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### RESEARCH ARTICLE

#### CHARACTERISTICS OF ANTIMICROBIAL PEPTIDES ORIGINATED FROM INVERTEBRATES INCLUDING INSECT.

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#### Abstract

Most AMPs discovered in invertebrates were found in arthropods including insect, and antibacterial activity was discovered by specifically high frequency against Gram positive bacteria. The length of peptides discovered the highest amount in 31~40 peptides, and it maintained comparatively higher levels in 11~50 peptides. The secondary structure of peptides showed the highest value in alpha-helix, but very lower amounts in beta-sheet and coil form. As net charge was ranged from +1 to +7, it was observed to have a net charge slightly higher than general AMPs. Aliphatic index was observed to have the largest amount between 70 and 80, which more than half of the peptides were found to form a stable structure. Therefore, it is suggested that AMPs found in invertebrates have characteristics similar to AMPs discovered in all the organisms.

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#### Introduction:-

All the organisms secrete primary protective substances for their own defense. One of such protective substances is antimicrobial peptides (AMPs). It is known that AMPs play a role as an important protective tool in insect to have not an acquired defensive mechanism, and have the function to induce AMPs as a defense substance against externally invading organisms. Cellular and humoral innate immune mechanisms are retained in animal kingdom (Lazzaro, 2008), and it is regulated by a signal pathway activated by various stimuli. Cellular immune responses of insect induce phagocytosis, nodulation and encapsulation (Schmidt and Theopold, 2001; Nappi et al., 2004; Lamprou et al., 2005; Sideri et al., 2008), whereas humoral response is associated with pro-phenol oxidase proteolytic cascade (Bulet et al., 2008; Bulet and Stocklin, 2005).

AMPs are generally composed of cationic substances in low molecular weight, and perform their functions by destroying cell membrane of microorganism (Bulet and Stocklin, 2005). The function of AMPs is not limited to direct killing of microorganisms, but related with action of immunomodulation and with regulation of endosymbiotic bacteria (Easton et al., 2009; Login et al., 2011). AMPs are classified within three groups according

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to structure and nonspecific richness of specific amino acids: (1) alpha-helical peptides lacking cysteine residues; (2) beta-sheet globular structure stabilized by intramolecular disulfide bridges; and rich peptides to maintain a large proportion of nonspecific proline or glycine residues.

In this review, as we investigated various properties of invertebrate and arthropoda including insect-derived AMPs discovered to date, we supported a basic data to classify the characteristics of insect-derived AMPs.

#### **Datadase for AMPs mining:-**

AMPs used in this review were analyzed by 702 peptides (Apr 2017) registered in InverPep (<http://ciencias.medellin.unal.edu.co/>).

#### **Classification of AMPs according to activity and their physical properties:-**

Most of the 702 AMPs studied in invertebrates have been found by arthropoda including insect. The number of AMPs discovered in Arthropoda was 583 AMPs and equivalent to 83% among total AMPs (Fig. 1). In the remaining phylum, Mollusca, Nematoda and Chordata showed 6.8, 3.3 and 3.0%, respectively. Arthropoda is included by insects, spiders and crustaceans, and 80% or more of existing species are included in arthropod animals.

The antimicrobial activity was accounted for the highest proportion as 520 AMPs against Gram positive bacteria (Fig. 2). The next activity against fungi was kept high amount as 202 AMPs. However, the activity against Gram negative bacteria was very low as 65 AMPs. In an uninduced state, although the antimicrobial activity of insect AMPs was detected by high numbers against Gram positive bacteria, it was hardly detected in Gram negative bacteria (unpublished data). However, when compared to AMPs that are common in nature to have low frequency activity of Gram positive bacteria (Yanmei et al., 2012), AMPs in insects maintain very specific phenomena. While such aspects are surviving in nature, it is assumed that insects are well-exposed to pathogenic Gram positive bacteria, but well-unexposed to Gram negative bacteria.

AMPs originated from insects were found to have not only antibacterial and antifungal properties but also parasite, insect, virus, cancer cell and chemostatic activities. These activities were observed at a very low frequency when compared to antibacterial and antifungal properties, but presented by the possibility to confer its various functionalities.

Generally, well-known AMPs are composed of sizes of 10 kDa or less, and it is known that they were composed of almost 20 to 40 residues (Dimopoulos et al., 1997; Reddy et al., 2004; Wang, 2013). The number of peptides observed in this review was high frequencies between 11~20 and 31~40 residues (Fig. 3). Even though numbers were observed by relatively low amounts between 21 and 30 residues, it is assumed that peptides corresponding to between 11 and 40 residues are excavated by more amounts as AMPs. This result shows a pattern that coincides with the high number of generally found AMPs between 11 and 40 residues (Wang, 2013; APD by July 2017). The number of AMPs at 40 residues or more tended to decrease step by step. Therefore, it is presumed to exhibit a common feature that AMPs in insects maintain higher amount in 11 to 40 peptides.

