CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF HEMAGGLUTININS FROM DIFFERENT PARTS OF GARLIC PLANT (ALLIUM SATIVUM)

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SUMMARY

Aqueous extracts from bulb, leaf and root of garke plant collected at Vanhung-Vannink, Khanbhos were examined for hemagglutination activity using native and enzyme-treated different animal and human crythrocytes. All extracts agglutinated at least one type of crythrocytes itseld. Activity was detected in extracts from garlie bulb, leaf and root with enzyme-treated rabbit crythrocytes. The hemagglutinins of active species were examined for sugar-binding specificity with various monosacchardes and glycoproteins, pH, temperature stability, and effect of divalent cations using ammonium sulfate precipitates prepared from their extracts. Activity of the hemagglutinins was not inhibited by monosacchardes, but was mibilited by some glycoproteins tested. The inhibition profiles with glycoproteins were different depending on hemagglutinin species, activity of hemagglutinins from garlie leaf and bulb was inhibited by tigh-mannose type *N*-glycans, whereas activity of hemagglutinins from garlie to was inhibited by complex type *N*-glycans, indicating that each part of garlie plant contains different lectins. On the other hand, the activities of hemagglutinins were stable over a wide range of pH and temperature, and independent of the presence of divalent cations. Hemagglutinins from garlie cations used in the heaterion. *Enterobacter cloacae*, bud did not affect other bacteria examined. These obtained results suggest that the parts of garlie plant may be good sources of useful lectus for many biological applications.

Keywords: Alluum sattvum, Annbacterial activity, Carbohydrate-binding specificity, Garlic plant, Hemagglutinins, Lectins, Stability.

INTRODUCTION

Lectins are carbohydrate-binding proteins that specifically recognize diverse sugar structures and thus mediate various biological processes, viz., cellcell and host-pathogen interactions, and serum glycoprotein turnover besides innate immune responses (Vijayan, Chandra, 1999). Lectins are known to occur in most of the organisms ranging from viruses and bacteria to plants and animals (Lis, Sharon, 1998). They represent a heterogenous group of oligomeric proteins that vary widely in size, structure and molecular organization. Lectins are found predominantly as storage proteins in planta. Commonly plant lectins are found in abundance in seeds and bulbs, although they may also be found in the leaves of some plants. The content of lectin varies in different organisms. The high yields of lectins from different sources may facilitate mass production. Application of lectins is possible depending on their properties. The anti-fungal and anti-insect activities of lectins can be made use in the control of pathogens. The production of anti-tumor and anti-viral drugs based on lectins may also be feasible (Lam, Ng, 2011).

