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Original Article

Alanine as natural biopesticide from *Mirabilis jalapa* and its interaction with glutamate as an inhibitor in insects immune system

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Abstract

Alanine is a secondary metabolic secretion from *Mirabilis jalapa.* It is used as a modulation of natural pesticide compound. Alanine is predicted to act as an inhibitor compound in the defense mechanisms in the insect body. This study investigated the modeling structures and the protein receptor from alanine in the immune system mechanism and their optimization of the effectiveness as biopesticides to the insects. This research was conducted using an in silico modeling through the reverse docking stage, including the collection of 3D structures of natural compounds, prediction of target protein, receptor profiling, clarification of the potential of alanine compounds based on mode of action with PyRx 0.8 software, and interaction visualization between alanine compounds with the target protein using PyMOL and LigPlus$^+$ software. The results showed that the alanine compounds from *M. jalapa* plants have targeted well the glutamate receptors in the insects. Both alanine and glutamate compounds have the same interactions with the glutamate proteins receptor shown by the Van der Waals interaction, e.g. the hydrogen bonding on certain amino acids has binding affinity of -15.889 kJ/mol. The interaction between alanine and glutamate affects the signal transduction cascade at the cellular level due to a neuromuscle and olfactory inhibitor. In addition, the continuous interruption of signal transduction affects the dysfunctional immune system of the insect body leading to their mortality.

Keywords: alanine, glutamate, insect immune system and *Mirabilis jalapa*.

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Introduction

Plants are generally synthesizing various forms of primary and secondary metabolites. The regulation of the primary synthesis produces carbohydrates, lipids, proteins, and nucleic acids, which are used by the plant to grow. On the other hand, the secondary synthesis produces the lipid precursor and aromatic amino acid which have the bioactivity and protective capability from the disturbance of various pest and disease towards the plant and the environment (Adeyemi, 2010). Both primary and secondary metabolites have several application and benefits for the plant itself, as medicinal material, and other ecological balancing functions (Daniel *et al*., 1999).

The secondary metabolite compounds have been studied recently (Wink, 2016., Mohamed, 2017; Delgoda, 2008; Pavarini, 2012; and Zhong, 2011). There have been many studies regarding their benefits, including various applications for botanical insecticides. The use of natural synthesis pesticide has a safe impact and easy to use by the farmers (Rattan, 2010). Phenol is one of the secondary metabolic secretion from *Mirabilis jalapa*. The processing of plant secondary metabolites as the modulation of natural pesticide compounds from phenol is now widely conducted. It is used to control the insect population in the nature, therefore, the ecological balance can be maintained.

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M. jalapa, as one of the wild herbaceous plants widely spread in Indonesia, produces secondary metabolites which can be utilized as biopesticide (Maulina, 2014; Suryani, 2014).

Several previous studies Maulina (2014) and Suryani (2014) revealed that the leaf extracts from *M. jalapa* are able to control the pest population in terms of decreasing the immune system. Alanine is one of secondary metabolite produced from *M. jalapa*. The leaves of *M. jalapa* are rich of alanine content (Taylor, 2015). In study by Daniel (1999), alanine is a single compound which is able to interact with other compounds and has a significant role in plant various synthesis. The synthesis of phenylalanine compounds in the shikimate pathway is the starting point for phenol synthesis in plants (Heldl, 2015).

Alanine is a phenol compound that has an important role for the control of herbivorous insects because the bioactivity of this alanine peptide acts as a natural toxin. As an active compound, it offers a benefit as natural pesticides. It acts as an inhibitor that disturb the signaling mechanisms in the insect metabolism processes.

Accurate information related to the structure, bonding of alanine and receptor, and interaction both of them in insect bodies is very critical to understand. It is fundamental to determine the application of *M. jalapa* as biopesticide. Some previous studies have suggested that *M. jalapa* potentially serves as a herbal medicine for human, including as anti-bacterial, anti-inflammatory, anti-cancer, anti-fungal, and anti-diuretic (Gogoi, 2016; Walker *et al.*, 2013; Zachariah, 2011). In addition, it is also potential to treat several diseases such as diarrhea, dycentery, and abdominal colic (Walker *et al.*, 2013; Naveed, 2010;

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Neugart, 2017). However, to the best of authors' knowledge, there is no previous research which revealed the use of *M. jalapa* as a natural pest control agent. This kind of immune system approach is believed to potentially offer an environment-friendly pest control.