The secondary structures of insect AMPs were accounted for the most proportion as 376 (53.6%) in the unknown structural peptides. The alpha-helix was the highest ratio at 128 peptides (18.2%) among the known structures, but ciclopeptide and coil form were observed at low frequencies (Fig. 4). Beta-sheet of 22 peptides was observed less frequent ratio than that of rich form. From such a result, it is suggested that alpha-helix maintains an optimal structure to induce antimicrobial action on membrane. However, it is presumed that beta-sheet and coil form are relatively difficult to show their functions via interaction on membrane. On the other hand, it is presumed that ciclopeptide is less frequent due to difficulty in forming the structure itself.

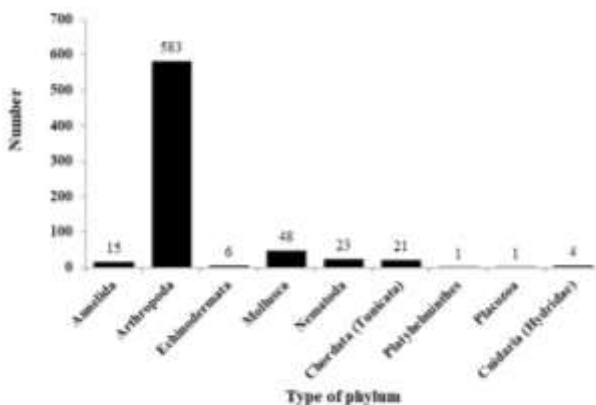
#### **Classification depending on chemical properties:-**

Net charge of AMPs in insect showed all high proportions from +1 to +7 (Fig. 5). This result is somewhat different from the aspect shown in the net charge of generally discovered AMPs. In the general case, net charge displays high values in +1 to +4 (Wang, 2013; APD by July 2017). Therefore, it is assumed that similar levels from +1 to +7 are specific phenomenon of AMPs in invertebrate.

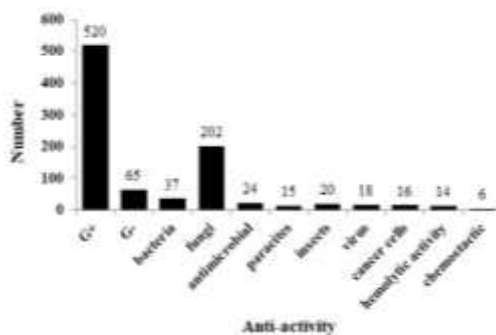
Wide distribution of net positive charge also induced wide distribution of pI value (Fig. 6). The pI values in this review showed a high distribution from 8 to 12. Aliphatic index showed the highest numerical value between 70 and

80, showed a tendency to decrease gradually around this region (Fig. 7). Overall peptides have been shown to maintain a relatively high structural stability with a numerical value of 55 peptides or more above 70% in aliphatic index.

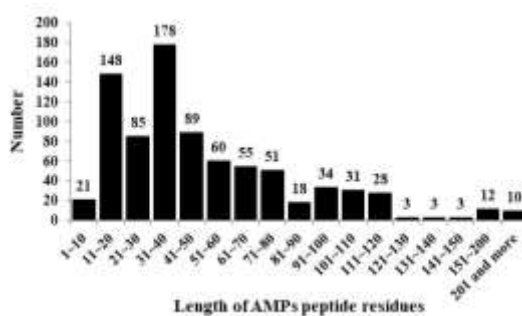
The residues of hydrophobic amino acids maintained the highest value between 30 and 40%, whereas less than 10% or more than 60% showed low frequencies (Fig. 8). In addition, the frequency of commonly known hydrophobic residues in AMPs presented the similar trend as 40 to 60% (Wang, 2013; APD by July 2017). It is assumed that these phenomena have a deep connection with net charge and pI value.



**Fig. 1:- AMPs originated from invertebrate.** Total AMPs employed for this study was 702 peptides obtained from InverPep database.



**Fig. 2:- Functional classification of AMPs originated from invertebrate.** Some AMPs appeared in partially overlapped activities such as antimicrobial activity including antibacterial and antifungal activities. These were indicated by separated number for each activity.



