveyed, extracts from 8 species agglutinated at least one type of erythrocytes tested (Table 1). Extract from *Tridacna squamosa* showed strong activities with trypsin- or papain-treated human A, B and O erythrocytes, whereas it did not agglutinate sheep and horse erythrocytes, even when erythrocytes were treated by enzyme. The extract from *Geloina coaxans* agglutinated all types of erythrocytes tested and showed also strong activities with trypsin- or papain-treated horse erythrocytes following by human A, B and O erythrocytes. Extract from *Atrina pectinata* had relatively activities with enzyme-

treated animal erythrocytes, but no haemagglutination was observed with the types of human erythrocytes irrespective of treatment with enzymes. In particular, the extracts from *Crassostrea bilineata*, *Spondylus squamosus* and *Periglypta crispate* did not agglutinate any type of human, rabbit, sheep and horse erythrocytes. Thus, the haemagglutination titers of the bivalve extracts were higher with animal erythrocytes than with human erythrocytes. These results were similar to those of screening for haemagglutination activity from Philippine marine invertebrates, which showed some haemagglutination activity against human red blood cell tested from bivalves (Mojica *et al.*, 2005).

Table 1. Haemagglutination activity of extracts from some Vietnam marine invertebrates.

Species	Haemagglutination titer of marine invertebrate extracts ^a																	
	Rabbit			Sheep			Horse			Human A			Human B			Human O		
	N _b	T ^c	P ^d	N	T	P	N	T	P	N	T	P	N	T	P	N	T	P
Mollusca																		
Bivalves																		
Arcidae																		
<i>Anadara antiquate</i> (Linnaeus, 1758)	- ^e	-	-	-	4	2	2	2	2	-	-	-	-	-	-	-	-	-
<i>Tegillarca nodifera</i> (Martens, 1860)	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-	-
Cardiidae																		

<i>Vasticardium elongatum</i> (Bruguière, 1789)	-	-	-	-	2	2	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tridacna squamosa</i> (Lamarck, 1819)	8	8	8	-	-	-	-	-	-	6	25	51	6	25	2	64	51	51
Corbiculidae										4	6	2	4	6	5	6	2	2
<i>Geloina coaxans</i> (Gmelin, 1791)	4	4	4	4	8	3	6	12	12	1	64	64	6	64	1	64	12	12
Mytilidae						2	4	8	8	6			4		2		8	8
<i>Perna viridis</i> (Linnaeus, 1758)	2	2	2	2	4	4	4	8	8	-	2	2	2	4	4	4	4	4
Ostreidae																		
<i>Crassostrea bilineata</i> (Röding, 1798)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pinnidae																		
<i>Atrina pectinata</i> (Linnaeus, 1767)	2	4	4	2	8	8	1	32	32	-	-	-	-	-	-	-	-	-
Spondylidae																		
<i>Spondylus squamosus</i> (Schreibers, 1793)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Veneridae																		
<i>Meretrix lyrata</i> (Sowerby, 1851)	-	-	-	-	2	2	-	-	-	-	-	-	-	-	2	-	-	-
<i>Periglypta crispata</i> (Deshayes, 1854)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gastropods																		
Babyloniidae																		
<i>Babylonia areolata</i> (Link, 1807)	8	8	16	4	6	6	8	32	32	2	2	2	-	-	-	2	2	2
Bursidae																		
<i>Tutufa rubeta</i> (Linnaeus, 1758)	4	32	32	1	1	6	3	64	32	8	64	64	4	4	4	16	12	12
Conidae																		
<i>Conus vexillum</i> (Gmelin, 1791)	4	4	8	3	6	6	1	64	16	4	8	4	4	4	4	4	4	4
Fascioliariidae																		
<i>Pleuroploca trapezium</i> (Linnaeus, 1758)	8	8	16	1	1	3	1	32	32	1	32	32	8	8	16	8	64	64
Haliotidae																		
<i>Haliotis ovina</i> (Gmelin, 1791)	-	-	-	-	-	-	4	16	16	-	-	-	-	-	-	-	-	-
Strombidae																		
<i>Harpago chiragra</i> (Linnaeus, 1758)	2	4	4	4	1	3	-	-	-	2	8	8	2	2	4	4	16	16

<i>Lambis lambis</i> (Linnaeus, 1758)	2	4	4	-	2	2	-	-	-	-	-	-	-	-	-	-	-	
Teligudae																		
<i>Tectus conus</i> (Gmelin, 1791)	6	51	51	3	6	6	-	-	-	8	64	64	3	32	32	12	51	51
	4	2	2	2	4	4							2			8	2	2
<i>Tectus pyramis</i> (Born, 1778)	-	16	16	4	4	4	-	-	-	4	8	8	-	-	-	2	4	4
Turbinidae																		
<i>Turbo petholatus</i> (Linnaeus, 1758)	-	4	4	-	-	-	8	16	16	2	2	2	-	-	2	-	-	-

Note: ^aHemagglutination activity was expressed as a titer, the reciprocal of the highest dilution showing positive hemagglutination. ^b Native erythrocytes; ^c Trypsin-treated erythrocytes; ^d Papain-treated erythrocytes; ^e Negative agglutination.