In this study, we predicted the mechanisms of immune systems and their molecular pathways, which lead to the signaling disorder by generating the second messenger that consequently destroy the defense immune systems of the insect. Therefore, this study investigated the modeling structures and the receptor bonds of alanine in insect immune system mechanism. In addition, the purpose of this study was to determine the optimization of the effectiveness of biopesticide from *M. jalapa*. Through the modeling of the alanine receptor structure, it is expected that the mode of action from its bond, acting as an inhibitor compound, can be clearly observed. Hence, the application of *M. jalapa* plant as a biopesticide for the insect pests, through the approach of weakening mechanisms that occur against insect body defense system, can be clearly measured

Methods

This research was conducted using in silico modeling referring to the method developed by Pangastuti *et al.* (2016, 2018) and Ilmawati *et al*. (2017).

Retrieval of Sample:

The compounds in *M. jalapa* was obtained from a Clavilia database (Clavillia Plant Database, 2004) of protein structures in the Raintree Nutrions. Table 1 represents the chemical compounds of the leave of *M. jalapa.*

In the study by Kastritis (2012), the flavonoid is peptide chains as a natural toxin that potential for biopesticide. Based on Table 1, the flavonoid compounds include alanine, leucine, trigonelline and tryptophan. Among those compounds, alanine is the most abundant compound. Therefore, alanine was selected as a specific sample for analysis. In addition, the compositions of alanine were obtained from the compounds database of PubChem.

Molecular Docking Process

The molecular reverse docking is used to observe the interactions of alanine with a target protein. It was identified using: (a) collection of 3D natural compounds structure, (b) predictions of target protein, (c) receptor profiling, and (d) clarification of the potential of alanine compounds based on mode of action using PyRx 0.8 software.

Initially, collection of natural compounds structure from PubChem was used to identify the biological activity from small molecule (Wang *et al.*, 2012). The PubChem was confirmed by three databases that are connected with NCBI's information systems, including PubChem substance, PubChem compound, and PubChem BioAssay. Therefore, 3D structure of the alanine can be obtained.

Predictions of target protein was identified using PharmMapper, Superpred , and Swiss Target Prediction. They were used to identify the bioactive molecule in the insect body. It was performed in order to observe the molecular mechanism that is fundamental as phenotype and their site biological activity.

Moreover, receptor profiling process was obtained using two online databases: UniProt and RSCB Protein Data Bank. The 2D and 3D structures are required to further analyze the receptor proteins.

Table 1. The chemical compounds contained in the leave of *M. jalapa*

Compound	Chemical Type	Ref.
Alanine	Proteid	K07026 K01698
Arab inito l, 2-ca rbo xy:	Carbohydrate	K22890
Campesterol	Steroid	K01698
Citric acid	Alkane to c4	K01698
Dotriacontane, n:	Alkane c5 or more	K01698
Glycine	Proteid	K07026 K01698
Hentriacontane, n:	Alkane c5 or more	K01698
Heptacosane, n:	Alkane c5 or more	K01698
Hexacosan-1-ol	Alkanol c5 or mo re	K01698
Hexacosane, n:	Alkane c5 or more	K01698
Leucine	Proteid	K07026 K01698
Lignoceric acid	Lipid	K01698
Nonacosane,n:	Alkane c5 or more	K01698
Octacosane, n:	Alkane c5 or more	K01698
Pentacosane, n:	Alkane c5 or more	K01698
Pentatriacontane, n:	Alkane c5 or more	K01698
Tartaric acid	Alkane to c4	K01698
Tetracosane, n:	Alkane c5 or more	K01698
Tetratriacontane, n:	Alkane c5 or more	K01698
Triacontane, n:	Alkane c5 or more	K01698
Tricosan-12-one	Alkano ne c 5 or more	K01698
Tricosane, n:	Alkane c5 or more	K01698
Trigonelline	Proteid	W01270
Tritriacontane, n:	Alkane c5 or more	K01698
Tryptophan	Proteid	K07026 K01698

Note: The grey column shows the compound containing protein.