Most commonly consumed plant foods contain lectins, and many of these foods are eaten raw, while others contain lectins that are active even after cooking and processing. A delicate equilibrium exists within the alimentary canal between lectins, dictary saccharides, immunoglobulins, viruses, bacteria and host cells (Kilpatrick, 1999). Garlic is well known across the centuries. It was used as a medicine by early civilizations (Rivilin, 2006). Garlic is widely consumed as an important spice all over the world. The health-beneficial effects of garlic (Allium sativum) have long been known and garlic has a reputation for its efficacy as an anti-microbial, anti-oxidant, anti-carcinogenic, anti-mutagenic, antihypertensive and immuno-modulatory agent (Corzo-Martinez et al., 2007). The anti-cancer effects of garlic are being extensively researched and most of these effects have been attributed to the organosulfur commounds such as dially sulfide, dially disulfide, diallyl trisulfide. S-allyleysteine or S., allylmercaptocysteine (Ross, Milner, 2006), Recent findings have shown that the anti-tumor effect of allyl sulfur compounds may be related to their antiinflammatory as well as immune-stimulatory properties (Iciek et al., 2009). Furthermore, one of the most important direct defense responses in plants against the attack by phytophagous insects is the production of insecticidal pentides or proteins. One particular class of entomotoxic proteins present in many plant species is the group of carbohydratebinding proteins or lectins (Vandenborre et al., 2011). Among the plant lectins, lectins exhibiting either mannose or mannose/glucose sugar binding affinity have been interested greatly, including Galanthus nivalis agglutinin. Concanavalin A and Pisum sativum agglutinin, which revealed palpable antimetabolic effects towards members of the homopteran insects both under in vitra (Rahbe et al., 1995) as well as in planta conditions (Powell et al., 1995; Gatehouse et al., 1996). Among the mannosebinding lectures. G. nivalis agglutinin (GNA) has been widely studied and introduced into different plants, viz, rice, wheat and tuber crops (Nagadhara et al., 2003, 2004; Gatchouse et al., 2003). Transgenic plants expressing GNA showed significant entomotoxic effects as evidenced by insect bioassays under controlled conditions (Couty et al., 2001). Similarly, bioassays based on artificialdiet-feeding system, using mannose-specific lectin from Allium sativum agglutinin showed antimetabolic effects towards green leafhopper and brown planthopper insects (Majumder et al., 2004). Expression of garlic lectins in tobacco conferred resistance against tobacco aphid and cotton leaf worm, respectively (Sadeghi et al., 2007, 2008). The transgenic rice lines expressing garlic leaf lectin gene was also reported for exhibiting increased resistance against green leafhonner and brown planthopper pests (Saha et al., 2006). Recently, the gene encoding lectin from garlic leaf was introduced into transgenic indica rice and exhibited surpassing resistance against green leafhopper. brown planthopper and whitebacked planthopper insects (Yarasi et al., 2008). Thus, garlic plant may be a dominant sources of useful lectins for basic research and application in biomedicine and transgenic plants.

Garlic plant Allium sativum has been cultivate in many countries as the valuable sources of many and medicine. In Vietnam, garlic plant has be cultivating extensively at Bacninh, Vinhol Haiduong, Lyson-Quangngai, Ninhhoa-Khanha Ninhhai-Ninhthuan with the production of 2.000 to per year. Those garlic species may contribute as source of not only attractive spice but also onbioactive compounds for biochemical and medicina uses. On the other hand, in the harvested process is garlic bulbs were only collected, whereas their ba and root were nearly all removed, where they me contain useful bioactive compounds for variant applications. The objective of this research was a biochemically characterize the hemagglutining and biological activity obtained from different parts of garlic plant for future applications.

MATERIALS AND METHODS

Materials

Garlic plants Allium sativum were collected # Vanninh District, Khanhhoa Province, Vietnamin March, 2014. After collection, the bulb, leaf and row was cut, separately, and were kept at - 20 °C until being used to extract protein. Blood from rabbit and chicken was obtained from the Institute of Vaccine Nhatrang, Vietnam, and human A-, B-, and O-type blood was obtained from Khanhhoa General Hospital, Vietnam. Transferrin, fetuin, asialo-fetuin, porcine stomach thyroglobulin and boym submaxillary mucin were purchased from Sigma fst Louis, MO). Yeast mannan was from Nakeral Chemical (Kvoto, Japan), All other chemicals used in this study were of the highest purity available. So species of human pathogenic bacteria Staphylococcus aureus, Pseudomonas aeruemos Escherichia coli, Enterobacter cloace, Bacillui cereus and Streptococcus facalis were obtained from Institute of Pasteur-Nhatrang. Three species of shrimp pathogenic vibrios, Vibrio alginolvica Vibrio parahaemolyticus and Vibrio harvevi wet obtained from Institute of Aquaculture Research No 3, Nhatrang, Victnam.

Preparation of extracts and ammonium sulfatt

A 100 g sample of each part of garlic plant (bulk leaf and root) was homogenized for 1 min in a bleade with 6 volumes of 0.02 M phosphate buffer, plf 7/ containing 0.85% NaCl (PBS), and kept # 4°C for 12 h with occasionally stirring. After filtration through a cheese cloth, the filtrate was centrifuged at 8.000 rpm for 10 min. The supernatants were stored at -20 °C until used. Hemagglutination tests were carried out with erythrocytes from human and various animals in a native state or enzyme-treated with trypsin and napain. 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. The precipitates were recovered by centrifugation at 6.000 rom for 30 min, dissolved in a small volume of PBS, and thoroughly dialyzed against the same buffer overnight. The nondialvzable fraction was recovered as ammonium sulfate precipitates used for hemagglutination-inhibition, stability and biological activity tests.