Gastropods

The extracts from 10 gastropod species surveyed agglutinated at least one type of erythrocytes tested (Table 1). The extract from *Tectus conus* agglutinated strongly with enzyme-treated rabbit and human A erythrocytes, but showed weak haemagglutination with sheep and human B, O erythrocytes. The extracts from *Haliotis ovina* and *Lambis lambis* agglutinated enzyme-treated animal erythrocytes, but showed no haemagglutination with human erythrocytes. The extract from *Tutufa rubeta* agglutinated strongly with enzyme-treated human A erythrocytes, but showed weak haemagglutination with the other types of erythrocytes, indicating that several gastropod species possessed human blood type specific lectins. The similar results had been reported from the survey for Philippine gastropod haemagglutination activity, which showed some agglutination activity against human red blood cell tested (Mojica *et al.*, 2005). The results suggest that Vietnam marine invertebrates may be good sources of useful lectins for many biological applications.

Sugar-binding specificities

From the 4 active species, carbohydrate-binding specificity of each extract was examined for haemagglutination–inhibition test with a series of sugars and glycoproteins using ammonium sulfate precipitates prepared from each extract. The haemagglutination activities of two extracts from *Tutufa rubeta* and *Pleuroploca trapezium* were not inhibited by any of the monosaccharides and glycoproteins examined (Table 2). On the other hand, haemagglutination activities of extract from *Tectus conus* were inhibited by porcine stomach mucin and its asialo derivative bearing O-glycans, but no inhibited by other glycoproteins bearing high mannose, complex and hybrid type N-glycans, such

as fetuin, transferin and thyroglobulin, indicating that this invertebrate species contains, at least, a lectin specific for O-glycans. Interestingly, extract from *Tridacna squamosa* appears to recognize the non-reducing terminal N-acetyl-D-galactosaminyl residue(s) of O-glycans, because its activity was strongly inhibited by porcine stomach mucin and its asialo derivative as well as by N-acetyl-D-galactosamine, D-galactose and N-acetyl neuraminic acid. The porcine stomach mucin contains O-linked carbohydrate structures sharing the core 1 Gal β 1–3GalNAc disaccharide, which can be substituted by N-acetylglucosamine branches terminated with fucose(α 1–2)-Gal (human blood group H), GalNAc(α 1–3)[Fuc α 1–2]Gal (human blood group A), or GlcNAc(α 1–4)-Gal at their non-reducing ends. Tn (GalNAc(α 1)-Ser/Thr) and T (Gal β (1–3)GalNAc α 1-Ser/Thr) antigens are also present in the porcine stomach mucin (Van Halbeek *et al.*, 1982; Karlsson *et al.*, 1997). In fact that elimination of sialic acid residues from porcine stomach mucin did not change inhibitory potential of parental glycoprotein, clearly indicating that the presence of N-acetyl neuraminic acid (NeuAc) as a terminal residue of the carbohydrate did not affect the haemagglutination activity of *T. squamosa*. The data suggest that extract from *T. squamosa* recognized preferentially the terminal GalNAc/Gal units in porcine stomach mucin (Figure 2) and this invertebrate species contains, at least, a lectin specific for O-glycans.

Inhibition by mucins bearing O-glycans that is related to GalNAc/Gal or GalNAc/NeuAc binding specificity reported for many lectins from marine invertebrates, such as tridacin from *Tridacna tridacna maxima* (Baldo *et al.*, 1978), PLC from Abalone *Haliotis laevigata* (Weiss *et al.*, 2000), PPL from Oyster *Ptereria penguin* (Naganuma *et al.*,

2006), BRL from Barnacle *Balanus rostratus* (Muramoto, Kamiya, 1986) and NnL from Jellyfish *Nemopilema nomurai* (Imamichi, Yokoyama, 2010). All of them bound GalNAc/Gal substituted through 1-3, 1-4, or 1-2 linkages in O-linked mucin-type glycans.

Table 2. Hemagglutination–inhibition test of marine invertebrate extracts with sugars and glycoproteins.