The last stage was performed using the Vina Wizard feature which is integrated in PyRx 0.8 software. It was minimized to obtain the most suitable conformation before the interaction with the target protein. Information obtained from KEGG Pathway Enrichment analysis was

clarified to identify the action mechanism of alanine compounds towards the target protein concerned, the activator or inhibitor. The natural compound and the control inhibitor compounds (inhibitor compounds or target protein activator) were used as a ligand in this step. Overall, the natural compound and the control inhibitor compounds (inhibitor compounds or target protein activator) were used as a ligand in this step.

Molecular Interaction and Visualization

The molecular interaction and visualization of the docking results were analyzed using PyMOL and LigPlus^{+} software. In this stage, a profiling visualization of an interaction between alanine compounds with the target protein was conducted. Therefore, all of the alanine and receptor that interact directly can be observed. In addition, the new alanine receptor was analyzed for their structure and function mechanisms in the insect body metabolism.

Results

Collections of Three Dimensional Structure of Alanine Compound

Fig 1 shows the visualization of 3D structures of natural compounds obtained from the PubChem database in Sybil data files format (*.sdf).

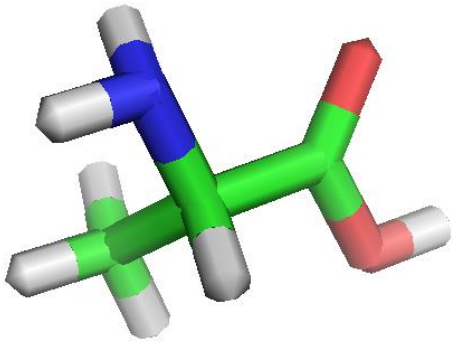

Figure 1. 3D structure of alanine compounds visualized through PyMol software (PubChem. DL-Alanine, 2017)

Prediction Result of Target Protein as a Receptor in the Insect Body

The target protein compounds were obtained through several prediction with the target proteins in PharmMapper webserver. PharmMapper predicts the target protein based on the similarity of pharmacophore between the compounds and the protein target (query-structure). The similarity was matched using both drug and other non-drug compounds that have been analyzed previously in vitro and in vivo (Liu *et al.*, 2010). Furthermore, SuperPred and Swiss Target Prediction webservers were used in order to predict the target protein interaction of a compound, based on structural similarity between the structure of the target compounds (query structure) with the approved drug structure and non-drug compounds that have been analyzed in vitro and in vivo (Dunkel *et al.*, 2008; Gfeller *et al.*, 2014).

The target proteins which have been identified using PharmMapper, SuperPred, and Swiss Target Prediction were further complemented by a number of other information including Gene ID, synonym names, molecular function and the involvement of each protein which was related to the biological processes within the insect body by referring to the information provided in the Uniprot database (Tab 2). Based on the data analysis with the same homology, it was clarified that glutamate protein shows the best performance as the receptor of alanine compounds. Fig 2 represents a visualization structure of alanine and glutamate protein as the receptors in the insect body. The 3D modeling structure of target protein was obtained from PharmMapper, SuperPred, and Swiss Target Prediction.

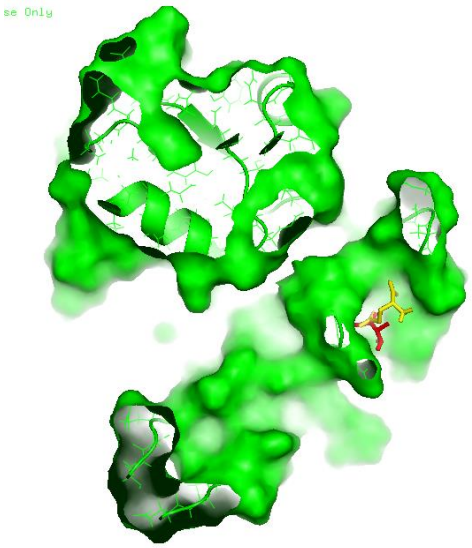

Figure 2. 3D structure of glutamate protein receptor (UniProt, Glutamate receptor 1, 2017)

Visualization of Interaction Between Alanine Compounds with Glutamate Target Protein

After collecting the 3D structure of alanine compounds, control inhibitors and target proteins, the molecular docking was performed using PyRx 0.8 to obtain the best binding pose with the lowest affinity bindings. The compounds with the lowest affinity bindings were saved in protein data bank (pdb) file format and ready for 3D visualization using PyMol and visualized into 2D scheme using Discovery Studio software.