Preparation of a 2% suspension of native and 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 papaintreated erythrocytes were prepared as follows. Onetenth 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 (Hung *et al.*, 2009a).

Hemagglutination assay

Hemagglutination assays were carried out using a microtiter method in a 96-well microtiter V-plate (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% crythrocyte suspension was added and the mixtures gently shaken and incubated at room temperature for 2 h. Hemagglutination was observed macroscopically and judged as positive in the case that more than 50% of crythrocytes in the well were agglutinated. Hemagglutination activity was expressed as a titer, the reciprocal of the highest two-fold dilution exhibiting positive hemagglutination. The assay was carried out in triplicate for each test solution.

Hemagglutination-inhibition test

Hemagglutnation-inhibition tests were carried out using ammonium sulfate-precipitates according to the method previously described (Hung *et al.*, 2009a). First, 25 μ L of serially two-fold dilutions of sugar or glycoprotein were prepared in saline. To each well, an equal volume of a hemaggluting solution with a hemagglutination titer of four 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 rabbit 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 hemagglutination was achieved. The assay was performed in duplicate per sugar compound The following sugars and elycoproteins were tested: the monosaccharides Dglucose, D-mannose and N-acetylneuraminic acid (sialic acid): and the glyconroteins transferrin. asialo-transferrin, fetuin, asialo-fetuin, yeast mannan, porcine stomach thyroglobulin, boyine submaxillary mucin, and asialobovine submaxillary mucin. Asialo-transferrin and asialobovine submaxillary mucin were prepared by hydrolyses of their parent staloglycoproteins with 0.05 M HCl for 1 h at 80°C followed by dialysis against saline overnight.

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

To examine the effects of divalent cations on hemagglutination activity, a 1 mL aliquot of a hemagglutinin solution was dialyzed at 4°C overnight against 100 mL of 50 mM EDTA in PBS. The non-dialyzable fraction was recovered and hemagglutination 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 a hemagglutinin solution was beated at various temperatures (from 30 to100 °C) for 30 min. immediately then cooled on ice. and hemagglutination activity was determined as above. To examine the effect of pH, a 1 mL aliquot of a hemagglutinin solution was dialyzed at 4°C overnight against 0.05 M buffers of various pH (from 3 to10) and then dialyzed against saline to eliminate the pH effect. The non-dialyzable fractions were assayed for hemagglutination activity. The following buffers were used; acetate buffer for pH 3, 4 and 5, phosphate buffer for pH 6 and 7, Tris-HCl buffer for pH 8 and carbonate buffer for pH 9 and 10. Hemagglutination activity was determined with trypsin-treated rabbit erythrocytes (Hung et al., 2009a).

Protein contents

Protein contents were determined by the method of Lowry *et al.* (1951) using bovine scrum albumin as a standard.

Screening on antibacterial activity

The antibacterial assay of ammonium sulfateprecipitates from each part of garlic plant was performed by the agar disc diffusion method (Parekh, Chanda, 2006). The bacteria were grown in medium containing nutrient broth and yeast malt broth, and incubated overnight in a shaker 37 °C. The cell concentration of bacteria was determined by measuring the suspension turbidity at 600 nm, and converted to colony forming units (105-106 CFU/mi) using a calibration curve. As a positive reference. ampicillin solution (1.0 mg/mL) in a medium was examined in the same way. As a negative control without both precipitate and ampicillin was incubated in the similar way. The disc (0.6 cm) was saturated with 20 uL of the test compound or ampicillin. allowed to dry and then placed on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm) surrounding bacterial growth.

