Journal of Physics: Conference Series

PAPER • **OPEN ACCESS**

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To cite this article: Dina Maulina *et al* 2018 *J. Phys.: Conf. Ser.* **1093** 012009

Identification of Peptides Compounds from *Mirabilis jalapa* L. (Caryophyllales: Nyctaginaceae) Potentially as a Biopesticide

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Abstract. Mirabilis Antiviral Protein (MAP) is a specific protein found in the *Mirabilis jalapa*. The MAP has many promising benefits, one of them is that it can be used as biopesticides. However, the structure and visualization of protein from MAP have not been identified. This study investigated the modelling structures of MAP compounds which potentially become bioinsecticide for insect and the interaction between bonding ligand-receptor in the surface cell. This research used In silico models that refers to the BIOPEP database with the bioinformatics tools methods. The screening of MAP compounds was conducted through hydrolysis steps by 30 enzymes to become new peptides. The new Antimicrobial Peptides (AMPs) were analyzed by a multidimensional statistical analysis using four predicted algorithms, Support Vector Machines (SVM), Random Forest (RF), a Neural Network Artificial (ANN) and Discriminant Analysis (DA) that were available in the Anti-Microbial Peptides Collection (CAMP Database). The results were identified six new types of AMPs from 449 AMPs during the in-silico proteolysis process toward *M. jalapa*. This peptide predicted the function become antimicrobials by inactivating the ribosomes. There were obtained three of the best peptide structures from *M. jalapa* that have the potential as toxin compounds (biopesticides). Through the visualization of the three AMPs, models of biopesticide structures are WIFKTVEEIKLVMGLLKSS, IKLVMGLLKSS, and ITNIRTKVA with the residual category 6-22, and the characterization of molecular weight was 561,7 to 2971,4 g/mol. The AMPs fulfill the Boman Index which the measurement of protein-peptide affinity to build the biological interactions, ranging from -0,51 to 2,98. Further, these renewable peptides can be used to determine the pathways that occur in targeted insect pests as biopesticides.

Keywords: Antimicrobial peptides (AMPs), biopesticide, mirabilis antiviral protein (MAP), *Mirabilis jalapa*.

1. Introduction

The various research about the benefits of secondary metabolite compounds from plants has been studied recently [1-3]. There have been many studies regarding their benefits in pharmacology, medicine and including applications for botanical insecticides [4, 5]. The use of botanical insecticides which derived from natural substances from plant becomes one of the safest alternatives to reduce the number of pest populations. Their applications have not caused chemical residues that would be safe for the environment [6, 7].

One of the botanical insecticides which intended as biopesticides for insect pests is *Mirabilis jalapa* L. (Caryophyllales: Nyctaginaceae). *M. jalapa* has many natural chemical compounds containing triterpenes, proteins, flavonoids, alkaloids, and steroids, alanine, alpha-amyrins, arabinose, beta-amyrins, campesterol, daucosterol, and dopamine [8]. The results of the cDNA sequences of *M. jalapa* plants contain the Mirabilis Antiviral Protein (MAP) compounds [9]. It was potentially as repellent and antifeedant. The MAP has an antiviral and antiviroid substance that functions to eliminating the pathogens that damage the body [10]. The MAP has the ability as an insecticide substance and inactivating the ribosomes' target cells. The specific protein affects to weaken the defense mechanism function of the pest body both physiological and anatomical [11, 12].

The previous study showed that *M. jalapa* able to decreasing the body defense. Target pest by the change number and activity of hemocyte cells from the Spodoptera pest [13]. The exposed larva with *M. jalapa* extract in 0.8% (w/v) concentration for 24 hours experienced the decreased body function signed by decreased movements. The avoiding movement behavior from food sources, vomiting and diarrhea. It shows that the weakening of the insect's immune system caused by inactivating ribosomes which also causes the changes in the mechanism and metabolism of *Spodoptera litura* cells and lead to the changes in overall body activity [14]. The antifeedant compounds which contained in the extract of *M. jalapa* also cause metabolic changes in the function of the gastrointestinal organs.

A number of previous studies on many kinds proteins in *M. jalapa* plant have been widely conducted. However, the special antimicrobial peptides (AMPs) as potentially as a biopesticide and the mechanism in the insect body is not known. The interaction between AMPs with cell membranes in the insect body becomes essential, to further detections the pathway ligand AMPs and receptor binding.

The information related to the structure, visualization and interaction MAP is very critical to understand. It is fundamental to determine the application of *M. jalapa* as a biopesticide. Unfortunately, there is no previous research which revealed the AMPs of *M. jalapa* that potentially as a natural pest control agent. The purpose of this study was to investigate the modeling structures and determine the optimization of the peptides from *M. jalapa*. Moreover, the modeling of the structure of the MAP protein will be known how the cellular interaction occurs inside the insect's body.