Sugars and glycoproteins	Samples			
	<i>Tridacna squamosa</i>	<i>Tectus conus</i>	<i>Pleuroploca trapezium</i>	<i>Tutufa rubeta</i>
<i>Sugars (mM)</i>				
D-mannose	^a -	25.0	-	-
D-glucose	-	-	-	-
D-galactose	6.25	-	-	-
N-acetyl-D-mannosamine	12.5	-	-	-
N-acetyl-D-glucosamine	12.5	-	-	-
N-acetyl-D-galatosamine	3.12	-	-	-
N-acetyl neuraminic acid	6.25	-	-	-
<i>Glycoproteins (µg/mL)</i>				
Porcine stomach mucin	7.81	125.0	-	-
Asialo-Porcine stomach mucin	7.81	125.0	-	-
Fetuin	-	-	-	-
Asialo-Fetuin	-	-	-	-
Transferin	-	-	-	-
Asialo-Transferin	-	-	-	-
Yeast Mannan	-	-	-	-
Thyroglobulin	-	-	-	-

Note: - ^a Indicates no inhibition at 100 mM for monosaccharides and at 2000 µg/mL for glycoproteins.

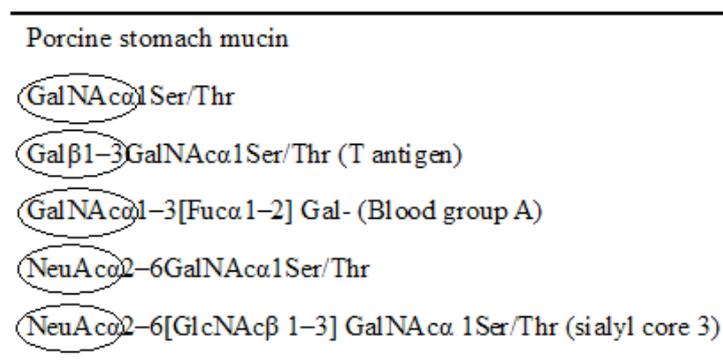


Figure 2. The putative epitopes recognized by haemagglutinin from *T. squamosa* in porcine stomach mucin are highlighted in oval. Gal: D-galactose; GalNAc: N-acetyl-D-galactosamine, NeuNAc: N-acetylneuraminic acid; GlcNAc: N-acetyl-D-glucosamine; Fuc: Fucose; Ser/Thr: Serine/Threonine.

Effects of divalent cations, pH, and temperature on haemagglutination activity

The effects of divalent cations, pH, and temperature on haemagglutination activity of the extracts from some active species listed in table 2 were examined. The haemagglutination activities of extracts from *T. rubeta* and *P. trapezium* were unchanged after being dialyzed against 50 mM EDTA and in the presence of 10 mM CaCl₂ or MgCl₂, indicating that haemagglutination activities of these extracts were not dependent on the presence of divalent cations. On the contrary, the haemagglutination activity of extracts from *T. squamosa* and *T. conus* were slightly decreased after being dialyzed by EDTA. Addition of CaCl₂ or

MgCl₂ at 10 mM concentration restored almost the total haemagglutination activity initially.

As shown in Figure 3a, the haemagglutination activities of *T. rubeta*, *P. trapezium*, *T. conus* and *T. squamosa* extracts were unchanged even when heated at 50°C for 30 min. However, their activities were significantly decreased as the incubation temperature exceeded 50°C and were completely lost by heating at 70°C for 30 min. Most of the marine invertebrate extracts examined maintained their activities over a wide range of pH values between 3 and 8 with a slight decrease in activity in more alkaline media, except for activity of *T. conus* extract was unchanged at a pH range between 3 and 10 (Figure 3b).