RESULTS AND DISCUSSION

Hemagglutination activity from different parts of garlic plant

Extracts from three parts of garlic plant showed relatively strong activities with enzyme-treated rabbit crythrocytes, but no hemagglutination with the other types of erythrocytes irrespective of treatment with enzymes (Table 1).

Sugar-binding specificities

From the 3 species, carbohydrate-binding specificity of each hemagglutination-inhibition test with a series of sugars and glycoproteins

using annmonium sulfate precipitates prepared from each extract. The hemagglutination activities of these hemagglutinitis were not inhibited by any of the monosaccharides examined. On the other hand, their hemagglutination activities were inhibited by glycoprotens bearing high manose N-glycans and complex N-glycans. The hemagglutinationinhibition profiles with a variety of glycoproteins dirffered depending on hemagglutinin species

(Table 2). The hemagelutination activity of garlie hulh and leaf was inhibited by yeast mannan and asialo-porcine thyroglobulin, both have high mannose N-glycans in molecular structures. Porcise thyroglobulin did not exhibit any inhibitory activity even at concentration of 2 mg/mL. This glycoprotein bears both high-mannose type (unit A-type) and complex type (unit B-type) oligosaccharides. Amonif the unit B-type, the major N-glycans contain at least 9 different structures consisting of mono- and disialvlated (a1-6) fucosvlated bi-, triantennary structures terminated either with $(\alpha 2-3)$ or $(\alpha 2-68)$ linked stalic acid or (B1-4)-linked galactose residue (Yamamoto et al., 1981). The elimination of sialic acid residues of porcine thyroglobulin enhanced inhibitory potential of parental glycoprotein, clearly inducating that the presence of sialic acid as a terminal residue affected the activity of the carbohydrate toward these hemagglutinins. The yeast mannan, which bears high mannose N-glycans with the $(\alpha 1-6)$ linkage in its backbone and $(\alpha 1-3)$ linkage in this side chains, showed strong inhibitory activity toward three hemagglutinins. The data indicate that the garlic bulb and leaf each contain at least a lectin specific for high-mannose type N-glycans, especially hemagglutinin from the garlic leaf showed the strongest high-mannose binding specificity among three hemagglutining examined (Table 2). Practical the mode of molecular recognition toward high mannose type N-glycans has also been intensively reported for lectins from various sources, such as lectin from bacterium Pseudomonas fluorescent (Sato et al., 2012), the legume lectin ConA (Naismith, Field, 1996), the monocot mannosebinding lectins (Barre et al., 1996), lectins from garlic bulb and leaf (Dam et al., 1998), lectins from the cultivated macroalgae (Hori et al., 2007; Hung et al., 2009b, 2011, 2014), and lectins from cyanobacteiria (Ziólkowska, Wlodawer, 2006). All them showed the different binding affinity in the structures of branched oligomannosides of highmannose type N-glycans. In contrast, the hemagglutinin from garlic root showed distinct carbohydrate binding specificity compared with that of the hemagglutinins from the garlic bulb and leaf. The hemagglutination activity of hemagglutinin from garlic root was inhibited by asialo-fetuin, porcine thyroglobulin and its derivative, but was not inhibited by yeast mannan even at concentration of 2 mg/mL. Fetun bearing both complex type N-glycans and O-glycans and porcine stomach mucin bearing O-glycans were no inhibitory, whereas asialo-fetuin was moderately inhibitory, indicating that that the

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presence of sialic acid as a terminal residue prevented interaction between hemagglutinin and fetuin (Figure 1). The data indicate that the garlic root contains, at last, a lectin specific for complex type N-glycans that can recognize the terminal Gal (β1-4) residues. The hemagglutination-inhibition profiles are diverse, depending on the originating species. The results suggest the presence of lectims specific for high mannose N-glycans and complex Nglycans in parts of garlic plant.

Table 1. Hemagglutination activities of extracts from parts of garlic plant. The hemagglutination activity is expressed as a titer that is the reciprocal of the highest two-fold dilution exhibiting positive agglutination.