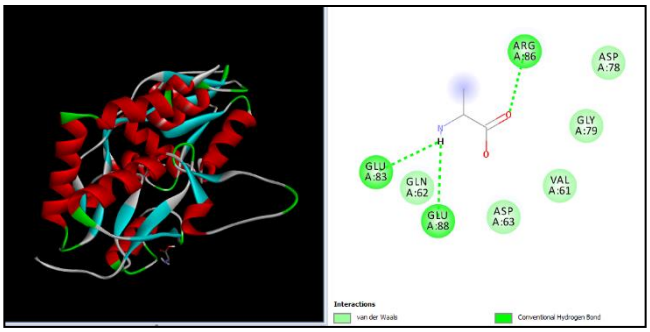

Figure 3. 2D visualization of alanine with glutamate receptor using Discovery Studio software.

The reverse docking is an analytical technique that is performed to determine the potential of a compound against the target protein in the body of a living organism (Zhang *et al.*, 2014; Zheng *et al.*, 2011). Through reverse docking technique, the potential of the compound can be detected through the site binding on the target protein compared with the commonly used control compounds. In addition, using a molecular docking using PyRx software, the potential of natural compounds as biopesticide with the affinity of binding to the target proteins can also be measured (Kastritis, 2004).

The binding affinity is an important aspect to be considered in molecular and macro molecular interactions (Seo *et al.*, 2014). A lower binding affinity indicates that a compound requires less energy to engage in its binding or interaction. In other words, the lower affinity value of binding increases the potential for binding with the target protein (Baker *et al.*, 2007; Tassa *et al.*, 2010). From molecular docking visualization using the LigPlus+ software, it is clear that the alanine compound interacts with glutamate through Van der Waals interaction and hydrogen bonding on certain amino acids with binding affinity of -15.889 kJ/mol (Tab 3).

Fig 4 shows 2D visualization illustrates the binding between the control compounds, which became the glutamate receptor protein ligand (glutamate with the alanine compounds). Moreover, Fig 5 shows the 2D visualization between glutamate with receptor protein. The visualization shows that alanine compounds and glutamate have the same interaction with glutamate receptor protein by hydrogen bonding on amino acid residues number of Ala95, Thr97, Arg102, Ser148, Thr149, Tyr179 and hydrophobic interactions such as at the residues number of Tyr66, Met96, Leu144, Gly147, and Glu198.

Table 2. Alanine target compounds prediction

Target	Uniprot ID	Gene Code	ChEMBL ID	By Homology	Probability	Number of sim. cmpds (3D)	Number of sim. cmpds (D2)	Target Class
Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	TDP1	CHEMBL1075138	No	0.9	1	2	Enzyme
Glutamate receptor ionotropic	NMDA 1	Q05586	GRIN1	CHEMBL2015	Yes	0.63	1	3 ion chanel
Complex	O75899& Q9UBS5	GABBR2&GABBR1	CHEMBL2111463	No	0.34	1	1	Membrane receptor & Membrane receptor
Sodium- and chloride-dependent GABA transporter 1	P30531	SLC6A1	CHEMBL1903	No	0.34	1	1	Transporter
Sodium- and chloride-dependent GABA transporter 3	P48066	SLC6A11	CHEMBL5208	No	0.34	1	1	Transporter
Sodium- and chloride-dependent taurine transporter	P31641	SLC6A6	CHEMBL5762	Yes	0.34	1	1	Unclassified
Sodium- and chloride-dependent betaine transporter	P48065	SLC6A12	CHEMBL3715	Yes	0.34	1	1	Transporter
Sodium- and chloride-dependent GABA transporter 2	Q9NSD5	SLC6A13	CHEMBL4535	Yes	0.34	1	1	Transporter
Gamma-aminobutyric acid receptor subunit gamma-2	P18507	GABRG2	CHEMBL1788	Yes	0.34	2	2	Ion channel
Gamma-aminobutyric acid receptor subunit gamma-1	Q8N1C3	GABRG1	CHEMBL4044	Yes	0.34	2	2	Ion channel
Gamma-aminobutyric acid receptor subunit gamma-3	Q99928	GABRG3	CHEMBL2739	Yes	0.34	2	2	Ion channel
Sodium- and chloride-dependent creatine transporter 1	P48029	SLC6A8	-	Yes	0.34	1	1	Transporter
Egl nine homolog 1	Q9GZT9	EGLN1	CHEMBL5697	No	0.3	1	1	Enzyme
Egl nine homolog 2	Q96KS0	EGLN2	CHEMBL3028	Yes	0.3	1	1	Enzyme
Microtubule-associated protein tau	P10636	MAPT	CHEMBL1293224	No	0.24	1	1	Unclassified