**Fig. 3:- Classification of invertebrate AMPs depending on peptide residues.** AMPs were classified in 10 units. However, 151 to 200 and above 201 were summarized to be tied up together due to small amount in each section.

	beta	ciclopeptido	coil	combine helix and beta	helix	nonhelixbeta	probably helix	rich	unknown	total
Number	22	1	1	58	128	2	23	91	376	702
Percent (%)	3.1	0.1	0.1	8.3	18.2	0.3	3.3	13.0	53.6	100

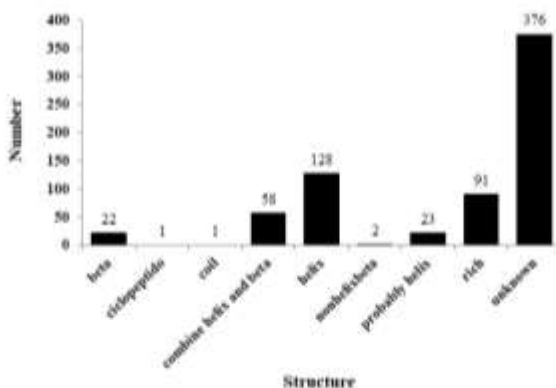


Fig. 4:- Classification of invertbrate AMPs depending on peptide structure. AMPs were classified depending on secondary structure of peptide.

Net charge	≥5	-5--0	0	0--g1	1--g2	2--g3	3--g4	4--g5	5--g6	6--g7	7--g8	8--g9	9--g10	total
Ratio (%)	1.6	5.1	2.1	10.0	10.8	13.0	11.5	11.0	12.7	9.7	3.8	3.3	5.4	100

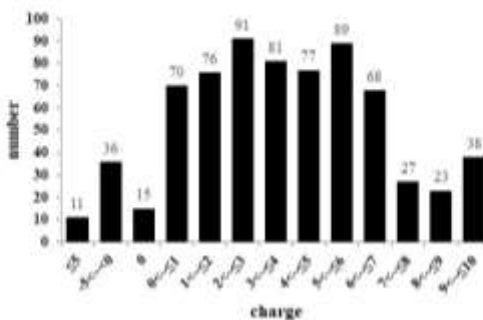


Fig. 5:- Classification of invertbrate AMPs depending on Net charge. AMPs were classified depending on net charge.

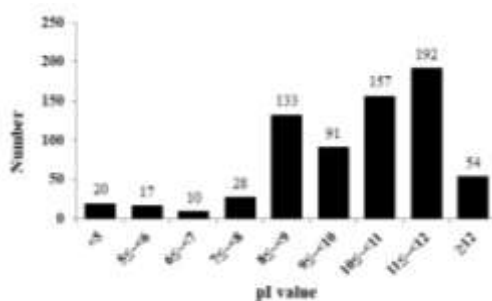
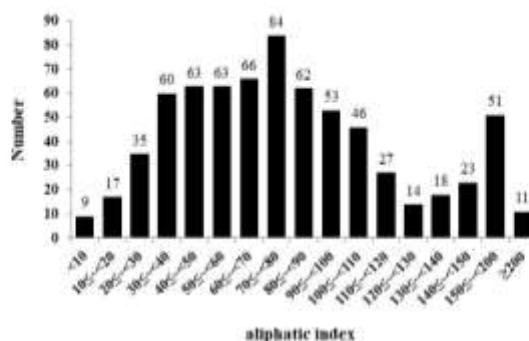
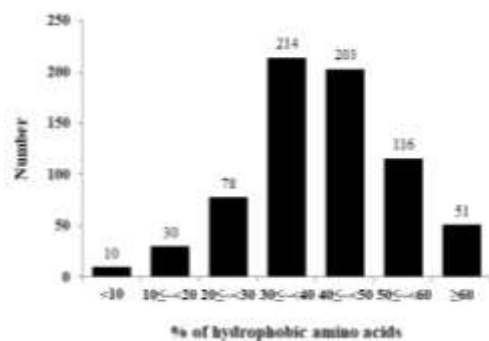


Fig. 6:- Classification of invertbrate AMPs depending on pI value. AMPs were classified depending on pI value.

Range	<10	10≤-20	20≤-30	30≤-40	40≤-50	50≤-60	60≤-70	70≤-80	80≤-90	90≤-100	100≤-110	110≤-120	120≤-130	130≤-140	140≤-150	150≤-200	≥200	total
Ratio (%)	1.3	2.4	5.0	8.5	9.0	9.0	8.4	12.0	8.8	7.5	6.6	3.8	2.0	2.6	3.3	7.3	1.6	100



**Fig. 7:- Classification of invertbrate AMPs depending on aliphatic index.** AMPs were classified depending on aliphatic index.



**Fig. 8:- Classification of invertbrate AMPs depending on hydrophobic amino acids.** AMPs were classified depending on hydrophobic amino acids.

### Conclusions:-

Most of AMPs found in invertebrate were found in arthropod including insect, and to be highly specific for Gram positive bacteria. AMPs showed the highest values by 11 to 40 in length, alpha helix in secondary structure, +1 to +7 in net charge and 70 to 80 in aliphatic index. Therefore, we suggest that AMPs found in invertebrate maintain similar characteristics to general AMPs.

### Acknowledgment:-

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