Species	Rabbit			Chicken			Human A			Human B			Human O		
	Na	Tb	Pc	N	т	P	N	т	P	N	Т	P	N	т	P
Garlic bulb	-	16	4	-	-	-	-	-	-	-	•	-	-	-	
Gerlic leaf	;	32	4							-					
Garlic root	- 1:	26 5	i12	-	-	•				-		-	-	-	

* Native erythrocytes. * Trypsin-treated erythrocytes. * Papain-treated erythrocytes. - No hemagglutination.

Table 2. Hemagglutination-inhibition test of the hemagglutinins from parts of garlic plant Values indicate the lowest concentration of sugar (mM) and glycoprotein (µg/mL) at which complete inhibition of hemagglutination (titler 4) was achieved.

Sugar & glycoprotein	Garlic bulb	Garlic leaf	Garlic root
Sugar (mM)			
Monosaccharides *			
Glucose			
Mannose			
Sialic acid			
Glycoprotein (µg mL*)			
Transferrin			
Fetuin			
Asialo-fetuin			250.0
Pocine thyroglobuiin			125 0
Asialo-pocine thyroglobulin	500.0	125 0	166 6
Yeast mannan	500 0	62.5	
Porcine stomach mucin			
Asialo-Porcíne stomach mucin			

* The monosaccharides examined are described in Materials and methods.

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





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

The effects of pH and temperature on hemagglutination activity of the hemagglutinins from some active species shown in Figure 2. Most of hemagglutination activities of these the hemagelutining were unchanged after being dialyzed against 50 mM EDTA and in the presence of 10 mM CaCl₂ or MgCl₂. Addition of CaCl 2 or MgCl₂ at 10 mM concentration restored almost the total hemagelutination activity initially. The hemagglutination activities of hemagglutinin from garlic bulb was also thermostable because its activity was unchanged by heating at 90 °C for 30 min, however, the activity slightly decreased a incubation temperature exceeded 90 °C (Figure 2a). On the other hand, the hemagglutination activities of hemagglutinin from garlic leaf and root were beat labile because the activities was significantly decreased as the uncubation temperature exceeded 50 °C and 60 °C, respectively (Figure 2a).

Three hemagglutinins from parts of garlic plan examined maintained their activities over a range of pH values between 5 and 8 with a slight diccrease a activity in more acidic and alkaline media. The activity of hemagglutinin from garlic bulb we unchanged at a pH range between 3 and 9 (Figure 24)



Figure 2. Effects of temperature (a) and pH (b) on hemagglutination activities of ammonium sulfate precipitates prepared 🐐

Antibacterial activity

The antibacterial activity of hemagelutinins from parts of garlic plant was examined with six human species of pathogenic hacteria Staphylococcus aureus. Pseudomonas aeruginosa. Escherichia coli, Enterobacter cloace, Bacillus cereus and Streptococcus facalis and 3 species of shrimp pathogenic marine bacteria Vibrio harvevi alginolyticus and V. parahaemolyticus. V. Hemagelutinin from garlic bulb inhibited the growth of Enterobacter cloacae with the diameter of the inhibitory zone was 11 mm at 74.76 mg/mL concentration, whereas hemagglutinin from garlic leaf showed the inhibitory zone of 12 mm at 10.82 mg/mL concentration, which was much lower than that of hemagglutinin from garlic bulb (Figure 3).

Three hemagelutining did not affect the growth of other bacteria tested. Standard ampicillin (1.0 mg/mL) was also used along with the hemagglutinins for comparison, where it showed the diameter of the inhibitory zone was 12 mm. The degrees of inhibition at 10.82 mg/mL concentrations of hemagglutinin from garlic leaf was comparable to that of ampicillin (1.0 mg/mL). although the chemical structures of these compounds are clearly distinct. The antibacterial activities have been reported for lectins from various biological sources, sush as lectin from Araucaria angustifolia seed showed antibacterial activity against Gram-negative and Gram-positive strains (Santi-Gadelha et al., 2006), lectin from Bufo arenarum skin had strong activity against Gram negative bacteria (Escherichia coli Kl?