2. Materials and Methods

This study used In silico method utilizing bioinformatics tools. The identification of AMPs analyzed the revealed type of protein data sequences. This research is referring to the method developed by Pangastuti *et al.* [15, 16] and Ilmawati *et al*. [17].

2.1. Retrieval of sample:

The peptides compounds in *M. jalapa* was obtained from a Clavilia database [18] of protein structures in the Raintree Nutritions. The cDNA for Mirabilis antiviral protein (MAP) peptide chains were saved in the BIOPEP and protein data bank (PDB).

2.2. Analysis of AMPs from Mirabilis jalapa:

The virtual screening is used to observe the new AMPs from *M. jalapa* as potential as a biopesticide. The analysis was identified by (a) searching for new proteins through FASTA sequences from MAP by the UniProt database [19], (b) predicting the proteins by cutting the structure using enzymes in the BIOPEP database (c) discovering antimicrobial peptides in the Anti-Microbial Peptide collection

(CAMP) database (d) using physicochemical properties in the Innovagen database [20] and antimicrobial database (APD2) [21] e) making prediction of new AMPs, and (f) determining the best protein.

Initially, the collection of the MAP FASTA sequence from the UniProt database to search the new of AMPs. Moreover, the database of sensory peptides and amino acids has an identical structure to the BIOPEP database of biologically active peptides. The information about sensory peptides and amino acids was inserted into the database using standard BIOPEP layouts for bioactive peptides. Information about the biological activity of sensory peptides. The sequence in the BIOPEP database to obtain a new peptide with peptide cutting process using 30 enzymes available in the database.

In addition, the new peptide analyzed by multidimensional statistical analysis as a support vector machine (SVM), random forest (RF), artificial neural network (JST) and discriminant analysis (DA) that available in the CAMP database. The Acquisition of AMPs was selected and compared to the data source through the new AMPs obtained.

The prediction of new AMP was analyzed with the following parameters, such as molecular weight, residual amount, extinction coefficient, iso-electric point, net cost at pH 7, and the predicted solubility using Innovagen and Boman Index using APD2 database. The last stage, the determination of the best protein structure which declared as a biopesticide compound from *M. jalapa* was visualized in 3D.

3. Results and Discussion

3.1. Results

The acquisition of information on the *M. jalapa* potentially as biopesticide was obtained the MAP sequence in the UniProt database. The BIOPEP data source would identify the MAP proteins contained in *M. jalapa*. Further, the peptide chains were cut by using the enzymes that produce polypeptide or peptide. Further, the pieces of polypeptide and peptide were analyzed to find out which polypeptide or peptide which has the function as Anti-Microbial Peptide by selecting to the CAMP database.

The results of the CAMP database by multidimensional statistical analysis supported vector machine (SVM), random forest (RF), artificial neural network (JST) and discriminant analysis (DA). The analysis results showed that there are 6 of 449 potential peptides as antimicrobial peptides. These AMPs compared with manual tools in the BIOPEP database to confirm the recency of the acquired AMP peptide. The six new AMP peptides from *M. jalapa* are summarized in Table 1, and the individual physicochemical AMP properties of *M. jalapa* was presented in Table 2.

There were three amino acids from the analysis according to the best characteristics (Seq. Id. 102, 113, and 395) with the residual category 6-22. This characterization is based on the molecular weight of 561.7 to 2971.4 g/mol. The positively charged peptides are the majority of antimicrobial peptides known as cationic antimicrobial peptides (CAP) [22], which have an isoelectric point close to 10, meaning that the AMP tends to precipitate and lose its biological function [23]. All those AMPs fulfill the Boman Index which the measurement of protein-peptide affinity and its ability to build the biological interactions, ranging from -0.51 to 2.98 for the AMP protein from *M. jalapa* [24]. According to the physicochemical nature of AMP, it was found that AMP with the sequence number ID 102, 113, and 395 is the best AMP (Figure 1).

Table 1. The new AMPs of *Mirabilis jalapa* with physicochemical properties support

seq. id	Sequence	SVM	RF	ANN	DA
98	LGIFRLENSIVNIYGKAGDV KKQAKFFLLAIQMVSEAARFKYISDKIP	0.527	0.5285	AMP	0.732
102	WIFKTVEEIKLVMGLLKSS	0.858	0.7505	AMP	0.681
113	IKLVMGLLKSS	0.982	0.5875	AMP	0.806
336	SKPSTTTATKCQLATSPVTISPWIFKTVE	0.982	0.5875	AMP	0.806
381	YGDLEKNGGLRKDNPLGIFRLEN	0.820	0.598	AMP	0.656
395	ITNIRTKVA	0.987	0.530	AMP	0.810

Super vector machine (SVM), random forest (RF), artificial neural network (ANN) and discriminant analysis (DA) multidimensional statistical analysis.