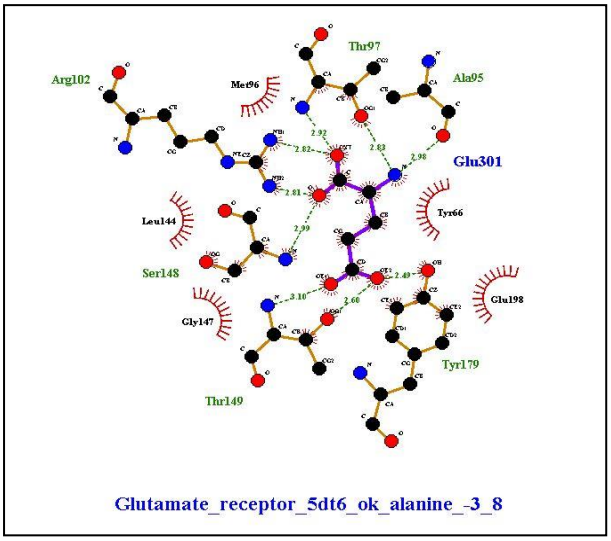

Figure 4. 2D interaction visualization between alanine and glutamate receptor using LigPlus^{+} software

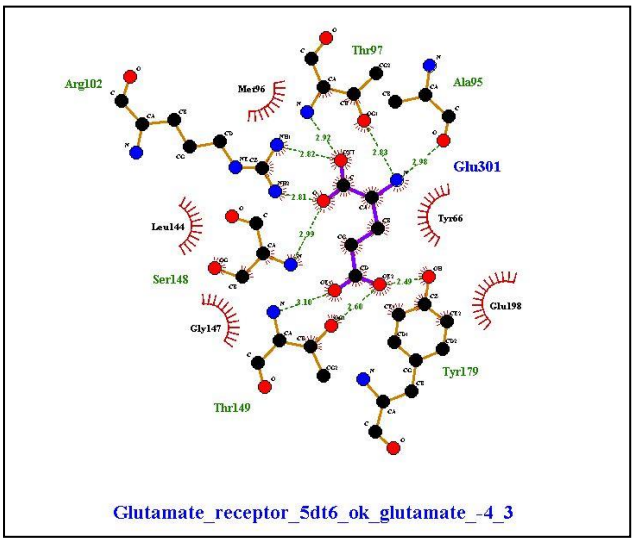

Figure 5. 2D visualization of interaction between glutamate and glutamate receptor using LigPlus^{+} software

Table 3. The similarity binding affinity the ligand and the glutamate receptor

Ligand	Receptor	Binding affinity (kJ/mol)
Glutamate	Glutamate_receptor_5dt6_ok_	-17.991
Glutamate	Glutamate_receptor_5dt6_ok_	-17.991
Glutamate	Glutamate_receptor_5dt6_ok_	-17.573
Glutamate	Glutamate_receptor_5dt6_ok_	-17.573
Glutamate	Glutamate_receptor_5dt6_ok_	-17.154
Glutamate	Glutamate_receptor_5dt6_ok_	-17.154
Glutamate	Glutamate_receptor_5dt6_ok_	-17.154
Glutamate	Glutamate_receptor_5dt6_ok_	-16.736
Glutamate	Glutamate_receptor_5dt6_ok_	-16.736
Alanine	Glutamate_receptor_5dt6_ok_	-15.889
Alanine	Glutamate_receptor_5dt6_ok_	-15.480
Alanine	Glutamate_receptor_5dt6_ok_	-15.062
Alanine	Glutamate_receptor_5dt6_ok_	-14.226
Alanine	Glutamate_receptor_5dt6_ok_	-14.226
Alanine	Glutamate_receptor_5dt6_ok_	-14.226
Alanine	Glutamate_receptor_5dt6_ok_	-14.226
Alanine	Glutamate_receptor_5dt6_ok_	-14.226
Alanine	Glutamate_receptor_5dt6_ok_	-14.226