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4100 and wild strains of Escherichua coli and Proteus morganii) and Gram positive bacteria (Enterococcus faecalis) (Riera et al., 2003) or lectin from Archidendron Jiringa seed showed activity against Bacillus subtilis, Staphylococcus aureus, and Candida albicans (Charungchutrak et al., 2011). Therefore, the mode of bacteriaaeglutination lectins are diverse, depending on the originating species. The result suggests that bacterium E. cloacae have mannoside or mannoside-like structure(s) on the cell surface which might respond as a receptor(s) for bemagglutinins. Thus, the garlic leaves are promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria.



Figure 3. Antibacterial activity of hemagglutinins from gartic leaf (a) and bulb (b) against Enterobacter cloace

CONCLUSION

Garlic plant Allium sativum has been widely cultivated in Vietnam as the valuable sources of spice and medicine. Hemagglutinins from parts of garlic plant had no affinity for monosaccharides but instead for glycoproteins bearing N-glycans. Their hemagelutination activity was stable over a wide range of pH and temperature and was not affected by divalent cations. Carbohydrate binding specificity of hemagglutining from parts of garlic plant showed distinction among them, hemagglutinins from garlic leaf and bulb recognized high-mannose type Nglycans, whereas hemagelutining from garlic root bond to complex type N-glycans, indicating that each part of garlic plant contains different lectins. Hemagelutinin from garlic leaf and bulb inhibited the growth of bacterium Enterobacter cloace, but did not affect other bacteria examined. These obtained results suggest that the parts of garlic plant may be good sources of useful lectins for many biological applications including as an anti-bacterial reagent.

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REFERENCES

Barre A, Van Damme EJM, Peumans WJ, Rouge P (1996) Structure-function relationship of monocot mannosebinding lectins. *Plant Physiol* 112: 1531-1540.

Charungchtrak S, Petsom A, Sangvanich P, Karnchanatat A (2011) Antriungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Nielsen. Food Chemistry 126: 1025-1032.

Corzo-Martmez M, Corzo N, Villamiel M (2007) Biological properties of omons and garlie *Trends Food Sci Tech* 18: 609–625.

Couty A, Down RE, Gatehouse AMR, Kaiser L, Pham-Delegue MH, Poppy GM (2001) Effects of artificial diet containing GNA and GNA expressing potatoes on the development of the apht parasitoid Aphidius ervi haiday (Hymenopter: Aphididae). Jo Jinsect Physiol 47: 1357-1366.

Dam TK, Bachyhawat K, Rani PG, Surolia A (1998) Garlic (Allium sativum) lectins bind to high mannose oligosaccharide chains. J Biol Chem 273: 5528-5535.

Gatchouse AMR, Down RE, Powell KS, Sauvion N, Rahbe Y, Newell CA, Merryweather A, Hamilton WDO, Gatchouse JA (1996) Transgenic potato plants with enhanced resistance to peach-potato aphild Myzus persicae. Entromol Exp Appl 79: 295-307.

Gatehouse JA, Reddy VD, Rao KV (2003) Transgenic indica rice resistant to sap sucking insects. *Plant Biotechnol J* 1: 231-240. Hung LD, Hori K, Nang HQ (2009a) Screening and preliminary characterization of hemagglutinins in Vietnamese manne algae, *J Appl Phycol* 21: 89-97.

Hung LD, Sato T, Shubata H, Hori K (2009b) Biochemical comparison of lectins among three different color strains of the red alga Kappaphycus alwarezii. Fish Sci 75: 723–730.

Hung LD, Sato Y, Hori K (2011) High-mannose N-glycanspecific lectins from the red alga Kappaphycus striatum (Carrageenophyte). Phytochemistry 72: 855-861.

Hung LD, Hirayama K, Ly BM, Hori K (2014) Purification, primary structure and biological activity of high-manose N-glycan-specific lectin from the cultivated *Euchsuma denticulatum*. J Appl Phycol. DOI 10.1007/s10811-014-0441-0.