The 3D visualization peptides result from the MAP individual above becomes a bright spot to understand the bounding among membranes that may occur inside the target insect body. The visual formation of specific proteins in *M. jalapa* plants is a reference for knowing the cellular pathway in weakening and controlling insect populations. The cellular pathway determination will be an important reference to knowing the further study and the synergism of these compounds in the insect body. Therefore, these findings indicate that *M. jalapa* plant as biopesticide that can be used in controlling insect pest populations which can be assessed through cellular levels.

Table 2. The physicochemical characterization of the AMPs from *Mirabilis jalapa*

Seq. ID	Sequence	Number of residues	Mass weight (g/mol)	Gravy	Boman index (kcal/mol)	Extinction coefficient ($M^{-1}cm^{-1}$)	Isoelectric point (pH)	Net charge at pH 7	Estimated solubility	Aliphatic index	Instability index
98	LGIFRLENSIVNIYGKAGDV KKQAKFFLLAIQMVSEAARFKYISDKIP	48	5426.45	0.18	0.86	2560	10.08	2	Good	109.79	12.95
102	WIFKTVEEIKLVMGLLKSS	19	2221.73	0.59	-0.08	5690	9.71	1	Good	133.16	25.59
113	IKLVMGLLKSS	11	1188.54	1.11	-0.82	0	10.73	2	Poor	168.18	3.45
336	SKPSTTTATKCQLATSPVTISPWIFKTVE	29	3105.61	0.08	0.94	5690	9.58	1	Poor	67.24	38.94
381	YGDLEKNGGLRKDNPLGIFRLEN	23	2612.93	-1.01	2.64	1280	0	-1	Good	84.78	8.13
395	ITNIRTKVA	9	1015.22	0.19	1.84	0	11.41	2	Good	130	31.58

The grey column shows the AMPs from the analysis according to the best characteristics.

3.2. Discussion

This study observed the MAP compound peptide *M. jalapa* which is an antimicrobial compound potentially as a biopesticide. There are three types of peptide sequences were found WIFKTVEEIKLVMGLLKSS, IKLVMGLLKSS, and ITNIRTKVA. AMPs of these findings are specific compounds of *M. jalapa* containing tannin, triterpene, flavonoids, isoflavones, alkaloids, coumarins, and proteins. This component was secondary metabolites with the specific function as antifeedant and repellent for insects.

Flavonoids are antibacterial compounds that have complex proteins extracellular structure and dissolved. This structure caused damage to the cells followed by the discharge of intracellular component [25]. Also, to their role in DNA-RNA inhibition and synthesis by intercalation or hydrogen bonding with a nucleic acid base accumulation. Flavonoids played a role in inhibitor energy metabolism according to absorb various active metabolites and macromolecular biosynthesis when the cells require sufficient energy.

Tannin is a compound that has a destructive mechanism in insect cells as an inhibitor with activates cell adhesion (the binding molecule on the host). The located of binding was on the surface of cells and enzymes to disrupt the transport of proteins inner layer of cells. Tannins have the receptor targets in the cell membranes polypeptides and capable of damaging it. Since tannins are parts of phenol compounds, it causes to readily be a soluble in the water.