Discussion

This study observed and found that in insect, alanine compounds of *M. jalapa* have glutamate target receptors. The binding of alanine and glutamate has similarity of interaction with the glutamate protein bonds on residues number of Ala95, Thr97, Arg102, Ser148, Thr149, Tyr179 and hydrophobic interactions among the residues number of Tyr66, Met96, Leu144, Gly147, and Glu198 (Fig 4 and 5). These receptor ligand bonds are used as proof that alanine compounds have the potential to interfere with the immune mechanisms in the insect body.

The previous research (Anis *et al.*, 1981; Feader, 1970; Kolodziejczyk *et al.*, 2008; Mishbach *et al.*, 2014) informed that the path of glutamate cascade in insects is a signaling pathway in the nervous system with the target at the regulatory center of neuromuscle and olfactory. Glutamate is a neurotransmitter that has the main AMPA

receptors, NMDA receptors, and metabotropic glutamate receptors (Leboulle, 2013). As a neurotransmitter, glutamate in the nervous system pathway affects the mechanism of the immune system. These relationships do not work directly, but they are mediated by the hormonal system (Adamo, 2006; Klowden, 2013). The nervous system generally generates electrical impulses and alters the chemical messages in the synapses and then passes them toward the next connected neuron. The neurotransmitter is bound directly to the receptors located in the post synapse directly, not through the blood vessels. This mechanism gives a number of local responses to the synapse and give the respond to endocrine cells.

Alanine on *M. jalapa* serves as a biopesticide which is capable of acting as an inhibitor for glutamate (referring to the visualization results in Fig 4 and 5) in the insect body by changing the conformation of glutamate structure as neurotransmitter. Alanine, as a glutamate inhibitor, effects the blocking glutamate in its original receptors in the insect body (Anis *et al*., 1981). This causes non-response to the muscle and olfactory excitatory pathways of insects (Kolodziejczyk *et al.*, 2008;.

The signal disturbance from pathway mechanism of neuromuscle effects the disruption of the mechanism of muscle movement throughout the insect (Anis *et al.*, 1981; Feader, 1970). This includes not only the dysfunctional in body movement, but also the interference to the muscle mechanisms in the respiratory system, digestion and blood circulation. These disruptions cause a decrease in motion activity leading to a paralyzed insect. This was clarified through the previous Maulina (2013) experimental test on the *Spodoptera larvae* which it stopped eating after 12 h exposure of *M. jalapa*. Cessation of feed consumption leads to malnutrition causing to metabolic disorders characterized by impaired physiological function, resulting in damage in tissue, organ and organism.

The same case also happened in the olfactory mechanism blocking glutamate which also resulted in disruption mechanism. An olfactory nerve cells disorder causes the malfunction in sensory transduction. Therefore, the potential action from stimulus does not work (Stengl *et al*., 1999). The effect of this condition is the failure to receive and continue the stimuli from source to the olfactory regulatory center. It leads to metabolic disorders and the breakdown of body resistance.

The disturbance to neuromuscle and olfactory responses make the insects become unadaptive to the environment; and these conditions causes the collapse of insect body defenses (Mishbach *et al.*, 2014). The weakening of various responses on the entry of foreign substances is an indication of a weakening of the insect defense system. The toxicity of the *M. jalapa* biopesticide leads to the death of the marked larvae, characterized by the changes of cadaver to become flaccid, the shrinking of the body size, and the body color become blackish and smelly (Maulina, 2013). These characteristic indicate a melanization phenomenon as a form of self-defense against the exposure to foreign matter.

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