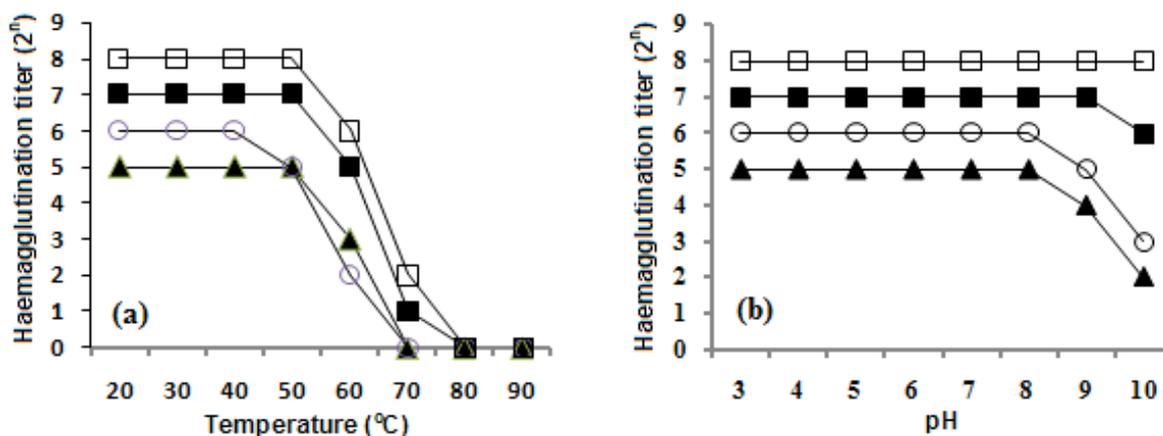


Figure 3. Effects of temperature (a) and pH (b) on haemagglutination activities of ammonium sulfate precipitates prepared from marine invertebrates. *Pleuroploca trapezium* (○), *Tutufa rubeta* (▲), *Tridacna squamosa* (■), *Tectus conus* (□). Values are means (there was no variance).

CONCLUSION

The 21 species of Vietnam marine invertebrates, including 11 bivalve and 10 gastropod species were examined for haemagglutination activity using native and enzyme-treated different animal and human erythrocytes. The 8 bivalve and 10 gastropod species were found to have haemagglutination activity toward at least one type of erythrocyte tested. A total of 86% of extracts from marine invertebrate species surveyed were active. The haemagglutination–inhibition tests indicated that extracts from *Tridacna squamosa* and *Tectus conus* might contain, at least, a lectin specific for O-glycans. On the other hand, the

haemagglutination activities of four marine invertebrate extracts were stable over a wide range of pH and temperature. Thus, Vietnam marine invertebrates could be promising sources of useful lectins for applications. The wealth of information obtained in the present study would be helpful for identification of new lectins and for clarification of the biological significance. Isolation and characterization studies on lectins from these marine invertebrates are currently underway.

Acknowledgements: This research was supported by Vietnam Academy of Science and Technology (VAST) under grant number VAST06.04/16-17. The

authors wish to thank MSc. Bui Quang Nghi – Institute of Oceanography, Vietnam for the identification of marine invertebrate samples.

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HOẠT TÍNH NGỪNG KẾT HỒNG CẦU CỦA CÁC DỊCH CHIẾT TỪ MỘT SỐ LOÀI ĐỘNG VẬT KHÔNG XƯƠNG SỐNG BIỂN VIỆT NAM

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TÓM TẮT

Dịch chiết từ 21 loài động vật không xương sống biển Việt Nam, bao gồm 11 loài động vật hai mảnh vỏ và 10 loài chân bụng, đã được kiểm tra hoạt tính ngưng kết máu với các dạng hồng cầu khác nhau của động vật và người ở dạng tự nhiên và dạng đã xử lý enzyme. 8 loài động vật hai mảnh vỏ và 10 loài chân bụng thể hiện hoạt tính ngưng kết với ít nhất một trong số các hồng cầu thử nghiệm. Tổng số 86% dịch chiết từ loài động vật không xương sống biển được dùng để khảo sát đã có hoạt tính ngưng kết với ít nhất một dạng hồng cầu. Dịch chiết từ 2 loài động vật hai mảnh vỏ (*Tridacna squamosa* và *Geloina coaxans*) và 3 loài chân bụng (*Tutufa rubeta*, *Pleuroploca trapezium* và *Tectus conus*) đã có hoạt tính ngưng kết mạnh với các dạng hồng cầu nhóm máu người A, B, O, thỏ và ngựa đã được xử lý enzyme. Trong thí nghiệm ức chế sự ngưng kết máu với các đường và các glycoprotein khác nhau, hoạt tính ngưng kết hồng cầu của hai dịch chiết từ *T. rubeta* và *P. trapezium* không bị ức chế bởi các đường đơn và các glycoprotein, trong khi đó hoạt tính của hai dịch chiết từ *T. squamosa* và *T. conus* bị ức chế mạnh bởi porcine stomach mucin, chỉ ra sự có mặt của lectin đặc hiệu cho O-glycan của các loài này. Hoạt tính ngưng kết hồng cầu của các dịch chiết từ *T. rubeta* và *P. trapezium* bền trong phạm vi rộng của nhiệt độ và pH, và không phụ thuộc vào sự có mặt của cation hóa trị hai, trái lại hoạt tính ngưng kết hồng cầu của hai dịch chiết từ *T. squamosa* và *T. conus* phụ thuộc nhẹ vào sự có mặt của cation

Journal of Biotechnology **15**(4): 691-701, 2017

hóa trị hai. Kết quả gợi ý rằng động vật không xương sống biển Việt nam có thể là một nguồn lectin giá trị cho sử dụng trong hóa sinh và y sinh.

Từ khóa: *Đặc tính liên kết carbohydrate; Độ bền; Động vật không xương sống biển; Hoạt tính ngưng kết hồng cầu; Khảo sát; Lectin*