Hori K, Sato Y, Ito K, Fujiwara Y, Iwamoto Y, Makino H, Kawakubo A (2007) Strict specificity for hugh-mannose type N-glycans and primary structure of a red alga *Eucheuma serra* lectin. Glycobiology 17: 479–491.

Iciek M, Kwiecien J, Wlodek L (2009) Biological properties of garlic and garlic-derived organosulfur compounds. Environ Mol Mutagen 50: 247-65.

Kilpatrick DC (1999) Immunological aspects of the potential role of dietary carbohydrates and lectins in human health. *Eur J Nutr* 38: 107–117.

Lam SK, Ng TB (2011) Lectins: production and practical applications. Appl Microbiol Biotechnol 89: 45-55.

Lis H, Sharon N (1998) Lectins carbohydrate-specific proteins that mediate cellular recognition. *Chem Rev* 98: 637-674.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275.

Majumder P, Banerjee S, Das S (2004) Identification of receptors responsible for binding of the mannose specific lectin to the gut epithelial membrane of the target insects. *Glycoconj* J 20: 525-530.

Nagadhara D, Ramesh S, Pasalu IC, Rao YK, Krishnaiah NV, Sarma NP, Bown DP, Gatehouse JA, Reddy VD, Rao KV (2003) Transgenic indica nce resistant to sap sucking insects. *Plant Biotechnol J* 1: 231-240.

Nagadhara D, Ramesh S, Pasalu IC, Rao YK, Sarna NP, Reddy VD, Rao KV (2004) Transgenic rice plants expressing the snowdrop lectin gene (gna) exhibit highlevel resistance to the whitebacked planthopper (*Sogatella furcifera*). Theor April Genet 109: 1399-1405.

Naismith JH, Field RA (1996) Structural basis of trimannoside recognition by Concanavalin A. J Biol Chem 271: 972-976.

Parekh J, Chanda S (2006). In vitro antimicrobial activities of extracts of Launaea procumbens Roxb. (Labiateae), Vitis vimifera L. (Vitaceae) and Cyperus rolundus L (Cyperaceae), Afr J Biomed Res 9: 89-93.

Powell KS, Gatebouse AMR, Hilder VA, Peumans W, Van Damme EJM, Boonjawat J, Horsham K, Gatebous JA (1995) Different antimetabolic effects of related plac lectin towards nymphal stages of *Nilaparvata lugen Entomol Exp Appl* 73: 61-65.

Rahbe Y, Sauvion N, Febvay G, Peumans WJ, Gateboas AMR (1995) Toxicity of lectins and processing of ingests proteins in the pea aphid Acyrthosiphon pisum. Enloss Exp Appl 76: 143-155.

Riera AS, Daud A, Gallo A, Genta S, Aybar M, Sanchers (2003) Antibacterial activity of lactose-binding lecting from *Bufo arenarum* skin. *Biocell* 27: 37-46.

Rivlin RS (2006) Is garlic alternative medicine? J. Nut 136: 713-715.

Ross SA, Milner JA (2006) Garlic: the mystical food is bealth promotion. In: Wildman REC, editor. Handbook of nutraceuticals and functional foods 2nd Edition. Boa Raton, FL: CRC Press, p 73–99.

Sadeghi A, Broeders S, De Greve H, Hernalsteens JF, Peumans WJ, Van Damme EJM, Smagghe G (2007) Expression of garile test lectin under the control of the phloem-specific promoter Asus1 from Arabidopsis thaliane protects tobacco plants against the tobacco aphild (Mya nicolanae). Pest Management Science 63. 1215-1223.

Sadeghi A, Smagghe G, Broeders S, Hernalsteens JP, De Greve H, Peumans WJ, Van Damme EJM (2008) Ectopically expressed leaf and bulb lections from gaits (Allium sativum L.) protect transgenic tobacco plant against cotton leafworm (Spodoptera liitoralia). Transgenic Res 17: 9-18.