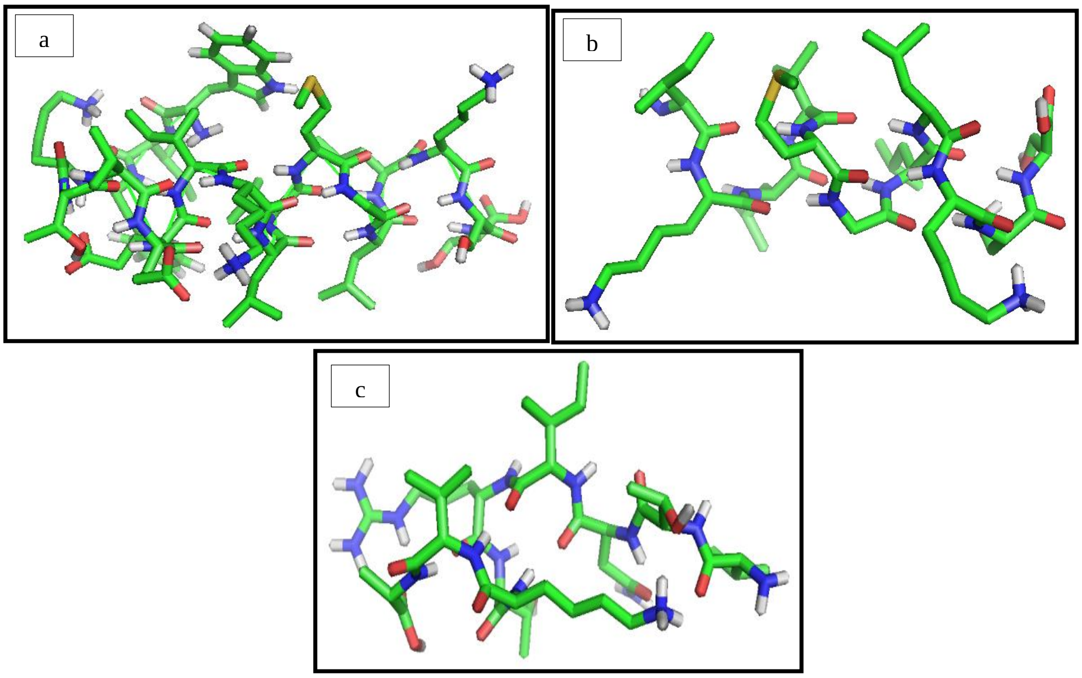

Figure 1. The AMPs sequence of *Mirabilis jalapa* visualized using PyMOL$^+$ software (a) Sequence 102 WIFKTVEEIKLVMGLLKSS (b) Sequence 113 IKLVMGLLKSS (c) Sequence 395 ITNIRTKVA.

The phenol compounds will attack a polar group (phosphate group). Additionally, the phospholipid molecule will break down into glycerol, carboxylic acids, and phosphoric acid. The phospholipid components were not able to recover the damaging cell membrane and impact to the cell membrane would be detrimental and experiencing growth barrier. The next stage, when the phospholipid layer was a very thin condition, phenol could penetrate easily and damage cell contents.

The mechanism of antimicrobial works in inhibiting the organism is varied and complex. Antibacterial compounds can work bacteriostatic ally, bacteriocidal, and bacteriologically [26]. The previous studies reveal that MAP is a compound that has the ability as a protein, also capable of disabling ribosomal activity in the target cells [10]. These compounds are allegedly damaged to target insects cell. Based on the previous review, the information obtained that MAP is antiviral and antiviroid substances for *M. jalapa* plants which eliminate pathogens that damage the body. The identification toward the active compounds contained shows that there are four main components of the MAP: 3,3methylenebis (4-hydroxycoumarin); N-D-alpha-phenyl glycine; Laminaribiitol; and 3-(4-(dimethylamino) cinnamoyl) -4hydroxycoumarin [27]. The potential of MAP compounds as biopesticides are proved by the results of studies showed a decreasing in physiological function indicated the cellular and humoral immune responses as parameters the decrease in hemocytic amount, phagocytosis activity, and lectin activity when exposed with *M. jalapa* [11, 14].

The function of *M. jalapa* as a biopesticide principally intended to regulate the number of pests with active MAP compounds contained therein which do not harm other non-target organisms. Based on the characteristics of insect mortality caused by the application of *M. jalapa*, it can be used as a reference in natural pest control. The larvacidal feature that causes death on insects, the content of flavonoids, saponins, and tannins from *M. jalapa* leaf extract is a repellent compound for insects. The saponins compounds, flavonoids, triterpenoids, alkaloids and fatty oils are larvicidal (toxic) for insects [28]. The content of these compounds can disrupt the function of nerves by inhibiting the acetylcholinesterase enzymes in breaking the neurotransmitters into acetic acid and choline, which affect in the coordination disorders of the larval muscle system [29, 30].

4. Conclusion

M. jalapa has six peptides with the potential as antimicrobials based on the CAMP database. According to the physicochemical characteristic of AMP, the three new peptides were found with the best characterization of *M. jalapa* plants that have the potential as a biopesticide; there were sequence ID 102 WIFKTVEEIKLVMGLLKSS, sequence ID 113 IKLVMGLLKSS, and sequence ID 395 ITNIRTKVA. These renewable peptides used to determine the pathways that occur in targeted insects as biopesticides.

Acknowledgments

The authors express their sincere thanks to Ms. Ardini Pangastuti, S. Pd., M.Si. for her assistance in In-silico methods. We would like to thank Doctoral Dissertation Research Grant, Directorate of Research and Community Service, Directorate General of Research and Development Strengthening of Research Ministry, Technology and Higher Education of Indonesia which has provided a funding for this research.

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