Saha P, Majumder P, Dutta I, Ray T, Roy SC, Das S (2006) Transgenic rice expressing *Allium sativum* lef lectin with enhanced resistance against sap-sucking inset pests. *Planta* 223: 1329-1343

Santi-Gadelha T, Gadelha CAA, Aragão KS, Oliveira CC, Mota MRL, Gomes RC (2006) Purification and biologial effects of *Araucaria angustifolia* (Araucariaceae) sted lectin. Biochem Biophys Res Commun 350: 1050-1055.

Sato Y, Mornmoto K, Kubo T, Yanaguhara K, Seyana T (2012) High manose-binding antiviral lectin PFL fora *Pseudomonas fluorescens* Pf0-1 promotes cell death of gastric cancer cell MKN28 via interaction with α2-integra *PLos One* 7: e45922.

Vandenborre G, Smagghe G, Van Damme EJM (2011) Plant lecturs as defense proteins against phytophagous insects. *Phytochemistry* 72: 1538-1550.

Vijayan M, Chandra N (1999) Lectins, Curr Opin Struct Biol 9: 707-714.

Yamamoto K, Tsuji T, Irimura T, Osawa T (1981) The

structure of carbohydrate unit B of porcine thyroglobulin J Biochem 195: 701-713.

Yarasi B, Sadumpati V, Immanni CP, Vudem DR, Khareedu VR (2008) Transgenic rice expressing Allium sativum leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. BMC Plant Biology doi:10.1186/1471-2229-8-102.

Ziółkowska NE, Włodawer A (2006) Structural studies of algal lectins with anti-HIV activity. Acta Biochim Pol 53: 617-626.

MÔ TẢ ĐẶC TÍNH VÀ HOẠT TÍNH SINH HỌC CỦA HEMAGGLUTININ TỪ CÁC BỘ PHẬN CỦA CÂY TÔI (*Allium Sativum*)

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TÓM TẤT

Dịch chiết từ các phần củ, là và rể của cây toị được thu ở Huyện Vạn Ninh, Tinh Khánh hoà đá được kiểm ra hoạt tỉnh ngung kết mảu với hồng của người và động vật ở cả dạng tri nhiện và đugg đã được xử lý enzyme. Tất cả địch chiết thu được đã ngung kết với it nhất một đạng hồng cầu đưở được sử dụng. Hoạt tính mạnh đã được phát thiện trong các địch chiết từ củ, là vì rể của tôi với hồng cầu đưở được xử lý enzyme. Tất cả địch chiết trừ củ, là và rể của tôi với hồng cầu đưở được xử lý enzyme. Đảng các đường đơn và các glycoprotein khác nhau, khủ săng bên với nhiệt độ, PH và ảnh hương của caton hóa trị hai đến hoạt tính quơng kết hông của. Hoạt tình của các hemzgultimin không bị và chiế bởi các đường đơn các đường đơn và các để bị của chiết của dực chiến sử hệng nguyên tri như của hemaggiturin trừ là và tế chiết thủ trừ cả trả các chiết thủa thếng bị các đường đơn những đảo lực chế bởi nguyên để bị từ chiết của thực thực vào mấu hemaggiturini. Hoạt tính của hemaggiturini trừ là và là bị thủa chế ôn Aguyên dang hịch-mannoace, trong khi để hoạt tính của hemaggiturini trừ là và là bi thư chế để việt triể các các chiết thuế triể các là chiết thủa triể được vào các chiết thủa các hemaggiturini bến trong một phạm vì của nhiệt độ, pH và không phư nhưởc vào catton hóa tri hải. Các hemaggiturini bến trong một phạm vì của nhiệt độ, pH và không *hu* choác ter cloace, nhưng không ức chế sự phát triển của sác vi khuẩn khác đấ dược kiếm tra. Kết quả thư được trong nghiên cứu đã chủ đác chiến gia chế triển các

Từ khóa: Allum sativum, Cây tôi, Đặc tỉnh liên kết Carbohydrate, Đô bền, Hoạt tính kháng khuẩn, Hemagglutının, Lectin

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